Identification, Characterization and Mapping of *LrCen*, a New Seedling Leaf Rust (*Puccinia triticina*) Resistance Gene in Spring Wheat (*Triticum aestivum*)

by

Marley Boyce

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ABSTRACT

Leaf rust (*Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex Desmaz. f. sp. *tritici*)) is an economically significant pathogen affecting wheat (*Triticum aestivum* L.) crops globally. Genetic resistance is the most cost effective and environmentally friendly means to control for rust diseases. Rapid evolution of new races of *P. triticina* means that new sources of resistance need to be identified. With advances in molecular technologies, the identification of markers flanking resistance genes has become an effective means of integrating one or more genes into a breeding program through marker assisted selection.

The objective of this study was to characterize and map two putatively novel sources of leaf rust resistance in spring wheat. The first was a leaf rust resistance gene (*Lr*) identified in a screening of *Aegilops taschii*, the D genome progenitor of spring wheat. The second was an unknown *Lr* gene identified in the Thatcher-*Lr1* (Tc-*Lr1*) near-isogenic differential lines (NIL), RL6003, with the appearance of a new avirulent race of leaf rust, TDBG. This gene was temporarily designated as *LrCen* and believed to be introduced from the *Lr1* donor, Centenario. A phenotypic and genotypics analysis of North American and some foreign germplasm was performed to observe the distribution of *LrCen* and confirm the source of introduction into the Thatcher-*Lr1* NIL. Additionally, *LrCen* was mapped to the long arm of chromosome 7A, a similar genetic location as previously mapped *Lr20*. This relationship was further explored to distinguish *LrCen* from *Lr20*.

The resistance identified in *Ae. tauschii* was transferred to a synthetic hexaploid creating the resistant parent 98-223-27. Subsequently, a double haploid (DH) mapping population was developed from the cross Thacher/98-223-27 (Z01M). Phenotyping of 167 DH lines with *Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex Desmaz. f. sp. *tritici*) race BBBD revealed a single hypersensitive leaf rust resistance gene segregating at the seedling stage. Two-point linkage analysis of the genotypic segregation of adult plant resistance (APR) gene *Lr34*, described by the diagnostic marker Indel 11, and the seedling

resistance phenotypes identified linkage between the two genes. Haplotyping of chromosome 7DS in the Z01M DH population and subsequent mapping confirmed the localization of the seedling resistance gene distal to *Lr34*.

The novel seedling resistance identified in RL6003, *LrCen*, was isolated in a Thatcher background and DH population was generated from the cross Tc-*LrCen*/Sumai3-*lr34*. 180 DH individuals were phenotyped using *LrCen* avirulent race, TDBG, and fit the expected 1:1 segregation for a single gene. Parental lines and 92 DH lines were genotyped using the Illumina Infinium assay with the iSelect 90K wheat SNP array. Two-point linkage was conducted between the phenotypes and genotypes. A data base search using the linked marker sequences provided a putative mapping location of the long arm of chromosome 7A. This mapping location was supported by genotyping of the 7AL chromosomal region with microsatellite markers localized to 7AL in published consensus maps. *Gwm344* (9.5 cM) and *cfa2240* (0.6 cM) were identified as SSR markers flanking *LrCen*.

In a study of wheat germplasm the origin of *LrCen* appears to trace back to the susceptible parent Thatcher, not Centenario as was initially proposed. This study suggests heterogenous distribution of *LrCen* in Thatcher and many of the cultivars that have been developed with Thatcher in their pedigree. Mapping of *LrCen* to chromosome 7AL, the same general region as previously characterized seedling leaf rust resistance gene *Lr20,* prompted studies on the relationship of the two genes. RL6092 (Tc-*Lr20* NIL) and a number of a NILs developed from this line, an F_2 population (Tc/RL6092-B), and a number of genotypes previously characterized as carrying *Lr20* were used in combination with Tc-*LrCen* to study this region of chromosome 7AL. The preliminary study revealed that the two genes appear distinct from one another. Further mapping efforts are required to localize *Lr20* and more predictive flanking markers are required to further confirm these results.

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CHAPTER 1

GENERAL INTRODUCTION

In a world with an ever-increasing population size demanding more resources than ever, food production is being challenged to double by 2050 (Ray et al. 2013). Breeding for biotic and abiotic stress resistance is essential to address the effects of climate change and to minimize impact on the environment. Wheat leaf rust, *Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex Desmaz. f. sp. *tritici*), has long been recognized as the most common and widespread disease affecting wheat yields (Samborski 1985). Yield losses due to leaf rust are generally low in comparison to some other crop diseases, averaging 5% to 25%, the prevalence of the disease and its worldwide distribution put it among the most important diseases affecting wheat (Oelke and Kolmer 2005, Chu et al. 2009).

While more than 60 sources of genetic leaf rust resistance have been discovered to date, the ability of the *P. triticina* to evolve quickly, and almost solely through asexual recombination, has made *P. triticina* the successful pathogen it is today (McIntosh et al. 2012). This success has required researchers to constantly discover and incorporate novel sources of resistance. The majority of genes deployed for leaf rust resistance to date provide a strong source of race-specific resistance, but tend to be broken down quickly by the emergence of new virulent races of the pathogen (Herrera-Foessel et al. 2012). The key to effective incorporation of leaf rust resistance into a breeding program is the identification of strong and sustainable sources of resistance. To date, at least four resistant loci conferring race non-specific adult plant resistance (APR) have been identified including *Lr34* (Dyck 1987), *Lr46* (Singh et al. 1998), *Lr67* (Hiebert et al. 2010), and *Lr68* (Herrera-Foessel, 2012). Studies indicate that the most effective examples of sustainable resistance to leaf rust have been achieved through stacking a slow-rusting gene with one or more race-specific genes effective against prevalent virulent races of leaf rust in the growing region (Roefls 1988, Dyck 1991, German and Kolmer 1992, Singh 1992), or alternatively through stacking of multiple slow-rusting genes (Herrera-Foessel et al, 2012).

The discovery, localization, and incorporation of resistance genes has been accelerated with the introduction of sophisticated genome-specific, localized, microsatellite (Roder et al. 1998a) and single nucleotide polymorphism (SNP) systems (Neelam et al. 2012; Semagn et al. 2013). The hexaploid nature of the wheat genome makes genetic studies more complex. However, the more than 1000 microsatellite markers developed so far (Roder et al. 1998a, Guyomarc'h et al. 2002, Somers and Isaac 2004, Sourdille et al. 2004, Song et al. 2005) and localized on a high-density microsatellite map of wheat (Somers et al. 2004) and the recent development of the iSelect wheat 90K SNP array (Wang et al. 2014), provide an abundant set of markers broadly distributed across the entire genome.

Molecular mapping of traits of interest identifies molecular markers flanking or linked to a genomic region carrying the gene. Linkage studies in breeding populations can then be used to identify genotypes carrying the desired alleles through marker-assisted selection (MAS). While MAS is an excellent tool that can be used to efficiently and effectively select for desirable traits in a breeding program, it can be expensive to apply in many smaller breeding programs. Using MAS for parent development and selection can be a more affordable alternative use of MAS in such programs (Collard et al. 2005; Moose and Mumm 2008; Xu and Crouch 2008).

Understanding host-pathogen interaction is essential when attempting to provide a sustainable source of genetic resistance. While the gene-for-gene model (Flor 1971) can explain the interaction of many host-pathogen combinations in wheat; horizontal resistance and regulatory loci that have been identified in the wheat-leaf rust pathosystem all challenge this theory and complicate the implementation of durable resistance. This difficulty to achieve durable resistance to disease is one factor contributing to the average annual rates of yield increase from genetic improvement in wheat being estimated at only 0.35% per year prior to 1990 and 0.67% per year from 1990 to present (Thomas and Graf 2014). At this rate, wheat yields will fall short of the doubled demand by 2050 (Ray et al.

2013). However, more optimistic results were observed in an on-farm study across the Canadian Prairies from 1991 to 2012 where average yields rose by 1.40% annually when genetic improvement was combined with other management practices (Thomas and Graf 2014). This prediction is proof that strong scientific efforts must continue in wheat breeding programs and farm management practices for all yield-limiting factors present today and in the future.

The objectives of this study were to: (i) genetically map a resistance gene indentified in an accession of *Ae. taschii* in the double haploid (DH) population 98-223-27/Thatcher (ii) determine the inheritance and map the resistance gene, *LrCen*, identified in the Tc-LrCen/Sumai3-lr34 DH population and identify linked DNA markers (iii) explore the relationship between seedling resistance genes *LrCen* and *Lr20* which map to chromosome 7AL and are resistant to *P. triticina* race TDBG and (iv) determine the distribution of newly mapped *LrCen* within a selection of North American and world-wide germplasm to trace lineage back to a potential origin within Canadian germplasm.

CHAPTER 2

LITERATURE REVIEW

2.1 The Crop - Wheat

Wheat (*Triticum aestivum* L.) is an important cereal food crop worldwide; where cereal crops account for 50% of global caloric intake (FAO statistical yearbook 2013). In Canada, record yield production of 37.5 million tonnes was documented in 2013, of which 27.3 million tonnes was spring wheat; this represents a 38% increase over 2012 yields (components of Statistics Canada catalogue no. 11-001-X). Canada Western Red Spring (CWRS) wheat is the largest class of wheat grown in the Canadian prairies, with average production near 15 million metric tonnes annually. Desireable end-use quality characteristics of CWRS allow it to be exported to over 60 markets annually (Dexter et al. 2006).

Common wheat is an allohexaploid species originating from the interspecific hybridization of three different diploid species resulting in three sub-genomes that are referred to as the A, B and D genomes. Early evidence suggested *Triticum monococcum* L. (2*n* = 14, AA) as the progenitor species of the A sub genome of tetraploid and hexploid wheat (Gill and Kimber 1974). However, studies of a similar diploid species, *Triticum urartu* Thumanian *ex* Gandilyan, found in the same geographic areas as *T. monococcum* provided evidence that the two species were very closely related. This led some to believe that *T. monococcum* and *T. urartu* may be the same species or, a more accepted theory, that *T. urartu* is the donor of the A genome of common wheat (Konarev 1983; Kerby and Kuspira 1987; Dvorak et al. 1993). While the identity of the B genome progenitor of wheat is unclear, many studies have suggested *Triticum speltoides* L. (2*n* = 14, BB) as the early donor (Kimber and Riley 1963; Talbert et al. 1995; Daud and Gustafson 1996). Other possibilities were summarized by Sears (1977) including: the donor species may now be extinct, the donor species has not been discovered, or the B genome is the result of an introgression of two or more distinct parental species. In the 1920s and 1930s numerous

Aegilops species were proposed as possible progenitors of the D genome (Kerby and Kuspira 1987). While other species have been considered, much evidence supports *Aegilops tauschii* Coss. (2*n* = 14, DD) as the progenitor for hexaploid wheats (*T. aestivum*) (Dubcovsky and Dvorak 2007).

The convergence of two or more species previously adapted to different environments allows domesticated polyploid species to mirror the adaptation advantages seen in natural polyploid species, resulting in the ability to thrive within a broader range of environmental conditions (Dubcovsky and Dvorak 2007). Some important adaptations that have allowed polyploid wheat to thrive as a modern crop include: loss of spike shattering, loss of tough glumes (free threshing), increased seed size and number of tillers, erect growth habits, reduced seed dormancy, improved tolerance to soil pH and salinity, greater opportunity for disease resistance, and the potential for new food products (Jantasuriyarat et al. 2004; Dubcovsky and Dvorak 2007). Conversely, polyploid species are limited in initial genetic diversity because only a small number of plants contribute to their genetic background, creating what is known as the polyploidy bottleneck (Stebbins 1950). The most severe bottleneck is observed in the D-genome of *T. aestivum* where studies indicate diversity values less than 15% of those observed in the wild relative *Ae. Tauschii* (Talbert et al. 1995; Dvorak et al. 1998; Talbert et al. 1998).

Despite the wide range of environments conducive to wheat production, wheat yield is highly variable from season to season due to a number of biotic and abiotic production constraints. Without proper management, many of these constraints can be detrimental to wheat crops worldwide. Wheat is affected by a wide variety of biotic factors namely diseases and insects.

Wheat diseases range from obligate parasites, the most prevalent being wheat leaf rust (*Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex Desmaz. f. sp. *tritici*)) and powdery mildew (*Blumeria graminis* DC Speer f. sp. *tritici*) (Saari and Prescott 1985; Gill et al. 1986); to residue borne and non-obligate, semi-biotrophic pathogens which represent such diseases as tan spot (*Pyrenophora tritici-*

repentis Drechsler) and fusarium head blight (*Fusarium graminearum* Schwabe) (Duveiller et al. 2007). Wheat is also affected by a series of soilborne root rots and nematodes which cause the most significant losses under drought stressed conditions; as well as some viral pathogens such as barley yellow dwarf virus (BYDV) which is transmitted by aphids and found nearly everywhere wheat is grown (Sharma et al. 1995; Duveiller et al. 2007). Oerke *et al.* (1994) estimated that the annual global average yield loss due to the diseases of wheat is 12.4%, however estimates from developing countries are more difficult to obtain and may alter this number slightly (Oerke et al. 1994; Duveiller et al. 2007).

A number of insects have been described as pests of wheat, but overall they are not a prime cause of significant yield losses unless populations reach very high levels. There are, however, examples of insects that, in the absence of host resistance, will result in substantial losses in crop yield and end-use quality. Across Canada, as well as most other wheat growing regions of the world, wheat midge (*Sitodiplosis mosellana* Géhin) (Allen 1955) has become a chronic pest affecting spring wheat crops annually. Wheat midge overwinters in the soil and emerges as single generation in late June or early July (Doane et al. 1987; Elliott et al. 2011). Females lay eggs under glumes and along floret ridges; larvae development within florets results in reduced kernel filling or kernel abortion (Mukerji et al. 1988). Yield loss severity due to wheat midge is dependent on synchrony between egg-laying and anthesis; florets affected by eggs laid post anthesis are dramatically less affected than florets affected during spike emergence to flower (Alberta Agriculture and Rural Development; http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex2507). This makes early seeding of wheat crops or seeding of early maturing cultivars relatively effective methods to reduced losses to wheat midge (Elliott et al. 2011). Under the pressure of higher population numbers that may develop over a number of years, alternate methods of control may be required including: *Macroglenes penetrans*, a wasp capable of controlling up to 40% of wheat midge infestations (Olfert et al. 2009); insecticide applications at heading (Elliott 1988); and seeding of midge tolerant varieties that carry the

Sm1 resistance gene (McKenzie et al. 2002). The Hessian fly (*Mayetiola destructor* Say), found in North Africa, the Mediterranean region, and West Asia, is another example of an insect that, in the absence of host resistance, will cause significant yield losses by halting tiller growth and kernel filling, and inducing lodging (Gill et al. 1986). Insects may also have indirect effects on wheat production as they often act as vectors in the transmission of disease, as is the case with the aphid and barley yellow dwarf virus (BYDV) (Duveiller et al. 2007).

Abiotic factors also pose major production constraints on wheat yields and are often more difficult to manage genetically. Major abiotic stresses that affect wheat include, but are not limited to: extreme temperatures, drought/ flood conditions, photon irradiance, nutrient deficiencies, and inorganic solute concentrations. Production management can be limited by the fact that these stresses are often related to one another, for example, extreme high temperatures in combination with high sunlight may impose drought and light stress on plants, while also subjecting the plants to mineral toxicity in the dry soil. It has been observed that a plant's ability to defend against each additional stress may be decreased due to reduced physiologic functioning within a stressed plant (Tester and Bacic 2005). Breeding for resistance to such abiotic factors is also complicated by the complexities introduced by genotype by environment interactions. However, introgression of these traits from land races to commercial cultivars by marker-assisted selection have demonstrated some promising results and will be very important in breeding programs to support high yields under the less than favourable growing conditions expected in the future (Dubcovsky 2004).

2.2 The Pathogen

2.2.1 Wheat leaf rust

Wheat leaf rust, caused by the basidiomycete *Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex Desmaz. f. sp. *tritici*), is the most common and widespread disease of wheat worldwide (Kolmer 1996;

Oelke and Kolmer 2005). Infection on susceptible wheat plants occurs primarily on the leaf blade of infected plants, but will sometimes spread to the sheath under the correct conditions (Roelfs et al. 1992). Yield reduction as a result of leaf rust infection ranges from 5% to 25% depending on crop stage, environmental conditions, and genetics of the cultivar infected (Chu et al. 2009). However, losses of up to 65% have been observed under severe epidemic conditions (Saari and Prescott 1985; Singh et al. 2011). Leaf rust reduces the economic value of wheat by diminishing seed yield and quality through reduced floret set and grain shrivelling (Roelfs et al. 1992).

Wheat leaf rust is well adapted to a range of different climates (Kolmer 1996). Environmental conditions are a key component in both inoculum movement and spore germination and control the appearance and severity of leaf rust outbreaks from year to year and location to location (Roelfs et al. 1992; McCallum et al. 2007).

Despite low levels of sexual recombination in nature, the ability of *P. triticina* to evolve asexually and overcome host resistance contributes to its success as a disease of wheat worldwide (Dyck and Kerber 1985; Bolton et al. 2008). Annually, western Canadian losses in wheat due to leaf rust were estimated at $88 million from 2001 to 2005 (McCallum et al. 2007). While these statistics are 10 years old and may not reflect the losses observed today through increased use of fungicide, they demonstrate the widespread issue that this pathogen represents for wheat production. Along with the use of fungicides and early seeding of wheat crops, the preferred method of rust control is through the use of genetically resistant host cultivars. Breeding for resistance offers an environmentally friendly alternative to the use of chemical pesticides. Providing resistance in cultivars does not add costs for producers.

2.2.2 Lifecycle, host range and epidemiology of *Puccinia triticina*

Puccinia triticina, is an obligate parasite of wheat and some other grasses. It is a heteroecious fungus with the asexual and sexual stages of its life cycle occurring on two taxonomically unrelated host species. *P. triticina* is macrocyclic, with five distinct spore types, each with varied impact on agriculture (Bolton et al. 2008). The primary host of *P. triticina* is common hexaploid wheat, *T. aestivum*. Infections on the primary host are the result of asexual urediniospores and aeciospores which can germinate on, and infect, the telial host. The resulting production of urediniospores continues the infection cycle. Also, *P. triticina* has been found to occur naturally on tetraploid durum wheat (*T. turgidum* ssp. *Durum*), wild emmer (*T. dicoccoides*), domesticated emmer wheat (*T. dicoccum*), triticale (X Triticosecale) and common goatgrass (*Ae. cylindrical*) in the southern Great Plains of the USA. Additionally, some isolates identified in Israel are specific to *Ae. speltoides,* a related diploid species (Yehuda et al. 2004). While each of these species acts as a host to *P. triticina,* the host species specificity of *P. triticina* races can be grouped. Isolates that infect durum wheats and *Ae. speltoides* appear to have a limited host range and differ genetically from races that infect common wheat. This high level of host specificity in *P. triticina* isolates, indicates that the two groups may be considered as two different *formae speciales* (Yehuda et al. 2004; Goyeau et al. 2006; Ordonez and Kolmer 2007; Bolton et al. 2008).

Teliospore development within uredinia on the host survive hot, dry conditions and signal the beginning of the sexual portion of the pathogen's life cycle. Sexual recombination of wheat leaf rust occurs during meiosis in the production of basidiospores. Sexual reproduction can occur on the alternate hosts *Thalictrum speciosissimum* L. (meadow rue) and *Isopyrum fumaroides* L. *Th. speciosissimum* is native to southern Europe and southwest Asia, however, there are related species found naturally within North America (Levine and Hildreth 1957; Kolmer 2013). The sexual stage of the leaf rust lifecycle is of little significance to the epidemiology of this disease as these secondary hosts tend to be isolated

geographically, or are resistant to infection, leading to relatively little, to no, sexual recombination worldwide (Bolton et al. 2008; Kolmer 2013).

Leaf rust infections of wheat crops across North America, as well as most other places worldwide, are the result of asexual urediniospores. Urediniospores infect winter wheat crops as well as volunteer wheat crops in the fall in the southern United States. They over-winter on the plants through the crop's dormancy period as the pathogen's spores are able to survive the same environmental conditions as the wheat leaf. This is referred to as a "green bridge" (Roelfs et al. 1992). In the following season, environmental cues initiate urediniospore release from the uredinia that land on host crops in the surrounding area. In the presence of free water the urediniospores germinate and a germ tube extends along the leaf surface until encountering a stomatal opening where an appressorium develops over the stomata followed by penetration via a penetration peg (Roelfs et al. 1992; Bolton et al. 2008). In the event of a compatible host-pathogen interaction, haustoria develop within the living host cells and the infection spreads locally throughout the host leaf tissue. A resistant host will inhibit or slow the rate of haustorium development resulting in an incompatible reaction (Roelfs et al. 1992). Where living host tissue is available, uredinial infections have the ability to cycle continuously producing large numbers of secondary inoculum that can then be wind dispersed up to hundreds of kilometres to infect on a continental scale.

Canadian*P. triticina* inoculum is urediniospores transported via wind currents from the Southern United States where winter wheat crops seeded in the previous growing season act as a green bridge. With increased temperatures in spring and summer, winter wheat crops in Texas and the Gulf Coast states begin to mature. Uredinia can exploit their macrocyclic nature by producing inoculum on spring wheat crops throughout the eastern and southern states and moving to the southern and northern Great Plains region of the United States and Canada (Kolmer 2013). Urediniospores are then wind-dispersed and travel in large numbers in northbound wind currents reaching Canada in June and leading to significant

infections in late July and early August, giving early seeded crops an advantage (Roelfs et al. 1992; McCallum et al. 2007).

2.2.3 Population genetics – physiologic specialization

An understanding of the genetic variability of *P. triticina* populations and their distribution worldwide is essential to the success of wheat breeding programs and the deployment of appropriate sources of genetic resistance (Kolmer 1996; McCallum et al. 2010). Traditionally, variation in collections of isolates for both leaf and stem rust were classified based on infection type in a set of wheat cultivars and varieties each containing an undefined set of resistance genes. As more information became available on the genetics of rust resistance a differential set of near-isogenic lines (NILs) was developed for several leaf rust resistance (Lr) genes, with each NIL containing a single Lr gene in a Thatcher background (Dr Peter Dyck, CRC-AAFC, Winnipeg Canada). Virulence surveys are conducted annually in the United States, Canada and Australia as well as other countries to monitor the virulence structure in the local or regional *P. triticina* populations and to detect new races that may present problems in the future. Since its acceptance by the North American Wheat Leaf Rust Research Workers Committee in 1986, virulence surveys conducted in North America have used a nomenclature system based on the combination of high and low infection types of each leaf rust isolate on the aforementioned NILs. Virulence combinations are assigned based on virulence and avirulence patterns of the culture on four sets of four, NILs (set 1: *Lr1, Lr2a, Lr2c,* and *Lr3*; set 2: *Lr9, Lr16, Lr24,* and *Lr26*; set 3: *Lr3ka, Lr11, Lr17,* and *Lr30*; set 4: *LrB, Lr10, Lr14a,* and *Lr18*) resulting in a four letter code. This code may be followed by a hyphen and a list of supplemental differentials with ineffective host genes (Long and Kolmer 1989). North American virulence surveys detect more than 50 races of *P. triticina* annually (McCallum et al. 2007; Kolmer 2013).

A number of marker types including, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP) and simple sequence repeat (SSR) polymorphism markers have been used to classify cereal rust populations and to study species origins and subsequent spread of rust. Genetic studies have revealed six distinct groups of *P. triticina* races (identified as NA-1 through 6) based on identical or highly related SSR genotypes in combination with virulence to significant leaf rust resistance genes. Groups NA-3 and NA-5 represent 95% of the leaf rust population and occur throughout the Great Plains and southern and eastern states of the USA (Kolmer 2013). Comparisons to world-wide isolate collections show high levels of similarity between isolates collected in Uruguay, Argentina, Chile, Brazil (Ordonez et al. 2010), and North American (Ordonez and Kolmer 2007) in groups NA-3 and NA-5. As well, isolates collected from durum wheats across Europe, South America, Mexico and the Middle East demonstrated nearly identical SSR genotypes, suggestive of common ancestry and intercontinental migration of the pathogen. These genetic studies also support the hypothesis that sexual recombination is not a significant evolutionary factor for *P. triticina.* This is based on heterozygosity levels that exceed those expected through random mating (clonal organisms maintain diversity at a single locus at higher rates than sexually reproducing organisms), high levels of linkage disequilibrium between SSR markers, and an observed correlation between virulence and SSR genotypes, which are all characteristic of a highly clonal species (Balloux et al. 2003; Kolmer 2013).

The previously mentioned high levels of population variability can be attributed to the genetics of the wheat – leaf rust pathosystem. Despite low levels of sexual recombination in *P. triticina* populations, evolution of new virulent races is essential for the survival of the obligate parasite. The use of diverse wheat cultivars across North America and worldwide, each containing different combinations of resistance has been primarily responsible for driving the distribution of virulence in *P. triticina* populations. Long distance wind transport of *P. triticina* spores selected in one region may result in the introduction of races with new virulences in adjacent regions where corresponding resistance has not

been deployed by breeding programs (Kolmer 2013). Evidence of this was demonstrated by McCallum et al. (2010) in the 2007 virulence survey. The appearance of isolates virulent to leaf rust resistance gene *Lr9* in British Columbia and Alberta, which had not been detected in this region between 1998 and 2006, suggest that selection for this phenotype on cultivars in the USA was likely responsible for the appearance in western Canada (McCallum et al. 2010). The dominance of races TDBG (56.2 % and 61.0% of virulence phenotypes obtained in Canada in 2006 and 2007 respectively) and TDBJ (18.7% and 15.2% of virulence phenotypes obtained in Canada in 2006 and 2007 respectively) across the Canadian Prairies as well as north central parts of the USA directly south of Manitoba and Saskatchewan also support this model of pathotype distribution (Kolmer et al. 2009; McCallum et al. 2010).

2.3 Leaf Rust Resistance

2.3.1 Sources of wheat leaf rust resistance

To date there are 63 leaf rust resistance genes in wheat mapped to a chromosomal location and given a unique designation (McIntosh et al., 2012; Herrera-Foessel, 2012). Leaf rust resistance genes are utilized by wheat breeding programs to develop wheat cultivars with genetic resistance to *P. triticina* infection that comes at no extra cost to the producer or the environment. The obligate parasitic nature of the pathogen has encouraged rapid evolution of *P. triticina* virulence under the selective pressures of the host resistance that are deployed across the world. Leaf rust resistance genes are categorized in two broad classes, seedling resistance genes and adult plant resistance (APR) genes. While seedling genes can be detected at both seedling and adult plant stages, conferring an all-stage resistance phenotype, they tend to be race specific, and for this reason more easily broken down (Lagudah 2010). Typical to other plant disease resistance genes, seedling resistance genes have conserved motifs and code for proteins with nucleotide binding site (NBS) and leucine rich repeat (LRR) domains. Examples of effective seedling resistance genes commonly used in resistant cultivars are *Lr21, Lr16* and *Lr14a* (McCallum et al. 2010;

Neelam et al. 2012). Exceptions to this rapid breakdown of race-specific seedling genes have been observed, virulence reports on seedling resistance gene *Lr32* have been reported worldwide, but at a very low frequency (Kerber 1987; Singh 1991; McIntosh et al. 1995; Thomas et al. 2010).

Adult plant resistance genes, often referred to as field resistance, are expressed at the post-seedling stage. The APR category includes both genes that express race-specific resistance as well as the slow rusting race non-specific resistance genes. Race-specific APR genes function similar to seedling genes characterized by a hypersensitive reaction conditioning a very low infection type on the host (Kolmer 2013). Examples of such genes are *Lr12* and *Lr13.* Alternatively, race non-specific APR genes confer moderate resistance to all races of leaf rust to the host plant but not through the classical hypersensitive response (Singh et al. 2011). Race non-specific resistance typically offers partial resistance to many different races, referred to as a race non-specific APR phenotype. Race non-specific phenotypes describe a host plant that exhibits a longer latent period, increased colony abortion as a result of cell necrosis, and fewer and smaller uredinia produced overall (Caldwell 1968; Lagudah 2010). Race non-specific, durable resistance is observed in *Lr34, Lr46, Lr67,* and *Lr68* (Singh et al. 1998; Hiebert et al. 2010; Herrera-Foessel et al. 2012).

Of the above mentioned genes, *Lr34,* has proven very useful and long lasting despite the rapid evolution of the pathogen. Studies of this resistance have been important in understanding the mode of action responsible for the durability and broad, race non-specific resistance that has allowed it to provide lasting resistance (German and Kolmer 1992; Hiebert et al. 2010; McCallum et al. 2012). Durable resistance to leaf rust has been rare, however, certain combinations have proven to stand the test of time, including *Lr34*, *Lr34+Lr13* and *Lr34+Lr12* (German and Kolmer 1992). Such combinations of *Lr34* with race specific genes appear to be so successful due to an apparent ability of *Lr34* to enhance the resistance conditioned by the other leaf and stem rust host genes (German and Kolmer 1992; Kolmer 1996). *Lr34,*

which appears to have early origins in the South American cultivar Frontana and land races primarily of Chinese origin (Lagudah et al. 2009; Lagudah 2011), is part of a multi-fungal pathogen resistant single gene locus that also confers resistance to stripe rust (*Yr18,* Singh, 1992), stem rust (Hiebert et al. 2010), powdery mildew (*Pm38,* Spielmeyer et al. 2005), and barley yellow dwarf virus (*Bdvl1,* Singh 1993). Therefore, the *Lr34* locus has advantages that make it one of the most versatile and important of all leaf rust resistance genes (McCallum et al. 2012). A similar multi-fungal resistance has been observed in association with slow-rusting *Lr46* locus which co-segregates with stripe rust resistance gene *Yr29* and powdery mildew resistance gene *Pm39* (Lillemo et al. 2008; Lagudah 2011). It remains to be determined whether these are the result of a single gene, or a closely linked set of genes. Both *Lr34* and *Lr46* also co-segregate with a gene for leaf tip necrosis, *Ltn1* and *Ltn2* respectively, a phenotype often used to putatively indicate the presence of the leaf rust resistance gene within a line (Rosewarne et al. 2006; Lagudah 2011). Preliminary studies by Spielmeyer et al. (2013) indicate that recently characterized APR gene *Lr67* is also a multiple pathogen resistance locus/ gene cluster.

Since its deployment in the Canadian cultivar 'Glenlea' in 1972, *Lr34* has provided a reliable source of resistance to many successful Canadian cultivars (McCallum and DePauw 2008; McCallum et al. 2010). Within Western Canada some of the more commonly used leaf rust resistance genes incorporated into commercial cultivars are *Lr10, Lr13, Lr14a, Lr16, and Lr21* (McCallum et al. 2007; McCallum et al. 2012). One of the most newly discovered leaf rust resistance gene was identified and mapped by Herrera-Foessel et al. (2012) and was designated *Lr68*. It has been mapped to chromosome 7BL as a new adult plant leaf rust resistance gene conferring race non-specific resistance to the host plant and is thought to have originated from the same Brazilian cultivar as the popular APR gene *Lr34*, Frontana. *Lr68* demonstrates a similar additive gene effect as is seen in *Lr34*. The combination of *Lr68* and *Lr34* has provided consistently higher and more stable slow rust resistance to the host plant. Common wheat cultivar "Parula" contains

at least three known race non-specific genes: *Lr34, Lr46,* and *Lr68*. In combination, these genes provide highly effective, long-term resistance (Herrera-Foessel et al. 2012).

Along with common wheat (*T. aestivum*), sources of leaf rust resistance have been characterized in lower-ploidy level relatives of the hexaploid species including the immediate diploid progenitor species *Aegilops tauschii* (e.g. *Lr21;* Rowland and Kerber 1974). These progenitors are ideal donors in a breeding program because they do not pose barriers to recombination. In addition to progenitor species, related species are also used for the diversification of rust resistance. These include: *Aegilops elongatum* (e.g. *Lr24;* Schachermayr et al. 1995), and *Aegilops umbellulata* (e.g. *Lr9;* Sears 1961; Schachermayr et al. 1994) and common rye, *Secale cereale* (e.g. *Lr26;* Browder 1980; Kolmer 2013). Genes from wild relatives can be introgressed into wheat through wide crosses and special manipulation to facilitate recombination with wheat DNA. Wild relatives provide many new sources of resistant germplasm which may contribute to durable resistance; however, these alien translocations are often accompanied by deleterious traits that are difficult to remove.

2.3.2 Genetics of the host-parasite interaction

Understanding the genetic relationship between wheat and *P. triticina* is essential to develop breeding strategies that will be effective in the long-term. To date, 63 leaf rust resistance genes have been identified in wheat. Continued evolution of the pathogen requires a constant search for new and more durable sources of resistance. Two research strategies are novel gene discovery and gene stacking (to be discussed in more detail in the breeding section). Both are effective; yet require an understanding of the genetics controlling virulence and avirulence in the pathosystem. Within *P. triticina* avirulence/ virulence is conditioned by a number of independently segregating loci that are widely distributed throughout the estimated 16-18 chromosomes of the pathogens genome. These loci function

independently and in combination to further complicate the genetic interaction in the wheat-*P. triticina* pathosystem (Dyck and Samborski 1974; Leonard and Szabo 2005).

The majority of leaf rust resistance genes discovered to date confer race specific type resistance. In general, for each resistance gene present in the host, there is a corresponding genetic locus in the pathogen that conditions virulence and avirulence based on the alleles present in a particular isolate. This is known as the gene-for-gene model and is common to many plant-pathogen pathosystems (Samborski and Dyck 1968; Flor 1971; Kolmer 1996). An incompatible reaction (resistant) will only be expressed by the host if it is homozygous or heterozygous for a dominant resistance gene (RR or Rr) for which the pathogen has a corresponding avirulence (AA or Aa). Regardless of the presence of a resistance gene within the host, if the pathogen has a corresponding virulence (aa) a compatible reaction (susceptible) will occur. In this way the pathosystem is codependent. The gene-for-gene model is evidence of a coevolution between the host and *P. triticina* whereby the evolution of the pathogen is not random, but guided by the genetics of the host. Genes that function in this manner tend to be those that are highly effective in the short term, but are overcome quickly by the pathogen through selection of virulent races. Recent examples of this include the important Canadian wheat cultivars AC Barrie and Superb (McCallum et al. 2007).

Although the gene-for-gene theory explains the mode of action for many host-pathogen interactions, there are exceptions to this rule. For example, race non-specific resistance (horizontal resistance), and the appearance of regulatory loci that alter the expression of an apparent resistance source. *P. triticina* mutation studies conducted by Statler (1985, 1987) revealed two results: a gain in virulence to different Lr genes, but also the opposite whereby genes changed from virulent to avirulent. In his work with flax, Flor (1971) established that, in general in the gene-for-gene model, host resistance was dominant to susceptibility and pathogen avirulence was dominant to virulence. In the first case, a

change from avirulence to virulence, can be explained simply by a mutation in the dominant avirulence gene resulting in a loss of function of the avirulence alleles and in turn the creation of virulence alleles that are not recognized by the host resistance. The second change from virulence to avirulence is more complex and departs from this classical model introducing a new variable in the genetics of this pathosystem interaction. Possible explanations for this shift from virulence to avirulence are that the mutation induced a change in the virulence gene that is now recognized by the host resistance or alternatively, the mutation occurred at a regulatory locus that may have otherwise inhibited avirulence in *P. triticina* (Statler 1985, 1987; Bolton et al. 2008).

Allelic sets may also diverge from the classical gene-for-gene model. In the case of *Lr2a, b,* and *c,* a single recessive gene in *P. triticina* is responsible for avirulence. However, a 3 X 3 table demonstrating reaction phenotypes for each of the three alleles acting independently against three different isolate types ((1) low on *Lr2a*, (2) intermediate on *Lr2a*, and (3) high on *Lr2a*) reveals that a dominant gene at an independent locus in the pathogen is differentially affecting the expression of avirulence to the three alleles (Dyck and Samborski 1974; Kolmer 1996). Isolates expressing low infection types on *Lr2a* are consistently low on *Lr2b* and *Lr2c,* while those expressing an intermediate infection type on *Lr2a* have higher infection types on *Lr2b* and *Lr2c,* and isolates that are virulent to *Lr2a* are always virulent on *Lr2b* and *Lr2c* (Dyck and Samborski 1974; Kolmer 1996).

Samborski (1963) demonstrated that the expression of avirulence in *P. triticina* is also affected by gene/ allele dosage. In studies performed using the resistant line Transfer (*Lr9*) a heterozygous *P. triticina* isolate with a single dominant gene controlling avirulence led to an intermediate host resistant infection type, while the same cultivar inoculated with selfed progeny of the same *P. triticina* isolate that was homozygous dominant for the avirulence alleles, resulted in a lower host resistance infection type (Samborski 1963; Bolton et al. 2008). Allele dosage in the host also affects the resulting infection type

such that a heterozygous individual may display an infection type intermediate to that of a homozygous resistant and homozygous susceptible individual (Kolmer 1992). Further studies indicate that avirulence/ virulence in *P. triticina* is, in some cases, conditioned by different numbers of genes as well as differently expressed genes (dominant and recessive) that segregate independently from one another, while host resistance in wheat is primarily epistatic (Samborski and Dyck 1976; Kolmer 1992). While there may be more than one resistance gene present in a cultivar that confers resistance to the same race, the gene expressing the lowest reaction phenotype masks the effects of less effective resistance sources. Exceptions to this have been demonstrated. *Lr34* has a unique ability to enhance resistance conditioned by other genes when they are combined in a single cultivar, resulting in a lower overall infection type. Another example of gene interactions such as complimentary gene action was described by Singh and McIntosh (1984) in which *Lr27* and *Lr31*, found in cultivar Gatcher, provided resistance only when present together (Singh and McIntosh 1984a, b; Kolmer 1996).

Optimal rust infection occurs between 15 and 20°C. At temperatures ≤10°C restricted infection development may occur and at temperatures ≥25°C pronounced chlorosis may occur (Kolmer 1996). Temperature studies have revealed that some leaf rust resistance genes are affected by varying temperature conditions (Pretorius and Kemp 1990; Pretorius et al. 1994). Genes *Lr18, Lr14a, Lr30, Lr15,* and *Lr11* showed lower infection types at lower temperatures and higher infection types at higher temperatures. The opposite was observed for genes *Lr16, Lr17,* and *Lr23*. These studies also revealed that the temperature response was highly dependent on the *P. triticina* isolate and the growth stage of the plant making it hard to classify genes according to temperature sensitivities (Dyck and Johnson 1983; Kolmer 1996).

2.4 Disease rating scale

Phenotypic disease assessment for the purpose of research is conducted in uncontrolled field settings, as well as indoor controlled environments and greenhouses. Leaf rust can be rated at both the seedling and adult plant stage, depending on experimental design and research goals. The host-pathogen interaction in the wheat-leaf rust pathosystem is highly influenced by internal and external factors including: environment, host stage, host nutrition, host tissue, inoculum density, time, host resistance genetics, and pathotype genetics and aggressiveness (Roelfs et al. 1992). It is essential to develop standard conditions in order to moderate these effects as much as possible.

Field studies are usually evaluated near the end of the growing season at the adult plant stage with primary ratings based on the pustule type and infection severity or percentage of flag leaf tissue covered by pustules. To evaluate pustule type, a scale ranging from total immunity with very minor to no necrotic flecking to total susceptibility (S) appearing as large uredinia with no chlorosis is used. Intermediate to these ratings are: resistant (R), appearing as very small pustules surrounded by necrosis; moderately resistant (MR), a small to medium pustule size surrounded by a chlorotic/necrotic border; and moderately susceptible (MS), represented by medium sized pustules surrounded by some chlorosis (Roelfs et al. 1992). This scale can be referenced visually in the 'Rust Scoring Guide' produced by the Research Institute for Plant Protection (IPO) (Accessible as a download from the CIMMYT webpage at: http://repository.cimmyt.org/xmlui/handle/10883/1109). Infection severity is evaluated using a modified Cobb scale outlined by Peterson et al. (1948). The diagrammatic scale is based on the arbitrary maximum value of 37% (approximately one third) of leaf tissue being infected representing the upper limit for underlying mycelium development to be 100% destructive to the plant. Lower severities are evaluated in decreasing intervals of ten (Peterson et al. 1948).

Indoor phenotypic evaluation can be performed at both seedling and adult plant stages. Seedling tests are preferred because they require less space and time. Testing of adult plant resistance genes must be done at later stages of plant development for accurate assessment. Resistant host responses (low infection type due to avirulent *P. triticina* isolate) range from "0" representing complete immunity with no macroscopic sign of infection, ";" hypersensitive flecking with no visible uredinia, "1" very resistant with only small uredinia surrounded by necrosis, to "2" small to medium uredinia surrounded by necrosis/ chlorosis. A resistant host response may also be rated as "X" representing a heterogeneous distribution of variably sized uredinia from necrotic flecks to medium sized pustules surrounded by chlorosis/necrosis. Susceptible host responses (high infection type due to virulent *P. triticina* isolate) range from "3", moderately susceptible with medium sized uredinia surrounded by chlorosis to "4", fully susceptible with large uredinia and no chlorosis (Long and Kolmer 1989; McCallum et al. 2010). These ratings may be adjusted by using characters such as a minus (-) to represent an individual in a category with uredinia on the lower limit or a plus (+) to indicate uredinia on the upper limit of a particular category, multiple – or + may be used to further differentiate infection types (Roelfs et al. 1992).

2.5 Cultural disease control

Early efforts to eradicate the alternate hosts of the wheat stem rusts were successful in reducing and even eliminating the sexual lifecycle thereby reducing sexual recombination and subsequent evolution of the pathogen in North America. The eradication of common barberry (*Berberis vulgaris* L.) began across North America in 1918 and lasted until 1975. This effort reduced the disease to a minor threat that was controlled effectively through genetic resistance. The recent emergence of the highly virulent Ug99 in East Africa could jeopardize the current state of control, although not a current threat in North America it has had devastating effects in affected regions and has begun to spread (Pretorius et al. 2000; Jin et al. 2008). Eradication of alternate hosts of leaf rust, however, has not been effective. The

inherent alternate host resistance to *P. triticina* infection and geographic isolation already greatly reduce contact with the pathogen, yet the pathogen continues to evolve asexually in the absence of significant frequencies of sexual reproduction (Roelfs et al. 1992; McCallum et al. 2007; Kolmer 2013).

Similar to other cereal rusts, early seeding of spring wheat crops is an effective method to reduce leaf rust infection. As previously outlined, *P. triticina* inoculum is produced in the southern regions of the United States and travels north with wind currents. Significant levels of inoculum reach northern areas of the US and Canada in June. If seeded early enough, the crop may mature prior to severe infection (Samborski 1985; McCallum et al. 2007). In a similar fashion, early maturing spring wheat crops are less likely to be affected by late-season inoculum (Roelfs et al. 1992).

With a lack of an alternate host for over-wintering of *P. triticina* spores, tillage of fields to bury crop residues and volunteer wheat plants in inoculum-producing areas of the southern US helps to reduce the green bridge that carries spores from season to season. With the extensive annual growth of winter wheat crops in these inoculum producing regions, namely the central and southern Great Plains (Sweeney et al. 2000), total elimination of the greenbridge is impossible and therefore tillage is only a method to reduce initial inoculum levels and must be used in combination with other cultural techniques to be effective (Zadoks and Bouwman 1985; Roelfs et al. 1992).

Plant nutrition and the use of fertilizers in plant nutrient management have a long history of providing wheat yield advantages to producers. In addition, improved plant health leads to reduced incidence of disease (Sweeney et al. 2000). An inverse relationship between an increase in phosphorus (P) levels and a decrease in incidence of wheat root take-all (*Gaeumannomyces graminins* (Sacc.) Arx and Oliver var. *tritici* Walker) and powdery mildew (*Erysiphe graminis* D.C.) has been documented (Boquet and Johnson 1987; Brennan 1989, 1995). A similar study contrasting P and potassium (K) levels with leaf rust severity indicate that while P increased yield by increasing heads m^{-2}, severity was not significantly

affected. In contrast, K fertilizers that are typically provided in a potassium chloride (KCl) form, showed moderate suppressive effects on leaf rust severity of up to 10% in susceptible cultivars. It is also important to note that while plant health has demonstrated some ability to improve the plants' tolerance to disease, it has also been shown that the denser canopies that result from nutrient rich plants can lead to decreased sunlight exposure and increased humidity, favouring the development of infection that does occur on these plants (Burdon and Chilvers, 1982). Although effects of nutrients on improving tolerance of plants to disease are inconsistent, providing a healthy base for plant growth can be combined with other control measures to provide some level of disease protection.

Chemical fungicide use on wheat has risen in many regions of Canada over the past decade primarily to combat the re-emergence of fusarium head blight (FHB). Across North America, FHB has resulted in severe epidemics from Quebec to Saskatchewan in Canada, as well as, over 26 states in the US. FHB is caused by fungal species of *Fusarium*, predominantly *F. graminearum* in Canada, which infects crops at the flowering stage and can be devastating to grain yield and quality (Windels 2000). *F. graminearum* may also produce a mycotoxin, deoxynivalenol (DON), which affects end-use quality, and food and feed safety. These factors contribute to the economic value of chemical input costs incurred to the farmer to ensure adequate and useable grain yields (Windels 2000). Effective FHB control can be achieved through the application of fungicides if applied at the appropriate time. Common fungicides used for FHB control in Western Canada are Folicur (active ingredient (AI): tebuconazole), Prosaro (AI: prothioconazole and tebuconazole), Caramba (AI: metaconazole) and Proline (AI: prothioconazole) and are also an effective option in the control of cereal rust diseases. These fungicides are also effective for control of leaf rust. However, due to differences in growth stage application timing for the control of different diseases; it is often not economically viable for farmers to perform multiple applications throughout a growing season to protect against the earlier or later arrival of rust spores. For this reason, other methods of control tend to be favoured for the rust diseases, most notably, genetic resistance

(McCallum et al. 2007). Repeated chemical applications also increase the chance for fungal pathogens to develop resistance, thereby reducing fungicide efficacy. Chemicals may also have negative environmental impacts, making it important for producers to consider many factors before choosing this method of control.

2.6 Wheat breeding for disease resistance

Breeding crop plants to improve certain aspects of their habit began as early domestication of plant species. Early breeding efforts over the first 10,000 years of food grain production resulted in yields of one billion tons by 1960. In only 40 years since, plant breeding efforts, such as the introduction of dwarf genotypes in wheat and maize combined with improved agronomic practices, doubled food grain production to two billion tons in 2000 (Poehlman 1987; Lynch 2007). Plant breeding objectives today are diverse and include increased yield, improved end-use quality, and tolerance to biotic and abiotic stresses.

Durability of genetic resistance to plant pathogens is essential to the success of breeding programs. Durable disease resistance can only be achieved through knowledge of pathogen epidemiology and population structure (i.e. virulence frequencies and virulence combinations) in combination with an understanding of wheat genetics and the available sources of host resistance (Stuthman et al. 2007; Azzimonti 2013). Complete or qualitative resistance conferred by major resistance genes in the host results in an incompatible reaction with a specific avirulent race of the pathogen, but tends to demonstrate poor durability under field conditions (Parlevliet 2002).

Quantitative resistance provides a reduction in disease severity and is associated with a few to many genes, each providing partial effects. Such quantitative resistance has been demonstrated to provide a more durable resistance from season to season and is favoured in breeding programs today (Stuthman et al. 2007; Azzimonti 2013). Durable disease resistance can also be achieved through gene stacking or pyramiding. This involves the integration of a number of genes, often a durable resistance

source with one or more additional effective genes, within a single cultivar which results in broad and more durable resistance (Dubcovsky 2004; McCallum et al. 2007). In the wheat – leaf rust pathosystem additive gene action has been described in such situations (Bjarko and Line 1988). The Canadian wheat cultivar, Glenlea, is an example of successful gene stacking combining the slow-rusting gene *Lr34* with *Lr1* and a third gene linked or allelic to *Lr13* (Dyck et al. 1985). Glenlea was grown in the Canadian prairies for over 30 years and still provides an adequate level of resistance (McCallum et al. 2007).

In order to harness all genetic potential available to crop species, Vavilov (1940), discussed the potential locked within the DNA of crop relatives and the importance of exploiting these genetic resources. This insight lead to the creation of gene banks, collections of living seed 'exotics' that include races and species closely related to cultivated crop species. This effort is conducted worldwide and has resulted in more than 700 documented seed collections with an estimated 2.5 million entries (Plunknett et al. 1987). This effort has begun to shift the paradigm for germplasm evaluation from screening for recognizable phenotypes, to localization of important genetic loci through identification, and mapping of key genes that can be easily incorporated through marker assisted breeding techniques (Tanksley 1997).

2.7 Molecular analysis and genetic mapping

2.7.1 Molecular markers

Molecular marker technologies are continuously evolving and improving. The newest generation of molecular markers have simplified protocols, reduced time to results, reduced cost per data point, increased throughput, increased species specificity, and increased distribution across associated genomes. These molecular advances have the potential to initiate a second "Green Revolution", oftened referred to as the "technology revolution", essential to maintain food demands in the future (Dubcovsky 2004). For the purposes of crop improvement, molecular markers can be used as chromosome landmarks to facilitate the selection of chromosome regions carrying desired traits in a more cost and time effective

manner. This selection process is especially beneficial for the accumulation of a number of genes, often used to provide effective and a broad range of disease resistance. Additionally, molecular markers can be used to identify and incorporate genes for traits that are difficult to measure or are highly affected by environment and difficult to accurately phenotype (Dubcovsky 2004; Collard et al. 2005).

There are a number of molecular marker classes, each with their own unique properties. Choice of marker type depends on a number of factors including: availability for the species of study, cost, convenience, and intended application. Commonly used marker types are restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), microsatellites or simple sequence repeats (SSRs), sequence-tagged sites (STS), amplified fragment length polymorphisms (AFLPs), and single nucleotide polymorphisms (SNPs) (Gupta et al. 1999b; Gupta and Varshney 2000; Poczai et al. 2013).

The first molecular marker system developed was RFLP analysis and is a co-dominant, hybridization-based marker type. First developed for human genome mapping; RFLPs were later adopted for mapping of plant genomes (Botstein et al. 1980). RFLP analysis is time-consuming and labour-intensive and has had relatively little use in *T. aestivum* studies due to low the frequency of polymorphic RFLPs in the hexaploid genome (Tanksley et al. 1989; Gupta et al. 1999b). Both RAPD (Williams et al. 1990) and AFLP (Vos et al. 1995) markers use a polymerase chain reaction (PCR)-based system reducing time and cost and increasing productivity. RAPDs are a dominant maker type that utilizes a single primer sequence to amplify discrete random sequence. As with RFLPs, low levels of detectable polymorphisms within the hexaploid wheat genome, along with lack of reproducibility, has made them of lesser use in wheat research (Gupta et al. 1999b). Despite being a dominant marker type, AFLP markers have been more successful in the study of *T. aestivum* where research has been done in assessing genetic diversity (Barrett and Kidwell 1998; Barrett et al. 1998), construction of genetic maps (Penner et al. 1998), and the identification of diagnostic or closely linked markers for different agronomic traits (Goodwin et al. 1998;

Hartl et al. 1998; Gupta et al. 1999b). AFLP use a two-step approach which involves use of specific restriction enzymes to digest the template DNA followed by the ligation of double-stranded adapters that provide primer-binding sites for amplification through PCR. A selected subset of fragments can be amplified using the sequence specific primer-binding site to provide a large number of bands to score for polymorphisms (Vos et al. 1995; Gupta et al. 1999b).

STS markers are a generally co-dominant, PCR-based system. Unlike the above described marker types, STS markers require some prior sequence knowledge which can be a limitation to their availability for some crops, but provides them with a level of species specificity that RFLP, RAPD and AFLP markers lack (de Vincente and Fulton 2003). A STS is a short (approximately 200-300 bases long) and unique sequence of DNA in a specific locus on the representative species genome. The marker system utilizes a pair of primers complementary to the unique tagged sequence, designed by sequencing a mapped low-copy RFLP probe, to selectively amplify the desired region; the product is then analyzed using gel electrophoresis (Doggett 1992; Gupta et al. 1999b). STS primers use long sequences and making the detection of allelic variation more reproducible than the original RFLP markers (de Vincente and Fulton 2003).

SSRs or microsatellite markers are regions of simple sequence repeats of one to six base pairs, most commonly two to three bases in length. SSRs are ubiquitous, abundant and evenly distributed within eukaryotic genomes. However, the frequency of microsatellites varies dependent on the species (Gupta et al. 1999b; Gupta and Varshney 2000; de Vincente and Fulton 2003). SSRs have quickly become the most widely used type of STS, identifying polymorphisms in the length of the repetitive region by a PCR-based amplification with primers designed from the DNA flanking regions of the microsatellite. Flanking regions tend to be highly conserved reducing non-specific binding and increasing species specificity of microsatellite markers (Roder et al. 1998a; Roder et al. 1998b; Gupta et al. 2002). Microsatellites are

generally co-dominant. In polyploidy species, microsatellites may be single, genome-specific products representing a known locus, or amplify multiple loci as a consequence of sequence homeology across the multiple sub genomes. This can add a level of complexity to the marker's profile, requiring a way to differentiate allelic products from each sub genome (Gupta and Varshney 2000). Information for microsatellite distribution is available for many species including wheat, through construction of genetic and physical maps (Roder et al. 1998a; Roder et al. 1998b; Gupta et al. 2002). Microsatellites have been used extensively in hexaploid wheat research as they provided the highest frequency of polymorphism of any previously developed marker type to date. The high levels of polymorphism exhibited by microsatellite markers provides higher resolution in measuring genetic distance and an improved ability to determine population structure (Roder et al. 1998b; Van Inghelandt et al. 2010; Semagn et al. 2013).

SNPs refer to a single base pair change in a DNA sequence observed between two members of a species or between two homologous chromosomes. Typically the single base change has the alternative of one of two possible nucleotides at a known position (Neelam et al. 2012; Semagn et al. 2013). SNPs are the most abundant source of biallelic polymorphisms and have begun to replace SSRs for research in species for which extensive sequencing has been completed; however, SSRs maintain some advantages over SNPs as previously discussed (Van Inghelandt et al. 2010; Semagn et al. 2013). A series of SNP genotyping platforms are available from a number of companies each combining a different set of chemistry, detection method, and reaction format to accommodate different research needs (Chen and Sullivan 2003; Grover and Sharma 2016). These genotyping platforms are continuously evolving alongside the continuous progress of high-throughput genomic technologies. Population analysis by SNP markers can be performed through a variety of marker-dense, genome-wide multiplexing platforms as well as on a more localized basis through uniplex genotyping platforms that use smaller numbers of pre-selected SNPs (Semagn et al. 2013).

Multiplex SNP platforms are the highest throughput direct genotyping system available to researchers. Chip-based technology allows screening of hundreds to millions of SNPs in a single assay (Gupta et al. 1999b). The number of SNPs is dependent on the number identified for the species of interest. Development of multiplex SNP assays requires substantial time for assay development including the identification of SNPs that provide adequate genome-wide coverage (Gupta et al. 1999a). Although availability of such multiplex assays was originally low in plant species, recent developments in the technology and reduction in cost have made these platforms more accessible to plant researchers (Gupta et al. 1999a; Semagn et al. 2013). Two widely used chip-based genotyping arrays are the GeneChip microarray from Affymetrix (Santa Clara, CA, USA; www.affymetrix.com) and the wide spectrum of BeadArray technologies offered by Illumina including the Infinium High Density array used in this research (San Diego, CA, USA; www.illumina.com). Multiplex genotyping is most useful in large-scale studies with fewer sample numbers requiring large quantities of wide-spread genotypic data. Multiplex genotyping may be less useful for directed studies including crop improvement where trait loci are often fixed, reducing the value of such a large and dispersed dataset (Low et al. 2006; Neelam et al. 2012; Semagn et al. 2013).

Wheat researchers have a 90K iSelect genotyping chip available from Illumina (Wang et al. 2014). It is important to note, most SNPs act as dominant markers in polyploid species making them suitable probes to screen inbred varieties or populations (e.g. near isogenic lines and doubled haploid populations), but more problematic for screening heterozygous materials (e.g. F_2 populations) (Allen et al. 2011).

Where the wide-spread, high-density marker systems are not required, uniplex SNP genotyping systems offer an affordable and easy-to-use alternative with the same abundance of available polymorphisms. Such platforms include TaqMan offered by Life Technologies (Burlington, ON, CAN;

www.lifetechnologies.com) and Kompetitive Allele Specific PCR or KASP developed by Kbioscience for in-house genotyping at LGC Genomics (Teddington, Middlesex, UK; www.lgcgenomics.com). The KASP assay is cost- and time-effective with pre-mixed reagents and flexible experimental design options for 96-, 384-, and 1,536-well plate format assays (KASP genotyping chemistry – User guide and manual, http://www.lgcgenomics.com/genotyping/kasp-genotyping-reagents/?download_file=22_1_kasp_manual.pdf&download_cat=downloads). SNPs are overall fairly transferable from one genotyping platform to the next; LGC Genomics reports a 98-100% assay design success rate and a 93-94% conversion to successful working assay rate (LGC Genomics applications note, http://www.kbioscience.co.uk/reagents/KASP_Taqmancomparison.pdf; Semagn et al. 2013). Semagn et al. (2014) demonstrate a high degree of accuracy for the KASP system with a mean rate of 0.7% for mismatches among positive controls (in the same plate). Many mismatches were scoring errors resulting from the stringent parameters set in the automatic allele calling and could be corrected by manual scoring (reduced to <0.5%). Use of KASP technology in plant breeding is relatively new but has already demonstrated a value with the development and validation of a diagnostic SNP for the presence of leaf rust resistance gene *Lr21* that provides a quicker and cheaper, breeder-friendly tool for marker assisted selection (MAS) (Neelam et al. 2012).

2.7.2 Mapping populations

In order to create a linkage map and successfully locate a gene of interest, a mapping population segregating for the gene(s) of interest must be created. Populations are typically the result of sexual recombination occurring between two parental lines pre-selected for a difference of one or more gene(s) of interest. The more distantly related the parental lines, the higher rate of polymorphic markers a researcher can expect in the resulting population. The most commonly used population types in mapping studies are F_2, backcross (BC), recombinant inbred lines (RILs), and doubled haploid (DH) populations. Each of these population types have their own unique advantages and disadvantages. Selection of

population type is dependent on budget, time and labour availability, species of study, pollination system, and population size available (Collard et al. 2005).

F_2 populations, derived from the self-pollination of F_1 hybrids, and BC populations, derived from pollination of F_1 hybrids with one of the parental lines, are attractive to researchers due to ease of development, cost and time efficiency, and high proportion of heterozygous loci (Collard et al. 2005). Both F_2 and BCF_1 populations have the disadvantage of a single plant representing each genotype. As a result, replication over time and space cannot be conducted and observations made on a single plant are not reliable for many traits. This can be overcome through cloning (where available) or self-pollination of individual plants creating F_2-derived F_3 families in the case of F_2 plants (Zhang and Xu 2004). Observations of BC populations can be conducted at any generation with the knowledge that each generation will increase the percentage of elite alleles segregating within the population. Generally five or six backcrosses are sufficient to fix the recurrent parent (RP) alleles within the population making observations more reliable (http://passel.unl.edu/pages/informationmodule.php?idinformationmodule=1031263034&topicorder=4&maxto=7 [Accessed: March 2014]). The use of marker-assisted selection (MAS) in BC breeding has been demonstrated to accelerate the recovery of the RP genome (Frisch et al. 1999). However, in the case of both F_2 and BC studies, each of these additional steps (creation of F_3 families and multiple BC generations) is an added time and labour commitment. Additionally, the use of dominant marker systems and phenotype accuracy can be complicated by the presence of heterozygous loci making MAS less effective (Allen et al. 2011).

RIL populations are a series of homozygous lines, each with a unique genetic make-up composed of chromosomal segments from the parental lines, developed through repeated inbreeding beginning with the F_2 individuals. RIL populations are homozygous at nearly all loci (approximately 94% homozygous

for F_6-derived populations and approximately 97% for F_7-derived populations) and are particularly useful in the mapping of quantitative traits (Mansur et al. 1996). The elimination of heterozygotes facilitates phenotyping of partially dominant traits and makes genotyping by means of dominant and co-dominant markers systems possible. RIL populations also have more opportunity for recombination through repeated generations of self-pollination, an added advantage over DH populations, while marker density needs to be higher in order to assemble linkage groups (Bernardo 2003; Smith et al. 2008). DH populations are also 'true breeding' or homozygous populations and can be reproduced without genetic recombination occurring. Studies with DH populations can be performed using both dominant and co-dominant marker types. They are highly useful in the mapping of quantitative traits as they provide unlimited opportunity for replications over time and space (Liu et al. 2002). DH populations provide a faster means of reaching homozygocity compared to RILs and each DH represents a unique combination of recombination between the parental genomes (Collard et al. 2005). However, DH production is very labour intensive and low production efficiency has required much research to optimize production for each species (Liu et al. 2002). Additionally, DH populations can only be developed in species amenable to tissue culture, limiting their utility in some crop improvement breeding programs (Collard et al. 2005).

2.7.3 Marker assisted selection

Marker assisted selection (MAS) provides an indirect method for the selection of target traits using molecular markers that are closely linked to gene(s) of interest or derived from the actual gene sequence (perfect markers). MAS can effectively reduce time and costs for trait integration in breeding programs. In addition, markers can be used for identification and quantification of genetic variation within genetic resources. However, the application of MAS is still quite expensive and remains unaffordable for many breeding programs to implement on a large scale (Collard et al. 2005; Moose and Mumm 2008; Xu and Crouch 2008). MAS can be applied to many different types of traits, making it a

versatile tool in plant breeding programs that require incorporation of an array of traits. Additionally, MAS speeds up the detection of alleles that are transferred to an individual through natural chromosome recombination and therefore does not face the same public scrutiny as use of transgenic and other newly developed trait introgression technologies (Dubcovsky 2004).

The national wheat MAS consortium, MASwheat (http://maswheat.ucdavis.edu [Accessed: February 25, 2014]), was developed by wheat researchers and breeders from 12 public programs across the United States and funded by the USDA Initiative for the Future of Agriculture and Food Systems. A similar program also operates in Australia (Australian National Wheat Molecular Marker Program – NWMMP). The MASwheat consortium was developed to enable public breeding programs to incorporate MAS capacities within their current programs and to transfer new developments in wheat genomics and biotechnology to U.S. wheat production (Dubcovsky 2004).

MAS can be useful for leaf rust resistance breeding. Leaf rust resistance genes may be epistatic when more than one effective Lr gene is present in a given wheat line. Phenotypic identification of individual leaf rust resistance genes is difficult when two genes express resistance to the same *P. triticina* isolates. Use of MAS can facilitate incorporation of multiple resistance genes with similar expression into commercial cultivars (Tanksley 1997; Kolmer 2013). The constant evolution of improved marker technologies and their implementation in breeding programs provide researchers with the ability to characterize and select the best parental genetics, make better crosses, and improve selection of progeny containing valuable traits. MAS can be used for selection of individuals within a population with useful stacks of Lr genes that are effective against a broad range of *P. triticina* races.

CHAPTER 3

IDENTIFICATION, CHARACTERIZATION AND MAPPING OF A NEW LEAF RUST (*Puccinia triticina*) RESISTANCE GENE TRANSFERRED FROM *Aegilops tauschii* Coss. TO COMMON WHEAT (*Triticum aestivum*)

3.1 Abstract

Leaf rust, caused by *Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex Desmaz. f. sp. *tritici*), is a wide-spread disease of wheat that affects grain yield and quality. The preferred method of leaf rust control in wheat is through integration of genetic resistance. A source of leaf rust resistance was identified in *Aegilops tauschii*, the D genome progenitor of wheat, was transferred to RL5866, a synthetic hexaploid (RL5866=Tetra-Canthatch x *Ae. tauschii* RL5767). A double haploid (DH) population was generated by crossing the resistant line 98-223-27 (98-223-27=Thatcher-Lr34*3/RL5866) with Thatcher. A set of 167 DH lines segregating for the gene of interest and *Lr34* were inoculated with *P. triticina* race BBBD. A single hypersensitive leaf rust resistance (Lr) gene segregated at the seedling and adult stages. Testing with a perfect marker for *Lr34* suggested that the unknown Lr gene is linked to *Lr34*. This was supported by initial microsatellite marker haplotyping of chromosome 7DS in 98-223-27. No seedling Lr genes from the primary gene pool have been mapped to chromosome 7D, thus the Lr gene in 98-223-27 may represent a new gene if the putative location on 7DS is confirmed.

3.2 Introduction

Wheat is among the most important crops worldwide, grown on more than 200 million hectares of land and contributing to the employment of more than 1 billion people in developing countries (FAO, 2008). In North America, wheat is among the top three crops produced, recently surpassed by canola for the number one crop in Canada with more than 23,166,000 MT produced in 2010 (FAOSTAT, 2010). Of

the various diseases that affect wheat crops worldwide, leaf rust, caused by the pathogen *Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex Desmaz. f. sp. *tritici*) is the most common and widespread (Oelke and Kolmer 2005). Yield reduction caused by leaf rust typically ranges from 5% to greater than 15%, depending on crop stage, environmental conditions, and genetics of the cultivar infected (Chu et al. 2009). Today, breeding for resistance to diseases and other biotic and abiotic factors is of the utmost importance in the agricultural industry.

Evolution of new virulences in *P. triticina* has reduced the effectiveness of some of the known resistance genes. While some of these defeated resistance genes still contribute to effective resistance in combination with other resistance genes (McCallum and Thomas 2011), these changes in the pathogen make the discovery of new sources of resistance important for effective control of disease outbreaks. New gene discovery is not limited to *Triticum aestivum* L. Broadening the search to progenitor and related species introduces the possibility to identify resistance genes that are unlikely to be present in the host species (Sears 1956). Identification of resistance genes in progenitors and related species of wheat has resulted in the successful transfer of a number of sources of resistance, including the first alien transfer from *Aegilops umbellulata*, *Lr9* (Sears 1956, Chhuneja et al. 2007). Progenitor species identified with novel sources of resistance pose no barriers to recombination and are thus more suitable than non progenitors for use in commercial wheat cultivars. *Aegilops taschii* Coss., the D-genome progenitor of common wheat, has provided a number of characterized leaf rust resistance genes including: *Lr21* (allelic to *Lr40*) (Kerber and Dyck 1969, Huang and Gill 2001), *Lr22a* (Rowland and Kerber 1974), *Lr41*, *Lr42*, and *Lr43* (Cox et al. 1994). Adult plant resistance gene *Lr22a* is unique in that it demonstrates high levels of resistance typically exhibited by race-specific seedling resistance genes, different from most APR genes which demonstrate slow-rusting, race nonspecific phenotypes (Hiebert et al. 2007). While *Lr22a,* has not been extensively incorporated in North American cultivars, no confirmed reports of virulence have been

reported and offer interesting genetic options for use in gene pyramiding in North American cultivars (Huang and Gill 2001, Hiebert et al. 2007).

Researchers at the Cereal Research Centre – Agriculture and Agri-Food Canada (CRC-AAFC) have produced populations with leaf rust resistance derived from *Aegilops tauschii* (= *Ae. squarrosa*, = *T. tauschii*) accession RL5767. This line showed a hypersensitive reaction to leaf rust at the adult plant stage that appeared to be distinct from *Lr22* (Hiebert, personal communication).

The objective of this study was to: (i) characterize the (adult plant) leaf rust resistance gene derived from *Ae. tauschii*, (ii) genetically map the (adult plant) leaf rust resistance gene and identify usable flanking markers for marker assisted selection, and (iii) determine the interaction between the new (adult plant) leaf rust resistance gene and *Lr34*.

3.3 Materials and Methods

3.3.1 Population development

Two DH populations, Z01M and Z02M, that were previously developed by Drs. Kerber and Hiebert of CRC-AAFC were used in this study. These populations were developed as follows. Dr. Kerber conducted an extensive screening of *Aegilops tauschii (= Ae. squarrosa, = T. tauschii)* accessions, the D genome progenitor of common hexaploid wheat, for new sources of leaf and stem rust. The D genome progenitor was targeted as a source of resistance because it poses no barriers for recombination which is important for use in breeding. *Ae. tauschii* RL5866 showed a hypersensitive resistance to leaf rust at the adult plant stage which appeared to be distinct from *Lr22*. A synthetic hexaploid, RL5866, was derived from a cross of diploid (DD) *Ae. tauschii* RL5767 and tetraploid (AABB) Tetra-Canthatch (Kerber unpublished).

The resistance carried in RL5866 was transferred to Thatcher-*Lr34* (Tc-*Lr34*) by recurrent backcrossing and selection to generate the line 98-223-27. Dr. Hiebert then crossed 98-223-27 to a leaf

rust susceptible line Thatcher (Tc) as well as Tc-*Lr34*. F_1 plants from the Tc/98-223-27 population were used to create a double haploid (DH) population, Z01M, where *Lr34* and the gene of interest were both segregating using the maize pollination/embryo rescue method (Thomas et al. 1997). Following the same methods, a second DH population was developed from the Tc-*Lr34*/98-223-27, Z02M, where the gene of interest was segregating but *Lr34* was fixed (Hiebert, unpublished).

3.3.2 *P. triticina* controlled environment inoculations

A single plant from each of the 188 DH lines from the Z01M DH population (Thatcher/98-223-27 segregating for the gene of interest and *Lr34*) and two replicates of each parental line were germinated in six 4x8 root trainers containing Sun Gro Horticulture Sunshine Professional Growing Mix 5 (Agawam, MA, USA; www.sungro.com). Plants were grown for eleven days prior to inoculation in a growth chamber under 16 light and 8 dark hours at 20˚C and 18˚C respectively. The mapping population was reduced to 167 DH lines based on heterozygocity from marker screenings, off-types from phenotypic screenings and lack of seed germination.

Single spore inoculum was developed via the method described by McCallum and Seto-Goh (2003). To determine the virulence pattern of the spores used as inoculum, inoculations were performed on the set of 16 differential lines (Table 4.1) and infection types were observed. The isolate used was classified as race BBBD by technician Pat Seto-Goh (CRC-AAFC). Spore viability was tested by spreading a single layer of spores across a 2% water agar petri plate left overnight in a dark, cool environment. The spores were then observed under a microscope the following day to assess germination.

Seedling inoculations were performed with single spore isolates of *P. triticina* race BBBD (race 1), described as avirulent to most leaf rust resistance genes (Long and Kolmer 1989). Vacuum dried urediniospores isolated using the method described above (stored at 4˚C) were removed from

refrigeration, released from the vacuum by opening glass tubes, and heat shocked at 40˚C for five minutes in a water bath. Eleven day old seedlings were inoculated by suspending urediniospores in light mineral oil and then the spore-oil suspension was sprayed onto the leaves as previously described by McCallum and Seto-Goh (2003). Post inoculation, plants were left for approximately 30 minutes to allow the oil to volatilize, leaving the spores bound to the leaves. Inoculated seedlings were then incubated in a dew chamber overnight at 100% relative humidity under dark conditions at approximately 20˚C. The following day, plants were removed from the dew chamber and air dried before being returned to the controlled environment previously described.

After seedling ratings were performed (see below), plants were treated with Tilt fungicide (Syngenta Canada Inc., Guelph, ON; www.syngenta.ca) to prevent contamination with powdery mildew before adult plant inoculations and then transplanted to 3.8 liter pots (3 DH lines/pot) containing three parts bulk soil to one part Sun Gro Horticulture Sunshine Professional Growing Mix 5 (Agawam, MA, USA; www.sungro.com). Plants were watered as needed and treated with a water-soluble 20-20-20 (NPK) fertilizer on a once weekly schedule. The same inoculation process was repeated on the population at the adult plant stage at the onset of boot stage/ head emergence, GS40-49 (Zadoks et al. 1974).

At the same time, the process outlined above was repeated on 94 DH lines from the Z02M DH population (Thatcher-*Lr34*/98-223-27). Controlled environment inoculations and ratings were attempted in four single plant replications at different times for both Z01M and Z02M DH population lines.

In addition, at head emergence, GS40-49 (Zadoks et al. 1974), glassine bags were placed over one spike per plant to ensure self-pollination. This provided the self-pollinated seed source for the 2012 field trial.

3.3.3 Disease assessment

Phenotypic evaluation of infection type was performed on the first and second leaves 12 days post inoculation (DPI) for seedlings and on the flag leaf at 16 DPI for adult plants. Infection types were classified following the leaf rust rating scale described by Long and Kolmer (1989) using specific modifications outlined by Roelfs et al. (1992).

Infections types from 0 (fully resistant – no sign of infection) to 2 (small to medium sized pustules surrounded by chlorosis/ necrosis) were considered as resistant host responses, while infection types 3 to 4 (medium to large pustules with little to no visible chlorosis) were considered as susceptible host responses. Phenotypic ratios were tested for goodness of fit using Chi-Square analysis.

3.3.4 Field phenotyping of Z01M DH population

Field trails were conducted on the University of Manitoba campus in Winnipeg in 2012 and 2013 and in Portage La Prairie in 2013 using a randomized complete block design (RCBD) with two replicates of each experimental line in the Z01M population. In the 2012 field trail, each 0.6 meter row was seeded with twenty self-pollinated (SP) seeds, where available. Entries that did not have twenty SP seeds available were either substituted with open-pollinated (OP) seeds or winter wheat to reduce weed competition. The field design consisted of 6 rows, leaf rust spreader rows consisting of 55% Morocco, 35% Thatcher (both highly susceptible to leaf rust), and 10% Wolfe barley (a marker to identify spreader rows) were incorporated alternating between rows 3 and 4 in each range to avoid straight rows of spreader across the trial in an attempt to evenly distribute inoculum. Susceptible check Thatcher, resistant check McKenzie, a number of each of the parental lines (Thatcher, Thatcher+*Lr34*, and 98-223-27) and a positional check winter wheat line (CDC Falcon) were incorporated randomly into the trial.

In 2012 and 2013 inoculations were performed at the three to four leaf stage and again at the five to seven leaf stage. Inoculations were done using the 2012 and 2013 epidemic mix, respectively. The epidemic mix is a representative and proportional mixture of those virulence phenotypes found in Canada during the previous year (source: CRC – AAFC). One gram of spores was suspended in 1.5 L of a light mineral oil (Bayol 55, Imperial Oil Canada, Toronto, ON, Canada) and sprayed onto spreader rows. Observations for infection development began around the onset of anthesis, based on the first appearance of flowering within the field (integrated checks were the first to flower). Ratings were perfomed using a combination of pustule classification ranging from fully susceptible (S) to fully resistant (R) (Roelfs et al. 1992; Research Institute for Plant Protection webpage at: http://repository .cimmyt.org/xmlui/handle/10883/1109) and severity evalution using a modified Cobb scale (Peterson et al. 1948). The 2012 trial was hand harvested keeping replicates separate, and threshed to provide seed for the 2013 season.

3.3.5 DNA extraction

The following protocol was used for DNA extraction for marker analysis. Leaf tissue (four pieces approximately 2.5-3.8 cm) was collected from the third or fourth leaf (youngest, uninfected tissue), after infection types were assessed on the first and second leaf. Tissue was placed into two 96-well collection microtube extraction plates with glass beads to macerate tissue following freeze-drying. Tissue was lyophilized on the Christ Alpha 1-4 LD plus (Montreal Biotech Inc., Kirkland, QC, 3551 St-Charles BLVD. Suite 506) overnight and subsequently stored at -20°C. Immediately prior to extraction tissue was macerated to a fine powder using the Qiagen TissueLyser II (Qiagen, Missassauga, ON; www.qiagen.com) for 10 minutes, changing the plate orientation after 5 minutes. DNA was extracted using a modified ammonium acetate extraction protocol (Chao and Somers, http://maswheat.usdavis.edu/PDF/DNA0003.pdf, accessed February 2012) based on procedures of

Pallotta et al. (2003). DNA was quantified using Hoechst 33258 stain on a fluorimeter and stock DNA was then diluted to a 15 ng/µl working solution.

3.3.6 Molecular analysis

Characterization of D genome in the Z01M parental lines and DH population

Two replicates of the parental lines, 98-223-27 and Thatcher, and a subset of 6 resistant and 6 susceptible randomly selected DH lines from the DH population were screened using markers linked to known resistance genes on the D genome (Table 3.1). A set of 19 microsatellite (SSR – simple sequence repeat) markers and one primer pair specific to *Lr1*, were screened against the test population for polymorphisms. For the *Lr1* primer pair F233/R712, additional check lines Glenlea and Thatcher-*Lr1* were used to confirm positive reactions.

Additional screening of all 167 DH individuals and two replicates of the parental lines of the Z01M DH population was done with molecular marker Indel 11 (Table 3.1), a perfect marker for *Lr34*, to determine the segregation of *Lr34* within the population.

Table 3.1 Forward and reverse primers for molecular markers used to screen the Z01M population and parental lines for known leaf rust resistance genes on the D genome of *Triticum aestivum*.

Resistance gene (location)	Molecular marker	Annealing Tm (°C)	Forward Sequence	Reverse Sequence	Reference
Lr34 (7DS)	Indel 11 (caIND11)	61	GTCTCCCAATCTGCATGCTC	TACCTCCCAAAAGCCAGTTG	Dakouri et al. 2010
***Lr22* (2DS)**	*gwm296*	61	AATTCAACCTACCAATCTCTG	GCCTAATAAACTGAAAACGAG	Roder et al. 1998
***Lr32* (3D)**	*wmc43*	61	TAGCTCAACCACCACCCTACTG	ACTTCAACATCCAAACTGACCG	Somers et al. 2004
	barc135	52	ATCGCCATCTCCTCTACCA	GCGAACCCATGTGCTAAGT	Song et al. 2005
***Lr21* (1DS)**	*Lr21* marker	55	CGCTTTTACCGAGATTGGTC	TCTGGTATCTCACGAAGCCTT	Huang and Gill 2001
***Lr39* (1D)**	*gwm210*	61	TGCATCAAGAATAGTGTGGAAG	TGAGAGGAAGGCTCACACCT	Roder et al. 1998
***Lr67* (4D)**	*cfd71*	60	CAATAAGTAGGCCGGGACAA	TGTGCCAGTTGAGTTTGCTC	Guyomarc'h et al. 2002
	cfd23	60	TAGCAGTAGCAGCAGCAGGA	GCAAGGAAGAGTGTTCAGCC	Guyomarc'h et al. 2002
***Lr19* (7DL)**	*gwm44*	61	GTTGAGCTTTTCAGTTCGGC	ACTGGCATCCACTGAGCTG	Roder et al. 1998
***Lr24* and *Lr60* (1DS)**	*wmc432*	51	ATGACACCAGATCTAGCAC	AATATTGGCATGATTACACA	Somers et al. 2004
	barc119	51	CACCCGATGATGAAAAT	GATGGCACAAGAAATGAT	Song et al. 2005
	barc149	51	ATTCACTTGCCCCTTTTAAACTCT	GAGCCGTAGGAAGGACATCTAGTG	Song et al. 2005
	gdm126	60	TCCATCATATCCGTAGCACA	CGTGGTTGATTTCAGGAGGT	Somers et al. 2004
	cfd15	60	CTCCCGTATTGAGCAGGAAG	GGCAGGTGTGGTGATGATCT	Guyomarc'h et al. 2002
***Lr1* (5DL)**	F233 and R712 (primer pair)	65	AGTCTGCACAATCTTTTCCGG	ATCTGTAGTTGGTCCACCAAGG	Cloutier et al. (2007)
***Lr2* (2DS)**	*cfd65*	60	AGACGATGAGAAGGAAGCCA	CCTCCCTTGTTTTTGGGATT	Guyomarc'h et al. 2002
	cfd56	60	TTGCATAATTACTTGCCCTCC	CTGGTCCAACTTCCATCCAT	Guyomarc'h et al. 2002
	cfd51	60	GGAGGCTTCTCTATGGGAGG	TGCATCTTATCCTGTGCAGC	Guyomarc'h et al. 2002
	cfd36	60	GCAAAGTGTAGCCGAGGAAG	TTAGAGTTTTGCAGCGCCTT	Guyomarc'h et al. 2002
	wmc111	61	ATTGATGTGTACGATGTGCCTG	CATGTCAATGTCATGATGAAGC	Somers et al. 2004
	gwm261	61	CTCCCTGTACGCCTAAGGC	CTCGCGCTACTAGCCATTG	Roder et al. 1998

Genotyping of Z01M and Z02M surrounding the Lr34 locus

The three parental lines of the Z01M and Z02M DH populations, 98-223-27 (resistant parent for both), Thatcher, and Thatcher-*Lr34* were genotyped with 35 microsatellite markers surrounding the *Lr34* locus on the short arm of chromosome 7D (Table 3.2).

Microsatellite markers determined to be polymorphic between the resistant and susceptible parents of the DH populations (Table 3.2), were used to genotype half of the Z01M population (94 individuals) to be used in a two-point linkage analysis with phenotypic data collected from controlled environment and field experiments.

Table 3.2 Results for microsatellite marker genotyping of the 7DS loci associated with the genomic region of *Lr34* on the parental lines of the Z01M and Z02M double haploid (DH) populations.

Microsatellite marker	Marker result[1]	Annealing Tm (°C)	Forward Sequence	Reverse Sequence	Reference[2]
barc121	M*	51	ACTGATCAGCAATGTCAACTGAA	CCGGTGTCTTTCCTAACGCTATG	A
barc126	P	51	CCATTGAAACCGGATTTGAGTCG	CGTTCCATCCGAAATCAGCAC	A
barc154	M*	51	GTAATTCCGGTTCCACTTGACATT	GGATGGGCAGCTTCAAGGTATGTT	A
barc172	P	51	GCGAAATGTGATGGGGTTTATCTA	GCGATTTGATTTAACTTTAGCAGTGAG	A
barc219	M*	55	GCGATCCCACAATGCATGACAACTTC	GGACGTCCGATCGAATTGGTTT	A
barc352	M*	60	CCCTTTCTCGCTCGCCTATCCC	CTGTTTCGCCCAATCTCGGTGTG	A
barc5	M*	51	GCGCCTGGACCGGTTTTCTATTTT	GCGTTGGGAATTCCTGAACATTTT	A
barc87	M	51	GCTCACCGGGCATTGGGATCA	GCGATGACGAGATAAAGGTGGAGAAC	A
cfd14	P	60	CCACCGGCCAGAGTAGTATT	TCCTGGTCTAACAACGAGAAGA	B
cfd21	P	60	CCTCCATGTAGGCGGAAATA	TGTGTCCCATTCACTAACCG	B
cfd26	M	60	TCAAGATCGTGCCAAATCAA	ACTCCAAGCTGAGCACGTTT	B
cfd30	M*	60	AATCGCACAACAATGGTTCA	GCCTCTCCTCTCTGCTCCTT	B
cfd31	M	60	GCACCAACCTTGATAGGGAA	GTGCCTGATGATTTTACCCG	B
cfd41	M	60	TAAAGTCTCAGGCGACCCAC	AGTGATAGACGGATGGCACC	B
cfd46	P	60	TGGTGGTATAGTCGTTGGAGC	CCACACACACACACCATCAA	B
cfd66	M	60	AGGTCTTGGTGGTTTTGGTG	TTTTCACATGCCCACAGTTG	B
gdm145	M	55	TGAAGGACAAATCCCTGCAT	TCCCACCTTTTTGCTGTAGA	C
gpw1142	M*	60	TACTTGTGCGAGGTTCTCGG	CCTCCCATTCACCTCCTCTT	D
gpw128	M*	60	AGGAAACATGTCGAACCGAG	TGCAATCCTAAACGTGGTTG	D
gpw7265	M*	60	ATGATGATGCACGCAATGTT	CAGTAGGTTTCCCTGGTGGA	D
gpw299	P*	60	CCCTCGGTTATAGTGGGAAA	GCTCGGTGCTACTGGAAAAC	D
gpw4092	M*	60	TGAAAATGATTGCACACTTGC	ATCCAAGCCTTTGTTTGCTG	D
gwm130	M	61	AGCTCTGCTTCACGAGGAAG	CTCCTCTTTATATCGCGTCCC	E
gwm295	N/A	61	GTGAAGCAGACCCACAACAC	GACGGCTGCGACGTAGAG	E
gwm295	N/A	61	GTGAAGCAGACCCACAACAC	GACGGCTGCGACGTAGAG	E

gwm437	P	61	GATCAAGACTTTTGTATCTCTC	GATGTCCAACAGTTAGCTTA	E
wcmc463	M	51	GATTGTATAGTCGGTTACCCCT	ATTAGTGCCCTCCATAATTGTG	C
wmc121	P	61	GGCTGTGGTCTCCCGATCATTC	ACTGGACTTGAGGAGGCTGGCA	C
wmc42	M	51	GCCCTTGGTCCTGGGGTGAGCC	GCCTCATCCAGAGAGCCTGCGG	C
wmc438	M	61	GACCGTTGGGCTGTATAGCATT	CTCTGACAGTGGTGGAGCTTGA	C
wmc450	M	61	GCAGGACAGGAGGTGAAGAAG	AGGCGTTGCTGATGACACTAC	C
wmc606	M	66	CCGATGAACAGACTCGACAAGG	GGCTTCGGCCAGTAGTACAGGA	C
wmc698	M	51	GTGAAGGGAGAGCTAGCAA	ACAGTTGGCCCAGCTAGTA	C
wmc827	M*	61	ACGGTGACCTCAGTGCTCAC	ATGCTTGCCTCAGCAAAACC	C
wmc94	P	51	TTCTAAAATGTTTGAAACGCTC	GCATTTCGATATGTTGAAGTAA	C

[1]M = monomorphic, P = polymorphic, N/A = no amplification, * = poor resolution, [2]A = Song et al. 2005, B = Guyomarc'h et al. 2002, C = Somers et al. 2004, D = Sourdille et al. 2004, E = Roder et al. 1998

3.3.7 PCR amplification

PCR reactions for all microsatellite markers were performed in 384-well plates; each reaction contained 5.0 µl of template DNA diluted to 15.0 ng/µl (75.0 ng DNA/ reaction), 2.0 µl ddH_2O, 1.0 µl 1X PCR buffer (Applied Biosystems, Streetsville, Ontario, Canada), 1.5 mM $MgCl_2$, 0.8 mM dNTPs, 1.8 pmols M13 labelled primer (FAM/HEX/NED), 0.2 pmols forward primer, 2.0 pmols reverse primer and 1U *Taq* DNA polymerase for a final volume of 10.0 µl per reaction. PCR reactions were carried out under the following conditions: denaturation at 93°C for 2:00 min followed by 30 cycles of 94°C for 1:00 min, 0.5°C/s to annealing temperature (Tm), Tm for 0:50 min, 0.5°C/s to 72°C, 72°C for 1:00 min followed by an final extension step of 72°C for 5:00 min and held infinitely at 4°C. Annealing temperatures were determined by the specific marker and ranged from 51°C to 61°C (Tables 3.1, 3.2). PCR fragment analysis was performed on an ABI 3100 genetic analyzer (Applied Biosystems, Streetsville, Ontario, Canada) using a three colour M-13 primer dye set (FAM, HEX and NED) as described by Somers et al. (2004). Marker output from the ABI was displayed using Genographer version: 2.1 (Montana State University).

The PCR protocol for *Lr1* forward and reverse primers, F233 and R712 (Table 3.1) followed the methods decribed by Cloutier et al. (2007). PCR reactions were carried out under the following conditions: denaturation at 94°C for 5:00 min followed by 35 cycles of 94°C for 0:30 min, 65°C for 0:30 min, 72°C for 0:30 min followed by a final extension step of 72°C for 10:00 min and held indefinitely at 15°C (Cloutier et al. 2007). 25.0 µl PCR products and 5.0 µl 6X loading buffer were loaded on a 1.0% agarose gel stained with 0.43 µg/mL ethidium bromide in 0.5X TAE buffer for 90 minutes at 80V. Fragment size was determined by including 5.0 µl of Invitrogen Low DNA Mass Ladder by Life Technologies (www.lifetechnologies.com). Agarose gel images were photographed during visualization on a UV transilluminator.

3.4 Results

3.4.1 Controlled environment tests of reaction to *P. triticina*

The phenotypic segregation ratio for seedling resistance to *P. triticina* race BBBD in controlled environment inoculations of the Z01M DH population fitted a 1:1 ratio in three replicates ($p = 0.486$; Table 3.3). The adult plant screening of the first replicate of the Z01M DH population demonstrated a 3:1 ratio ($p = 0.264$) which fitted the expected segregation of two genes including the gene of interest and *Lr34*, the adult plant resistance gene known to be segregating within this population. Four repeated controlled phenotypic screenings were attempted to confirm adult plant ratings however, powdery mildew contamination by the time plants reached adulthood in the second screening resulted in little to no infection. The third replicate was attempted earlier at an intermediate growth stage in attempt to avoid the powerdy mildew results were inconsistent to no infection. A fourth adult plant screening was attempted with little, to no, disease development, results could not be repeated.

The lack of disease infection in controlled environment testing was investigated. Spore germination problems were ruled out. Leaf wetness interruptions were eliminated based on the sealed dew chamber system used (Stuckey and Zadoks 1989) and soil conditions including disease and nutrient deficiencies were unlikely causes as plants did not appear to exhibit symptoms of moisture or nutrient deficiencies. Unconducive growth chamber conditions were suspected as the most likely source of limited infection development, as the growth chamber being used was switched after first set of inoculations. While the exact source is not known; lights, air conditions, such as moisture levels, temperature, or pollutants could be possible explanations (University of Sydney 2003).

The first replicate of seedling inoculations performed on 93 lines of the Z02M DH population resulted in all lines demonstrating susceptibility with infection types (ITs) between ‘3’ and ‘4’. Since both susceptible and resistant checks showed a susceptible reaction, there may have been a problem

with the inoculum, potentially a mislabelled vial of inoculum. The same set of 93 DH lines produced a 38 resistant to 48 susceptible segregation ratio at the adult plant stage, which fitted the expected 1:1 segregation ratio (p = 0.281) for a single leaf rust resistance gene, 7 lines could not be evaluated due to poor plant development and/or lack of inoculum. The second replicate of inoculations was performed on self-pollinated seeds that resulted from the first planting. The seedling screening demonstrated a differential resistance reaction between the first and second leaf of most lines within the population, whereby the first leaf was usually more susceptible and the resistance was expressed more clearly on the second leaf to emerge (phenotypic ratings were based on second leaf ratings). In the second replicate there were 54 resistant and 38 susceptible lines, conforming to the expected 1:1 ratio (p = 0.096) for a single seedling resistance gene, one line was eliminated due to no germination. In this screening the susceptible parent (Thatcher-*Lr34*) also showed a resistant phenotype indicating the potential for: an introduction of an unknown source of seedling resistance through the susceptible parent, a seeding error, or a labelling error while harvesting seed. A second set of consistent phenotypic results to confirm the initial findings was not achieved due to the same disease and environmental factors experienced with the Z01M population.

Table 3.3. Phenotypic segregation ratios for resistance to *Puccinia triticina* race BBBD in the double haploid (DH) mapping populations Z01M and Z02M in two replications performed under controlled environmental conditions.

Population	Replicate	Stage	Resistant lines	Susceptible lines	X^2 (p-value)	Expected ratio
Z01M	1	Seedling	88	79	0.486	1:1
		Adult	119	48	0.264	3:1
	2	Seedling	88	79	0.486	1:1
		Adult	N/A	N/A	N/A	3:1
Z02M	1	Seedling	0	93	ERROR	1:1
		Adult	38	48	0.281	1:1
	2	Seedling	54	38	0.096	1:1
		Adult	N/A	N/A	N/A	1:1

N/A = no results

3.4.2 Field phenotyping of Z01M DH population

Phenotypic results from 2012 field trials conducted on the University of Manitoba campus are not reported due to very low levels of precipitation and high heat throughout the growing season resulting in stunted plant growth, little to no tillering, and slow disease development. The trial was also planted directly beside a stem rust experiment; high levels of stem rust at the time of rating concealed the low levels of leaf rust that did develop.

Phenotypic reactions for the 2013 field trials conducted on the University of Manitoba campus and Portage la Prairie research site resulted in variable distribution of phenotypes, especially intermediate and susceptible ratings (Appendix 8.1). In field studies, intermediate reactions consist of infection types that could not be positively classified as resistant or susceptible based on infection severity percentages and pustule types observed. Due to the variability of the field data, means were created across the replicates and locations. Classification of means was in 3 broad categories: susceptible (pustule types predominantly described as S and MS, severity 40% or greater), intermediate (pustule types predominantly described as MS, I and MR, severity ratings ranged from 15-50%), and resistant (pustule types predominantly MR and R, severity ratings ranged from 0-20%). Any lines for which a mean could not be created was eliminated from the reported data set (Appendix 8.1). Comparison of the means of field data collected in 2013 field trails with APR reaction types from a controlled environment (grouped using the same criteria as the field data) supported the APR ratings for which only a single replicate of data is available.

Table 3.4. Comparison of phenotypic reaction means observed in 2013 field trials conducted in Winnipeg and Portage La Prairie inoculated with an epidemic mix of *Puccinia triticina* races and adult plant resistance (APR) reactions in controlled environment for double haploid (DH) mapping population Z01M.

Data source	Susceptible lines	Intermediate lines	Resistant lines
APR controlled environment	31	86	51
2013 Field trial	40	82	46

3.4.3 Molecular analysis

Characterization of D genome in Z01M parental lines and DH population

The results molecular screening of the Z01M DH population parental lines (98-223-27 and Thatcher) and a subset of 6 resistant and 6 susceptible DH lines screened with 19 microsatellite markers and one primer pair linked to known resistance genes on the D genome were monomorphic, revealing no linkage to loci tested and confirming the unknown source of resistance was due to a novel resistance locus (Table 3.1). Screening of the entire DH population with the *Lr34*-specific marker Indel 11 determined that *Lr34* was present in 96 of the 167 lines observed (3 lines had ambiguous results in repeated screenings). These results did not fit the expected 1:1 ratio (p-value = 0.03) for the segregation of a single gene in a DH population.

Genotyping of Z01M and Z02M surrounding the Lr34 locus

The results of screening the three parental lines of both Z01M and Z02M populations (Thatcher, Thatcher+*Lr34*, and 98-223-27) with 35 microsatellite markers surrounding the *Lr34* locus on chromosome 7D identified eight polymorphic markers between the susceptible parents Thatcher and Thatcher+*Lr34* and the resistant parent 98-223-27 (Table 3.2). These polymorphic markers were then used to screen 93 DH lines from the Z01M population, 6 lines were removed as heterozygotes and 1 as an off-type phenotypically, leaving a mapping population of 86 DH lines. A two-point linkage analysis

was performed against the seedling stage phenotypic infection types observed in the controlled environment. Many SSR markers had poor resolution and could not be scored on the DH population. Microsatellite markers *wmc121* (recombination fration (RF) = 0.34) and *cfd21* (RF = 0.28) failed a chi-square test for independent segregation from *Lr34* marker Indel 11 (p = 0.003 and 0.0001, respectively) and are therefore segregating as expected on chromosome 7DS. Microsatellite marker *wmc121* segregated independently from seedling resistance (RF = 0.43, p = 0.19) while *cfd21* failed a chi-square test for independent segregation from seedling resistance (RF = 0.38, p = 0.03) and is therefore linked to the gene of interest. A genetic linkage map for the seedling resistance and associated SSR markers was constructed using MapDisto Version 1.7.5 mapping software (Lorieux 2012) and subsequently aligned with mapping locations described by published microsatellite maps (Figure 3.1) (Roder et al. 1998, Guyomarc'h et al. 2002, Somers et al. 2004). The seedling resistance mapped 12.0 centimorgans distal to *Lr34*. Microsatellite marker gwm295 was used as a landmark for Indel 11 as it does not appear on consensus maps. Gwm295 has repeatedly been described in association with QTL for the *Lr34/Yr18* region; while not diagnostic for breeding purposes it represents a general mapping location that appears on consensus maps and is associated with Indel 11 (Suenaga et al. 2003, Bossolini et al. 2006, Kolmer et al. 2008). Mapping order was consistent with those observed on consensus maps (Roder et al. 1998, Guyomarc'h et al. 2002, Somers et al. 2004).

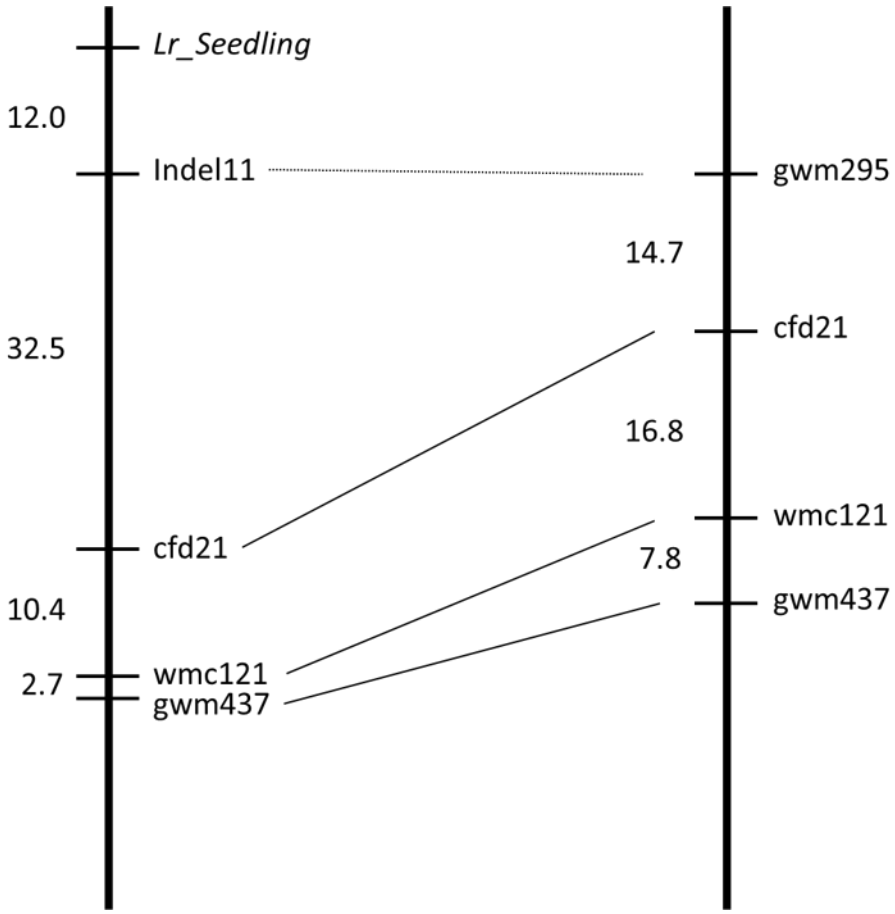

Figure 3.1. Linkage map displaying the seedling leaf rust resistance gene and associated microsatellite markers on the short arm of chromosome 7D, mapped on 86 individuals of the Z01M (Thatcher/98-223-27) doubled haploid population (left) compared to publiched consensus mapping locations (right). *Puccinia triticina* race BBBD was used for phenotyping. Distances are reported in centimorgans (cM).

3.5 Discussion

Phenotypic screening at the seedling stage of the Z01M population revealed a 1:1 segregation ratio ($p = 0.486$) for resistant to susceptible lines (Table 3.3). This result was contrary to what was expected, as initial observations made on the resistant line (by Dr. Kerber) demonstrated a hypersensitive response at the adult plant stage only. The appearance of resistance at the seedling stage indicated that the gene of interest may be a seedling gene rather than an APR gene as initially thought, or that an additional source of resistance was introduced at some point in the development of the DH population. Resistant parent, 98-223-27, demonstrated the expected resistant phenotype and

Thatcher, the susceptible parent, demonstrated a susceptible reaction. A possible explanation for the variable phenotypic results observed could be the introgression of a second resistance gene into resistant parent 98-223-27, likely originating from *Ae. tauschii*. Alternatively, an unknown source of seedling resistance could have been introduced by the susceptible parent Thatcher or during the recurrent backcross stages with Thatcher-*Lr34* before the creation of the DH population. While phenotypic screening of Thatcher plants used in this study with BBBD revealed a susceptible reaction at the seedling stage, this was not the exact seed source of Thatcher used in the development of the DH population. Chapter 5 will discuss evidence for previously unknown heterogeneous distribution of resistance genes in the Thatcher cultivar.

The first replicate of adult plant phenotyping of the Z01M population with leaf rust race BBBD in a controlled environment resulted in a 3:1 ratio which fits the expected segregation of two genes; the gene of interest as well as *Lr34*, the APR gene known to be segregating within this population. In this experiment a distinct mesothetic reaction was observed in 19 lines that exhibited a mixture of necrotic flecking and susceptible pustules. This phenotype was classified as a resistant reaction. Initially it was predicted that the mesothetic reaction was due to the presence of *Lr34* on its own in a line, as all of these lines were susceptible at the seedling stage and the unknown gene was suspected to provide the unexpected seedling resistance observed. *Lr34* has also been described as producing larger pustules on younger tissue (the base of the flag leaf or infection on younger flag leaves) with progressively smaller pustules and necrotic flecking appearing on older tissues near the tip of the leaves which could be described as mesothetic. However, results from screening with the *Lr34* marker, Indel 11, determined that none of the lines expressing the mesothetic reaction carried *Lr34,* indicating it may represent a unique phenotypic expression of an unknown gene in the absence of *Lr34*. In field studies, these genotypes generally displayed higher infection types ranging from fully susceptible to intermediate resistance under the pressure of the *P. triticina* 2013 epidemic mix. There were also six lines that were

susceptible at the seedling stage and resistant at the adult plant stage (may have been mesothetic but displayed a higher number of necrotic flecks resulting in a more resistant classification) that did not carry *Lr34* based on Indel 11. This resistance could be the result of the APR gene that was first described by Kerber indicating that a third, unknown, seedling resistance gene may have been introduced during the creation of the DH population. There were also three DH lines that were scored as susceptible at the adult plant stage, but were positive for *Lr34* based on Indel 11. Oelke and Kolmer (2005) described an intermediate to high infection type (33+/23) on *Lr34*. This may explain the erroneous classification of the DH lines. A number of repeated screenings were attempted but variability in infection types recorded for each line due to poor infection establishment in the controlled environment and contamination with the highly destructive disease powdery mildew (Tilt application was unsuccessful in controlling the disease) resulted in no consistency with the initial phenotypic segregation observed.

Comparisons of the phenotypic data collected from the field study of the Z01M population in 2013 and the adult plant phenotypes observed in the controlled environment was complicated due to the aforementioned intermediate and moderate levels of resistance and susceptibility exhibited in field trails under pressure of the epidemic mix of the pathogen. Overall, field infection types tended to be slightly higher than those observed in the controlled experiments. This could be explained by the use of the epidemic mix in the field which provides many other races of *P. triticina* that could be virulent to the gene(s) in question, reducing the resistance observed in the field as compared to those in the controlled environment with a single race. This variability also includes many of the lines that were described as having a mesothetic or intermediate infection type which can introduce rating errors and could be mistaken for a susceptible or resistant reaction, depending on the level of infection. The means created across the locations and replicates provided a dataset that could be compared with the controlled APR reactions. Means of the field results supported the results observed in a controlled environment at the adult plant stage but were not sufficient as a second replicate of data.

All 93 DH lines, the resistant parent, and the susceptible parent of the Z02M DH population showed susceptible reactions (IT of '3' or greater) at the seedling stage in the first replicate of controlled environment inoculations. These results were as expected for an adult plant resistance gene. However, the results of the Z01M population screening suggested the unknown gene was in fact a seedling resistance gene and therefore these results were considered erroneous and could have been caused by the use of a mislabelled tube of rust inoculum being used. The same plants were inoculated at the adult plant stage with race BBBD and produced a 38 resistant to 48 susceptible segregation ratio, which fit the expected 1:1 segregation ratio (p-value = 0.281) for a single gene. Leaf rust race BBBD is described as having an intermediate infection type (2/3+) on APR gene *Lr34* (Oelke and Kolmer 2005), which is fixed in the Z02M population. This intermediate infection type may have been rated as a susceptible reaction. The second replicate of inoculations was performed on self-pollinated seeds harvested from the first planting. The seedling screening demonstrated a differential resistance reaction between the first and second leaf of most lines within the population, whereby the first leaf was usually more susceptible and the resistance was expressed more clearly on the second leaf. The results of the second replicate of inoculations supported the hypothesis that a mislabelled source of inoculum was used in the first replicate. There were 54 resistant and 38 susceptible lines, which fit the expected 1:1 ratio (p = 0.096) for single gene segregation. These infection types correlated 82% of the time with the adult plant resistance segregation in the first replicate of inoculations. Despite being sprayed with Tilt, the adult plants in this population developed powdery mildew before adult plant inoculations could be performed and were not suitable for testing.

Gene postulation by genotyping with molecular markers known to be linked to the gene in question is an effective method to confirm a known source of resistance in a line, as well as confirm a novel source of resistance based on its genetic location (Hiebert et al. 2010, Vanzetti et al. 2011, Abdelbacki et al. 2013). In an exploration of *Aegilops tauschii* , the D genome progenitor of wheat, Dr.

Eric Kerber identified an accession RL5767 as a carrier of novel adult plant resistance. A synthetic hexaploid, RL5866, was developed (Tetra-Canthatch/RL5767) that expressed a hypersensitive response at the adult plant stage. Monomorphic results from screening with molecular markers linked to resistance genes on the D genome (Table 3.1) in the two DH populations (Z01M and Z02M) created from this synthetic hexaploid suggest they are unlikely to carry any of the currently characterized leaf rust resistance genes on the D genome.

The presence of *Lr34*, an APR gene also carried on the D genome, believed to segregate in the DH population Z01M was confirmed by a screening of the population with the gene-specific marker Indel 11. It was shown that 96 of the 167 lines in the population carried *Lr34*. This deviation from the expected ratio could be the result of skewing, referred to as segregation distortion, observed after the double haploid production system that may have favoured the survival of gametic genotypes carrying favourable alleles near the *Lr34* locus (Nakagahra 1972). Alternatively, this could be due to random or sampling error where a null hypothesis is true even when the statistical analysis rejects it as was observed by Thomas et al. (2010) in the mapping of *Lr32*.A two-point linkage analysis was performed with the genotypic marker data and phenotypic seedling resistance data and revealed an 8.3% recombination frequency (RF) between the segregation of seedling resistance and presence of *Lr34*. This low RF putatively indicates linkage of the gene of interest to *Lr34* providing a potential mapping location on the short arm of chromosome 7D. This result is further support of a third, APR, gene segregating in the population. If the DH population was segregating for only the two linked genes (the unknown seedling resistance and *Lr34*), it should not fit the 3:1 segregation ratio expected for two independent genes.

To confirm the putative linkage of the seedling resistance with *Lr34*, parental lines Thatcher, Thatcher+*Lr34*, and 98-223-27, were genotyped with 35 microsatellite markers (Table 3.2) surrounding

the *Lr34* locus on chromosome 7D. Polymorphic markers on chromosome 7D represent potential chromosomal locations of the gene of interest. The eight polymorphic markers from the parental screening were selected to test on 94 DH lines from the Z01M population. Genetic output was used in a two-point linkage with the seedling phenotypic infection types observed in the controlled environment. A genetic map describing the location of the unknown seedling resistance relative to *Lr34* (Indel 11) and three linked microsatellite markers, *cfd21*, *wmc121,* and *gwm437* was generated. The linkage and genetic location of the novel source of seedling resistance distal to *Lr34* on the short arm of chromosome 7D was confirmed (Figure 3.1). Mapping order was consistent with those observed on consensus maps and genetic distances were relative to those in the mapping populations as well (Roder et al. 1998, Guyomarc'h et al. 2002, Somers et al. 2004). A similar comparison of correlation of recombination distances and marker distribution along the consensus map (Roder et al. 1998) was used to confirm the identification of a single gene, *Lr32*, and its location on chromosome 3DS (Thomas et al. 2010).

Phenotypic and genotypic analysis of the Z01M population revealed 25 lines that were susceptible at the seedling stage and resistant at the adult plant stage, but were negative for the presence of *Lr34.* This result indicates a potential third gene segregating in the population. F_2 population development with representatives of each of the various phenotypic (seedling and adult plant resistance reactions) and genotypic (Indel 11 marker data) combinations and subsequent analysis of F_2-derived F_3 families, could offer a way to characterize and map the various components contributing to leaf rust resistance in the Z01M DH population.

Due to the limited timeframe of a Master of Science project it became unlikely that a clear conclusion could be reached on this project. The inability to repeat the initially observed phenotypes, troubles with achieving rateable infection levels, the appearance of a potential third gene in one

population, and the inability to trace back where it was introduced lead to my decision to stop pursuing this project and to concentrate my efforts on an alternate project that I had started when these issues started to appear. The initial observation of a 91.7% co-segregation of seedling resistance in the Z01M DH population and APR gene *Lr34* was confirmed through genotyping with microsatellites surrounding this locus on chromosome 7D. The seedling *Lr* gene was genetically mapped 12.0cM distal to *Lr34* (Figure 3.1). The appearance of a 1:1 segregation ratio in the seedling stage of both Z01M and Z02M DH mapping populations supports the presence of an unknown seedling resistance gene segregating in both populations, contrary to the initial findings of Dr. Kerber that indicated an APR gene. The 3:1 segregation ratio of resistant to susceptible lines at the adult plant stage in the Z01M DH mapping population supports that two genes are segregating. While it was initially predicted that these genes were the unknown seedling gene and *Lr34* (known to segregate in this population), the close linkage identified between these two genes contradicts this hypothesis and suggests a third gene in the population. This is further supported by the appearance of DH lines in the Z01M population that were susceptible to BBBD at the seedling stage, but resistant at the adult plant stage, and known to be negative for the presence of *Lr34* through screening with Indel 11. Until repeatable adult plant phenotyping is achieved in this population, the third APR gene remains a putative conclusion that could be studied further through the development of F_2-derived F_3 families for phenotypic and genotypic observation. Additionally, a new cross of a susceptible line with a DH line identified as carrying only the unknown APR gene may provide clarity.

CHAPTER 4

IDENTIFICATION, CHARACTERIZATION AND MAPPING OF *LRCEN*, A NEW SEEDLING LEAF RUST *(P. triticina*) RESISTANCE GENE IN SPRING WHEAT (*T. aestivum*)

4.1 Abstract

Wheat leaf rust, caused by *Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex Desmaz. f. sp. *tritici*), is the most widespread disease of wheat worldwide and causes average annual yield losses of 5 to 25%. The emergence of a new predominant race of leaf rust, TDBG, in the 2004 Canadian virulence survey led to the identification of a second leaf rust resistance gene segregating in the Thatcher-*Lr1* near-isogenic differential line, RL6003, which produced an unusual mesothetic infection type. This gene was subsequently isolated in a Thatcher background and temporarily designated as *LrCen* (Tc-*LrCen*). A cross was made with a susceptible parent (Tc-*LrCen*/ Sumai3-*lr34*) and a doubled haploid (DH) mapping population was generated from the hybrids. Parental lines and 180 double haploid (DH) individuals were phenotyped with race TDBG and a 1:1 ratio was observed in the DH population. Parental lines and 94 DH individuals were genotyped with the Illumina Infinium assay using a custom iSelect 90K wheat SNP array. Two-point linkage between the phenotype and polymorphic SNP markers identified linked markers. A BLAST search of linked SNP sequences was performed against the Wheat Survey Sequence providing a putative chromosomal location of 7AL. Subsequent mapping with microsatellite markers confirmed *LrCen* was located on the long arm of chromosome 7A flanked by *gwm344* (9.5 cM) and *cfa2240* (0.6 cM) as well as a group of co-segregating SNPs also at a genetic distance of 0.6 cM. When the SNP sequences were converted to the kompetitive allele specific PCR (KASP) markers they were found to be dominant, making them less useful for marker assisted selection in populations with heterozygotes. *LrCen* mapped distal to *Lr20*; the only other *Lr* gene previously identified on chromosome 7AL.

4.2 Introduction

Wheat leaf rust is the most wide-spread disease of common and durum wheat and occurs nearly everywhere it is grown (Kolmer 1996). Wheat leaf rust is caused by the basidiomycete *Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex Desmaz. f. sp. *tritici*) and is a major production constraint of wheat in North America, causing annual yield losses ranging from 5 to 25% and higher losses under epidemic conditions (Anikster et al. 1997; McCallum et al. 2010). In Canada, leaf rust infections are caused by uredinospores that are dispersed via wind currents from the southern United States, where they overwinter on emerging winter wheat crops and volunteer plants known as a "green bridge" (Roelfs et al. 1992). Severity of leaf rust infection varies from year to year and is highly influenced by climate. It is often not economical for producers to apply costly fungicides. Crop rotation, a common cultural practice in combating disease, is not effective against the wind transported spores. The preferred method of leaf rust control is through breeding for genetic resistance, as it comes at no additional cost to producers and reduces the environmental impacts associated with fungicide application. While genetic resistance has been achieved through various combinations of *Lr* genes, *P. triticina* is an obligate parasite and rapid evolution of the pathogen is required for its survival to overcome host resistance genes (McIntosh et al. 2010; Herrera-Foessel et al. 2012) making the search for new sources and combinations of resistance important. More than 60 races of leaf rust are observed in North American virulence surveys annually. While most of these races are observed from year to year, the emergence of new virulent races often leads to the breakdown of resistance genes (McCallum et al. 2007; McCallum et al. 2010). Resistance has been overcome by the pathogen in a number of Canadian wheat cultivars including Katepwa (1980s), AC Barrie (1990s), and Superb (2000s); each of which was widely grown during the indicated periods, but was inevitably overcome by the evolutionary pressures they exerted on the pathogen population (McCallum et al. 2008).

Dr. Peter Dyck, formerly of the CRC – AAFC developed a series of near isogenic wheat lines (NILs) in a Thatcher (Tc) background, each containing a single leaf rust resistance (Lr) gene. Crosses between Thatcher and the donor parent wheat lines were followed by five backcrosses coupled with selections for resistance in each generation. Following the final backcross, homozygous resistant individuals were selected from selfed families. These differential lines are used extensively world-wide in virulence surveys, genetic studies of resistance genes, and host/parasite interaction experiments (McCallum and Hiebert 2012). Because of the rapid evolution of *P. triticina*, annual virulence surveys are conducted in North America and other wheat growing regions to monitor virulence patterns and plan breeding efforts accordingly. The emergence of a number of isolates with a new virulence pattern collected in 2004 (now known as TDBG and some isolates of TDBJ (Long and Kolmer 1989)) produced an unusual mesothetic infection type with some individuals of the Thatcher-*Lr1* NIL, RL6003 (pedigree: Thatcher*^{6}/Centenario; Dyck and Samborski 1968). Further phenotyping of these lines with *P. triticina* race BBBD, avirulent to *Lr1*, demonstrated uniform resistance suggesting avirulence of race TDBG was due to a secondary resistance gene previously unnoticed until the emergence of avirulence in race TDBG. This seedling resistance gene was temporarily designated as *LrCen,* as it was thought to have been introduced via the *Lr1* donor parent, Centenario (McCallum et al. 2005). Further research on this gene is required.

The objective of this study was to: (i) determine the inheritance of *LrCen*, (ii) genetically map this resistance gene in the Tc-LrCen/Sumai3-lr34 double haploid population segregating for resistance, and (iii) identify flanking DNA markers.

4.3 Materials and Methods

4.3.1 Population development

A DH population, Tc-LrCen/ Sumai3-lr34, was used in this study. A cross was made between a selection of RL6003 (a NIL of Thatcher carrying *Lr1* and *LrCen*) with resistance to *P. triticina* race TDBG (avirulent to *LrCen* and virulent to *Lr1*) and the susceptible cultivar Thatcher. F_2 progeny were evaluated with leaf rust races TDBG and BBBD (avirulent to *Lr1*) and an individual expressing a low infection type under disease pressure from TDBG but a high infection type under pressure of race BBBD was selected as containing only the *LrCen* resistance gene, Thatcher (Tc)-*LrCen*, and was crossed to universally susceptible line Sumai3-*lr34* (Sumai3^{*6}/Thatcher). F_1 plants from the Tc-LrCen/Sumai3-lr34 population were used to create a double haploid (DH) population (Hiebert, unpublished) using the maize pollination method (Thomas et al. 1997).

4.3.2 *P. triticina* inoculations

A set of 188 DH lines from the Tc-LrCen/ Sumai3-lr34 population and two replicates of each parental line were germinated in six 4x8 root trainers containing Sun Gro Horticulture Sunshine Professional Growing Mix 5 (Agawam, MA, USA; www.sungro.com). Plants were grown for eleven days prior to inoculation in a controlled environment under 16 light and 8 dark hours at 20˚C and 18˚C respectively. At head emergence, GS40-49 (Zadoks et al. 1974), glassine bags were placed over one spike per plant to ensure self-pollination to provide a seed source for additional study.

Single spore inoculum was developed via the method described by McCallum and Goh (2003). Spore viability was tested by spreading a single layer of spores across a 2% water agar petri plate left overnight in a dark, cool environment. The spores were then observed under a microscope the following day to ensure successful germtube development. Ratings were performed to determine virulence patterns based on infection types observed across the 16 differential lines (Table 4.1).

Seedling inoculations were performed with single spore isolates of the *LrCen* avirulent *P. triticina* race TDBG. Vacuum dried urediniospores isolated using the method described above (stored at 4˚C) were removed from refrigeration, left at room temperature to rehydrate for a period of 3 hours (Roelfs et al. 1992) and heat shocked at 40˚C for five minutes in a water bath. Eleven day old seedlings were inoculated using the oil suspension misting system described previously by McCallum and Seto-Goh (2003). Post inoculation, plants were left for approximately 30 minutes to allow the oil to volatilize, leaving the spores bound to the leaves. Inoculated seedlings were then placed in a dew chamber overnight at 100% relative humidity under dark conditions at approximately 20˚C. The following day, plants were removed from the dew chamber and air dried before being returned to the controlled environment previously described.

After seedling ratings were performed, plants were treated with Tilt fungicide (Syngenta Canada Inc., Guelph, ON; www.syngenta.ca) to prevent contamination with powdery mildew before adult plant inoculations and then transplanted to one liter pots (3 plants/pot) containing three parts bulk soil to one part Sun Gro Horticulture Sunshine Professional Growing Mix 5 (Agawam, MA, USA; www.sungro.com). Plants were watered as needed and treated with a water-soluble 20-20-20 fertilizer on a once weekly schedule. The same inoculation process was repeated on the population at the adult plant stage at the onset of boot stage/ head emergence, GS40-49 (Zadoks et al. 1974).

Table 4.1 Single-gene near isogenic line (NIL) differential sets used in the nomenclature to designate leaf rust (*P.triticina)* races in wheat (Long and Kolmer 1989).

	Single-gene NIL			
Set 1	Lr1	Lr2a	Lr2c	Lr3
Set 2	Lr9	Lr16	Lr24	Lr26
Set 3	Lr3ka	Lr11	Lr17	Lr30
Set 4	LrB	Lr10	Lr14a	Lr18

4.3.3 Disease assessment

Phenotypic evaluation of infection type was performed on the first and second leaves 12 days post inoculation (DPI) for seedlings, and on the flag leaf at 16 DPI for adult plants. Infection types were classified following the leaf rust rating scale described by Long and Kolmer (1989) using specific modifications outlined by Roelfs et al. (1992).

Host infection types range from "0" representing complete immunity with no macroscopic sign of infection, ";" hypersensitive flecking with no visible uredinia, "1" very resistant with only small uredinia surrounded by necrosis, "2" small to medium uredinia surrounded by necrosis/ chlorosis, "3", moderately susceptible with medium sized uredinia surrounded by chlorosis to "4", fully susceptible with large uredinia and no chlorosis. A host IT can also be scored as "X" representing a heterogeneous distribution of variably sized uredinia from necrotic flecks to medium sized pustules surrounded by chlorosis/necrosis (Long and Kolmer 1989; McCallum et al. 2010). These ratings may be adjusted by using characters such as a minus (-) to represent an individual in a category with uredinia on the lower limit or a plus (+) to indicate uredinia on the upper limit of a particular category. Multiple – or + may be used to further differentiate infection types (Roelfs et al. 1992). Infections types from 0 to 2 were considered as resistant host responses, while infection types 3 to 4 were considered as susceptible host responses. Additionally, a rating of X was given to individuals exhibiting heterogeneous distribution of variably sized uredinia from necrotic flecks to medium sized pustules surrounded by chlorosis/necrosis. Infection types rated as X were considered resistant. Data were tested for goodness of fit to a 1:1 segregation ratio expected for a single gene in a DH poplation using Chi-Square analysis.

4.3.4 DNA extraction

The following protocol was used for DNA extraction for microsatellite, Kompetitive Allele Specific PCR (KASP), and sequence-tagged sites (STS) marker analysis. Leaf tissue (four pieces

approximately 2.5-3.8 cm) was collected from the third or fourth leaf (youngest, non-infected tissue), after infection types were assessed on the first and second leaf. Tissue was placed in two 96-well collection microtube extraction plates with glass beads at the base for tissue lysing. Tissue was lyophilized on the Christ Alpha 1-4 LD plus (Montreal Biotech Inc., Kirkland, QC, 3551 St-Charles BLVD. Suite 506) overnight and subsequently stored at -20°C. Immediately prior to extraction, tissue was macerated to a fine powder using the Qiagen TissueLyser II (Qiagen, Missassauga, ON; www.qiagen.com) for ten minutes, flipping orientation after five minutes. DNA was extracted using a modified ammonium acetate extraction protocol (Chao and Somers, http://maswheat.usdavis.edu/PDF/DNA0003.pdf, accessed February 2012) based on procedures of Pallotta et al. (2003) (Pallotta et al. 2003; Hiebert et al. 2010). DNA was quantified using Hoechst 33258 stain on a fluorometer and stock DNA was then diluted to a 15 ng/µl working solution.

The following protocol was used for DNA extraction for Illumina Infinium assay analysis. Leaf tissue (approximately 50 mg wet weight) was collected from seedlings at the three to four leaf stage (youngest, non-infected tissue), after infection types were assessed on the first and second leaf. Tissue was placed into two 96-well collection microtube extraction plates with a 4mm tungsten carbide bead at the base for tissue lysing. Tissue was lyophilized on the ChristAlpha 1-4 LD plus (Montreal Biotech Inc., Kirkland, QC, 3551 St-Charles BLVD. Suite 506) overnight and subsequently stored at -20°C. Immediately prior to extraction, tissue was macerated to a fine powder using the Qiagen TissueLyser II (Qiagen, Missassauga, ON; www.qiagen.com) for 2 minutes, flipping orientation after 1 minute. DNA was extracted with the Qiagen DNeasy 96 Plant Kit (Qiagen, Mississauga, ON, www.qiagen.com) and quantified on a fluorometer using PicoGreen stain (Life Technologies Inc, Burlington, ON; www.lifetechnologies.com). Stock DNA was then diluted to a 50 ng/µl working solution.

4.3.5 Association mapping and preliminary SSR screening

Association mapping

The parental lines used in the creation of the experimental Tc-LrCen/Sumai3-lr34 DH population (Tc-*LrCen* and Sumai3-*lr34*) and Thatcher were previously included in a genome-wide association study (GWAS) of 256 wheat cultivars with 336 microsatellite markers (SSRs) (Roder et al. 1998; Somers et al. 2004; Song et al. 2005) representing 372 loci selected based on genome coverage and simplicity of marker profile to facilitate association mapping experiments (D.J. Somers, unpublished data). McCallum (unpublished) conducted phenotyping of all lines included in the initial SSR screening with a number of informative *P. triticina* races including: BBBD, TDBG (avirulent on *LrCen, Lr16* and *Lr22a*), MBDS (avirulent on *Lr16*), and MGBJ (virulent on *Lr16,* avirulent on *Lr2a*). These phenotypic data were used to select lines most likely to express a low infection type due to the presence of *LrCen* with no epistatic effects with resistance genes *Lr16* and *Lr22,* to which race TDBG is also avirulent (Table 4.2). *Lr2a* lines were included as a second verification of the association mapping software. This set of informative lines, as well as lines known to carry *Lr16, Lr22a* and *Lr2a*, which were used as checks, were selected for association mapping with TASSEL version 3.0 – Trait Analysis by Association, Evolution and Linkage software (Cornell University, Ithaca, NY; www.maizegenetics.net). For this study, microsatellite data from the Somers GWA study and phenotypic data from McCallum were analyzed using TASSEL. Microsatellites with the highest F-statistic and the most significant P-value were selected from the TASSEL output to screen Tc-*LrCen* and Sumai3-*lr34* and three resistant bulks and three susceptible bulks (created using individuals from the experimental DH population) for linkage analysis. Each of the three resistant and susceptible bulks was composed of five unique resistant or susceptible DH lines respectively. PCR and fragment analysis (with an ABI 3100 genetic analyzer, Applied Biosystems, Streetsvill, ON, Canada) were performed as described by Somers et al. (2004).

Table 4.2 Postulated *LrCen* lines*, Lr16,* and *Lr2a* lines used in association mapping of leaf rust resistance gene *LrCen* based on the infection types of three informative *Puccinia triticina* isolates generated in the lab of McCallum (unpublished).

Line	TDBG	MBDS (Avr *Lr16*)	MGBJ (Avr Lr2a)	*Lr* gene
Domain	11-n	1-2-	3	16
Invader	1-n	1+	3-	16
Prodigy	11+	1+	3-	16
BW278	1+	1+	3+	16
RL4137	1+	;1-	3+	16
BW310	1+-n	11-	3	16
98B19_N22	1-n	1-	3+	16
98B25-AS6D01	1+-n	11-	3-	16
98B34-T4B	1+	1-	3	16
98B50-H4D	1+	1-	3	16
BW346	1+-n	1+	3-	16
Snowbird	1+n	1-	3+	16
98B19-AG2C01	1+-n	1-	3	16
98B19-AG2C06	1+-n	1-	3	16
Kanata	1+-n	1+	3+	16
BW278	1+-n	1+	3-	16
94B_F26	1+-n	1+	3-	16
94B43-BLW4	1+n	1+	1+3-	16
Bronze	1+-n	1+	3-	16
BW346	1+	1+	3	16
BW833	1+	1+	3-	16
McVey	;1=	1+	3-	16
PT211	1+	1-	3+	16
SD2980	1+n	1-	1+3-	16
Kenyon	1+n	1+	1+	16
Minto	2-	;1-	;1=	22a
Superb	3+	0;	0;	2a
BW293	3+	;	;	2a
ND2710	3+	0	0;	2a
98B69_H36	3+	0;	;	2a
ALSEN	3+	0	;	2a
98B25-AU5A01	3+	;	;	2a
98B25-DF2B01	3+	0;	;	2a
98B25-DF2B04	3+	;	;	2a
98B43-BW4E	3+	;	;	2a
Grandin	3+	;	;	2a
Apr-37	3+	;	;	2a
94B35-1923A	3+	;	;	2a

NORSEMAN	3=	0	;	2a
Benito	3+	0;	;	2a
Lancer	;1-3-X	3	3+	Cen*
Park	;1-3-X	3-	3	Cen
Garnet	;1-3X	3+	3+	Cen*
Marquis	;1=	3	3+	Cen
00H01_P61	;1-3-X	0	0;	Cen
96B42-E3C	;1-3-X	3	3	Cen
ND739	X-	0	;	Cen
8901-DL1C	;1-3-X	;	;	Cen*
EATONIA	;1-3-X	3+	3	Cen*
BW608	;1=X	3+	3-	Cen*
BW621	;1=3-X	3+	3	Cen*
BW666	;1=3-X	3+	3-	Cen*
BW710	;1=3-X	3+	3+	Cen*
BW828	;1=3-X	;	;	Cen
CANUCK	;1=3-X	3+	3+	Cen*
CUNNINGHAM	;1-X-	;	0;	Cen*
DM5637_B8	;1-X-	;	;	Cen*
JANZ	;1-3-X-	0	0	Cen*
LEADER	;1-3-X-	3+	3	Cen*
ND643	;1-3-X	0	;	Cen*
PT555	;1-3-X-	;	0	Cen
Cypress	;1-X-	3+	3	Cen*
Renfrew	;1=n	3+	3+	Cen
Coronation	;1-3-X	3+	3	Cen
Lake	;1=3-X	3+	3-	Cen
Lee	;1=X-	3	3-	Cen
Bro_White	;1=X-	3+	3+	Cen
Preston	;1=X-	3+	3+	Cen
RedBobs	;1=X-	3+	3+	Cen
RedFife	;1=X-	3+	3+	Cen
Regent	;1=X-	3+	3+	Cen
Renown	;1=	3+	3+	Cen
Stanley	;1=3-X-	3+	3+	Cen
Suprime	;1=3-X-	3+	3+	Cen
WhiteFife	;1-X-	3+	3+	Cen
Apex	;1-X-	3+	3+	Cen
Rescue	;1=3-X-	3+	1+3-	Cen*

* = Chapter 5 generated DNA marker data that support the presence of *LrCen*, those unmarked are less likely to carry the gene.

Preliminary SSR screening

Using the same 336 SSR markers used in the Somers (unpublished) genome-wide SSR screening, comparisons were also made between Tc-*LrCen* and Thatcher as well as Tc-*LrCen* and Sumai3-*lr34* (Hiebert, unpublished). From this, 34 polymorphic markers (Table 4.3) were identified representing regions that could harbour *LrCen.* These 34 polymorphic markers were screened on the Tc-LrCen/Sumai3-lr34 DH parental lines as well as three resistant bulks and three susceptible bulks (each bulk was a mixture of DNA from 5 unique resistant lines or 5 unique susceptible lines, respectively) to test for linkage to *LrCen*. Markers showing putative linkage to *LrCen* were then screened on Tc-*LrCen*, Sumai3-*lr34,* and 94 DH lines, a subsample of the original 188 DH lines, for linkage analysis. These 94 lines represent the first plate of DNA extracted from the entire population. This subset was selected as an initial exploratory set that was large enough to demonstrate possible linkage, while being time and cost efficient. PCR and fragment analysis (with an ABI 3100 genetic analyzer, Applied Biosystems, Streetsvill, ON, Canada) were performed as described by Somers et al. (2004).

Table 4.3 Markers polymorphic on Thatcher, Thatcher-LrCen, and Sumai3-lr34 from genome-wide microsatellite screenings (Somer, unpublished and Hiebert, unpublished) selected for screening the Thatcher-LrCen/Sumai3-lr34 double haploid (DH) population

Locus	Chromosome	Annealing Tm (°C)	Forward Sequence	Reverse Sequence	Reference
barc108	7A	50	GCGGGTCGTTTCCTGGAAATTCATCTAA	GCGAAATGATTGGCGTTACACCTGTTG	A
barc148	1A/1D	52	GCGCAACCACAATGTATGCT	GGGGTGTTTTCCTATTTCTT	A
barc18	2B	52	CGCTTCCCATAACGCCGATAGTAA	CGCCCGCATCATGAGCAATTCTATCC	A
barc54	6D	60	GCGAACAGGAGGACAGAGGGCACGAGAG	GCGCTTTCCCACGTTCCATGTTTCT	A
barc67	3A	52	GCGGCATTTACATTTCAGATAGA	TGTGCCTGATTGTAGTAACGTATGTA	A
cfa2147	1B	61	TCATCCCCTACATAACCCGA	ATCGTGCACCAAGCAATACA	B
cfa2219	1A	61	TCTGCCGAGTCACTTCATTG	GACAAGGCCAGTCCAAAAGA	B
cfa2250	5A	61	AGCCATAGATGGCCCTACCT	CACTCAATGGCAGGTCCTTT	B
cfa2257	7A	61	GATACAATAGGTGCCTCCGC	CCATTATGTAAATGCTTCTGTTTGA	B
cfd175	7D	61	TGTCGGGGACACTCTCTCTT	ACCAATGGGATGCTTCTTTG	C
cfd223	3D	61	AAGAGCTACAATGACCAGCAGA	GCAGTGTATGTCAGGAGAAGCA	C
cfd6	3B	61	ACTCTCCCCCTCGTTGCTAT	ATTTAAGGGAGACATCGGGC	C
cfd73	2D	61	GATAGATCAATGTGGGCCGT	AACTGTTCTGCCATCTGAGC	C
gwm156	5A	51	CCAACCGTGCTATTAGTCATTC	CAATGCAGGCCCTCCTAAC	D
gwm193	6B	61	CTTTGTGCACCTCTCTCTCC	AATTGTGTTGATGATTTGGGG	D
gwm257	2B	61	AGAGTGCATGGTGGGACG	CCAAGACGATGCTGAAGTCA	D
gwm335	5B	61	CGTACTCCACTCCACACGG	CGGTCCAAGTGCTACCTTTC	D
gwm340	3B	61	GCAATCTTTTTTCTGACCACG	ACGAGGCAAGAACACACATG	D
gwm369	3A	61	CTGCAGGCCATGATGATG	ACCGTGGGTGTTGTGAGC	D
gwm400	7B	61	GTGCTGCCACCACTTGC	TGTAGGCACTGCTTGGGAG	D
gwm471	7A	61	CGGCCCTATCATGGCTG	GCTTGCAAGTTCCATTTTGC	D
gwm498	1B	61	GGTGGTATGGACTATGGACACT	TTTGCATGGAGGCACATACT	D
wmc177	2A	61	AGGGCTCTCTTTAATTCTTGCT	GGTCTATCGTAATCCACCTGTA	E
wmc24	1A	51	GTGAGCAATTTTGATTATACTG	TACCCTGATGCTGTAATATGTG	E
wmc336	1A	61	GTCTTACCCCGCGATCTGC	GCGGCCTGAGCTTCTTGAG	E
wmc44	1B	61	GGTCTTCTGGGCTTTGATCCTG	TGTTGCTAGGGACCCGTAGTGG	E

wmc477	2B	61	CGTCGAAAACCGTACACTCTCC	GCGAAACAGAATAGCCCTGATG	E
wmc517	7B	61	ATCCTGACGTTACACGCACC	ACCTGGAACACCACGACAAA	E
wmc594	3A	51	CCCCTCACTGCCG	ATATCCATATAGTACTCGCAC	E
wmc632	3B	61	GTTTGATTGGTCGTTCCTGGTC	AACAGCGAATGGAGGGCTTTAG	E
wmc698	7D	51	GTGAAGGGAGAGCTAGCAA	ACAGTTGGCCCAGCTAGTA	E
wmc809	7A	61	CAGGTCGTAGTTGGTACCCTGAA	TGAACACGGCTGGATGTGA	E
wmc824	7D	61	CCGATGAACTTAAAAGTACCACCTG	CATGGATTGACACGATTGGC	E
wmc9	7A	51	AACTAGTCAAATAGTCGTGTCCG	GTCAAGTCATCTGACTTAACCCG	E

A = Song et al. 2005, B = Sourdille et al. 2004, C = Guyomarc'h et al. 2002, D = Roder et al. 1998, E = Somers and Isaac 2004

4.3.6 Illumina Infinium HD Assay Ultra

The Illumina Infinium (San Diego, CA, USA; www.illumina.com) iSelect 90K wheat SNP chip (Wang et al. 2014) was used to genotype the first 94 DH individuals from the Tc-LrCen/Sumai3-lr34 population plus each parental line (Tc-*LrCen* and Sumai3-*lr34*). The remainder of the population was screened using a subset of informative markers after the preliminary map was created. Marker filtering was performed using Illumina GenomeStudio to identify SNPs polymorphic between the parental lines; any ambiguous or uninformative SNPs (ie. low call rates or those that detected polymorphic SNPs at more than one locus) were removed from the dataset before analysis. Filtered marker data was exported to Microsoft Excel 2010 and a two-point linkage analysis was performed between the infection types obtained through phenotypic evaluation with race TDBG (i.e. *LrCen*) and the polymorphic SNPs. Recombination fractions (RF) were calculated for each polymorphic SNP (Appendix 8.2). SNPs were then sorted based on RF, eliminating any SNPs with an RF value greater than, or equal to, 0.5 as an initial threshold to identify putatively linked SNPs. The reduced set of markers was then imported into MapDisto Genetics Software Version 1.7.5 (Mathias Lorieux; www.mapdisto.free.fr) for linkage analysis. This analysis removed any SNPs with an RF above 0.38. To assess chromosome location of markers demonstrating linkage to *LrCen*, SNPs were compared to a previously constructed map created with the iSelect 90K wheat SNP chip (Cabral et al. 2014). Additionally, a basic local alignment tool (BLAST) search was performed of the sequences surrounding the SNPs in the Wheat Survey Sequence (http://www.wheatgenome.org/Projects/IWGSC-Bread-Wheat-Projects/Sequencing/IWGSC-Chromosome-Survey-Sequencing).

4.3.7 Linkage mapping

Marker screening

A BLAST search revealed a putative chromosomal location on the long arm of 7A. Directed chromosomal screening of the Tc-LrCen/Sumai3-lr34 DH population was used to identify markers on chromosome 7AL putatively linked to *LrCen*. Initial screening was performed on the same 94 DH individuals from the *LrCen* population, plus each parental line (Tc-*LrCen* and Sumai3-*lr34*) used for Illumina Infinium genotyping. Screening was performed using one STS marker and 30 SSRs (Table 4.4). Recombination fractions were calculated for each marker. Markers with RF values less than 0.5 were selected for putative linkage to *LrCen*. The second plate of 94 DH individuals from the *LrCen* DH population and a set of parental lines were then screened with SSR markers demonstrating putative linkage.

Linked SNPs from the iSelect 90K wheat SNP chip were converted to the KASP markers (Teddington, Middlesex, UK; www.lgcgenomics.com). Source sequences for SNP markers from the 90K iSelect array were used to design two allele specific forward primers and one common reverse primer for the KASP assay (LCG Genomics - KBioscience, Teddington, Middlesex, UK; www.lgcgenomics.com) (Appendix 8.2). A group of KASP markers were selected for screening on the parental lines, plus seven resistant and seven susceptible lines, to ensure successful conversion, marker quality, and marker robustness. Using the MapDisto output of the Illumina Infinium iSelect data, 44 robust KASP markers, one representative marker of each group of co-segregating SNPs linked to *LrCen,* and a higher density of markers for the groups most closely linked to *LrCen* as indicated in Appendix 8.2, were selected to screen the second plate of the *LrCen* DH population.

Table 4.4 Microsatellite markers on chromosome 7AL used to screen the Thatcher-LrCen/Sumai3-lr34 double haploid (DH) population

Locus	Annealing Tm (°C)	Linkage to *LrCen*	Forward Sequence	Reverse Sequence	Reference
gwm63	60		TCGACCTGATCGCCCCTA	CGCCCTGGGTGATGAATAGT	A
gwm282	55	yes	TTGGCCGTGTAAGGCAG	TCTCATTCACACACAACACTAGC	A
gwm344	55	yes	CAAGGAAATAGGCGGTAACT	ATTTGAGTCTGAAGTTTGCA	A
gwm332	60	yes	AGCCAGCAAGTCACCAAAAC	AGTGCTGGAAAGAGTAGTGAAGC	A
gwm276	55		ATTTGCCTGAAGAAAATATT	AATTTCACTGCATACACAAG	A
wmc790	61		AATTAAGATAGACCGTCCATATCATCCA	CGACAACGTACGCGCC	B
wmc273	51	yes	AGTTATGTATTCTCTCGAGCCTG	GGTAACCACTAGAGTATGTCCTT	B
wmc633	61	yes	ACACCAGCGGGGATATTTGTTAC	GTGCACAAGACATGAGGTGGATT	B
wmc525	61	yes	GTTTGACGTGTTTGCTGCTTAC	CTACGGATAATGATTGCTGGCT	B
wmc809	61		CAGGTCGTAGTTGGTACCCTGAA	TGAACACGGCTGGATGTGA	B
wmc607	61		ATATATGCCCATGAAGCTCAAG	GATCGAGCTAAAGCTGATACCA	B
wmc488	61		AAAGCACAACCAGTTATGCCAC	GAACCATAGTCACATATCACGAGG	B
wmc65	61		TGGATGGGAAGGAGAATAAGTG	ATCCAACCGGAACTACCGTCAG	B
wmc596	61		TCAGCAACAAACATGCTCGG	CCCGTGTAGGCGGTAGCTCTT	B
wmc603	61		ACAAACGGTGACAATGCAAGGA	CGCCTCTCTCGTAAGCCTCAAC	B
cfa2019	60		GACGAGCTAACTGCAGACCC	CTCAATCCTGATGCGGAGAT	C
cfa2257	60		GATACAATAGGTGCCTCCGC	CCATTATGTAAATGCTTCTGTTTGA	C
cfa2240	60	yes	TGCAGCATGCATTTTAGCTT	TGCCGCACTTATTTGTTCAC	C
cfa2040	60	yes	TCAAATGATTTCAGGTAACCACTA	TTCCTGATCCCACCAAACAT	C
cfd6	60		ACTCTCCCCCTCGTTGCTAT	ATTTAAGGGAGACATCGGGC	D
barc253	50		GGGAAGACACGACACGACTC	TCGTAAGATTACCTCGGATGAAGAA	E
gpw3127	60		TGTTAGCAGAATAGACCCACCC	CATCATCCTCAATGCCACAG	C
gpw2252	60		GTGGTGCTATTGTGTGGGTG	CCAGATCGAGGTGAATCCAT	C
gpw4100	60	yes	AGCGACTTCTTAACTGTTAC	CTATCAAGGCGACTAGATT	C
gpw2308	60	yes	GGAGGAACCGAATCCAGAGT	GAGGCCGATCACATAAAGGA	C
gpw2338	60	yes	TAGGCAATTCGACAGGGATG	AAGAGTTGGCGGAAGTAGCA	C
gpw4124	60	yes	TGTGTGACAAGCGCCAGTTT	GTTCTGCTTGGAACTAGGATGG	C
gpw5281	60		TTCGTTCATATTGTCCCGGT	TAGTGCTACAGATCCGCCG	C
gpw356	60		GCTCTTGGACCTGTGTTTTC	TGGCAATCATGGCTTATCAA	C

gpw2083	60	AGATGGTAACTGGGCAATGG	GAATTGGGGGTCAGCATCTA	C
STS638	61	GCGGTGACTACACAGCGATGAAGCAATGAAA	GCGGTGACTAGTCCAGTTGGTTGATGGAAT	F

A = Roder et al. 1998, B = Somers and Isaac 2004, C = Sourdille et al. 2004, D = Guyomarc'h et al. 2002, E = Song et al. 2005, F = Neu et al. 2002

PCR amplification

PCR reactions for all microsatellite markers were performed in 384-well plates. Each reaction contained 5.0 µl of template DNA diluted to 15.0 ng/µl (75.0 ng DNA/ reaction), 2.0 µl ddH_2O, 1.0 1X PCR buffer (Applied Biosystems, Streetsville, Ontario, Canada), 1.5 mM $MgCl_2$, 0.8 mM dNTPs, 1.8 pmols M13 labelled primer (FAM/HEX/NED), 0.2 pmols forward primer, 2.0 pmols reverse primer and 1U *Taq* DNA polymerase for a final volume of 10.0 µl per reaction. Primer sequences used and references are outlined in Table 4.3 and Table 4.4. PCR reactions were carried out under the following conditions: denaturation at 93°C for 2:00 min followed by 30 cycles of 94°C for 1:00 min, -0.5°C/s to annealing temperature (Tm), Tm for 0:50 min, 0.5°C/s to 72°C, 72°C for 1:00 min followed by an final extension step of 72°C for 5:00 min and held infinitely at 4°C. Annealing temperatures were determined by the specific primer sequence and ranged from 51°C to 61°C (Tables 4.3 and 4.5). PCR fragment analysis was performed on an ABI 3100 genetic analyzer (Applied Biosystems, Streetsville, Ontario, Canada) using a three colour M-13 primer dye set (FAM, HEX and NED) as described by Somers et al. (2004). Marker output from the ABI was displayed using Genographer version: 2.1 (Montana State University).

The PCR protocol for STS638 (Table 4.4) was adapted and optimized from the protocols outlined by Hu et al. (1997) and Neu et al. (2002). Optimized PCR reactions were performed in 96-well plates. Each reaction contained 75 ng of template DNA, 12.5 µl ddH_2O, 1X PCR buffer (Applied Biosystems, Streetsville, Ontario, Canada), 2.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.02 pmols forward primer, 0.2 pmols reverse primer and 1U Taq DNA polymerase for a final volume of 25.0 µl per reaction. PCR reactions were carried out under the following conditions: denaturation at 94°C for 2:00 min followed by 35 cycles of 94°C for 1:00 min, 61°C for 1:00 min, 72°C for 1:00 min followed by a final extension step of 72°C for 6:00 min and held indefinitely at 4°C (Hu 1997; Neu et al. 2002). PCR products were separated by loading 25.0 µl of PCR products and 5.0 µl 6X loading buffer on a 1.2% agarose gel stained with 0.43 µg/mL ethidium bromide in 0.5X TAE buffer and running the electrical current for 2 to 3 h at 80V.

Fragment size was determined by including 5.0 µl of Invitrogen Low DNA Mass Ladder by Life Technologies (www.lifetechnologies.com). Agarose gel images were photographed during visualization on a UV transilluminator.

PCR reactions for KASP markers were performed in 384-well plates. Each reaction contained 30.0 ng of genomic DNA, 2.5 µl KASP 2X Mastermix (contains universal fluorescent FRET reporting dyes (FAM/HEX), KlearTaq hot-start DNA polymerase, ROX passive reference dye, buffer, $MgCl_2$ and dNTPs), 0.07µl primer assay mix (two forward and one reverse primer), and 0.5 µl ddH_2O for a total volume of 5.0 µl per reaction. PCR reactions were carried out under the following conditions: denaturation at 94°C for 2:00 min followed by 35 cycles of 94°C for 1:00 min, 61°C for 1:00 min, 72°C for 1:00 min followed by a final extension step of 72°C for 6:00 min and held indefinitely at 4°C. Post-PCR fluorescence profiling of SNPs was performed on a BMG Labtech FLUOstar Omega microplate reader (Ortenberg, Germany; www.bmglabtech.com) and genotyping analysis was performed using LGC Genomics KlusterCaller software (Teddington, Middlesex, UK; www.lgcgenomics.com).

Linkage analysis

Linkage analysis of microsatellite, STS, iSelect 90K wheat SNP, and KASP markers was performed using MapDisto genetics software Version 1.7.5 (Mathias Lorieux; www.mapdisto.free.fr) and a genetic linkage map of the chromosome was constructed using the default settings. Microsatellite markers that were closely linked to *LrCen* and displayed multiple PCR products were tested using nullitetra cytogentic stock lines (Sears 1954, 1966), Chinese Spring, and Tc-*Lr20* informative lines to identify the chromosome location for each product.

4.4 Results

4.4.1 Phenotypic population characterization

The experimental DH population of 188 individuals, generated from the Tc-LrCen/ Sumai3-lr34 cross, plus two replicates of each parental line were evaluated phenotypically with avirulent *P. triticina*

isolate TDBG to determine segregation of resistance. Five individuals were eliminated from the population due to a lack of repeatable phenotype, reducing the experimental population to 183 DH lines.

The observed segregation ratio of the remaining 183 DH individuals was 103:80 (resistant to TDBG : susceptible to TDBG) at both seedling and adult plant stages, which fit the 1:1 segregation ratio expected for a single leaf rust resistance gene segregating in a DH population, $\chi^2 = 2.89$ (P-value = 0.089). Dominant /recessive allelic interactions could not be determined because a DH population was used. Therefore, the dominance nature of the resistance gene could not be confirmed using the Tc-LrCen/ Sumai3-lr34 DH population.

The observed seedling and adult plant phenotypes have been summarized in Figure 4.1. Observed parental infection types for resistant parent, Tc-*LrCen,* demonstrated necrotic flecking and few small uredinia surrounded by necrosis; ratings ranged from ";$1^{=}$" to "$1^{=}$", as described previously (Figure 4.1B and E). Typically, the seedling and adult plants expressed the same level of resistance. However, in some cases it was observed that the adult plant expressed a slightly lower infection type than the seedling plant. Susceptible parent Sumai3-*lr34* exhibited medium to large uredinia with minimal chlorosis; infections types ranged from 3^{+} to 4 (Figure 4.1C and F). Similar to the resistant parent, infections types expressed by adult and seedling Sumai3-l*r34* plants were similar at both stages. Resistant individuals of the DH population exhibited two distinct phenotypic classes; a similar low reaction IT to the resistant parent Tc-*LrCen* (; - $1^{=}$) and a heterogeneous "X" reaction described previously by McCallum et al. (2005), commonly observed in the discovery of *LrCen* in the Tc-*Lr1* differential line. All susceptible plants in the population displayed high ITs ranging from 3^{+} to 4.

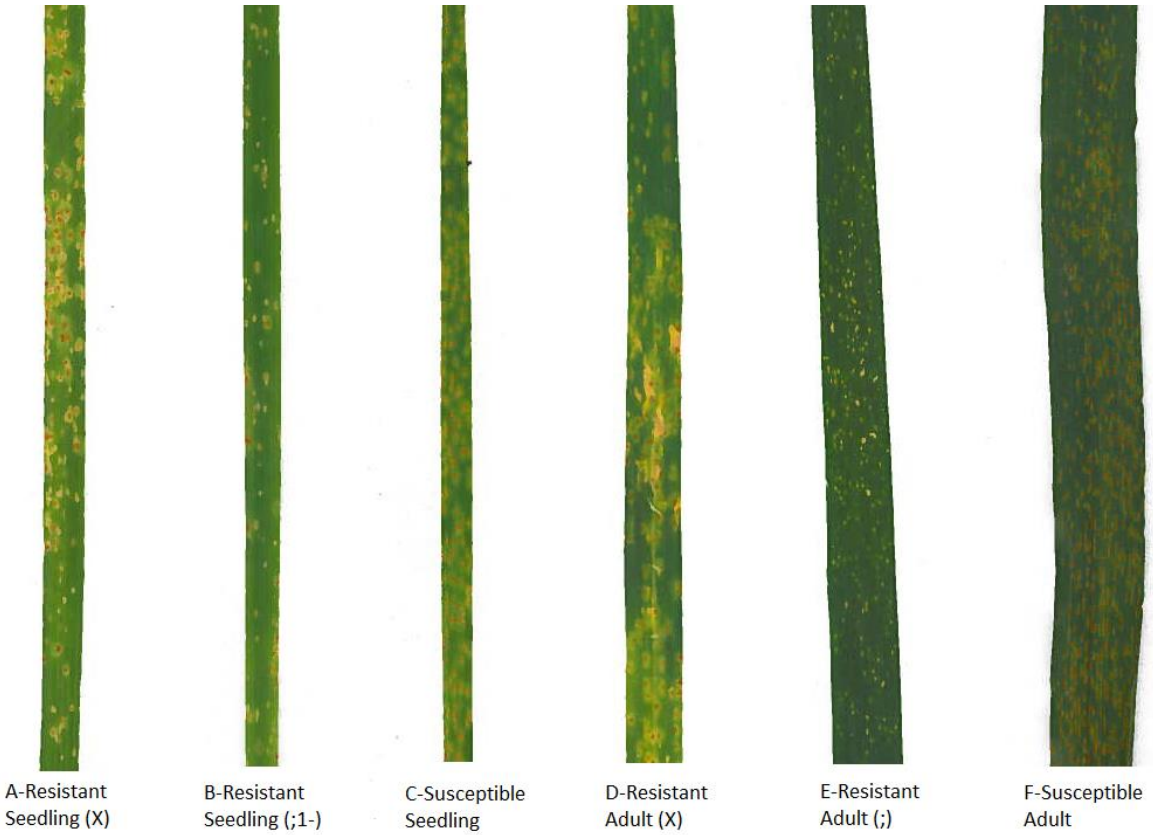

Figure 4.1 Differential reactions observed at the seedling and adult plant stages in the Tc-*LrCen/*Sumai3-*lr34* doubled haploid population when inoculated with P*uccinia triticina* race TDBG, avirulent to *LrCen*.

4.4.2 Association mapping

Results of the association mapping of 37 presumed *LrCen* lines, 25 *Lr16* lines, and 14 *Lr2a* lines (Table 4.2) using TASSEL version 3.0 software (Cornell University, Ithaca, NY; www.maizegenetics.net) indicated that the putative mapping location for the resistance gene *LrCen* was chromosome 1A. This was based on the TASSEL output providing the highest F-statistic and the most significant P-values for *barc148, gwm164, wmc432, wmc278,* and *wmc469* (Table 4.5). Lines carrying *Lr16, Lr22a* and *Lr2a* were used as reference points to ensure TASSEL output was being properly interpreted. Previously mapped chromosome locations for leaf rust resistance genes *Lr16* on the short arm of chromosome 2B (McCartney et al. 2005) and *Lr22a* on the short arm of chromosome 2D (Hiebert et al. 2007) were correctly identified by the association mapping software. Analysis of PCR products revealed association

mapping was unsuccessful; no linkage was observed between markers on chromosome 1A and phenotypes of the bulks.

Parental lines, and three resistant and three susceptible bulks were subsequently screened with a total of 34 primer pairs that demonstrated polymorphisms between Tc-*LrCen*, Sumai3-*lr34* and Thatcher from the genome-wide SSR screening (Somers, unpublished; Hiebert, unpublished) (Table 4.3). These represented potential genomic regions for the *LrCen* resistance gene. An encouraging pattern was observed with microsatellite *wmc177* on chromosome 2A, however, further screening of the first 94 DH individuals indicated no linkage to the phenotypic data.

Table 4.5 Microsatellite markers predicted to be linked to *LrCen* based on association mapping

Locus	Chromosome	F-statistic	P-value	Parental Screening Result
barc148	1A	17.48	1.98E-14	polymorphic
gwm164	1A	16.79	1.57E-12	monomorphic
wmc432	1A	13.95	9.30E-11	monomorphic
wmc278	1A	11.45	1.16E-06	monomorphic
wmc469	1A	10.79	1.11E-12	monomorphic

4.4.3 Illumina Infinium HD Assay Ultra

Among the 81,000 SNPs screened on the genome-wide iSelect 90K wheat SNP chip (Wang et al. 2014), 13,894 polymorphic SNPs were detected between the DH parental lines, Tc-*LrCen* and Sumai3-*lr34*, using GenomeStudio software (Illumina Inc.; http://bioinformatics.illumina.com/informatics/sequencing-microarray-data-analysis/genomestudio.html). Identification of linked SNPs was performed by calculating the recombination fraction (RF) (# recombinant gametes/total # of gametes) between each SNP and the phenotype, followed by a chi-square test to determine the threshold of statistically significant RF values. Sorting SNPs based on RF found that 133 SNPs were linked to the gene of interest in 17 co-segregating groups or linkage bins (Appendix 8.2). Three individuals from the DH population were removed from

further analysis due to inconsistencies in marker genotyping, further reducing the DH population to 180 individual lines. Linkage analysis of polymorphic markers associated with phenotypic data for the 94 DH lines was performed using MapDisto Version 1.7.5 (Mathias Lorieux; www.mapdisto.free.fr) (parameters - LOD min: 3, rmax/d max: 0.3).

Of the 133 SNP markers linked to *LrCen,* 13 were present on a previously constructed genetic map (Cabral et al. 2014) (Appendix 8.2). All 13 SNPs were previously mapped to the same linkage group and were tentatively assigned to chromosome 7A. Both BS00023003_51 and wsnp_Ra_c71121231834 mapped to the same linkage group as microsatellite marker *gwm344,* which has been previously mapped to the long arm of chromosome 7A (Roder et al. 1998). A BLAST search was also performed with linked SNP sequences against the Wheat Survey Sequence (http://www.wheatgenome.org/) as a secondary means of determining putative chromosomal location. Of the 133 sequences BLAST searched, 101 (76%) had the best match to sequences from chromosome 7AL, supporting the previous findings; while 14 (11%) and 15 (11%) had chromosome 7AL as a second and third best match respectively. The remaining 3 (2%) sequences had no matches in the Wheat Survey Sequence.

4.4.4 Linkage analysis and genetic mapping

Along with the 133 SNP sequences putatively located on the long arm of chromosome 7AL, 30 microsatellite markers (Table 4.4) previously mapped to 7AL were selected to screen the DH population and parental lines. Genetic linkage was established between *LrCen* and 12 SSRs (Table 4.4). A final linkage map was generated by combining microsatellite and SNP scores. SNP markers that co-segregated in linkage blocks were reduced to a single representative SNP per linkage block (Appendix 8.2), and population phenotypic data scored using *P. triticina* race TDBG. The completed linkage map generated a linkage group spanning 64.5 cM (Figure 4.2). *LrCen* was flanked by microsatellite markers, distally by *cfa2240* and a group of co-segregating SNP makers at a distance of 1.1 cM and proximally

(relative to the centromere) by *gwm344* at a distance 5.0 cM. Both *gwm344* and *cfa2240* amplified alleles in the resistant parent, Tc-*LrCen* (150 bp, 302 bp, respectively), and susceptible parent, Sumai3-*lr34* (147 bp, 307 bp). Resulting marker location and order were consistent with a series of microsatellite maps used in the selection of appropriate markers including the deletion bin map (Somers, unpublished), International Triticeae Mapping Initiative (ITMI) map (Roder et al. 1998), and consensus map (Somers et al. 2004).

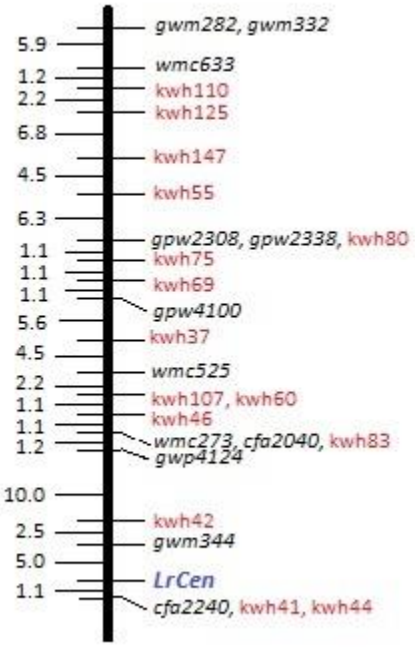

Figure 4.2 Linkage map displaying leaf rust resistance gene *LrCen* (blue) and associated microsatellite markers (black) and SNP markers (red) on the long arm of chromosome 7A, mapped on180 lines of the Tc-*LrCen*/Sumai3-*lr34* doubled haploid population. *Puccinia triticina* races TDBG and some isolates of TDBJ are avirulent to *LrCen.* Distances are reported on the left in centimorgans (cM).

Three single-product microsatellite markers linked to *LrCen, gwm344, gpw2308,* and *wmc633* were selected for screening on a panel of informative lines to validate their location on chromosome 7A. The hexaploid characteristic of wheat, introduces the added complexity of sequence homœology observed across the three sub-genomes (A, B, and D). As a result, microsatellites often amplify multiple

alleles across the sub-genomes as was demonstrated by tightly linked SSR, *cfa2240.* To ensure the allele size scored for *cfa2240* was in fact associated with the A sub-genome, it was also included in the analysis. Markers were screened against the following lines: nullisomic 7A tetrasomic 7B (N7AT7B), N7AT7D, N7BT7A, N7DT7B (Sears 1954, 1966), Tc-*LrCen*, Sumai3-*lr34*, and Tc-*Lr20* (the *Lr20* differential line, RL6092). Each of the four microsatellites amplified no product, for single PCR product markers, or no product of the allele size predicted to be on chromosome 7A in the case of multiple PCR product markers, for both nullisomic 7A lines (N7AT7B and N7AT7D) where chromosome 7A is absent, while the nullisomic 7B and 7D lines carried the expected allele size observed in the parental lines. These results confirmed the original hypothesis that alleles being scored for each of the SSRs were located on chromosome 7A (Figure 4.3). The *Lr20* differential line was included as *Lr20* has also been putatively mapped to the long arm of chromosome 7A. This relationship is explored further in the following chapter. However, preliminary results indicated that the *LrCen* resistant locus is distinct from *Lr20*.

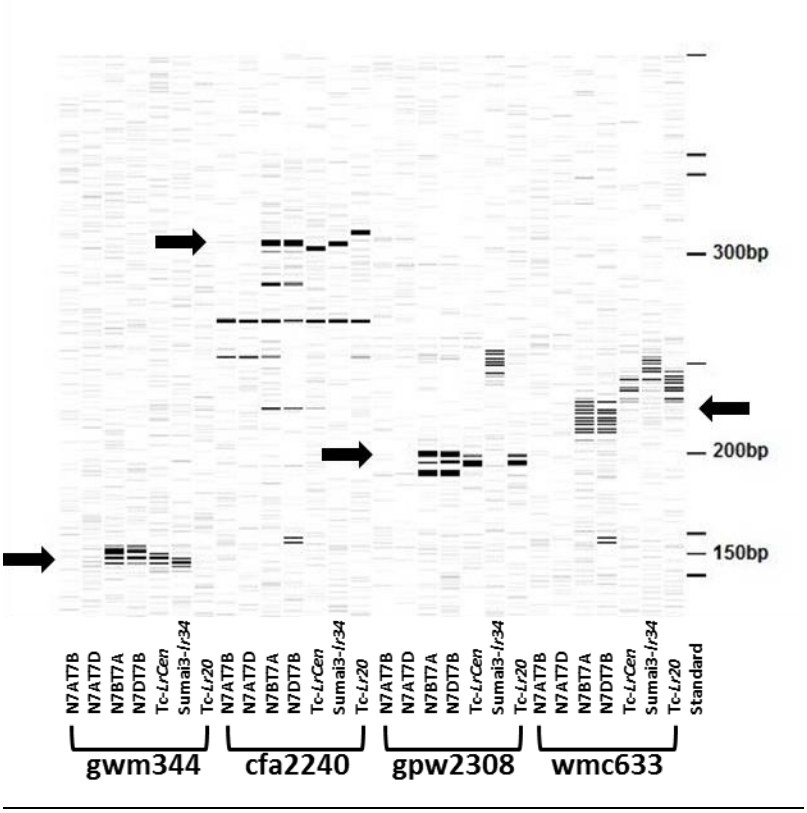

Figure 4.3 Observed PCR reactions for four microsatellite markers linked to leaf rust resistance gene *LrCen* mapped to the long arm of chromosome 7A screened on the following lines: nulli7A-tetra7B (N7AT7B), nulli7A-tetra7D (N7AT7B), nulli7B-tetra7A (N7BT7A), nulli7D-tetra7B (N7DT7B), Thatcher-*LrCen* (Tc-*LrCen*), Sumai3-*lr34*, and RL6092 (Thatcher-*Lr20* or Tc-*Lr20*), to confirm 7A location.

4.5 Discussion

The appearance of an unusual mesothetic phenotype associated with *P. triticina* race TDBG led to the screening of the Thatcher-*Lr1* NIL, RL6003 (Dr. Peter Dyck; CRC-AAFC), with *P. triticina* races BBBD (avirulent to *Lr1* and virulent to *LrCen*) and TDBG. This testing revealed a second unknown resistance gene segregating in the previously believed single gene line, RL6003, and was temporarily designated as *LrCen* (McCallum et al. 2005). *LrCen* was subsequently isolated and a doubled haploid mapping population was generated from a cross with Sumai3-*lr34*; the results of this mapping study confirm the identification of a new seedling leaf rust resistance gene, also referred to as 'all-stage resistance', mapped to the long arm of chromosome 7A. While not available at the time of mapping, the

subsequent publication of a consensus SNP genetic linkage map describing approximately 50% of the SNP sequences present on the array, confirm mapping to chromosome 7AL (Wang et al. 2014). Of the 133 SNPs linked to *LrCen* on the high-density 90K SNP array, 119 of them were mapped in the consensus map, of which 110 were mapped to chromosome 7A (the other 9 being mapped to 7B, consistent with BLAST results). The BLAST results located on 7B are most likely the result of duplicated (homoeologous and paralogous) genes that are a common source of complication when genotyping polyploid species (Wang et al. 2014). Furthermore, 72 of the 110 markers mapped to chromosome 7A were further mapped to the long arm, while the other 38 were not mapped specifically to an arm of the chromosome. The 133 sequences of the linked SNPs were converted to KASP markers. Phenotypic evaluations of 180 DH individuals and two replicates of each parental line were performed using avirulent race TDBG and segregation for *LrCen* fit the expected 1:1 phenotypic ratio for a single resistance gene. In order to rule out the possibility that *LrCen* was *Lr20*, which was reported to be on chromosome 7AL, preliminary genetic analysis of the DH population was conducted with STS638, an STS marker converted from random amplified polymorphic DNA (RAPD) probe UBC638 that was previously shown to be tightly linked with leaf rust resistance gene *Lr20* (542 bp fragment) (Hu 1997; Neu et al. 2002). A lack of amplification for the STS638 allele representing *Lr20* from this analysis indicate that *LrCen* is distinct from *Lr20* and represents a new gene or new allele of a gene. This relationship is discussed further in the following chapter. Also present on the long arm of chromosome 7A are previously mapped resistance genes *Pm1* (powdery mildew) and *Sr15* (stem rust). Both of these genes also mapped to the distal end of the chromosome with *Lr20* and are thought to be part of a tight cluster of unique genes, or a single gene with multiple-pathogen specificity (Watson and Luig 1966; Sears and Briggle 1969; McIntosh 1977; Neu et al. 2002).

Association mapping of *LrCen* using a set of Canadian cultivars believed to carry *LrCen,* based on phenotypic evaluations with a set of informative *P. triticina* races, revealed a putative location on

chromosome 1A. Subsequent screening of the DH population with microsatellite markers on 1A demonstrated that association mapping attempts were unsuccessful. This failure was further explained with the results of chapter 5, where Canadian wheat germplasm believed to carry *LrCen* underwent genetic evaluation with the tightly linked microsatellite marker *cfa2240* and SNP markers. Based on marker haplotypes, many of the lines tested did not appear to carry *LrCen,* contrary to the results of phenotypic evaluation and gene postulation. These results indicate that *LrCen* cannot be identified on a purely phenotypic basis. It is possible that other narrowly effective leaf rust resistance genes providing TDBG resistance exist throughout Canadian germplasm and have gone unnoticed until the recent emergence of the avirulent race in the early 2000s. These genes may have been present in the association mapping study resulting in the erroneous mapping location. Another possibility is that *LrCen* is located on a transposable element, explaining why it appears through phenotypic evaluation, but cannot be located through genetic evaluation with markers for a specific chromosomal region. Alternatively, *LrCen* may be an allele of a gene at a particular locus and there may be other, similar alleles that are found in different regions of the wheat genome.

LrCen, a race-specific seedling resistance gene, displayed a narrow range of resistance effective against one of the predominant races observed in North American virulence surveys, TDBG. TDBG was the most predominant race in the 2005 to 2007 virulence surveys of Manitoba and Saskatchewan accounting for 56.2% and 61.0% of isolates collected in 2006 and 2007, respectively. TDBJ, a similar virulence phenotype, was the second most common race accounting for 18.7% (2006) and 15.2% (2007) of isolates collected and some isolates of TDBJ are also avirulent to *LrCen* (McCallum et al. 2010). TDBG isolates were also avirulent to seedling resistance gene *Lr14a* located on the long arm of chromosome 7B (Herrera-Foessel et al. 2008) prompting allelism studies for the two genes. The results for an F_2 population from the cross Tc-*LrCen*/ Tc-*Lr14a* (RL6013) inoculated with avirulent race TDBG indicated

two independent, dominant genes segregating, confirming that *LrCen* is distinct from *Lr14a* (McCallum, unpublished).

Initial screening of RL6003 with *P. triticina* race TDBG identified a second segregating resistance gene that displayed a mesothetic reaction with variably sized uredinia distributed across the leaf surface (McCallum et al. 2005). This distinct phenotype was thought to be indicative of the presence of *LrCen,* however, further evaluation of the DH population with TDBG indicated that infection types displayed by individuals with *LrCen* ranged from a nearly immune response of ";1⁼" to the originally observed mesothetic "X" reaction (Figure 4.1).

While *LrCen* avirulent isolates represented 35% of all isolates collected in Canada in 2004 and has remained a significant portion of the isolates collected since then (McCallum, unpublished), the resistance conferred by *LrCen* is relatively insignificant to other virulence phenotypes. Therefore, *LrCen* must be used in combination with other, more broadly effective, genes in order to provide effective field resistance. Stacking of *LrCen* with race non-specific adult plant resistance (APR) genes *Lr34*, *Lr46*, and *Lr67* is an attractive option for breeders as gene combinations incorporating one, or more, of these APR genes with one, or more, important race-specific genes has proven effective in the past (Singh et al. 1998; Dubcovsky 2004; McCallum et al. 2007; Hiebert et al. 2010; McCallum et al. 2011). Each of these slow-rusting APR genes is also part of a multi-fungal resistance locus that, upon incorporation, provides effective resistance to multiple pathogens, further increasing their importance in gene stacking programs (Hiebert et al. 2010; Herrera-Foessel et al. 2011; McCallum et al. 2011).

Stacking leaf rust resistance genes through phenotypic evaluation of progeny has been difficult due to the effects of temperature, growth stage, epistasis, and infection type variability affecting the expression of resistance. As well, the need for multiple, informative races of a pathogen, the inability to screen for genes controlling multiple pathogens on a single plant, and the time and space requirements

of these extensive experiments increase the difficulty of identifying genes present through evaluation of infection types. The identification of tightly linked molecular markers flanking a resistance gene can be useful in eliminating many of these factors through the application of marker assisted selection in breeding programs. The markers flanking *LrCen* were *cfa2240* along with a group of co-segregating SNPs from the Infinium assay located distal to the gene at a distance of 1.1 cM and *gwm344* at a distance of 5.0 cM (Figure 4.2). Based on proximity to the gene, ease of use, and time efficiency, SNPs converted to KASP markers were originally thought to be the best option for screening for the presence of *LrCen*. Evaluation of an F_2 population believed to be segregating for *LrCen* indicated that the KASP markers were dominant. Dominant markers can be useful in F_2 populations when they are linked in coupling with a desireable allele, but would not be effective in identifying heterozygous individuals within the population, which are common when breeders are screening progeny in conventional breeding populations (Tan and Fu 2007). Therefore, marker type is important when selecting markers appropriate for marker assisted selection (MAS). Recent studies involving marker assisted selection show encouraging results in enabling the introgression of a number of resistance genes for a range of pathogens into a single cultivar that can be tested through molecular identification of flanking markers, reducing the need for timely and sometimes ineffective phenotypic evaluations (Huang et al. 1997; Dubcovsky 2004; Collard et al. 2005; Xu and Crouch 2008).

In summary, the second gene discovered in the *Lr1* differential line RL6003 designated as *LrCen* was mapped distally on the long arm of chromosome 7A. While not broadly effective, *LrCen* provides resistance to one of the predominant North American *P. triticina* race, TDBG, and some other isolates with similar virulence phenotypes. Incorporation of *LrCen* in combination with other broadly effective genes in Canadian cultivars could provide a high level of genetic resistance, reducing producers' need for application of expensive, potentially unhealthy, and environmentally harmful fungicides. While the narrow resistance provided by the gene may not make it a primary target in breeding programs, retention

of this gene when already present could be beneficial. The flanking microsatellite markers *cfa2240* (using the 302bp allele specific to chromosome 7A and linked to *LrCen*) and *gwm344* can be used as indicators of the presence of *LrCen* in marker assisted breeding efforts.

CHAPTER 5

THE DISTRIBUTION OF *LrCen* IN WHEAT GERMPLASM AND EXPLORATION OF THE RELATIONSHIP BETWEEN TWO SEEDLING LEAF RUST RESISTANCE GENES LOCATED ON CHROMOSOME *7AL*, *LRCEN* AND *LR20*

5.1 Abstract

Genetic resistance is the preferred method for controlling wheat leaf rust (*Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex Desmaz. f. sp. *tritici*)). It provides an effective protection throughout the entire growing season at no cost to the producer and with no impact on the environment. A previously unknown seedling Lr resistance gene, *LrCen,* identified in *Lr1* NIL RL6003 mapped to the long arm of chromosome 7AL, similar to *Lr20*. Annual virulence surveys also identified an unknown source of leaf rust resistance in the *Lr20* NIL, RL6092. An F_2 population was generated from a cross of Thatcher to an accession of RL6092 known to carry two sources of leaf rust resistance, one of which was postulated to be *LrCen* based on the infection type observed when inoculated with race TDBG (avirulent on *LrCen*). Molecular and phenotypic studies of the F_2 population confirmed at least two distinct sources of leaf rust resistance based on independent segregation of resistance to races TDBG and BBBD. Neither source was linked to the markers linked to *LrCen (cfa2240)* and *Lr20* (STS638) on chromosome 7AL. If *Lr20* is present in the accession RL6092, none of the races used in this study were able to identify it. Phenotypic and molecular studies of a diverse set of germplasm postulate the presence of *LrCen* in 36 wheat genotypes, highly concentrated in North American lines. Pedigree studies support the introduction of *LrCen* by way of Thatcher or one of Thatcher's parents.

5.2 Introduction

Since the Canadian Wheat Boom from 1880 to 1910 (Ward 1994), wheat cultivation has continued across the prairies, contributing to the financial and cultural development of western Canada (McCallum and DePauw 2008). The development of wheat cultivars grown in Canada has utilized diverse genetic resources including wild relatives and progenitor species of modern common hexaploid wheat (*Triticum aestivum*) to improve agronomic traits, quality factors, and to introduce genetic resistance to disease and insect pests through traditional breeding methods. A number of contact and systemic fungicides are available to combat the various diseases of wheat observed annually across the prairies. However, variability in disease incidence and severity from year to year, input and associated application costs, variable commodity market prices, and environmental concerns have all contributed to genetic resistance being the preferred method of disease control in wheat when available.

Wheat leaf rust is the most common and widespread disease of wheat. Effective genetic control of leaf rust has been achieved through the discovery of more than 63 leaf rust resistance genes and the integration of genes useful in defence against local pathogen populations (McIntosh et al. 2010; Herrera-Foessel et al. 2012) in wheat breeding programs. The rapid evolution of the wheat leaf rust pathogen, *Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex Desmaz. f. sp. *tritici*), has challenged many of the available sources of genetic resistance, rendering some ineffective against the current pathogen population, while others have proved to be more durable sources of resistance (Samborski 1985; Martens and Dyck 1989; McCallum et al, 2007). The ever-changing state of the *P. triticina* population genetics across North America has led to the development of cultivars with a range of leaf rust resistance genes or combinations of genes. Some genes are well-known, while others remain less studied, or even unknown, as was the case with the discovery of *LrCen*.

Molecular and phenotypic studies have postulated the distribution of many seedling and adult plant leaf rust resistance genes in worldwide germplasm (McIntosh et al. 2012). Interestingly, seedling Lr gene *Lr20*, like *LrCen*, has been mapped to the long arm of chromosome 7A. Dakouri et al. (2013) describe TDBG as avirulent on *Lr20*, and used this as one informative race to phenotypically predict, based on infection types observed*, Lr20* to be among the most common genes in the world wide germplasm with emphasis on Ethiopian germplasm.

A number of lineage studies have been conducted over the years since wheat cultivation began in Canada to trace the origin of wheat pedigrees. McCallum and Depauw (2008) provided an extensive overview of many earlier pedigree studies and supplemented these studies with more modern pedigrees and the incorporation of the diversified market classes in to which wheat cultivars are placed based on end-use quality characteristics. Throughout its history, Canadian wheat production has been dominated by the Canadian Western Red Spring (CWRS) class of wheat known for its superior bread-making and baking qualities. The genetic basis for many of the CWRS cultivars stems from the introduction of the cultivar Red Fife to Canada from Poland in 1870 (Campbell and Shebeski 1986). Despite some agronomic issues, the superior end-use quality of the grain produced by Red Fife made it the predominant variety grown across the Canadian prairies until the introduction of Marquis. Marquis was developed from a cross between Red Fife and Hard Red Calcutta to retain the end-use quality of Red Fife and introduce earlier maturity and higher yield (Morrison 1960). In 1939, concerns about stem rust out breaks led to the replacement of Marquis with the stem rust resistant variety Thatcher (Marquis/Iumillo Durum//Marquis/Kanred), developed at the University of Minnesota (Hayes et al. 1936). Today, Thatcher is used extensively in the study of the leaf rust pathogen (*Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex Desmaz. f. sp. *tritici*)) and as a parent in mapping leaf rust resistance genes due to its susceptibility to leaf rust. The progression of CWRS cultivars followed this evolutionary path maintaining Red Fife end-use quality traits through agronomically improved offspring, namely Marquis,

while adding genetics for other desirable agronomic and genetic resistance traits. This resulted in most cultivars grown through to the 1950s and 1960s being closely related (van Beuningen and Busch 1997). Subsequent cultivars included Selkirk for combined leaf and stem rust resistance (Martens and Dyck 1989), and Canthatch (predominantly Thatcher background) with added stem rust resistance to a new virulent race (Campbell 1963). Thatcher has remained a key component in the background of new cultivars using other parents as donors for disease resistance and improved agronomics (McCallum and DePauw 2008). Based on this knowledge of variety lineage, some assumptions relating to agronomics, the presence of resistance genes, etc. can be made.

This study explores the relationship between seedling resistance genes *LrCen* and *Lr20* which map to chromosome 7AL and are resistant to *P. triticina* race TDBG. The distribution of newly mapped *LrCen* within a selection of North American and world-wide germplasm will be evaluated to determine how frequently it appears and to trace lineage back to a potential origin within Canadian germplasm.

5.3 Materials and Methods

5.3.1 Plant materials

Genetic stocks

A set of informative lines was assembled, most originating from the 'single-gene' Thacher NILs RL6092 (*Lr20*) and RL6003 (*Lr1*) (Table 5.1). The *Lr20* NIL, RL6092 (pedigree: 'Thatcher'*6/'Timmo'), was selected, as it showed a similar avirulent resistance response to phenotypic screening with *P. triticina* race TDBG, as was previously described on *LrCen*. *Lr20* has also been mapped to the long arm of chromosome 7A in wheat (McIntosh et al. 2010), initiating studies on the relationship of *Lr20* and *LrCen*. A concurrent screening of RL6092 with *P. triticina* race PBDQ, virulent on *Lr20*, demonstrated heterogeneity within the line of at least two distinct *Lr* genes in the "single gene" differential NIL. In

order to observe each gene individually, crosses were made to Thatcher (Tc). A line believed to lack *Lr20*, was selected using a combination of race PBDQ (virulent on *Lr20*) and TDBG (avirulent on *Lr20*). RL6092-B is a selection of RL6092 carrying *Lr20* and a second unknown seedling resistance gene. RL6092-A is a similar line to RL6092-B however *Lr20* is believed to be absent, leaving a single-gene line carrying only the unknown resistance gene. RL6003, the original *Lr1* Thatcher NIL in which *LrCen* was first identified, the newly developed RL6003a carrying only *Lr1,* and Thatcher-*LrCen* (Tc-*LrCen*) were included to observe infection types of *LrCen* experimental lines alongside those of the *Lr20* experimental lines. Thatcher was included as a susceptible check, a TDBG-susceptible source, Little Club, was also included as Thatcher was predicted to be a possible donor of *LrCen* in the RL6003 NIL and could be the source of the second unknown gene in RL6092.

Thatcher/RL6092-B F_2 population

To further explore the relationship between *LrCen* and *Lr20*, a cross between Thatcher and an accession of the RL6092-B differential line, known to carry a second resistance gene, was used to develop an F_2 population (C. Hiebert, unpublished data). Phenotypic and genetic evaluation with DNA markers was conducted on three sets of 90 F_2 progeny (total of 270 F_2 progeny) and three replicates of each parental line (per set) to explore the hypothesis that *LrCen* was responsible for resistance to race TDBG in another, Thatcher NIL previously thought to carry a single-gene.

Lr20 source lines

A set of lines previously characterized to contain *Lr20* was assembled from the Plant Gene Resources of Canada (PGRC) genebank (Agriculture and Agri-Food Canada) including: two accessions of 'Norka' CN11207 and CN11208 (pedigree: 'Kubanka' pure line (Genetic Resources Information System for Wheat and Triticale – GRIS)), two accessions of 'Axminster' CN9574 and CN33630 (pedigree: 'Marquis'/? (GRIS)), 'Festival' CN10070 (pedigree: 'Pusa 3'//'Kenya W744'/'Baringa'), and 'Sappo'

CN45907 (pedigree: 'Weibulls-177-62'/'Weibulls-176-62' (GRIS)) (Neu et al. 2002). Each of these lines was used to study the relationship and to test for allelism between *Lr20* and *LrCen*.

Wheat Germplasm

In a previous genome-wide association mapping study of 276 *Triticum aestivum* genotypes (D.J. Somers, unpublished data), phenotypic evaluation of infection type was evaluated for a series of *P. triticina* races including: BBBD (highly avirulent – most Lr genes confer resistance), TDBG (avirulent on *LrCen and Lr16*), MBDS (avirulent on *Lr16* and *Lr2a*), and MGBJ (avirulent on *Lr2a*) (B.D. McCallum, unpublished data). Results of this study were used to select a set of genotypes, primarily North American, whose virulence phenotypes indicated the putative presence of *LrCen*. The selected genotypes along with genotypes appearing to have *Lr16* and *Lr2a* (*Lr16* also provides resistance to *P. triticina* race TDBG and could mask the presence of *LrCen*), and various other Canadian genotypes, were selected for genetic studies to determine the distribution of the newly characterized leaf rust resistance gene *LrCen,* in 185 wheat accessions (Appendix 8.3).

5.3.2 *Puccinia triticina* inoculations

Genetic stocks

Eight replicates of the lines listed in Table 5.1 were germinated in six 4x8 root trainers containing Sun Gro Horticulture Sunshine Professional Growing Mix 5 (Agawam, MA, USA; www.sungro.com) at a depth of approximately 2.5 cm. Plants were grown for eleven days prior to inoculation in a controlled environment under 16 light and 8 dark hours at 20˚C and 18˚C respectively, and watered as required.

Single spore inoculum was developed via the method described by McCallum and Goh (2003). Ratings were performed to determine virulence patterns based on infection types observed across the

16 differential lines (McCallum and Seto-Goh 2003). *P. triticina s*pore viability was tested by spreading a single layer of spores from a single source of a single race of the pathogen across a 2% water agar petri plate and left overnight in a dark, cool environment. The spores were then observed under a dissecting microscope the following day for successful spore germination as indicated by mycelium growth from a majority of the plated spores.

Seedling inoculations were performed with single spore isolates of the *LrCen* avirulent *P. triticina* race TDBG and the highly avirulent race BBBD that is virulent to *LrCen*. Vacuum dried urediniospores isolated using the method described above (stored at 4˚C) were removed from refrigeration, left at room temperature to rehydrate for a period of 3 hours (Roelfs et al. 1992) and heat shocked at 40˚C for five minutes in a water bath. Eleven day old seedlings were inoculated by spraying urediniospores suspended in light mineral oil as described previously by McCallum and Seto-Goh (2003). Post inoculation, plants were left for approximately 30 minutes to allow the oil to volatilize, leaving the spores bound to the leaves. Inoculated seedlings were then placed in a dew chamber overnight at 100% relative humidity under dark conditions at approximately 20˚C. The following day, plants were removed from the dew chamber and air dried before being returned to the controlled environment previously described.

Thatcher/ RL6092-B F_2 Populations

The sets of 90 F_2 individuals from a population created through the crossing Thatcher (Tc) to an accession of the RL6092-B NIL determined to carry a second unknown resistance gene (Tc/RL6092-B) were germinated in six 4x8 root trainers containing Sun Gro Horticulture Sunshine Professional Growing Mix 5 (Agawam, MA, USA; www.sungro.com). Plants were grown for eleven days prior to inoculation in a controlled environment under 16 light and 8 dark hours at 20˚C and 18˚C, respectively. Due to each F_2 individual representing a unique genetic recombination of the parental cross, the first set of 90 F_2

individuals were inoculated with two, single spore isolates on each plant for phenotypic observation of the two genes segregating within the population. The first set was inoculated with the highly avirulent *P. triticina* race BBBD on the first leaf and TDBG on the second leaf. Inoculations were performed using the methods described by McCallum and Seto-Goh (2003). In order to facilitate inoculations with two distinct *P. triticina* races, the second leaf was covered with a glassine bag during application of BBBD spores, glassine bags were left on for an hour long drying period. New glassine bags were then placed over the inoculated first leaf for the application of the TDBG spores and again left to dry for one hour before being placed overnight in the dew chamber. The second set of 90 F_2 individuals from the same cross were inoculated at the two leaf stage with *P. triticina* race MBPS (suggested to be avirulent on *Lr20;* B.D. McCallum, unpublished data) to observe the segregation of *Lr20*. The third set of 90 F_2 individuals was inoculated with race TDBG in order to provide a larger data set for the segregation of resistance to this race in the F_2 population.

Lr20 Source Lines

Two sets of four seeds per line, outlined in Table 5.4, were seeded in to two 4x5 growing trays filled with potting soil and topped with Captan fungicide (Southern Agricultural Insecticides, Inc.; www.southernag.com) to prevent fungal infection of seedlings pre-inoculation. Plants were germinated in a greenhouse under 16 light and 8 dark hours at 20˚C and 18˚C respectively, for 12 days. Seedling inoculations were performed with single spore isolates of *P. triticina* races TDBG and BBBD following the methods described previously by McCallum and Seto-Goh 2003.

Wheat Germplasm

The 185 genotypes included in Appendix 8.3 were germinated in 4x8 root trainers containing Sun Gro Horticulture Sunshine Professional Growing Mix 5 (Agawam, MA, USA; www.sungro.com). Plants were grown for eleven days prior to inoculation in a controlled environment under 16 light and 8

dark hours at 20˚C and 18˚C, respectively. Seedling inoculations were performed with single spore isolates of the *LrCen* avirulent *P. triticina* race TDBG following the methods described previously by (McCallum and Seto-Goh 2003).

5.3.3 Disease assessment

Phenotypic evaluation of infection type (IT) was performed on the first and second leaves 12 days post inoculation (DPI) for seedlings. ITs were classified following the leaf rust rating scale described by Long and Kolmer (1989) using specific modifications outlined by Roelfs et al. (1992) (McCallum et al. 2010).

ITs from 0 (fully resistant – no sign of infection) to 2 (small to medium sized pustules surrounded by chlorosis/ necrosis) were considered as resistant host responses, while ITs 3 to 4 (medium to large pustules with little to no visible chlorosis) were considered as susceptible host responses. Additionally, a rating of X was given to individuals exhibiting heterogeneous distribution of variably sized uredinia from necrotic flecks to medium sized pustules surrounded by chlorosis/necrosis. ITs rated as X were considered resistant. Data were tested for goodness of fit using Chi-Square analysis.

5.3.4 DNA extraction

After ITs were assessed on the first and second lead, tissue (four pieces approximately 2.5-3.8 cm) was collected from the third of fourth leaf (youngest, non-infected tissue) and placed into two 96-well collection microtube extraction plates with glass beads at the base for tissue maceration. Tissue was lyophilized on the Christ Alpha 1-4 LD plus (Montreal Biotech Inc., Kirkland, QC, 3551 St-Charles BLVD. Suite 506) overnight and subsequently stored at -20°C. Immediately prior to extraction, tissue was macerated to a fine powder using the Qiagen TissueLyser II (Qiagen, Missassauga, ON; www.qiagen.com) for ten minutes, flipping orientation of the 96-well plate after five minutes. DNA was

extracted using a modified ammonium acetate extraction protocol (Chao and Somers, http://maswheat.usdavis.edu/PDF/DNA0003.pdf, accessed February 2012) based on procedures of Pallotta et al. (2003). DNA was quantified using Hoechst 33258 stain on a fluorometer and stock DNA was then diluted to a 15 ng/µl working solution.

5.3.5 Marker screening

Screening with Lr20 marker STS638

In order to postulate the presence of *Lr20*, STS638, a marker linked to *Lr20* (Neu et al. 2002), was used to genotype each of the experimental lines/populations used in this study (Table 5.1 and Table 5.4) as well as the two plates (180 F_2 individuals) of the Thatcher/RL6092-B F_2 population with six replicates of each parental line (Table 5.2).

Microsatellite analysis of the 7AL chromosomal region of lines postulated to carry Lr20

To further characterize the region of chromosome 7AL represented by the *Lr20* marker (STS638), each of the lines from Table 5.1 that produced the expected 542 base pair (Hu et al. 1997; Neu et al. 2002) PCR product was genotyped with a series of 55 microsatellite markers (Appendix 8.4).

Molecular analysis of the Thatcher/ RL6092-B F_2 population for the distribution of Lr20 and LrCen with linked markers

To examine the hypothesis that the second gene providing resistance to *P. triticina* race TDBG segregating in the Thatcher/ RL6092-B F_2 population was *LrCen,* marker genotyping was performed. In total, 180 F_2 individuals and six replicates of each parental line were screened with co-dominant SSR marker *cfa2240* previously mapped at 1.1cM distal to *LrCen* and STS638, previously described by Neu et al. (2002) to be highly diagnostic of *Lr20.* A two-point linkage analysis was performed between the *cfa2240* marker results as well as the STS638 marker results and the infection types obtained through

phenotypic evaluation with race TDBG. A chi-square analysis was then performed to determine if the observed segregation of the *LrCen* type fragment (302bp) for *cfa2240* and TDBG resistance fit a model for independent assortment.

Genotyping wheat germplasm for presence of LrCen

A survey of DNA marker alleles was conducted to predict the distribution of *LrCen* within wheat germplasm based on phenotypic studies in a genome-wide association mapping study (D.J. Somers, unpublished data; B.D. McCallum, unpublished data). In total, 185 wheat genotypes, predominantly from Canadian germplasm, outlined in Appendix 8.3 were genotyped with microsatellite marker *cfa2240* and KASP marker *KWH41*, both tightly linked to *LrCen* at a distance of 1.1 cM (Chapter 4, Figure 4.2).

5.3.6 PCR amplification

PCR reactions for microsatellite markers were performed in 384-well plates. Each reaction contained 5.0 µl of template DNA diluted to 15.0 ng/µl (75.0 ng DNA/ reaction), 2.0 µl ddH_2O, 1.0 1X PCR buffer (Applied Biosystems, Streetsville, Ontario, Canada), 1.5 mM $MgCl_2$, 0.8 mM dNTPs, 1.8 pmols M13 labelled primer, 0.2 pmols forward primer, 2.0 pmols reverse primer and 1U *Taq* DNA polymerase for a final volume of 10.0 µl per reaction. Primer sequences used and references are outlined in Appendix 8.4. PCR reactions were carried out under the following conditions: denaturation at 93°C for 2:00 min followed by 30 cycles of 94°C for 1:00 min, 0.5°C/s to annealing temperature (Tm 51-61^{o}C, Appendix 8.4), Tm for 0:50 min, 0.5°C/s to 72°C, 72°C for 1:00 min followed by an final extension step of 72°C for 5:00 min and held infinitely at 4°C. PCR fragment analysis was performed on an ABI 3100 genetic analyzer (Applied Biosystems, Streetsville, Ontario, Canada) using a three colour M-13 primer dye set (FAM, HEX and NED) as described by Somers et al. (2004). Marker output from the ABI was displayed using Genographer version: 2.1 (Montana State University).

The PCR protocol for STS638 was adapted and optimized from the protocols outlined by Hu et al. (1997) and Neu et al. (2002). Optimized PCR reactions were performed in 96-well plates; each reaction contained 75 ng of template DNA, 12.5 µl ddH_2O, 1X PCR buffer (Applied Biosystems, Streetsville, Ontario, Canada), 2.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.02 pmols forward primer, 0.2 pmols reverse primer and 1U Taq DNA polymerase for a final volume of 25.0 µl per reaction. PCR reactions were carried out under the following thermocycler program: denaturation at 94°C for 2:00 min followed by 35 cycles of 94°C for 1:00 min, 61°C for 1:00 min, 72°C for 1:00 min followed by a final extension step of 72°C for 6:00 min and held indefinitely at 4°C (Hu et al. 1997; Neu et al. 2002). 25.0 µl PCR products and 5.0 µl 6X loading buffer were loaded on a 1.2% agarose gel (4.2 g of agarose in 350mL 0.5X TAE buffer) stained with 0.43 µg/mL ethidium bromide in 0.5X TAE buffer for 2 to 3 h at 80V. Fragment size was determined by including 5.0 µl of Invitrogen Low DNA Mass Ladder by Life Technologies (www.lifetechnologies.com). Agarose gel images were photographed during visualization on a UV transilluminator.

PCR reactions for the KASP marker *KWH41* (Appendix 8.2) were performed in 384-well plates; each reaction contained 30.0 ng of genomic DNA, 2.5 µl KASP 2X Mastermix (contains universal fluorescent FRET reporting dyes (FAM/HEX), KlearTaq hot-start DNA polymerase, ROX passive reference dye, buffer, $MgCl_2$ and dNTPs), 0.07µl primer assay mix (two forward and one reverse primer), and 0.5 µl ddH_2O for a total volume of 5.0 µl per reaction. PCR reactions were carried out under the following thermocycler program: denaturation at 94°C for 2:00 min followed by 35 cycles of 94°C for 1:00 min, 61°C for 1:00 min, 72°C for 1:00 min followed by a final extension step of 72°C for 6:00 min and held indefinitely at 4°C. Post-PCR fluorescence profiling of SNPs was performed on a BMG Labtech FLUOstar Omega microplate reader (Ortenberg, Germany; www.bmglabtech.com) and genotyping analysis was performed using LGC Genomics KlusterCaller software (Teddington, Middlesex, UK; www.lgcgenomics.com).

5.4 Results

5.4.1 Phenotypic population characterization

Genetic stocks

Phenotypic evaluation of a set of RL6092-derived and RL6003-derived lines outlined in Table 5.1 was conducted with *P. triticina* race BBBD, virulent to *LrCen,* and TDBG, avirulent to *LrCen,* to predict presence of leaf rust resistance genes in each of the lines. RL6092 (Thatcher*6/Timmo), the *Lr20* single gene isogenic differential line, showed a mesothetic reaction to TDBG putatively indicating the presence of *LrCen* in this line. RL6092-B, a selection of RL6092 known to contain two distinct leaf rust resistance genes had a low infection type for both races of *P. triticina* suggesting the presence of both *Lr20* and at least one other unknown gene. RL6092-A, a variant of RL6092 postulated to lack *Lr20* with only the second uncharacterized resistance gene displayed a high infection type with BBBD and an unexpected low infection type with TDBG, characteristic of the mesothetic reaction observed in lines carrying *LrCen*. Universally susceptible lines Thatcher and Little Club both had high infection types to both races of *P. triticina*. The *Lr1* single-gene NIL, RL6003a (isolated from RL6003) and the original RL6003, heterogeneous for *Lr1* and *LrCen,* were also included to provide a comparison of the mesothetic infection type expected with *LrCen*.

Table 5.1. Phenotypic infection types observed on a set of *Lr20* informative experimental lines inoculated with *Puccinia triticina* races BBBD and TDBG

Line (source)	Pedigree/description	BBBD IT	BBBD reaction	TDBG IT	TDBG reaction	STS638
RL6092 (CL108)	Tc*6/Timmo	3^+	S	X	R	+
RL6092 (CL108)	Tc*6/Timmo	3^+	S	X	R	+
RL6092 (CL108)	Tc*6/Timmo	3^+	S	X	R	+
RL6092 (CL108)	Tc*6/Timmo	3^+	S	X	R	+

RL6092-B (CL134)	08-865 RL6092 with 2 genes segregating	;1$^{=}$	R	;1^{-}	R	+
RL6092-B (CL134)	08-865 RL6092 with 2 genes segregating	;1$^{=}$	R	;1^{-}	R	+
RL6092-B (CL134)	08-865 RL6092 with 2 genes segregating	;1$^{=}$	R	;1^{-}	R	+
RL6092-B (CL134)	08-865 RL6092 with 2 genes segregating	;1$^{=}$	R	;1^{-}	R	+
RL6092-A (CL138)	08-865 RL6092 with single gene	3^{+}	S	X	R	+
RL6092-A (CL138)	08-865 RL6092 with single gene	3^{+}	S	X	R	+
RL6092-A (CL138)	08-865 RL6092 with single gene	3^{+}	S	X	R	+
RL6092-A (CL138)	08-865 RL6092 with single gene	3^{+}	S	X	R	+
Thatcher		3^{+}	S	3^{+}	S	-
Thatcher		3^{+}	S	3^{+}	S	-
Thatcher		3^{+}	S	3^{+}	S	-
Thatcher		3^{+}	S	3^{+}	S	-
Little Club		3^{+}	S	3^{+}	S	-
Little Club		3^{+}	S	3^{+}	S	-
Little Club		3^{+}	S	3^{+}	S	-
Little Club		3^{+}	S	3^{+}	S	-
Tc-LrCen (CL118)	Derived from Lr1 line	3^{+}	S	X	R	-
Tc-LrCen (CL118)	Derived from Lr1 line	3^{+}	S	X	R	-
Tc-LrCen (CL118)	Derived from Lr1 line	3^{+}	S	X	R	-
Tc-LrCen (CL118)	Derived from Lr1 line	3^{+}	S	X	R	-
RL6003a	Tc-Lr1	;	R	3^{+}	S	-
RL6003a	Tc-Lr1	;	R	3^{+}	S	-
RL6003a	Tc-Lr1	;	R	3^{+}	S	-
RL6003a	Tc-Lr1	;	R	3^{+}	S	-
RL6003	Tc-Lr1+LrCen	;	R	X	R	-
RL6003	Tc-Lr1+LrCen	;	R	X	R	-
RL6003	Tc-Lr1+LrCen	;	R	3^{+}	S	-
RL6003	Tc-Lr1+LrCen	;	R	X	R	-

IT = infection type, Tc = Thatcher, S = susceptible, R = resistant, + = *Lr20* allele product present, - = *Lr20* allele product absent

Thatcher/ RL6092-B F_2 Populations

Ninety individuals from an F_2 population from the cross Thatcher/ RL6092-B were inoculated with two *P. triticina* races; BBBD (virulent on *LrCen*) on the first leaf and TDBG (avirulent on *LrCen* and *Lr20*) on the second leaf to observe the segregation of the second unknown gene in the RL6092-B line, hypothesized to be *LrCen* (Table 5.2). The population segregated independently for resistance to BBBD and TDBG with a segregation ratio of 48:39 resistant to susceptible (three individuals were discarded due to poor germination) and 74:13 resistant to susceptible, respectively. While neither race fit the expected 3:1 ratio for a single gene providing resistance to TDBG, $\chi^2 = 4.69$ (P-value = 0.03) or BBBD, $\chi^2 = 18.24$ (P-value = 1.95E-05), pooling the segregation of both races resulted in 81 resistant to 6 susceptible individuals and conformed to the 15:1 segregation ratio expected for two dominant independent leaf rust resistance genes segregating in an F_2 population, $\chi^2 = 0.062$ (P-value = 0.803) (Table 5.3). A second set of 90 individuals from the same F_2 population segregated 59:21 resistant to susceptible (ten individuals were discarded for poor germination) when inoculated with *P. triticina* race MBPS (Table 5.2), which is reported to be avirulent to *Lr20* (B.D. McCallum, unpublished data), and fit a 3:1 ratio (Table 5.3). A third set of 90 F_2 plants was inoculated with *P. triticina* race TDBG and segregated 75 to 8 resistant to susceptible (seven individuals were discarded for poor germination) (Table 5.2), and fit a 15:1 ratio (Table 5.3).

Table 5.2. Phenotypic and molecular data observed on three sets of ninety individuals from the F_2 population Thatcher/RL6092-B

F_2 Set	Plant #	Line genotype	BBBD reaction	TDBG reaction	MBPS reaction	cfa2240	STS638
1	CQ5141	Thatcher	S	S	NA	B	B
1	CQ5142	RL6092-B	R	R	NA	A	A
1	CQ5143	F_2	S	R	NA	B	B
1	CQ5144	F_2	S	R	NA	A	A
1	CQ5145	F_2	R	R	NA	HET	A
1	CQ5146	F_2	S	R	NA	B	B

1	CQ5147	F_2	S	R	NA	HET	A
1	CQ5148	F_2	S	S	NA	B	B
1	CQ5150	F_2	S	R	NA	HET	A
1	CQ5151	F_2	S	R	NA	HET	A
1	CQ5152	F_2	S	R	NA	A	A
1	CQ5153	F_2	S	R	NA	HET	A
1	CQ5154	F_2	S	S	NA	HET	A
1	CQ5155	F_2	R	R	NA	A	A
1	CQ5156	F_2	R	R	NA	HET	A
1	CQ5157	F_2	S	R	NA	A	A
1	CQ5158	F_2	S	R	NA	HET	A
1	CQ5159	F_2	S	R	NA	B	B
1	CQ5160	F_2	S	S	NA	NA	NA
1	CQ5161	F_2	S	S	NA	HET	A
1	CQ5162	F_2	R	R	NA	B	B
1	CQ5163	F_2	R	R	NA	HET	A
1	CQ5164	F_2	R	R	NA	B	B
1	CQ5165	F_2	R	R	NA	A	A
1	CQ5166	F_2	R	R	NA	A	A
1	CQ5167	F_2	R	R	NA	HET	A
1	CQ5168	F_2	R	R	NA	HET	A
1	CQ5169	F_2	NA	R	NA	HET	A
1	CQ5170	F_2	S	R	NA	HET	A
1	CQ5171	F_2	R	R	NA	HET	A
1	CQ5172	F_2	S	S	NA	B	B
1	CQ5173	Thatcher	S	S	NA	B	B
1	CQ5174	RL6092-B	R	R	NA	A	A
1	CQ5175	F_2	R	S	NA	HET	A
1	CQ5176	F_2	S	R	NA	B	B
1	CQ5177	F_2	R	S	NA	HET	A
1	CQ5178	F_2	R	R	NA	HET	A
1	CQ5179	F_2	S	R	NA	A	A
1	CQ5180	F_2	R	S	NA	A	A
1	CQ5181	F_2	R	S	NA	B	B
1	CQ5182	F_2	R	R	NA	A	A
1	CQ5183	F_2	R	R	NA	HET	A
1	CQ5184	F_2	S	S	NA	A	A
1	CQ5185	F_2	R	R	NA	B	B
1	CQ5186	F_2	S	R	NA	A	A
1	CQ5187	F_2	R	R	NA	B	B
1	CQ5188	F_2	R	R	NA	A	A
1	CQ5190	F_2	R	R	NA	HET	A
1	CQ5191	F_2	R	R	NA	HET	A

1	CQ5192	F_2	S	R	NA	A	A
1	CQ5193	F_2	R	S	NA	NA	A
1	CQ5194	F_2	R	R	NA	HET	A
1	CQ5195	F_2	R	R	NA	B	B
1	CQ5196	F_2	R	R	NA	A	A
1	CQ5197	F_2	R	R	NA	HET	A
1	CQ5198	F_2	R	R	NA	B	B
1	CQ5199	F_2	S	R	NA	HET	B
1	CQ5200	F_2	R	R	NA	HET	A
1	CQ5201	F_2	S	R	NA	HET	A
1	CQ5202	F_2	R	R	NA	NA	B
1	CQ5203	F_2	R	R	NA	B	B
1	CQ5204	F_2	R	R	NA	B	B
1	CQ5205	Thatcher	S	S	NA	B	B
1	CQ5206	RL6092-B	R	R	NA	A	A
1	CQ5207	F_2	R	R	NA	B	B
1	CQ5208	F_2	S	R	NA	HET	A
1	CQ5209	F_2	S	R	NA	HET	A
1	CQ5210	F_2	S	R	NA	A	A
1	CQ5211	F_2	R	R	NA	A	A
1	CQ5212	F_2	R	R	NA	B	B
1	CQ5213	F_2	R	R	NA	HET	A
1	CQ5214	F_2	S	R	NA	HET	A
1	CQ5215	F_2	R	R	NA	A	A
1	CQ5216	F_2	R	R	NA	A	A
1	CQ5217	F_2	R	R	NA	HET	A
1	CQ5218	F_2	S	R	NA	HET	A
1	CQ5219	F_2	R	R	NA	B	B
1	CQ5220	F_2	R	S	NA	HET	A
1	CQ5221	F_2	S	R	NA	A	A
1	CQ5222	F_2	S	R	NA	B	B
1	CQ5223	F_2	R	S	NA	HET	A
1	CQ5224	F_2	S	R	NA	HET	A
1	CQ5225	F_2	S	R	NA	HET	A
1	CQ5226	F_2	S	R	NA	A	A
1	CQ5227	F_2	R	R	NA	B	B
1	CQ5228	F_2	S	R	NA	HET	A
1	CQ5229	F_2	S	R	NA	HET	A
1	CQ5230	F_2	S	R	NA	HET	A
1	CQ5231	F_2	S	R	NA	A	A
1	CQ5232	F_2	R	R	NA	HET	A
1	CQ5233	F_2	R	R	NA	HET	A
1	CQ5234	F_2	R	R	NA	HET	A

1	CQ5235	F_2	S	R	NA	A	A
1	CQ5236	F_2	R	R	NA	A	A
2	CQ5575	F_2	NA	NA	S	B	B
2	CQ5576	F_2	NA	NA	R	A	A
2	CQ5577	F_2	NA	NA	MR	HET	A
2	CQ5578	F_2	NA	NA	MR	B	B
2	CQ5579	F_2	NA	NA	S	B	A
2	CQ5581	F_2	NA	NA	R	A	A
2	CQ5582	F_2	NA	NA	MR	A	A
2	CQ5583	F_2	NA	NA	MR	HET	A
2	CQ5584	F_2	NA	NA	R	A	A
2	CQ5585	F_2	NA	NA	R	B	A
2	CQ5586	F_2	NA	NA	R	B	A
2	CQ5587	F_2	NA	NA	R	HET	A
2	CQ5588	F_2	NA	NA	S	HET	A
2	CQ5589	F_2	NA	NA	R	HET	A
2	CQ5590	F_2	NA	NA	MR	HET	A
2	CQ5591	F_2	NA	NA	S	B	B
2	CQ5592	F_2	NA	NA	MR	HET	A
2	CQ5593	F_2	NA	NA	R	A	A
2	CQ5594	F_2	NA	NA	S	HET	A
2	CQ5595	F_2	NA	NA	MR	B	B
2	CQ5596	F_2	NA	NA	R	HET	A?
2	CQ5597	F_2	NA	NA	R	HET	A?
2	CQ5598	F_2	NA	NA	MR	HET	A
2	CQ5599	F_2	NA	NA	S	B	B
2	CQ5600	F_2	NA	NA	S	B	B
2	CQ5601	F_2	NA	NA	MR	A	A
2	CQ5602	F_2	NA	NA	R	B	B
2	CQ5604	F_2	NA	NA	MR	HET	A?
2	CQ5605	F_2	NA	NA	S	HET	A?
2	CQ5606	F_2	NA	NA	S	B	B
2	CQ5607	Thatcher	NA	NA	S	B	B?
2	CQ5608	RL6092-B	NA	NA	R	A	A
2	CQ5609	F_2	NA	NA	MR	HET	A
2	CQ5610	F_2	NA	NA	MR	A	A
2	CQ5611	F_2	NA	NA	MR	HET	A
2	CQ5612	F_2	NA	NA	S	HET	A
2	CQ5613	F_2	NA	NA	MR	HET	A?
2	CQ5614	F_2	NA	NA	MR	HET	A
2	CQ5615	F_2	NA	NA	MR	HET	A
2	CQ5617	F_2	NA	NA	S	HET?	B
2	CQ5618	F_2	NA	NA	MR	HET	A

2	CQ5621	F_2	NA	NA	R	HET	A
2	CQ5623	F_2	NA	NA	MR	HET	A
2	CQ5624	F_2	NA	NA	MR	HET	A
2	CQ5625	F_2	NA	NA	MR	HET	A
2	CQ5626	F_2	NA	NA	S	B	B
2	CQ5627	F_2	NA	NA	S	A	A
2	CQ5628	F_2	NA	NA	MR	B	A?
2	CQ5629	F_2	NA	NA	R	HET	A
2	CQ5630	F_2	NA	NA	R	HET	A
2	CQ5633	F_2	NA	NA	R	HET	A
2	CQ5634	F_2	NA	NA	MR	HET	A
2	CQ5635	F_2	NA	NA	S	NA	A
2	CQ5636	F_2	NA	NA	MR	B	B
2	CQ5637	F_2	NA	NA	MR	A	A
2	CQ5638	F_2	NA	NA	MR	B	B
2	CQ5639	Thatcher	NA	NA	S	B	B
2	CQ5640	RL6092-B	NA	NA	R	A	A
2	CQ5641	F_2	NA	NA	S	A	A
2	CQ5642	F_2	NA	NA	S	B	B
2	CQ5643	F_2	NA	NA	R	B	B
2	CQ5644	F_2	NA	NA	MR	B	B
2	CQ5645	F_2	NA	NA	MR	NA	A
2	CQ5646	F_2	NA	NA	MR	B	B
2	CQ5647	F_2	NA	NA	S	HET	A
2	CQ5650	F_2	NA	NA	MR	HET	B
2	CQ5651	F_2	NA	NA	S	A	A
2	CQ5652	F_2	NA	NA	MR	B	B
2	CQ5653	F_2	NA	NA	R	B	B
2	CQ5654	F_2	NA	NA	S	B	B
2	CQ5655	F_2	NA	NA	MR	B	B
2	CQ5656	F_2	NA	NA	S	B	B
2	CQ5657	F_2	NA	NA	MR	HET	A
2	CQ5658	F_2	NA	NA	R	B	B
2	CQ5659	F_2	NA	NA	S	HET	B
2	CQ5660	F_2	NA	NA	MR	HET	B
2	CQ5661	F_2	NA	NA	MR	A	A
2	CQ5662	F_2	NA	NA	R	HET	A
2	CQ5663	F_2	NA	NA	R	HET	A
2	CQ5664	F_2	NA	NA	R	A	A
2	CQ5665	F_2	NA	NA	MR	A	A
2	CQ5666	F_2	NA	NA	MR	B	B
2	CQ5667	F_2	NA	NA	MR	B	B
2	CQ5668	F_2	NA	NA	S	HET	A

2	CQ5669	F_2	NA	NA	MR	HET	B
2	CQ5670	F_2	NA	NA	R	A	A
3	CQ5512	RL6092-B	R	NA	NA	NA	NA
3	CQ5515	F_2	R	NA	NA	NA	NA
3	CQ5516	F_2	R	NA	NA	NA	NA
3	CQ5517	F_2	R	NA	NA	NA	NA
3	CQ5518	F_2	R	NA	NA	NA	NA
3	CQ5519	F_2	R	NA	NA	NA	NA
3	CQ5520	F_2	R	NA	NA	NA	NA
3	CQ5521	F_2	R	NA	NA	NA	NA
3	CQ5522	F_2	R	NA	NA	NA	NA
3	CQ5523	F_2	R	NA	NA	NA	NA
3	CQ5524	F_2	R	NA	NA	NA	NA
3	CQ5525	F_2	R	NA	NA	NA	NA
3	CQ5527	F_2	R	NA	NA	NA	NA
3	CQ5528	F_2	R	NA	NA	NA	NA
3	CQ5529	F_2	R	NA	NA	NA	NA
3	CQ5530	F_2	R	NA	NA	NA	NA
3	CQ5531	F_2	R	NA	NA	NA	NA
3	CQ5532	F_2	R	NA	NA	NA	NA
3	CQ5533	F_2	R	NA	NA	NA	NA
3	CQ5535	F_2	S	NA	NA	NA	NA
3	CQ5536	F_2	S	NA	NA	NA	NA
3	CQ5537	F_2	R	NA	NA	NA	NA
3	CQ5538	F_2	R	NA	NA	NA	NA
3	CQ5539	F_2	R	NA	NA	NA	NA
3	CQ5540	F_2	R	NA	NA	NA	NA
3	CQ5542	F_2	R	NA	NA	NA	NA
3	CQ5544	RL6092-B	R	NA	NA	NA	NA
3	CQ5545	F_2	R	NA	NA	NA	NA
3	CQ5546	F_2	S	NA	NA	NA	NA
3	CQ5547	F_2	R	NA	NA	NA	NA
3	CQ5550	F_2	R	NA	NA	NA	NA
3	CQ5551	F_2	R	NA	NA	NA	NA
3	CQ5552	F_2	R	NA	NA	NA	NA
3	CQ5553	F_2	R	NA	NA	NA	NA
3	CQ5554	F_2	S	NA	NA	NA	NA
3	CQ5555	F_2	R	NA	NA	NA	NA
3	CQ5556	F_2	R	NA	NA	NA	NA
3	CQ5557	F_2	R	NA	NA	NA	NA
3	CQ5558	F_2	R	NA	NA	NA	NA
3	CQ5559	F_2	S	NA	NA	NA	NA
3	CQ5560	F_2	R	NA	NA	NA	NA

3	CQ5561	F_2	R	NA	NA	NA	NA
3	CQ5562	F_2	R	NA	NA	NA	NA
3	CQ5563	F_2	R	NA	NA	NA	NA
3	CQ5564	F_2	R	NA	NA	NA	NA
3	CQ5565	F_2	R	NA	NA	NA	NA
3	CQ5566	F_2	R	NA	NA	NA	NA
3	CQ5567	F_2	R	NA	NA	NA	NA
3	CQ5568	F_2	R	NA	NA	NA	NA
3	CQ5569	F_2	R	NA	NA	NA	NA
3	CQ5570	F_2	R	NA	NA	NA	NA
3	CQ5571	F_2	R	NA	NA	NA	NA
3	CQ5572	F_2	R	NA	NA	NA	NA
3	CQ5573	F_2	R	NA	NA	NA	NA
3	CQ5574	F_2	R	NA	NA	NA	NA
3	CQ5576	RL6092-B	R	NA	NA	NA	NA
3	CQ5577	F_2	R	NA	NA	NA	NA
3	CQ5578	F_2	R	NA	NA	NA	NA
3	CQ5579	F_2	S	NA	NA	NA	NA
3	CQ5580	F_2	R	NA	NA	NA	NA
3	CQ5581	F_2	R	NA	NA	NA	NA
3	CQ5582	F_2	R	NA	NA	NA	NA
3	CQ5583	F_2	R	NA	NA	NA	NA
3	CQ5584	F_2	R	NA	NA	NA	NA
3	CQ5585	F_2	R	NA	NA	NA	NA
3	CQ5586	F_2	R	NA	NA	NA	NA
3	CQ5587	F_2	R	NA	NA	NA	NA
3	CQ5588	F_2	R	NA	NA	NA	NA
3	CQ5589	F_2	R	NA	NA	NA	NA
3	CQ5590	F_2	R	NA	NA	NA	NA
3	CQ5591	F_2	R	NA	NA	NA	NA
3	CQ5592	F_2	R	NA	NA	NA	NA
3	CQ5593	F_2	R	NA	NA	NA	NA
3	CQ5594	F_2	S	NA	NA	NA	NA
3	CQ5595	F_2	R	NA	NA	NA	NA
3	CQ5596	F_2	R	NA	NA	NA	NA
3	CQ5597	F_2	R	NA	NA	NA	NA
3	CQ5598	F_2	R	NA	NA	NA	NA
3	CQ5599	F_2	R	NA	NA	NA	NA
3	CQ5600	F_2	R	NA	NA	NA	NA
3	CQ5601	F_2	S	NA	NA	NA	NA
3	CQ5602	F_2	R	NA	NA	NA	NA
3	CQ5603	F_2	R	NA	NA	NA	NA
3	CQ5604	F_2	R	NA	NA	NA	NA

3	CQ5605	F_2	R	NA	NA	NA	NA
3	CQ5606	F_2	R	NA	NA	NA	NA

S = susceptible, R = resistant, NA = no data available, A = PCR product size present for putative presence of *LrCen* (cfa2240, co-dominant marker) or *Lr20* (STS638, dominant marker), B = PCR product size negative for putative presence of *LrCen* (cfa2240, co-dominant marker) or *Lr20* (STS638, dominant marker), HET = heterozygous PCR product for *LrCen* allele (cfa2240), ? = weak resolution

Table 5.3. Chi-square and p-values reported for observed phenotypic ratios in the F_2 population Thatcher/RL6092-B

F_2 set	*Puccinia triticina* Race	Data type	Resistant lines	Susceptible lines	Segregation ratio tested (H_o)	P-value	χ^2	Degrees of freedom	Critical χ2 value	Conclusion
1	BBBD	observed	48	39						
		expected	65.2	21.7	3:1	1.9E-05	18.2	1	3.8	reject
	TDBG	observed	74	13						
		expected	65.2	21.7	3:1	0.03	4.6	1	3.8	reject
		expected	81.5	5.4	15:1	0.0008	11.2	1	3.8	reject
	BBBD/TDBG pooled	observed	81	6						
		expected	81.5	5.4	15:1	0.803	0.06	1	3.8	accept
2	MBPS	observed	59	21						
		expected	60	20	3:1	0.79	0.06	1	3.8	accept
3	TDBG	observed	75	8						
		expected	62.2	20.7	3:1	0.001	10.4	1	3.8	reject
		expected	77.8	5.1	15:1	0.20	1.6	1	3.8	accept
1 and 3	TDBG pooled	observed	149	21						
		expected	159.3	10.6	15:1	0.001	10.8	1	3.8	reject

Lr20 Source Lines

Using the informative races of *P. triticina,* BBBD and TDBG, spring wheat genotypes Axminster, Norka, Festival, and Sappo all described as having *Lr20* (Neu et al. 2002) were phenotyped to understand the relationship between *Lr20* and *LrCen.* Both race-specific seedling leaf rust resistance genes mapped to the long arm of chromosome 7A (Neu et al. 2002; Chapter 4, Figure 4.2). Infection types are described in Table 5.4, where Axminster, Sappo and some accessions of Norka (accession CN52322 was heterogeneous for resistance) appear resistant to TDBG, while Festival and other accessions of the Norka were susceptible. If these genotypes all carry *Lr20* as literature states (Neu et al. 2002), the presence of *Lr20* in these lines could not be validated using phenotypic observations with leaf rust race TDBG, which has been described as virulent on *Lr20* (Dakouri et al. 2013).

Table 5.4. Phenotypic infection types observed on cultivars suggested to carry *Lr20* and other informative lines inoculated with *Puccinia triticina* races BBBD and TDBG and screened with DNA marker STS638 linked to *Lr20*.

Name	BBBD IT	BBBD reaction	TDBG IT	TDBG reaction	STS638 reaction
Axminster (CN 9574)	3	S	;1-X	R	-
Axminster (CN 33630)	3	S	;$1^{=}$-1^{-}	R	-
Festival (CN 10070)	3	S	3^{+}	S	+
Norka (CN 11207)	;1^{-}	R	3^{+}	S	-
Norka (CN 11208)	0	R	X^{+}/3	S	+
Norka (CN 52322)	;1	R	X^{+}/3^{+}	HET	HET
Sappo (CN 45907)	3	S	;1^{-}	R	+
LrCen (CL118)	3	S	X^{-}	R	-
Little Club (CP963)	3	S	X^{+}	MR	-
Little Club (field source)	3	S	3^{+}	S	-
Thatcher (‘06 increase)	3	S	3^{+}	S	-
RL6092-B (CL138)	;1/3	HET	;$1^{=}$	R	+
Thatcher (McCallum source)	3	S	3^{+}	S	-

IT = infection type, S = susceptible, R = resistant, MR = moderate resistance, HET = heterogeneous

Wheat Germplasm

185 genotypes of wheat germplasm were selected based on previously described phenotypic responses (Appendix 8.3; B.D. McCallum, unpublished data) indicating the putative presence of one, or a combination of, *Lr16, Lr2a,* and *LrCen*. The phenotypic observations are outlined in Appendix 8.3 where 104 lines were resistant to race TDBG, eight lines were moderately resistant and 73 lines were susceptible.

5.4.2 Marker screening

Screening with Lr20 marker STS638

Neu et al. (2002) describe the dominant marker STS638, as being highly specific to the *Lr20* and *Pm1* gene locus. Results from screening of RL6092, RL6003, and the previously described variants created from these near-isogenic lines with STS638 are summarized in Table 5.1. Eight replicates of each line were tested to ensure no false positives were observed. RL6092, RL6092-B, and RL6092-A all amplified the 542bp PCR product corresponding to a positive identification of the *Lr20* gene while Tc-*LrCen,* RL6003, RL6003a, and susceptible check Thatcher and Little Club were all negative for the presence of *Lr20.* Lines that produced a 542bp fragment with *Lr20* linked marker STS638 were selected for further SSR genotyping of the 7AL locus. Thatcher was included as a negative control. A total of 55 SSR markers from chromosome 7AL (Appendix 8.4) were screened; however lines positive for STS638 had uniform SSR haplotypes on 7AL.

Each of the lines described as sources of *Lr20* (Neu et al. 2002) were screened with the *Lr20* linked marker STS638 in two replicates. Sappo and Festival produced a 542bp fragment suggesting they may carry *Lr20,* while Norka appeared to be heterogeneous for the presence of the 542pb fragment.

Axminster consistently produced no fragment for the dominant STS638 marker, suggesting it was negative for the presence of *Lr20* in two separate accessions (Table 5.4).

Thatcher/ RL6092-B F_2 population screening with tightly linked LrCen microsatellite marker cfa2240 and tightly linked Lr20 marker STS638

Ninety individuals from the Thatcher/*RL6092-B* F_2 population segregated independently for resistance to BBBD (48 resistant: 39 susceptible) and TDBG (74 resistant: 13 susceptible). This population was screened using microsatellite marker *cfa2240* previously demonstrated to be 1.1cM distal to newly identified leaf rust resistance gene *LrCen*, theorized to be a second gene present in the F_2 population. Screening of the same population was performed using the *Lr20* diagnostic marker STS638. While these markers showed close linkage to one another (RF = 0.061), a chi-square test ($\chi^2 = 2.61$, p-value = 0.106), was performed to confirm that the 302bp PCR fragment, representing the allele linked in coupling with *LrCen*, assorted independently of resistance to TDBG in the F_2 population. While no linkage was detected between the DNA markers and the observed BBBD and TDBG resistance, both markers followed the expected Mendelian segregation Table 5.5.

Table 5.5. Contingency table for segregation of molecular markers cfa2240 and STS638 linked to *LrCen* in the F_2 population Thatcher/RL6092-B inoculated with *Puccinia triticina* race TDBG, virulent on *LrCen*

Molecular Marker	Marker type	Data type	Homozygous for resistant allele size	Heterozygous	Homozygous for susceptible allele size	Segregation ratio tested (H_o)	P-value	χ^2	Degrees of freedon	Critical χ2 value	Conclusion
cfa2240	co-dominant	observed	43	80	52						
		expected	43.75	87.5	43.75	1:2:1	0.331	2.211	2	5.991	accept
STS638	dominant	observed	118		54						
		expected	129		43	3:1	0.053	3.752	1	3.841	accept

Wheat germplasm genotyping for presence of LrCen

A preliminary phenotypic evaluation of a collection of wheat genotypes revealed a large portion of germplasm that carried resistance to the relatively new *P. triticina* race TDBG often exhibited in the typical mesothetic reaction type observed with the presence of *LrCen.* A secondary phenotypic analysis and a genetic evaluation with KASP marker *KWH41* and SSR marker *cfa2240*, both tightly linked to *LrCen* with a genetic distance of 1.1cM, was performed to add confidence to a postulation of the distribution of *LrCen* within a subset of germplasm. While phenotypic assessment revealed 112 of 185 genotypes exhibited resistance to race TDBG (observed in a range of infection types from a fleck resistance to the characteristic mesothetic reaction type), genetic evaluation with SSR marker *cfa2240* and KASP marker *KWH41* postulated the presence of *LrCen* in only 36 of the TDBG resistant genotypes (Appendix 8.3). Twenty-one of the 185 genotypes had poor DNA quality or no DNA could be obtained and no data was available for SSR markers *cfa2240*, 12 of which were resistant to TDBG and could be positive for the *LrCen* allele (Appendix 8.3).

5.5 Discussion

Thatcher/RL6092-B F_2 Population

The emergence of a new predominant race of leaf rust in North America in 2004, TDBG, revealed a second leaf rust resistance gene segregating in the *Lr1* NIL (RL6003) (McCallum et al. 2005). This gene was temporarily designated as *LrCen* (McCallum and Hiebert 2012) and mapped to chromosome arm 7AL (Chapter 4, Figure 4.2). Further screening of the single-gene NIL differential set with TDBG and *Lr20* virulent race PBDQ revealed a similar low infection type in the *Lr20* NIL for TDBG and an unexpected low frequency of resistance to PBDQ. These results indicated the presence of at least one unknown gene segregating in the *Lr20* NIL. *Lr20,* like *LrCen,* has also been mapped to chromosome 7AL; however little has been described on the exact genetic location of *Lr20* on the 7AL

chromosome. TDBG virulence on *Lr20* had not been well studied at the time, which prompted the theory that the second source of resistance may be *LrCen,* based on the similar mesothetic infection type that was observed in the *Lr1* NIL. In order to determine if the second gene in this population could be *LrCen,* a set of lines, outlined in Table 5.1, was selected for phenotypic and genetic evaluation.

Phenotypic evaluation of an F_2 population from the cross Tc/RL6092-B with leaf rust races BBBD (48 resistant: 39 susceptible) and TDBG (74 resistant: 13 susceptible), a two-way contingency test (p-value = 0.148) confirmed the independent segregation of resistance to both *P. triticina* races confirming that there is more than one source providing resistance to *P. triticina* in the Tc/RL6092-B F_2 population. When data from the third set of 90 F_2 individuals inoculated with race TDBG only was considered on its own, the observed phenotypes were 75 resistant to 8 susceptible and fit the expected 15:1 segregation for two dominant genes (χ^2 = 1.625, p-value = 0.202), both providing resistance to race TDBG. However, when the phenotypes observed for TDBG resistance were pooled for both sets of 90 F_2 individuals a segregation ratio of 149 resistant to 21 susceptible was observed and failed to fit the same 15:1 ratio, χ^2 = 10.81 (P-value = 0.001). Explanations for the skewed genetic ratios could be due in part to the small population size for an F_2 experiment, where effects of environment tend to be more pronounced in F_2 populations due to a lack of replication (Bjarko and Line 1988). As well, scoring of the intermediate mesothetic infection types often observed with race TDBG and intermediate phenotypes observed in heterozygotes could also have resulted in scoring errors leading to the lack of consistency in the observed segregation ratio between plantings.

Two-point linkage analysis of this F_2 population unexpectedly revealed no linkage between the phenotypically observed TDBG infection types and the marker results for *Lr20* linked STS638 marker, described to by highly diagnostic of *Lr20* (Neu et al. 2002). Similarly, no linkage was detected with *LrCen* linked SSR marker *cfa2240.* This was not a surprise based on how closely the two markers co-segregated. Recent publications describe *Lr20* as resistant to race TDBG, making the lack of linkage of

resistance to TDBG and the DNA markers in the F_2 population more interesting (Dakouri et al. 2013). The observed low IT to TDBG for the *Lr20* line used here does not match the low IT pictured by McIntosh et al. (1995). This difference in IT expression suggests that the reference stock for *Lr20* used by McIntosh et al. in 2005 may carry a different resistance gene than the RL6092-B NIL being used in the current study and potentially a different gene than the aforementioned experiments conducted by Dakouri et al. (2013). PCR product segregation for *cfa2240* and STS638 indicated a tight linkage between the two markers with an RF value of 0.061. This confirms that the markers are producing PCR products from a similar region of the genome as would be expected for markers linked to genes both mapped to the distal end of chromosome 7AL. A lack of linkage between *cfa2240* and observed ITs for TDBG confirms that the source of TDBG resistance segregating in the F_2 population is not conferred by *LrCen*. The lack of linkage between the reported location of *Lr20* with linked marker STS638 and the infection types observed for TDBG and BBBD suggest that *Lr20* is also not the source of TDBG or BBBD resistance in the population. It seems likely, however, that *Lr20* is present in this population as STS638 was polymorphic between the parents Thatcher and RL6092-B in this region, but it is not providing the observed TDBG resistance. The lack of study of *Lr20* makes it hard to come to conclusions on the observed results. From this F_2 population it would appear that detecting the presence of *Lr20* by phenotype may be difficult. While reports indicate a low infection type (IT = 2) associated with TDBG and RL6092 (Dakouri et al. 2013), studies of the F_2 population created using this isogenic line failed to find any linkage between the phenotypes and DNA markers on chromosome arm 7AL.

The second set of 90 individuals from the same F_2 population inoculated with *P. triticina* race MBPS, also described as being low on RL6092 in a North American virulence survey (B.D. McCallum, unpublished data), was performed in efforts to confirm the presence of *Lr20* in the population. Again, no significant linkage was detected between the phenotypic and genotypic data. Furthermore, mapping of a single seed descent (SSD) population from the cross ‘Schomburgk’/’Yarralinka’, where ‘Schomburgk’

was described as amplifying the 542 bp fragment representative of a positive identification of *Lr20* with STS638 (Nu et al. 2002) and 'Yarralinka' amplifying no fragment, resulted in a recombination value of 7.1 cM (LOD score of 14.0) between *Lr20* and STS638 (Khan et al. 2005). This value indicates that the STS638 marker is not as closely linked to *Lr20* as originally thought and therefore not as diagnostic of *Lr20* as was previously described.

The conclusions that can be drawn from this experiment are that there are at least two independent seedling resistance genes segregating within the Tc/RL6092-B F_2 population and therefore carried by the *Lr20* NIL, RL6092. Observed phenotypes for one set of 90 F_2 individuals inoculated with race TDBG conforms to a 15:1 ratio suggesting that there may be two genes providing resistance to TDBG segregating within the population. However, pooled results for 170 F_2 individuals, a larger population size, did not fit the expected 15:1 ratio for resistance to TDBG. This can likely be explained by the variability in phenotyping of mesothetic ITs. This cannot be confirmed, but could be studied further with a larger F_2 population size or developing F_2-derived F_3 populations to observe more than one plant for more consistent phenotyping results. Neither seedling gene, identified through phenotypic evaluation with *P. triticina* races TDBG and BBBD, is linked to the region carrying *LrCen* or the region carrying *Lr20* as determined by screening with the population with linked DNA markers. The polymorphism observed with dominant marker STS638 between parental lines of the F_2 population in the region carrying *Lr20* suggests that *Lr20* is present in the population, however, none of the races used in phenotypic screenings (TDBG, BBBD, and MBPS) detected *Lr20*. This suggests that if *Lr20* is present in the population, none of the races used were virulent and further phenotypic testing with other *P. triticina* races may allow for the detection of *Lr20* and help to further identify the location of the gene. Alternatively, the marker is present in the population but *Lr20* is absent. This would be supported by the findings of Khan et al. (2005), describing a RF of 7.1 cM between STS638 and *Lr20*. *LrCen* is not

present in RL6092 unless it is located on a transposable element and has been inserted elsewhere in the genome.

Lr20 Lines

Phenotypic screening of four replicates of each line outlined in Table 5.1 with *P. triticina* race TDBG confirmed resistance in RL6092, RL6092-B, RL6092-A, Tc-*LrCen*, and RL6003 to this race which is avirulent on *LrCen* and has been described as avirulent on *Lr20* (Dakouri et al. 2013), but results of this study are inconclusive. The resistant reaction observed in the RL6092-A line, an accession of RL6092-B believed to be lacking *Lr20* (selections were made based on the assumption that *Lr20* was conferring resistance to BBBD), support the previous results observed in the Tc/RL6092-B F_2 population that *Lr20* is not providing resistance to race TDBG and that there is a second gene providing resistance to TDBG. It could not be concluded which lines in fact do carry *Lr20* as no races used in the study were able to resolve this. The RL6092-A line was selected for phenotypic assessment with races TDBG and PBDQ. The actual virulence/ avirulence of these races on *Lr20* should be questioned, based on the above observations and could be contributing to unexpected results. Susceptible checks Thatcher and Little Club, as well as the newly developed single-gene *Lr1* NIL, RL6003a, all demonstrated the expected susceptibility. Molecular analysis of these lines with the *Lr20* linked marker STS638 amplified the 542bp fragment representative of the presence of *Lr20* in RL6092, RL6092-B, and RL6092-A. The detection of the 542bp fragment in the RL6092-A accession, selected for the absence of *Lr20* through phenotypic evaluation with a combination of *P. triticina* races PBDQ and TDBG, suggests that the races used were unable to detect *Lr20* or the STS marker is not as tightly linked to *Lr20* as previously thought. This finding is consistent with the 7.1 cM recombination value observed by Khan et al. (2005). If *Lr20* was successfully removed from RL6092-A, then the false positive may have been the result of a single recombination event that occurred during the removal of *Lr20* from this accession.

The *Lr20* stock genotypes received from the PGRC revealed resistance to TDBG in two accessions of Axminster (CN9574 and CN33630), Sappo (CN45907), and one accession of Norka (CN11208), while a second accession of Norka (CN52322) appeared to be heterogeneous for resistance where one out of four plants had a low infection type. As TDBG has been described as avirulent on *Lr20,* this would phenotypically support the presence of *Lr20* in these lines, however, results of this study put in to question the avirulence of TDBG on *Lr20*. A third accession of Norka (CN11207) and Festival (CN10070) were both susceptible to TDBG. Genotyping of the four replicates of each of these lines with *Lr20* linked marker STS638 revealed the expected 542bp fragment in Sappo, the resistant accession of Norka (11208), and in one plant out of four of the heterogeneous accessions of Norka (CN33630) and Festival. If *Lr20* does in fact provide resistance to TDBG, these genotypic results would support the presence of *Lr20* in Sappo, Norka (CN11207), the TDBG resistant plant of the heterogeneous Norka line, and Festival. Festival was not resistant to TDBG and would further support that it may not be *Lr20* that is providing the resistance to TDBG. Negative genetic results for STS638 in TDBG resistant genotype Axminster also supports that the resistance to TDBG is the result of a gene other than *Lr20*. Alternatively, this lack of consistency with phenotypic and genotypic data could be due to a recombination event between the gene and the marker, as the marker has been demonstrated to be less diagnostic than previously claimed. Axminster accession CN9574 presented the 302bp PCR fragment linked with *LrCen* in three out of four plants when genotyped with *cfa2240*. This may explain the TDBG resistance in this line with the possible presence of *LrCen.* The overall lack of consistency in observed phenotypes and genotypes of these lines further supports the results observed above and suggests that further investigations are required into *Lr20* before any conclusions can be made about the relationship with *LrCen.*

Distribution of LrCen in Wheat Germplasm

After the discovery of leaf rust resistance gene *LrCen* found segregating in the *Lr1* NIL differential line RL6003, it was initially predicted to have been introduced through the parental line

Centanario, as Thatcher was used as a known susceptible background for the second parent. While other genes were also determined to provide resistance to TDBG including *Lr9*, *Lr3Ka*, *Lr3bg*, *Lr16*, *Lr11*, *Lr14a* and *b*, *Lr18*, *Lr20*, *Lr21*, and *Lr32* (Dakouri et al. 2013); a phenotypic study of a diverse subset of wheat genotypes used in the Somers (unpublished) association mapping population revealed a number of genotypes that did not appear to carry any of the characterized resistance genes, but did provide effective resistance to TDBG (McCallum and Hiebert 2012). This was postulated to be due to the presence of newly mapped *LrCen*. A total of 185 genotypes were selected to test this theory based on phenotypic results of the McCallum (2005, 2012) phenotypic study (Appendix 8.3). A set of Canadian genotypes was selected based on pedigree to provide insight on the possible origin of this, what appeared to be, broadly distributed seedling leaf rust resistance gene.

Phenotypic results of 185 wheat genotypes revealed resistance to *P. triticina* race TDBG in 112 genotypes. Each of the 185 genotypes was then genotyped using KASP marker *KWH41* and SSR marker *cfa2240*, both tightly linked to *LrCen* at a distance of 1.1 cM to help assess the postulation of *LrCen*. The results of this genotypic analysis revealed that 35 of the 112 resistant genotypes screened are postulated to carry *LrCen* (results for marker *cfa2240* are summarized Appendix 8.3, *KWH41* provided the exact same genotypic scores and was therefore omitted). The other 77 genotypes that expressed resistance to TDBG could carry one, or a combination of, the other seedling resistance genes known to provide some level of resistance to this race. A similar situation was observed in the RL6092-B NIL whereby a seedling resistance gene which was phenotypically similar to *LrCen* was detected, but was located elsewhere in the genome. Alternatively, it is possible that *LrCen* exists on a transposable element making genotypic selection for this gene more difficult and would require gene cloning to design a gene-specific marker for effective screening. While most of the genes that provide resistance to TDBG are described as expressing moderate infection types (2 to X), the range of infection types observed in this phenotypic assessment (; to 2,X) could be explained by a synergistic effect of multiple

genes providing the very low infection type reactions. This range in infection types was also exhibited in the segregating DH mapping population used to locate *LrCen* to chromosome 7AL. The mid-range infection types scored as 2 or X could sometimes be mistaken for a higher or susceptible reaction, which may have led to some lines being incorrectly classified as susceptible. This could be an explanation for the Australian genotype Janz that was phenotypically scored as susceptible, but genetically the marker suggests *LrCen* is present. Janz has the same pedigree as Australian genotype Cunningham that was registered ten years later. Cunningham showed a resistant infection type to TDBG that is consistent with *LrCen* and also carried the *cfa2240* allele that was previously shown to be linked in coupling with *LrCen,* indicating that the variation in phenotypic and genotypic results for Janz could also be due to a single recombination event that resulted in the removal of *LrCen* (from at least this accession of Janz) while still carrying the *cfa2240* allele that suggests the presence of *LrCen*.

It is also important to note that genotypes such as BW717 (*Lr14a*), BW807 (*Lr14a*/*Lr16*), and McVey (*Lr16*) were phenotypically presumed to carry TDBG resistant *Lr14a* or *Lr16* based on low infection types to one or both of *P.triticina* races MBDS (low on *Lr16*) and MGBJ (low on *Lr14a*) that was in turn providing them with TDBG resistance (Appendix 8.3; McCallum, unpublished). Had genotypic testing of these lines not been conducted for the presence of *LrCen,* it may have gone unnoticed in these genotypes. Epistatic effects exhibited by other *Lr* genes including *Lr14a* and *Lr16* may mask the presence of *LrCen* phenotypically, strengthening the importance of molecular confirmation in genetic characterization studies.

The results of this study reveal that although *LrCen* does not appear to be present in as many genotypes as was previously estimated, it is still widely distributed within the observed germplasm in both older and more modern lines. A pedigree analysis of many of the genotypes positive for *LrCen* reveals common lineage, suggesting that the introduction of *LrCen* into many modern genotypes was likely through Thatcher (McCallum and DePauw 2008; Genetic Resources Information System for Wheat

and Triticale (GRIS): www.wheatpedigree.net [Accessed: January 2015]; Biological and Biotechnology Sciences Research Council (BBSRC): https://www.jic.ac.uk/germplasm/docs/**Wheat**%20**Pedigree**%20data.xlsx [Accessed: January 2015]; North Dakota State University (NDSU): Variety development). Although Thatcher is considered a susceptible line and was used in the creation of the DH mapping population for *LrCen* (the accession used in the DH population was determined by molecular and phenotypic testing to be negative for the presence of *LrCen*), it is possible that being such an old cultivar, many accessions exist and that *LrCen* is heterogeneously distributed in some genetic stocks of the line, while others lack the gene due to a single recombination event. While Red Fife and Marquis, both predominant genotypes grown early in the history of the Canadian Western Red Spring class in Canada, and important components in the pedigree of Thatcher, carry resistance to TDBG, they were both negative for the molecular markers linked to *LrCen*. It is possible that one of these lines is also heterogeneous and *LrCen* was introduced into some accessions of the Thatcher background in this way. This would support why Thatcher is common to the lineage of many lines described as carrying *LrCen* as well as the Thatcher-*Lr1* NIL (RL6003) in which *LrCen* was discovered, while other genotypes with a Thatcher background do not carry the gene.

Another example of heterogeneous distribution of *LrCen* within accessions of the same genotype is Little Club. Early in the discovery of *LrCen,* McCallum and Hiebert (2012) recognized that races avirulent to *LrCen* were also commonly avirulent to another wheat genotype previously described as universally susceptible, Little Club. Allelism testing on over 400 F_2 progeny from the cross Tc-*LrCen*/Little Club revealed no segregation, confirming the presence of *LrCen* in the susceptible genotype. This is another case where the distribution of *LrCen* is heterogeneous, as two sources were tested during the described phenotyping studies with TDBG and both were susceptible. This could be the case with other genotypes, namely those with Thatcher in the background. Depending on what source of Thatcher was used in creating the crosses for specific accessions or sources of each genotype, the

distribution of *LrCen* may be even broader than is suggested here. These results support why association mapping efforts discussed in the mapping of *LrCen* were unsuccessful, as many lines believed to carry *LrCen* have been demonstrated to carry a similar gene that must appear at a different chromosomal location.

In summary, an F_2 population from the cross of Thatcher to an accession of RL6092, RL6092-B*,* initially believed to carry *Lr20* and a second unknown seedling resistance gene based on phenotypic selection showed resistance to three races of *P. triticina* (TDBG, BBBD and MBPS). Based on previous studies indicating a tight linkage of marker STS638 and *Lr20*, no linkage between phenotypic infection types and the marker indicated that none of the three races detected *Lr20.* It was also concluded that no resistance in the F_2 population mapped to chromosome 7AL, eliminating the possibility that *LrCen* was providing resistance to TDBG in this population. Phenotypic and genotypic studies indicate that there are at least two leaf rust resistance genes found in RL6092. Based on previous genetic information, including tight linkage with STS638 (Neu et al. 2002) and avirulence of TDBG on *Lr20* (Dakouri et al. 2013), neither of these genes appears to be *Lr20*. The results of this study disagree with the results of Dakouri et al. (2013) that describe avirulence of TDBG on Lr20. This may suggest that phenotypic gene postulation is not sufficient and more investigation is required into the *Lr20* NIL and genotypes postulated to carry *Lr20* to better characterize the *Lr20* gene. These inconsistencies make it difficult to form conclusions on the relationship of *Lr20* and *LrCen*. The confirmed mapping location of *LrCen* on the long arm of chromosome 7AL with tight linkage to SSR marker *cfa2240* postulated the presence of *LrCen* in 36 genotypes of wheat germplasm, primarily North American (NA). Pedigree studies suggest a likely origination for introduction into RL6003 and North American germplasm by Thatcher or a component of its pedigree. A phenotypic gene postulation study conducted on a diverse set of wheat genotypes for *Lr20* postulate a large distribution in South America, Africa, and Europe with

a relatively low presence in North American genotypes.

CHAPTER 6

GENERAL DISCUSSION

Wheat leaf rust studies can be traced back to 1815 where de Candolle designated it as *Uredo rubigo-vera*. While the pathogen we refer to today as *Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex Desmaz. f. sp. *tritici*) has undergone a number changes since its initial description, it has long been described as the most common and widespread disease affecting wheat (Roelfs 1988). As with most biotic and abiotic stresses affecting crops, genetic resistance is the preferred method of control, as it results in no additional cost to the producer or the environment. A novel source of resistance was identified to be segregating in the Thatcher-*Lr1* (Tc-*Lr1*) near-isogenic differential line, RL6003, following the emergence of a new virulent race of *P. triticina* TDBG. RL6003 had previously been described as a single gene line developed by Dr. Peter Dyck, used in the Thatcher background differential wheat set (Table 4.1) to describe virulence patterns of *P. triticina* races. This gene was temporarily designated as *LrCen* as it was believed to have been introduced via the *Lr1* donor, Centanario (McCallum and Hiebert, 2012). While the newly identified seedling resistance gene did not appear to provide a broad spectrum resistance, preliminary phenotypic screening of a diverse set of germplasm suggested that *LrCen* may be widely distributed. Other known resistance genes were also described as providing resistance to race TDBG including *Lr16* and *Lr22*. *LrCen* was distinct from these genes based on studies with avirulent P*. triticina* races.

An F_2 population was developed for a cross between a selection of the RL6003 selection demonstrating resistance to TDBG (suggesting presence of *LrCen*) and susceptible cultivar Thatcher to isolate the novel source of TDBG resistance. Thatcher-*LrCen* was then crossed to universally susceptible cultivar Sumai3-*lr34,* F_1 plants were used to create a double haploid (DH) population (Hiebert unpublished). This study utilized the 90K wheat SNP array (Wang et al. 2014) to identify a putative mapping location for the unknown seedling gene by identifying linked markers through a two-point

linkage analysis with phenotypic data collected by inoculation with TDBG and creating a linkage map of the region. Phenotypic studies of the Tc-*LrCen*/Sumai3-*lr34* DH population confirmed the segregation of a single seedling resistance gene with a ratio of 103 resistant to 80 susceptible individuals. The 90K wheat SNP array is powerful genetic tool developed recently for use in studies of genome patterns and diversity, determination of ancestral relationships and for studies such as this, marker-trait association and mapping (Wang et al 2014). In the present study the SNP array was used to quickly and effectively identify polymorphic regions in the resistant and susceptible parental lines and subsequently correlate these regions with the phenotypic data observed in the DH mapping population. Recombination fractions were calculated and 133 SNPs demonstrated linkage to the trait of interest. To date, nearly 50% of the SNPs on the 90K array have been genetically mapped (Wang et al. 2014). This information was not available at the time of the study, so a combination of a genetic map from a biparental cross (Cabral et al. 2014) and BLAST searches of SNP sequences demonstrating putative linkage against the Wheat Survey Sequence provided a mapping location on the long arm of chromosome 7A. While the results of the consensus SNP genetic linkage map published by Wang et al. (2014, Table S13) were not available at the time, referencing them today confirms the results of the current study. Of the 133 SNP markers demonstrating linkage to *LrCen* resistance, 90% of them were mapped in the consensus map, of which 92% were mapped to chromosome 7A (the other 9 being mapped to 7B, consistent with BLAST results). Moreover, 72 SNP sequences were further localized to the long arm, while the other 38 were not mapped specifically to an arm of the chromosome. The 133 sequences of the linked SNPs were converted to the KASPar marker system (Teddington, Middlesex, UK; www.lgcgenomics.com) for further screening purposes.

Based on the presumed mapping location on chromosome 7AL a set of 30 co-dominant microsatellite markers (SSR), localized to 7AL based on microsatellite genetic maps (Roder et al. 1998, Guyomarc'h et al. 2002, Somers and Isaac 2004, Sourdille et al. 2004, Song et al. 2005), were used to

further characterize this region of the parental and DH mapping lines. Genetic linkage was established between TDBG phenotypic data and 12 SSR markers using MapDisto Version 1.7.5 software. A linkage map was created using both SNP and SSR scores generating a final linkage group spanning 64.5 cM. Markers flanking the genomic region carrying *LrCen* were identified as SSR *cfa2240* and a group of co-segregating SNPs located distally at a distance of 1.1 cM and SSR *gwm344* located proximally at a distance of 5.0 cM. The PCR reaction for microsatellite marker *cfa2240* presented allele products for each of the three homoeologous chromosomes in the hexaploid genome of common wheat, this is an added complexity that can be encountered in mapping studies of hexaploid species. To eliminate doubt on chromosomal location of linked allele sizes being attributed to chromosome 7A, cytogenetically produced nullisomic lines: nullisomic 7A tetrasomic 7B (N7AT7B), N7AT7D, N7BT7A, N7DT7B (Sears 1954, 1966), and Tc-*LrCen*, Sumai3-*lr34*, and Tc-*Lr20* were screened with linked markers. Allele sizes scored for presence/absence of *LrCen* for *cfa2240* (302bp/307bp, respectively) and *gwm344* (150bp/147bp, respectively), were absent in both nullisomic 7A lines and restored in nullisomic 7B and 7D lines. This confirmed that PCR products being scored were located on chromosome 7A and therefore confirmed the genetic location of *LrCen* on chromosome 7AL.

Tc-*Lr20*, the *Lr20* differential line (RL6092), was included in the aforementioned screening of *LrCen* linked SSR markers because it has previously been mapped to chromosome 7AL, aside from this, *Lr20* has not been well studied (Sears and Briggle 1969, Neu et al. 2002). Screening of eight plants from the differential line RL6092 with TDBG were all resistant supporting observations by Dakouri et al. (2013) of *Lr20* providing TDBG resistance, although this finding has not been confirmed. In a concurrent screening of a number of plants from RL6092 with *P. triticina* race PBDQ, believed to be virulent on *Lr20*, resistance was observed at a low frequency indicating a second unknown source of resistance segregating in the population. The similarities in resistance to race TDBG and mapping location of *Lr20 and LrCen* prompted studies to explore the relationship between the two seedling resistance genes.

PCR results for *cfa2240* of the *Lr20* differential line produced a new larger allele size (315bp) as compared to those observed in the resistant and susceptible parental lines and individuals in the DH population used to study *LrCen*. This allele size was similar to that observed in genotypes Percy and Stanley in a germplasm screening and suggested that *LrCen* and the source of TDBG resistance in RL6092, believed to be *Lr20*, were distinct.

To further explore this relationship an F_2 population from the cross Tc/RL6092-B was evaluated with leaf rust race TDBG and BBBD. Phenotyping was performed on two groups of 90 F_2 individuals, the first group were inoculated at the two leaf stage where one leaf was inoculated with BBBD and the other with TDBG, the second group was inoculated at the same stage, but using only race TDBG. Results of the phenotypic screening provided some conflicting results, but independent segregation of TDBG and BBBD resistance confirmed more than one Lr gene segregated in the population. The segregation ratio of individuals inoculated with only TDBG was 75 resistant to 8 susceptible and fit the 15:1 segregation ratio that would be expected for the presence of two dominant genes ($\chi^2 = 1.625$, $p = 0.202$). When phenotypic data for TDBG from first set of F_2 individuals was pooled with the second set the segregation became 149 resistant to 21 susceptible and no longer fit the 15:1 ratio ($\chi^2 = 10.81$, $p = 0.001$). The small population size increasing effects of environment on the unrepeated results of F_2 studies (Bjarko and Line 1988) and the difficulty of scoring intermediate infection types of heterozygotes could both be factors in the inconsistency observed in the two sets of phenotyping. Repeating a similar inoculation on a larger set of F_2 individuals could help to confirm the segregation ratio for TDBG resistance. DNA was extracted from these lines and a genetic analysis was performed with STS638, a marker described as highly diagnostic of the presence of *Lr20*, SSR marker *cfa2240,* and a set of co-segregating SNPs with demonstrated tight linkage to *LrCen*. Both STS638 and *cfa2240* demonstrated no significant linkage to the TDBG phenotypes; this suggested that neither *Lr20* nor *LrCen* were responsible for the observed TDBG resistance. The fact that neither marker demonstrated linkage to the resistant

phenotype was not surprising based on their close linkage on 7AL (markers had an RF value of 0.07); however, the absence of linkage to *Lr20* was surprising. This population should segregate for *Lr20* as one of the parental lines originates from the *Lr20* differential line. This result does align with the data reported by Dakouri et al. (2013).

Results from this study do not support observations by other researchers that *Lr20* provides resistance to race TDBG (Dakouri et al. 2013) and STS638 is effective for identification of lines carrying *Lr20* (Neu et al. 2002). The results of this study suggest one or more of the following: (i) *Lr20* is not present in the F_2 population and therefore not present in that accession of the differential line, (ii) TDBG is virulent on *Lr20*, and/or (iii) STS638 is not as tightly linked to *Lr20* as previously reported. While this study is unable to determine conclusively which, if any, of these conclusions contributed to the results observed, it is important to note that the infection types were inconsistent with the low infection types pictured by McIntosh et al. (1995). This suggests the RL6092-B NIL carries a gene other than *Lr20* and this could have led to wrongly classifying *Lr20* as resistant to TDBG. Additionally, linkage studies by Khan et al. (2005) suggested a recombination value of 7.1 cM between *Lr20* and STS638. This result would also support that STS638 is not entirely diagnostic of *Lr20* as previously described (Neu et al. 2002). Even if linkage of STS638 to *Lr20* was not as tight as previously described, there should still have been linkage detected if *Lr20* was involved in the resistance. This is further support that TDBG is virulent on *Lr20* if it is in fact present in the differential line RL6092. While not many conclusions can be made, the evidence supports further characterization and mapping of *Lr20* is required to better understand the relationship of *LrCen* and *Lr20*. Results also revealed that SNP markers converted to the KASPar system were dominant. This suggests that although the KASPar markers offer a quick and efficient method of screening known homozygous lines including DH populations, NILs, etc., linked co-dominant microsatellites are a more useful tool in molecular studies where heterozygotes may be present and need to be identified such as this F_2 population.

To further understand *Lr20,* stock lines used in validating STS638 were ordered from the Plant Gene Resources of Canada and phenotyped using TDBG along with RL6092, RL6092-B (accession of RL6092 known to carry 2 genes), RL6092-A (RL6092 believed to have had *Lr20* removed), Tc-*LrCen*, RL6003 (with *LrCen*), RL6003a (without *LrCen*), Thatcher, and Little Club. Resistance was observed in stock lines Axminster, Sappo and one accession of Norka, as well as heterogeneously in a second accession of Norka, RL6092, RL6092-B, Tc-*LrCen*, and RL6003. These results were as expected and support previous literature (Neu et al. 2002, Dakouri et al. 2013). The resistant reaction observed in the RL6092-A line support previous observations suggesting that *Lr20* is not the source of TDBG resistance being carried in the NIL. These lines were subsequently screened with STS638 where amplification of the expected 542bp fragment in RL6092, RL6092-B, Sappo, the resistant accession of Norka and single resistant plant in the heterogeneous Norka accession were all as expected. Unexpectedly, RL6092-A also amplified the 542bp fragment, this is further support of a greater distance between *Lr20* and STS638 than previously described and could be the result of a single recombination event as *Lr20* was once present in the genetic background of this line. Alternatively, this could suggest that *Lr20* is present and all races used were virulent. TDBG-susceptible Festival also amplified the 542bp fragment where TDBG-resistant accessions of Axminster did not. These contradictory results further question if TDBG is in fact avirulent on *Lr20*, and/or that once again the presence of the 542bp fragment does not predict the presence of *Lr20.* The lack of TDBG resistance and no amplification of the 542bp fragment for STS638 in the third accession of Norka indicate that Norka is a variable cultivar and may not be reliable for marker validation. Again, no definitive conclusions can be made, but results do support the observations made in the F_2 population study suggesting that TDBG is virulent on *Lr20*. While more questions than answers came from studying the relationship of *Lr20* and *LrCen*, preliminary results do suggest two distinct genes.

The identification of a novel, and previously undetectable source of resistance appearing in what was believed to be a single-gene NIL raised the question on the origination of the gene and the distribution of the gene within related germplasm. Because each of the wheat leaf rust differential lines were created in the same 'susceptible' Thatcher background, and this novel TDBG resistance was not observed in all differential lines, it was initially assumed that introduction of the seedling resistance was from the *Lr1* donor Centanario. Phenotyping a diverse set of germplasm with a number of *P. triticina* races identified a number of lines that appeared to carry *LrCen* based on susceptible reaction types for isolates that could identify the presence of other genes known to provide TDBG resistance and also raised questions as to whether the introduction was really from the Centanario background (McCallum 2005, Hiebert and McCallum 2012). A diverse selection of 185 genotypes were phenotyped with TDBG and revealed 112 resistant lines that were subsequently genotyped using *LrCen* linked markers *cfa2240* and *KWH41* used to perform an analysis on potential origin of *LrCen.* Results for both markers were consistent in identifying 35 lines positive for the allele size indicative of *LrCen,* this suggested that *LrCen* was distributed at a much lower frequency throughout the germplasm than previously thought. Tracing back the pedigrees of the new and old genotypes of the lines that appeared to carry *LrCen* revealed a common lineage suggesting introduction through Thatcher (McCallum and DePauw 2008, Genetic Resources Information System for Wheat and Triticale (GRIS): www.wheatpedigree.net [Accessed: January 2015], Biological and Biotechnology Sciences Research Council (BBSRC): https://www.jic.ac.uk/germplasm/docs/wheat%20pedigree%20data.xlsx [Accessed: January 2015], North Dakota State University (NDSU): Variety Development). While Thatcher is considered a susceptible line, it is old and many genetic stocks exist, this study would suggest that *LrCen* is heterogeneously distributed in Thatcher. This also explains why not all lines with Thatcher in the background, including the wheat leaf rust differential NILs, carry *LrCen*. It is likely that heterogeneous distribution of *LrCen* in one of the predominant genotypes contributing to the pedigree of Thatcher, Red

Fife or Marquis, are the origin of the introduction into Thatcher. While both genotypes are resistant to TDBG, they do not amplify the allele size representative of *LrCen*, the observed genetic distance of 1.1 cM between the resistant region and the molecular markers provide opportunity for a recombination event making genetic confirmation of the presence impossible in these lines. While phenotypic resistance is not an effective means of screening for the presence of *LrCen* as is demonstrated throughout the study of the distribution of *LrCen* in diverse germplasm, the appearance of resistance in both genotypes that appear in the pedigree of Thatcher suggests that testing other stocks of these genotypes may help in determining the origin of *LrCen* and if it can be traced back to one of these two major progenitor lines.

The results of this study confirm the importance of genetic confirmation and marker assisted selection for the presence of a gene of interest in a breeding program. While not 100% accurate for imperfect markers; genotyping provides a much higher level of resolution for selection of traits than depending solely on phenotypic data. The identification of a novel source of leaf rust resistance, *LrCen*, in a NIL differential line believed to carry a single source of resistance; and the subsequent tracing of its origin back to an old cultivar, Thatcher, used as a susceptible background in the creation of the wheat leaf rust differential set widely-applied in virulence surveys and race nomenclature is a reminder of the genetic potential offered in the vast hexaploid genome of wheat. The appearance of new virulence in the leaf rust pathogen unlocks new resistance potential within the existing wheat germplasm, even genotypes previously characterized as susceptible. This study also underlines the importance of a thorough genetic study to identify flanking markers for effective detection and integration of the resistance gene to avoid confusion that may arise through identification with phenotyping only. If breeding programs are able to map, characterize and exploit this genetic potential it provides hope for sustainable resistance and wheat production for the future.

CHAPTER 7

Literature Cited

Abdelbacki, A., Soliman, N., Najeeb, M., Omara, R. 2013. Postulation and identification of resistance genes against *Puccinia triticina* in new wheat cultivars in Egypt using molecular markers. International Journal of Chemical, Environmental & Biological Sciences 1(1): 104-109.

Allen, A.M., Barker, G.L., Berry, S.T., Coghill, J.A., Gwilliam, R., Kirby, S., Robinson, P., Brenchley, R.C., D'Amore, R., McKenzie, N., Waite, D., Hall, A., Bevan, M., Hall, N., Edwards, K.J. 2011. Transcript-specific, single-nucleotide polymorphism discovery and linkage analysis in hexaploid bread wheat (*Triticum aestivum* L.). Plant Biotechnology Journal 9: 1086-1099.

Allen, W.R. 1955. The wheat midge, *Sitodiplosis mosellana* (Gehin). Annual Conference of Manitoba Agronomists, pp 28-29.

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

Azzimonti, G.L., Sache, I., Goyeau, H. 2013. Components of quantitative resistance to leaf rust in wheat cultivars: diversity, variability and specificity. Plant Pathol. DOI: 10.1111/ppa.12029.

Balloux, F., Lehmann, L., de Meetus, T. 2003. The population genetics of clonal and partially clonal diploids. Genetics 164: 1635-1644.

Barrett, B.A., Kidwell, K.K. 1998. AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci. 38: 1261-1271.

Barrett, B.A., Kidwell, K.K., Fox, P.N. 1998. Comparison of AFLP and pedigree-based genetic diversity assessment methods using wheat cultivars from the Pacific Northwest. Crop Sci. 38: 1271-1278.

Bernardo, R. 2003. Parental selection, number of breeding populations, and size of each population in inbred development. Theor. Appl. Genet. 107: 1252-1256.

Bjarko, M.E., Line, R.F. 1988. Heritability and number of genes controlling leaf rust resistance in four cultivars of wheat. Phytopathology 78: 457-461.

Bolton, M.D., Kolmer, J.A., Garvin, D.F. 2008. Wheat leaf rust caused by *Puccinia triticina*. Mol. Plant Pathol. 9: 563-575.

Boquet, D.J., Johnson, C.C. 1987. Fertilizer effects on yield, grain composition, and foliar disease of doublecrop soft red winter wheat. Agron. J. 79: 135-141.

Bossolini, E., Krattinger, S.G., Keller, B. 2006. Development of simple sequence repeat markers specific for the *Lr34* resistance region of wheat using sequence information from rice and *Aegilops tauschii*. Theor. Appl. Genet. 113: 1049-1062.

Botstein, D., White, R.L., Skolnick, M., Davis, R.W. 1980. Construction of a genetic linkage map in using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 314-331.

Brennan, R.F. 1989. Effects of superphosphate plus flutriafol on yield and take-all of wheat. Aust. J. Exp. Agric. 29: 247-252.

Brennan, R.F., 1995. Effects of levels of take-all and phosphorus fertilizer on the dry matter and grain yield of wheat. J. Plant Nutr. 18: 1159-1176.

Browder, L.E. 1980. A compendium of information about named genes for low reaction to *Puccinia recondite* in wheat. Crop Sci. 20: 775-779.

Burdon, J.J., Chilvers, G.A. 1982. Host density as a factor in plant disease ecology. Annual Review of Phytopathology 20: 143-166.

Cabral, A.L., Jordan, M.C., McCartney, C.A., You, F.M., Humphreys, D.G., MacLachlan, R., Pozniak, C.J., 2014. Identification of candidate genes, regions and markers for pre-harvest sprouting resistance in wheat (*Triticum aestivum* L.). BMC Plant Biol. 14: 340.

Campbell, A.B. 1963. Registration of Pembina and Canthatch wheats. Crop Sci. 3: 457-458.

Campbell, A.B., Shebeski, L. 1986. Wheat in Canada – past and present. In: Slinkard, A.E., Fowler D.B. (eds.) Canadian Wheat Production Symposium, Saskatchewan, Canada, pp 1-14.

Chen, X., Sullivan, P.F. 2003. Single nucleotide polymorphism genotyping: biochemistry, protocol, cost and throughput. Pharmacogenomics J. 3: 77-96.

Chhuneja, P., Kaur, S., Goel, R.K., Aghaee-Sarbarzeh, M., Dhaliwal, H.S. 2007. Introgression of leaf rust and stripe rust resistance genes from *Aegilops umbellulata* to hexaploid wheat through induced homoeologous chromosome pairing. In: Buck, H.T., Nisi, J.E., Salomon, N. (eds.) Wheat Production in Stressed Environments, Springer, Netherlands, pp 83-90.

Chu, C.G., Friesen, T.L., Xu, S.S., Faris, J.D., Kolmer, J.A. 2009. Identification of novel QTLs for seedling and adult plant leaf rust resistance in wheat doubled haploid population. Theor. Appl. Genet. 119: 263-269.

Cloutier, S., McCallum, B.D., Loutre, C., Banks, T.W., Wicker, T., Feuillet, C., Keller, B., Jordan, M.C. 2007. Leaf rust resistance gene *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large psr567 gene family. Plant Molecular Biology 65: 93-106.

Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B., Pang, E.C.K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142: 169-196.

Cox, T.S., Raupp, W.J., Gill, B.S. 1994. Leaf rust resistance genes *Lr41, Lr42,* and *Lr43* transferred from *Triticum tauschii* to common wheat. Crop Sci. 34(2): 339-343.

Dakouri, A., McCallum, B.D., Walichnowski, A.Z., Cloutier, S. 2010. Fine-mapping of the leaf rust *Lr34* locus in *Triticum aestivum* (L.) and characterization of large germplasm collections support the ABC transporter as essential for gene function. Theor. Appl. Genet. 121(2): 373-384.

Dakouri, A., McCallum, B.D., Radovanovic, N., Cloutier, S. 2013. Molecular and phenotypic characterization of seedling and adult plant leaf rust resistance in a world wheat collection. Molecular Breed. 32(3): 663-677.

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

de Vicente, M.C., Fulton, T. 2003. Using molecular technology in studies on plant genetic diversity. Illus. Nelly Giraldo. IPGRI, Rome, Italy and Institute for Genetic Diversity, Ithaca, New York, USA.

Dexter, J.E., Preston, K.R., Woodbeck, N.J. 2006. Chapter 6: Future of flour a compendium of flour improvement. In: Popper, L., Schafer, W., Freund, W. (eds.) Canadian Wheat, Agrimedia. Bergen, Dunne, Germany, pp 43-62.

Doane, J.F., Olfert, O., Mukerji, M.K. 1987. Extraction precision of sieving and brine flotation for removal of wheat midge, *Sitodiplosis mosellana* (Diptera: Cecidomyiidae), cocoons and larvae from soil. J. Econ. Entomol. 80: 268-271.

Doggett, N.A. 1992. The polymerase chain reaction and sequence-tagged sites. Los Alamos Sci. 20: 128-134.

Dubcovsky, J. 2004. Marker-assisted selection in public breeding programs: the wheat experience. Crop Sci. 44: 1895-1898.

Dubcovsky, J., Dvorak, J. 2007. Genome plasticity a key factor in the success of polyploid wheat under domestication. Science 316: 1862-1866.

Duveiller, E., Singh, R.P., Nicol, J.M. 2007. The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. Euphytica 157: 417-430.

Dvorak, J., di Terlizzi, P., Zhang, H.B, Resta, P. 1993. The evolution of polyploidy wheats: identification of the A genome donor species. Genome 36: 21-31.

Dvorak, J., Luo, M.C., Yang, Z.L., Zhang, H.B. 1998. The structure of *Aegilops tauschii* genepool and the evolution of hexaploid wheat. Theor. Appl. Genet. 97: 657-670.

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

Dyck, P.L., Samborski, D.J. 1968. Genetics of resistance to leaf rust in the common wheat varieties Webster, Loros, Brevit, Carina, Malakof and Centenario. Can. J. Genet. Cytol. 10: 7-17.

Dyck, P.L., Johnson, R. 1983. Temperature sensitivity of genes for resistance in wheat to *Puccinia recondita.* Can. J. Plant Pathol. 5: 229-234.

Dyck, P.L., Samborski, D.J. 1974. Inheritance of virulence in *Puccinia recondita* on alleles at the *Lr2* locus for resistance in wheat. Can. J. Genet. Cytol. 16: 323-332.

Dyck, P.L., Samborski, D.J., Martens, J.W. 1985. Inheritance of resistance to leaf rust and stem rust in the wheat cultivar Glenlea. Can. J. Plant Pathol. 7: 351-354.

Dyck, P.L. 1991. Genetics of adult-plant leaf rust resistance in 'Chinese Spring' and 'Sturdy' wheat. Crop Sci. 31: 309-311.

Elliot, B. 1988. Evaluation of insecticides for protection of wheat against damage by wheat midge, *Sitodiplosis mosellana* (Gehin) (Diptera: Cecidomyiidae). Can. Entomol. 120: 615-626.

Elliot, B., Olfert, O., Hartley, S. 2011. Management practices for wheat midge, *Sitodiplosis mosellana* (Gehin). Prairie Soils & Crop Journal 4: 8-13.

FAO Statistical Yearbook 2013. http://www.fao.org/docrep/018/i3107e/i3107e00.htm Accessed: May 2015.

Fetch, T.G. 2005. Races of *Puccinia graminis* on wheat, barley, and oat in Canada in 2002 and 2003. Canadian Journal of Plant Pathology 27: 572-580.

Flor, H.H. 1971. Current status of the gene-for-gene concept. Annual Review of Phytopathology 9: 275-296.

Frisch, M., Bohn, M., Melchinger, A. 1999. Comparison of selection strategies for marker-assisted backcrossing of a gene. Crop Sci. 39: 1295-1301.

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

Gill, B.S., Kimber, G. 1974. Giemsa C-banding and the evolution of wheat. Proc. Natl. Acad. Sci. USA 71: 4086-4090.

Gill, B.S., Raupp, W.J., Sharma, H.C., Browder, L.E., Hatchett, J.H., Harvey, T.L., Moseman, J.G., Waines, J.G. 1986. Resistance in *Aegilops squarrosa* to wheat leaf rust, powdery mildew, greenbug, and Hessian flu. Plant Dis. 70: 553-556.

Goodwin, S.B., Hu, X., Shaner, G.E. 1998. An AFLP marker linked to a gene for resistance to *Septoria tritici* blotch in wheat. In: Slinkard, A.E. (ed.) 9th Int. Wheat Genet. Symp. University Extension Press, Univ. of Saskatchewan, Saskatoon, pp 108-110.

Goyeau, H., Park, R.F., Schaeffer, B., Lannou, C. 2006. Distribution of pathotypes with regard to host cultivars in French wheat leaf rust populations. Phytopathology 96: 264-273.

Grover, A., Sharma, P.C. 2016. Development and use of molecular markers: past and present. Critical Reviews in Biotechnology, 36(2): 290-302.

Gupta, K., Balyan, S., Edwards, J., Isaac, P., Korzun, V., Roder, M., Gautier, M.F., Jourdier, P., Schlatter, R., Dubcovsky, J., De La Pena, C., Khairallah, M., Penner, G., Hayden, J., Sharp, P., Keller, B., Wang, C., Hardouin, P., Jack, P., Leroy, P. 2002. Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor. Appl. Genet. 105: 413-422.

Gupta, P.K., Roy, J.K., Prasad, M. 1999a. DNA chips, microarrays and genomics. Curr. Sci. 77: 875-884.

Gupta, P.K., Varshney, R.K. 2000. The development and use of microsatellite markers for genetics analysis and plant breeding with emphasis on bread wheat. Euphytica 113: 163-185.

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

Hartl, L., Mori, S., Schweizer, G. 1998. Identification of a diagnostic molecular marker for the powdery mildew resistance gene *Pm4b* based on fluorescently labelled AFLPs. In: Slinkard, A.E. (ed.) 9th Int. Wheat Genet. Symp. University Extension Press, Univ. of Saskatchewan, Saskatoon, pp 111-113.

Hayes, H.K., Ausemus, E.R., Stakman, E.C., Bailey, C.H., Wilson, H.K., Bamberg, R.H., Markley, M.C., Crim, R.F., Levine, M.N. 1936. Thatcher Wheat. Agricultural Experiment Bulletin. University of Minnesota.

Herrera-Foessel, S.A., Singh, R.P., Huerta-Espino, J., William, H.M., Garcia, V., Djurle, A., Yuen, J. 2008. Identification and molecular characterization of leaf rust resistance gene *Lr14a* in durum wheat. Plant Dis. 92: 469-473.

Herrera-Foessel, S.A., Lagudah, E.S., Huerta-Espino, J., Hayden, M.J., Bariana, H.S., Singh, D., Singh, R.P. 2011. New slow-rusting leaf rust and stripe rust resistance genes *Lr67* and *Yr46* in wheat are pleiotropic or closely linked. Theor. Appl. Genet. 122: 239-249.

Herrera-Foessel, S.A., Singh, R.P., Huerta-Espino, J., Rosewarne, G.M., Periyannan, S.K., Viccars, L., Calvo-Salazar, V., Lan, C., Lagudah, E.S. 2012. *Lr68*: A new gene conferring slow rusting resistance to leaf rust in wheat. Theor. Appl. Genet. 124: 1475-1486.

Hiebert, C.W., Thomas, J.B., Somers, D.J., McCallum, B.D., Fox, S.L. 2007. Microsatellite mapping of adult-plant leaf rust resistance gene *Lr22a* in wheat. Theor. Appl. Genet. 115: 877-884.

Hiebert, C.W., Thomas, J.B., McCallum, B.D., Humphreys, D.G., DePauw, R.M., Hayden, M.J., Mago, R., Schnippenkoetter, W., Spielmeyer, W. 2010. An introgression on wheat chromosome 4DL in RL6077 (Thatcher*6/PI 250413) confers adult plant resistance to stripe rust and leaf rust (*Lr67*). Theor. Appl. Genet. 121: 1083-1091.

Hu, X.Y., Ohm, H.W., Dweikat, I. 1997. Identification of RAPD markers linked to the gene *Pm1* for resistance to powdery mildew in wheat. Theor. Appl. Genet. 94: 832-840.

Huang, N., Angeles, E.R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadivel, N., Bennett, J., Khush, G.S. 1997. Pyramiding of bacterial blight resistance genes in rice: Marker-assisted selection using RFLP and PCR. Theor. Appl. Genet. 95: 313-320.

Huang, L., Gill, B.S. 2001. An RGA-like marker detects all known *Lr21* leaf rust resistance gene family members in *Aegilops tauschii* and wheat. Theor. Appl. Genet. 103(6-7): 1007-1013.

Jantasuriyarat, C., Vales, M.I., Watson, C.J.W., Riera-Lizarau, O. 2004. Identification and mapping of genetic loci affecting the free-threshing habit and spike compactness in wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 108: 261-273.

Jin, Y., Szabo, L.J., Pretorius, Z.A. 2008. Virulence variation within the Ug99 lineage. In: Appels, R., Eastwood, R., Lagudah, E.S., Langridge, P., Mackay, M., McIntyre, L., Sharp, P. (eds.) 11th International Wheat Genetics Symposium. Sydney University Press, Brisbane, Australia.

Kerber, E.R., Dyck P.L. 1969. Inheritance in hexaploid wheat of leaf rust resistance and other charaters derived from *Aegilops squarrosa*. Canadian Journal of Genetics and Cytology 11: 639-647.

Kerber, E.R. 1987. Resistance leaf rust in hexaploid wheat: *Lr32*, a third gene derived from *Triticum tauschii*. Crop Sci. 27: 204-206.

Kerby, K., Kuspira, J. 1987. The phylogeny of the polyploid wheats *Triticum aestivum* (bread wheat) and *Triticum turgidum* (macaroni wheat). Genome 29: 722-737.

Khan, R.R., Bariana, H.S., Dholakia, B.B., Naik, S.V., Lagu, M.D., Rathjen, A.J., Bhavani, S., Gupta, V.S. 2005. Molecular mapping of stem and leaf rust resistance in wheat. Theor. Appl. Genet. 111: 846-850.

Kimber, G., Riley, R. 1963. The relationships of the diploid progenitors of hexaploid wheat. Can. J. Genet. Cytol. 5: 83-88.

Kolmer, J.A. 1992. Virulence heterozygocity and gemetic phase disequilibria in two populations of *Puccinia recondita* (wheat leaf rust fungus). Heredity 68: 505-513.

Kolmer, J.A. 1996. Genetics of resistance to wheat leaf rust. Annual Review of Phytopathology 34: 435-455.

Kolmer, J.A. 2013. Leaf rust of wheat: Pathogen biology, variation and host resistance. Forests 4: 70-84.

Kolmer, J.A., Long, D.L., Hughes, M.E. 2009. Physiologic specialization of *Puccinia triticina* on wheat in the United States in 2007. Plant Dis. 79: 525-529.

Kolmer, J.A., Singh, R.P., Garvin, D.F., Viccars, L., William, H.M., Huerta-Espino, J., Ogbonnaya, F.C., Raman, H., Orford, S., Bariana, H.S., Lagudah, E.S. 2008. Analysis of the *Lr34/Yr18* rust resistance region in wheat germplasm. Crop Sci. 48: 1841-1852.

Konarev, V.G. 1983. The nature and origin of wheat genomes on the data of grain protein immunochemistry and electrophoresis. In: Sakamoto, S. (ed.) 6th International Wheat Genetics Symposium. Plant Germ-Plasm Institute, Faculty of Agriculture, Kyoto University, Kyoto, Japan, pp 65-75.

Lagudah, E.S. 2011. Molecular genetics of race non-specific rust resistance in wheat. Euphytica 179: 81-91.

Lagudah, E.S., Krattinger, S.G., Herrera-Foessel, S., Singh, R.P., Huerta-Espino, J., Spielmeyer, W., Brown-Guedira, G., Selter, L.L., Keller, B. 2009. Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. Theor. Appl. Genet. 119: 889-898.

Leonard, K.J., Szabo, L.S. 2005. Stem rust of small grains and grasses caused by *Puccinia graminis*. Mol. Plant. Pathol. 6: 99-111.

Levine, M., Hildreth, R.C. 1957. A natural occurrence of the aecial stage of *Puccinia rubigo-vera* var. *tritici* in the United States. Phytopathology 47: 110-111.

Lillemo, M., Asalf, B., Singh, R.P., Huerta-Espino, J., Chen, X., He, Z., Bjornstad, A. 2008. The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor. Appl. Genet. 116: 1155-1166.

Liu, W., Zheng, M.Y., Polle, E.A., Konzak, K.F. 2002. Highly efficient doubled-haploid production in wheat (*Triticum aestivum* L.) via induced microspore embryogenesis. Crop Sci. 42: 686-692.

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

Lorieux, M., 2012. MapDisto: fast and efficient computation of genetic linkage maps. Mol. Breed. 30: 1231-1235.

Low, Y.L., Wedren, S., Liu, J. 2006. High-throughput genomic technology in research and clinical management of breast cancer. Evolving landscape of genetic epidemiological studies. Breast Cancer Res. 8(3): 209.

Lynch, J.P. 2007. Turner review No. 14: roots of the second green revolution. Aus. J. Bot. 55: 493-512.

Mansur, L.M., Orf, J.H., Chase, K., Jarvik, T., Cregan, P.B., Lark, K.G. 1996. Genetic mapping of agronomic traits using recombinant inbred lines of soybean. Crop Sci. 36: 1327-1336.

Martens, J.W., Dyck, P.L. 1989. Genetics of resistance to rust in cereals from a Canadian perspective. Can. J. Plant Pathol. 11: 78-85.

McCallum, B.D., Seto-Goh, P. 2003. Physiologic specialization of wheat leaf rust (*Puccinia triticina*) in Canada in 2000. Can. J. Plant Pathol. 25: 91-97.

McCallum, B.D., Cloutier, S., Rampitsch, C., Bykova, N. 2005. Evidence for a second seedling resistance gene to leaf rust in the "Thatcher"-*Lr1* near-isogenic line RL6003. Can. J. Plant Pathol. 27: 472-473.

McCallum, B.D., Fetch, T., Chong, J. 2007. Cereal rust control in Canada. Aus. J. Agric. Res. 58: 639-647.

McCallum, B.D., DePauw, R.M. 2008. A review of wheat cultivars grown in the Canadian prairies. Can. J. Plant Sci. 88: 649-677.

McCallum, B.D., Somers, D.J., Humphreys, D.G, Cloutier, S. 2008. Molecular marker analysis of *Lr34* in Canada Western Red Spring wheat cultivars. In: Appels, R., Eastwood, R., Lagudah, E.S., Langridge, P., Mackay, M., McIntyre, L., Sharp, P. (eds.) 11th International Wheat Genetics Symposium, Brisbane, Australia.

McCallum, B.D., Seto-Goh, P., Xue, A. 2010. Physiological specialization of *Puccinia triticina* in Canada in 2007. Canadian Journal of Plant Pathology 32: 229-236.

McCallum, B.D., Humphreys, D.G., Somers, D.J., Dakouri, A., Cloutier, S. 2011. Allelic variation for the rust resistance gene *Lr34/Yr18* in Canadian wheat cultivars. Euphytica 183: 261-274.

McCallum, B.D., Thomas, J. 2011. Effectiveness of wheat leaf rust gene combinations derived from the cultivar Pasqua. XIX Plant and Animal Genome Conference, San Diego, CA, USA, January 15-19 2011.

McCallum, B.D., Hiebert, C.W. 2012. Characterization of wheat leaf rust resistance gene *LrCen*. XX Plant and Animal Genome Conference, San Diego, CA, USA, January 14-18 2012. (Poster)

McCallum, B.D., Hiebert, C., Huerta-Espino, J., Cloutier, S. 2012. Wheat leaf rust. In: Sharma, I. (ed) Disease Resistance in Wheat. Punjab Agricultural University, India, pp 33-62.

McCartney, C.A., Somers, D.J., McCallum, B.D., Thomas, J.B., Humphreys, D.G., Menzies, J.G., Brown, P.D. 2005. Microsatellite tagging of the leaf rust resistance gene *Lr16* on wheat chromosome 2BSc. Mol. Breed. 15: 329-337.

McIntosh, R.A. 1977. Nature of induced mutations affecting disease reaction in wheat. International Atomic Energy Agency, Vienna, Austria.

McIntosh, R.A., Wellings, C.R., Park, R.F. 1995. Wheat rusts: an atlas of resistance genes. CSIRO publishing, Australia.

McIntosh, R.A., Dubcovsky, J., Rogers, W.J., Morris, C.F., Appels, R., Xia, X. 2010. Catalogue of gene symbols for wheat: 2010 supplement.

McIntosh, R.A., Yamakazi, Y., Dubcovsky, J., Rogers, J., Morris, C., Somers, D.J., Appels, R., Devos, K.M. 2012. Catalogue of gene symbols for wheat. http://www.shigen.nig.ac.jp/wheat/komugi/genes/download.jsp Accessed: May 2015.

McKenzie, R.I.H., Lamb, R.J., Aung, T., Wise, I.L., Barker, P., Olfert, O. 2002. Inheritance of resistance to wheat midge, *Sitodiplosis mosellana*, in spring wheat. Plant Breeding 121: 383-388.

Moose, S.P., Mumm, R.H. 2008. Molecular plant breeding as the foundation for 21st century crop improvement. Plant Physiology 147: 969-977.

Morrison, J.W. 1960. Marquis wheat – a triumph of scientific endeavour. Agric. Hist. 34: 182-188.

Mukerji, M.K., Olfert, O., Doane, J.F. 1988. Development of sampling designs for egg and larval populations of the wheat midge, *Sitodiplosis mosellana* (Gehin) (Diptera: Cecidomyiidae), in wheat. Can. Entomol. 120: 497-505.

Nakagahra, M. 1972. Genetic mechanism on the distorted segregation of marker gene belonging to the eleventh linkage group in cultivated rice. Japanese J. of Breeding 22: 232-238.

Neelam, K., Brown-Guedira, G., Huang, L. 2012. Development and validation of a breeder-friendly KASPar marker for wheat leaf rust resistance locus *Lr21*. Molecular Breeding 31: 233-237.

Neu, C., Stein, N., Keller, B. 2002. Genetic mapping of the *Lr20-Pm1* resistance locus reveals suppressed recombination on chromosome arm 7AL in hexaploid wheat. Genome 45: 737-744.

Oelke, L.M., Kolmer, J.A. 2005. Genetics of leaf rust resistance in spring wheat cultivars Alsen and Norm. Phytopathology 95: 773-778.

Oerke, E.C., Dehne, H.W., Schonbeck, F., Weber, A. 1994. Crop production and crop protection: estimated losses in major food and cash crops. Elsevier, Amsterdam.

Olfert, O., Elliot, B., Hartley, S. 2009. Non-native insects in agriculture: strategies to manage the economic and environmental impact of wheat midge, *Sitodiplosis mosellana*, in Saskatchewan. Biol. Invasions 11: 127-133.

Ordonez, M.E., German, S.E., Kolmer, J.A. 2010. Genetic differentiation within the *Puccinia triticina* population in South America and comparison with the North American population suggests common ancestry and intercontinental migration. Phytopathology 100: 376-383.

Ordonez, M.E., Kolmer, J.A. 2007. Simple sequence repeat diversity of a worldwide collection of *Puccinia triticina* from durum wheat. Phytopathology 97: 574-583.

Pallotta, M.A., Warner, P., Fox, R.L., Kuchel, H., Jefferies, S.J., Langridge, P. 2003. Marker-assisted wheat breeding in the southern region of Australia. In: Pogna, N.E., Romano, M., Pogna, E.A., Galerio, G. (eds.) 10th International Wheat Genetics Symposium, Institutio Sperimentale per la Cerealcoltura, Rome, pp 789-791.

Parlevliet, J.E. 2002. Durability of resistance against fungal, bacterial and viral pathogens: present situation. Euphytica 124: 147-156.

Penner, G., Zirino, M., Kruger, S., Townley-Smith, F. 1998. Accelerated recurrent parent selection in wheat with microsatellite markers. In: Slinkard, A.E. (ed.) 9th Int Wheat Genet. Symp. University Extension Press, Univ. of Saskatchewan, Saskatoon, pp 131-134.

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

Plunknett, D.L., Smith, N.J., Williams, J.T., Anishetty, N.M. 1987. Gene banks and the world's food. Princeton Univ. Press, Princeton, NJ.

Poczai, P., Varga, I., Laos, M., Cseh, A., Bell, N., Valkonen, J.P.T., Hyvonen, J. 2013. Advances in plant gene-targeted and functional markers: a review. Plant Methods 9(6): 1-32.

Poehlman, J.M. 1987. Breeding field crops. Van Nostrand Reinhold Company, New York, USA.

Pretorius, Z.A., Kloppers, F.J., Drijepondt, S.C. 1994. Effect of inoculum density and temperature on three components of leaf rust resistance controlled by *Lr34* in wheat. Euphytica 74: 91-96.

Pretorius, Z.A., Singh, R.P., Wagoire, W.W., Payne, T.S. 2000. Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f. sp. *tritici* in Uganda. Plant Dis. 84: 203.

Ray, D.K., Mueller, N.D., West, P.C., Foley, J.A. 2013. Yield Trends are Insufficient to Double Global Crop Production by 2050. PLoS ONE 8(6): e66428.

Roder, M., Korzun, V., Gill, B.S., Ganal, M.W. 1998a. The physical mapping of microsatellite markers in wheat. Genome 41: 278-283.

Roder, M., Korzun, V., Wendehake, K., Plaschke, J., Tixer, M.H., Leroy, P., Ganal, M.W. 1998b. A microsatellite map of wheat. Genetics 149: 2007-2023.

Roelfs, A.P. 1988. Resistance to leaf and stem rusts in wheat. In: Simmonds, N.W., Rajaram, S. (eds.) Breeding Strategies for Resistance to the Rusts of Wheat. CIMMYT, Mexico, pp. 10-22.

Roelfs, A.P., Singh, R.P., Saari, E.E. 1992. Rust diseases of wheat. In: Hettel, G.P. (ed.) Concepts and Methods of Disease Management. CIMMYT, Mexico.

Rosewarne, G.M., Singh, R.P., Huerta-Espino, J., William, H.M., Bouchet, S., Cloutier, S., McFadden, H., Lagudah, E.S. 2006. Leaf tip necrosis, molecular markers and beta1-proteasome subunits associated with the slow rusting resistance genes *Lr46/Yr29*. Theor. Appl. Genet. 112: 500-508.

Rowland, G.G., Kerber, E.R. 1974. Telocentric mapping in hexaploid wheat of genes for leaf rust resistance and other characters derived from *Aegilops squarrosa*. Canadian Journal of Genetics and Cytology. 16: 137-144.

Saari, E.E., Prescott, J.M. 1985. World distribution in relation to economic losses. In: Roelfs, A.P., Bushnell, W.R. (eds.) The cereal rusts: diseases, distribution, epidemiology, and control. Academic Press, Orlando, Florida, pp 259-298.

Samborski, D.J. 1963. A mutation in *Puccinia recondita* Rob. ex. desm. f. sp. *tritici* to virulence on Transfer, Chinese Spring X *Aegilops umbellulata* Zhuk. Can. J. Bot. 41. 475-479.

Samborski, D.J. 1985. Wheat leaf rust. The Cereal Rusts 2: 39-59.

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

Samborski, D.J., 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., Messmer, M., Feuillet, C., Winzeler, M., Keller, B. 1995. Identification of molecular markers linked to the *Agropyron elongatum*-derived leaf rust resistance gene *Lr24* in wheat. Theor. Appl. Genet. 90: 982-990.

Schachermayr, G., Siedler, H., Gale, M.D., Winzeler, H., 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.

Sears, E.R. 1954. The aneuploids of common wheat. Mo. Agric. Exp. Stn. Res. Bull. 527: 58.

Sears, E.R. 1956. The transfer of leaf-rust resistance from *Aegilops umbellulata* to wheat. In: Genetics in Plant Breeding, Book-haven Symposia in Biology 1956, pp 1-22.

Sears, E.R. 1961. Identification of the wheat chromosome carrying leaf rust resistance from *Aegilops umbellulata.* Wheat Information Service 12: 12-13.

Sears, E.R. 1966. Nullisomic-tetrasomic combinations in hexaploid wheat. In: Lewis, D.R. (ed.) Chromosome manipulation and plant genetics, Oliver and Boyd, London, England, pp 29-49.

Sears, E.R. 1977. Crop resources. In: Seigler, D.S. (ed.) The origin and future of wheat. Academic Press Inc., New York, USA, pp 193-196.

Sears, E.R., Briggle, L.W. 1969. Mapping the gene *Pm1* for resistance to *Erysiphe graminis* f. sp. *tritici* on chromosome 7A of wheat. Crop Sci. 9: 96-97.

Semagn, K., Babu, R., Hearne, S., Olsen, M. 2013. Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. Molecular Breeding 33: 1-14.

Sharma, H.C., Ohm, H.W., Goulart, L., Lister, R. Appels, R., Benlhabib, O. 1995. Introgression and characterization of barley yellow dwarf virus resistance from *Thinopyrum intermedium* into wheat. Genome 38: 406-413.

Singh, A., Pallavi, J.K., Gupta, P., Prabhu, K.V. 2011. Identification of microsatellite markers linked to leaf rust adult plant resistance (APR) gene *Lr48* in wheat. Plant Breeding 130: 31-34.

Singh, R.P. 1991. Pathogenicity variations of *Puccinia recondita* f. sp. *tritici* and *P. graminis* in wheat growing areas of Mexico during 1998 and 1999. Plant Pathol. 39: 424-433.

Singh, R.P. 1992. 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., McIntosh, R.A. 1984a. Complementary genes for reaction to *Puccinia recondita* in *Triticum aestivum.* I. Genetic linkage studies. Can. J. Genet. Cytol. 26: 723-735.

Singh, R.P., McIntosh, R.A. 1984b. Complementary genes for reaction to *Puccinia recondita* in *Triticum aestivum.* II. Cytogenetic studies. Can. J. Genet. Cytol. 26: 736-742.

Singh, R.P., Mujeeb-Kazi, A., Huerta-Espino, J. 1998. *Lr46*: a gene conferring slow-rusting resistance to leaf rust in wheat. Phytopathology 88: 890-894.

Smith, J.S., Hussain, T., Jones, E.S., Graham, G., Podlich, D., Wall, S., Williams, S. 2008. Use of double haploids in maize breeding: implications for intellectual property protection and genetic diversity in hybrid crops. Mol. Breed. 22: 51-59.

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

Song, Q.J., Shi, J.R., Singh, S., Fickus, E.W., Costa, J.M., Lewis, J., Gill, B.S., Ward, R., Cregan, P.B. 2005. Development and mapping of microsatellite (SSR) markers in wheat. Theor. Appl. Genet. 110: 550-560.

Sourdille, P., Singh, S., Cadalen, T., Brown-Guedira, G.L., Gay, G., Qi, L., Gill, B.S., Dufour, P., Murigneux, A., 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(1): 12-25.

Statler, G.D. 1985. Mutations affecting virulence in *Puccinia recondita.* Phytopathology 75: 565-567.

Statler, G.D. 1987. Mutation studies with race 1, *Puccinia recondita*. Can. J. Plant Pathol. 9: 200-204.

Stebbins, G.L. 1950. Variation and evolution in plants. Columbia University Press, New York, USA.

Stuckey, R.E., Zadoks, J.C. 1989. Effect of interrupted leaf wetness periods on pustule development of Puccinia recondita f. sp. tritici on wheat. Netherlands Journal of Plant Pathology 9(1): 175-185.

Stuthman, D.D., Leonard, K.J., Miller-Garvin, J., Donald, L.S. 2007. Breeding crops for durable resistance to disease. In: Sparks, D. (ed.) Advances in Agronomy. Academic Press, London, UK.

Suenaga, K., Singh, R.P., Huerta-Espino, J., William, H.M. 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.

Sweeney, D.W., Granade, G.V., Eversmeyer, M.G., Whitney, D.A. 2000. Phosphorus, potassium, chloride, and fungicide effects on wheat yield and leaf rust severity. Journal of Plant Nutrition 23: 1267-1281.

Tan Y.D., Fu Y.X. 2007. A new strategy for estimating recombination fractions between dominant markers from an F_2 population. Genetics 175(2): 923-931.

Talbert, L.E., Blake, N.K., Storlie, E.W., Lavin, M. 1995. Variability in wheat based on low-copy DNA sequence comparisons. Genome 38: 951-957.

Talbert, L.E., Smith, L.Y., Blake, N.K. 1998. More than one origin of hexaploid wheat is indicated by sequence comparison of low-copy DNA. Genome 41: 402-407.

Tanksley, S.D. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277: 1063-1066.

Tanksley, S.D., Young, N.D., Paterson, A.H., Bonierbale, M.W. 1989. RFLP mapping in plant breeding: new tools for an old science. Biotechnology 7: 257-264.

Tester, M., Bacic, A. 2005. Abiotic stress tolerance in grasses: from model plants to crop plants. Plant Physiology 137: 791-793.

Thomas, J.B., Chen, Q., Howes, N. 1997. Chromosome doubling of haploids of common wheat with caffeine. Genome 40: 552-558.

Thomas, J.B., Graf, R.J., 2014. Rates of yield gain of hard red spring wheat in western Canada. Can. J. Plant Sci. 94: 1-13.

Thomas J.B., Nilmalgoda, S., Hiebert, C., McCallum, B.D., Humphreys, D.G., DePauw, R.M. 2010. Genetic markers and leaf rust resistance of the wheat gene *Lr32*. Crop Sci. 50: 2310-2317.

University of Sydney: Plant Pathology. 2003. Accessed: Aug 2015
http://bugs.bio.usyd.edu.au/learning/resources/PlantPathology/infection/disease_devpmt.html

USDA: Barberry Situation – Past, Present, Future. 2005. Accessed: May 2015
http://www.ars.usda.gov/Main/docs.htm?docid=9749

USDA: Ug99 – emerging virulent stem rust races. 2013. Accessed: May 2015
http://www.ars.usda.gov/Main/docs.htm?docid=14649

Van Beuningen, L.T., Busch, R.H. 1997. Genetic diversity among North American spring wheat cultivars: I. Analysis of the coefficients of parentage matrix. Crop Sci. 37: 570-579.

Van Inghelandt, D., Melchinger, A., Lebreton, C., Stich, B. 2010. Population structure and genetic diversity in a commercial maize breeding program assessed with SSR and SNP markers. Theor. Appl. Genet. 120: 1289-1299.

Vavilov, N.I. 1940. The theory of the origin of cultivated plants according to Darwin. Z. landw. VerWes. Bulg. 10(3): 1-31.

Vanzetti, L.S., Campos, P., Demichelis, M., Lombardo, L.A., Aurelia, P.R., Vaschetto, L.M., Bainotti, C.T., Helguera, M. 2011. Identification of leaf rust resistance genes in selected Argentinean bread wheat cultivars by gene postulation and molecular markers. Electronic Journal of Biotechnology 14(3).

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

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B., Maccaferri, M., Salvi, S., Milner, S., Cattivelli, L., Mastrangelo, A., Whan, A., Stephen, S., Barker, G., Wieseke, R., Pileske, J., Consortium IWGS, Lillemo, M., Mather, D., Appels, R., Dolferus, R., Brown-Guedira G., Korol, A., Akhunova, A.R., Feuillet, C., Salse, J., Morgante, M., Pozniak, C., Luo, M.C., Dvorak, J., Morell, M., Dubcovsky, J., Ganal, M.W., Tuberosa, R., Lawley, C., Mikoulitch, I., Cavanagh, C., Edwards, K.J., Hayden, M., Akhunov, E. 2014. Characterization of polyploidy wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnology Journal 12: 787-796.

Ward, T. 1994. The origins of Canadian wheat boom, 1880-1910. The Canadian Journal of Economics 27(4): 865-883.

Watson, I.A., Luig, N.H. 1966. *Sr15* – a new gene for use in the classification of *Puccinia graminis* var. *tritici*. Euphytica 15:239-250.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18(22): 6531-6535.

Windels, C.E. 2000. Economic and social impacts of fusarium head blight: changing farms and rural communities in the Northern Great Plaines. Phytopathology 90: 17-21.

WISP Consortium. Genotyping Theme. http://www.wheatisp.org/Documents/Genotyping_Theme.php Accessed: May 2015.

Xu, Y., Crouch, J.H. 2008. Marker-assisted selection in plant breeding: from publication to practice. Crop Sci. 48: 391-407.

Yehuda, P.B., Eilam, T., Manisterski, J., Shimoni, A., Anikster, Y. 2004. Leaf rust on *Aegilops speltoides* by a new forma specialis of *Puccinia triticina.* Phytopathology 94: 94-101.

Zadoks, J.C., Chang, T.T., Konzak, C.F. 1974. A decimal code for the growth stages of cereals. Weed Research 14: 415-421.

Zadoks, J.C., Bouwman, J.J. 1985. Epidemiology in Europe. In: Roelfs, A.P., Bushnell, W.R. (eds.) The Cereal Rusts: Diseases, Distribution, Epidemiology, and Control. Academic Press, Orlando, pp 329-369.

Zhang, Y.M., Xu, S. 2004. Mapping quantitative trait loci in F_2 incorporating phenotypes of F_3 progeny. Genetics 166: 1981-1993.

CHAPTER 8

Appendices

Appendix 8.1 Summary of controlled environment (*Puccinia triticina* race BBBD) and field phenotypic data (*Puccinia triticina* epidemic mix) and microsatellite genetic marker data on chromosome 7DS collected on the Z01M DH population

	Controlled Environment Ratings (BBBD)				Microsatellite Marker Data				The Point - Rep 1		The Point - Rep 2		Portage - Rep 1		Portage - Rep 2		
DH line	Seedling IT	Seedling Rxn	Adult IT	Adult Rxn	Lr34	wmc121	cfd21	gwm437	Severity	Pustule type	Severity	Pustule type	Severity	Pustule type	Severity	Pustule type	Avg Field Rxn
CP#481	;1-	R	R	R	A	A	A	A	30-50	MS	20-30	MS	15-20	I	20-40	MS	I
CP#482	;1-	R	M	R	A	A	A	N/A	10-15	MR	50-60	S	15-25	I	30-50	MS	I
CP#483	3	S	R	R	B	A	A	A	60	MS	20-30	I	DEAD		DEAD		I
CP#484	4	S	S	S	B	B	B	B	30	I	60	S	DEAD		DEAD		I
CP#485	1	R	R	R	A	B	B	B	10-15	MR	T-5	R	T	R/MR	T	R	R
CP#487	4	S	S	S	B	B	B	A	70	S	60	S	70	S	70	S	S
CP#488	;1-	R	R	R	A	A	A	A	10	R	5	MR	5	R/MR	T-5	R/MR	R
CP#489	3	S	S	S	B	B	B	B	70	S	60-70	S	70	S	70	S	S
CP#490	3	S	S	S	B	A	B	A	70	S	60-70	S	70	S	70	S	S
CP#491	4	S	S	S	B	A	A	A	70	S	60	S	70	S	70	S	S
CP#492	3	S	S	S	B	B	B	B	60	S	50-60	S	70	S	70	S	S
CP#493	3	S	S	S	B	B	B	B	50	S	70	S	70	S	DEAD		S
CP#494	;1-	R	R	R	B	B	B	B	10-15	MR	15	MR	DEAD		DEAD		R
CP#495	3-	S	MS	S	B	B	B	B	70	S	70	S	70	S	70	S	S
CP#497	1	R	M	R	B	B	B	B	30	I	20	I	DEAD		DEAD		I
CP#498	4	S	M	R	B	B	B	B	40	I	15-20	I	DEAD		DEAD		I
CP#499	;	R	R	R	A	A	N/A	A	5-15	MR	5-10	R	T	R	T	R	R
CP#500	1	R	M	R	A	B	B	B	50	MS	20-40	I	25-40	I	5-15	MR	I
CP#501	;1-	R	M	R	A	A	B	A	50-60	MS	20	I	5-10	MR	10-15	MR/I	I
CP#503	3-	S	S	S	B	B	B	B	70	S	70	S	70	S	70	S	S
CP#504	3-	S	S	S	B	B	B	B	60-70	S	40-60	MS	70	S	70	S	S

CP#505	;1-	R	R	R	A	B	A	B	10-15	MR	5-10	MR	5-10	MR	5	MR	R
CP#506	;1-	R	R	R	A	B	B	B	T-5	R/MR	T-5	R	T	R	T	R	R
CP#507	2	S	R	R	A	A	A	A	5-10	MR	T	R	T-5	R/MR	T	R	R
CP#508	;	R	R	R	A	A	A	A	10	MR	10-15	MR	T-5	R/MR	T	R	R
CP#509	;	R	R	R	A	A	N/A	A	15	R/MR	T	R	T	R	T	R	R
CP#510	3	S	S	S	B	B	B	B	20	I	15-30	I	DEAD		DEAD		I
CP#511	;1-	R	R	R	A	A	A	A	T	R	T-5	R/MR	T-5	R/MR	T	R	R
CP#512	;	R	M	R	A	B	N/A	B	60	MS	30-40	I	5-10	MR	T-5	R/MR	I
CP#513	;	R	R	R	A	B	B	B	10	MR	5	MR	T	R	T-5	R/MR	R
CP#514	;1-	R	R	R	A	A	A	B	5	MR	T	R	T	R	T	R	R
CP#515	3	S	MMS	S	B	B	B	B	50-60	MS	30	I	DEAD		DEAD		I
CP#516	;1--	R	M	R	A	B	B	B	60	MS	40	I	10-15	MR	5	R	I
CP#517	2	S	M	R	A	B	B	B	60	S	40-60	MS	20-40	MS	10-20	MR/I	I/S
CP#519	;1-	R	M	R	A	B	B	B	60	MS	60	S	30-60	MS	30-40	MS	I/S
CP#520	2	S	MS	S	B	B	B	N/A	60	S	30-40	I	DEAD		DEAD		I/S
CP#522	;1	R	R	R	A	A	A	A	20	MR	15	MR	T-5	R/MR	T	R	R/I
CP#523	2	S	R	R	A	A	A	A	5-10	MR	5	MR	T-5	R/MR	T	R	R
CP#524	;1--	R	M	R	A	B/N/A	A	B	30	I	20-30	I	15-40	I/MS	15-30	I	I
CP#525	3	S	MS	S	B	A	A	A	70	S	60-70S	S	70	S	70	S	S
CP#526	4	S	Variable	S	B	A	B	A	30	I	15	MR	DEAD		DEAD		I
CP#527	;1-	R	M	R	A	B	B	B	50-60	MS	20-40	I	20-40	I	15-40	I	I
CP#528	;1-	R	M	R	A	A	N/A	A	30-50	MS	20-30	I	5	MR	15-20	I	R/I
CP#529	2	S	X	X	B	A	A	A	15	MR	70	S	DEAD		DEAD		I
CP#530	;	R	M	R	A	A	A	A	30-40	MS	60	S	15	I	15	MR	I/S
CP#531	;1-	R	R	R	A	N/A	N/A	A	T-5	MR	T	R	T	R	T	R	R
CP#532	3-	S	MS	S	B	B	B	B	60	S	60	S	DEAD		DEAD		S
CP#533	;--	R	R	R	A	B	B	B	5-10	R	T	R	T-5	R/MR	5	MR	R
CP#534	;1-	R	R	R	A	B	A	B	10	MR	T-5	R/MR	T	R	T	R	R
CP#535	1	R	M	R	A	A	A	N/A	30-60	MS	30	I	10-15	MR	15-40	I	I
CP#536	3	S	S	S	B	A	A	A	70	S	70	S	70	S	70	S	S
CP#537	3	S	R	R	A	A	A	A	T-5	R/MR	5	MR	T	R	T	R	R
CP#538	1	R	M	R	A	B	A	B	60	S	10-15	I	15-25	I	20-40	I	I/S

CP#540	;1-	R	R	R	A	A	A	A	15	R	T-5	R	T	R	T	R	R
CP#541	3	S	X	X	B	B	B	B	60	S	30-40	I	30-40	MS	40-60		I
CP#542	;1	R	MMR	R	A	A	A	A	60	MS	10-20	I	5-10	MR	15-30	I	I
CP#543	;1--	R	R	R	A	A	A	A	30-50	I	15-20	I	5-20	I	15-20	I	I
CP#544	;1--	R	R	R	N/A	A	A	A	10	MR	5	R	T	R	T	R	R
CP#545	4	S	S	S	B	B	B	B	70	S	70	S	70	S	70	S	S
CP#547	2	S	M	R	A	A	A	N/A	60	MS	40-60	MS	20-30	I	15-30	I	I/S
CP#548	;1-	R	M	R	A	B	B	B	60	MS	60	MS	5-15	MR	20-40	MS	I
CP#549	2	S	S	S	A	A	A	A	60	S	30-40	I	10-15	MR	10-15	MR	I
CP#550	1	R	M	R	A	B	B	N/A	70	S	60	S	70	S	70	S	S
CP#551	2	S	M	R	A	A	A	A	60	S	30	MS	20-50	MS	15-40	I/MS	I/S
CP#552	3	S	Variable	S	B	B	B	B	70	S	70	S	70	S	70	S	S
CP#553	;1-	R	M	R	A	B	B	B	60-70	MS	50	I	15-25	I	15-30	I	I/S
CP#554	1	R	MR	R	A	A	N/A	N/A	20	MS	30-40	I	T-5	R/MR	T	R	I
CP#555	4	S	X	X	B	B	B	B	60	S	20	I	DEAD		DEAD		I/S
CP#556	;1-	R	R	R	A	B	B	B	T	R	T-5	R/MR	T	R	T	R	R
CP#557	4	S	X	X	B	B	B	B	20	I	10-15	MR	5	R/MR	DEAD		R/I
CP#558	1	R	R	R	A	A	A	A	T-5	MR	T	R	T	R	T	R	R
CP#559	4	S	S	S	B	N/A	N/A	B	70	S	70	S	70	S	70	S	S
CP#560	;1-	R	M	R	A	A	A	A	20	I	50-60	MS	15-20	I	10-15	MR/I	I
CP#561	;	R	M	R	A	A	A	A	15-20	I	20-30	I	20-40	I	15	MR/I	I
CP#562	3	S	S	S	B	A	A	A	70	S	60	S	70	S	70	S	S
CP#563	3	S	MS	S	A	A	A	A	40-60	I	5-10	MR	5-10	MR	15-20	MR/I	I
CP#564	;1	R	MMS	S	A	B	B	B	60	S	30-40	I	15-30	I	15	MR/I	I/S
CP#565	3	S	S	S	B	B	B	B	60	S	40-50	S	70	S	70	S	S
CP#566	1	R	M	R	A	A	A	N/A	60-70	S	60	MS	15-20	I	10-15	MR	I
CP#567	4	S	MS	S	B	B	B	N/A	40	I	20-40	I	DEAD		DEAD		I
CP#569	;	R	R	R	A	B	B	B	5-10	R/MR	T	R	T	R/MR	T	R	R
CP#570	1	R	M	R	A	A	A	A	50	MS	30-50	MS	5-15	MR	5-15	MR	I
CP#571	3	S	S	S	B	B	B	B	50	MS	40-50	MS	70	S	70	S	S
CP#572	4	S	X	X	B	A	B	A	50	MS	20-50	I	40-60	I	DEAD		I/S
CP#573	;1--	R	MMS	S	A	N/A	N/A	B	60	S	30-50	I	15-20	I	15-30	I	I/S

CP#574	3	S	X	X	B	B	B	B	50	MS	DEAD		DEAD		DEAD		I/S
98-223-27	;1	R	R	R	A	A	A	A	30	MR	5-15	MR	T	R	T	R	R
Thatcher	3/4	S	S	S	B	B	N/A	B	70	S	70	S	70	S	70	S	S
CP#577	;1	R	M	R	A	N/D	N/D	N/D	20	I	20-50	I	15-20	I	15-30	I	I
CP#578	;1-	R	M	R	A	N/D	N/D	N/D	30	I	20-30	I/MS	30-50	MS	15-20	MR/I	I
CP#579	;1-	R	R	R	A	N/D	N/D	N/D	T	R	5-10	MR	T	R	T	R	R
CP#580	3	S	S	S	B	N/D	N/D	N/D	20	I	40-60	MS	DEAD		DEAD		I/S
CP#581	2	S	M	R	A	N/D	N/D	N/D	50	MS	15	I	20-30	I	15-30	I	I
CP#582	1	R	R	R	A	N/D	N/D	N/D	5-10	MR	T-5	R/MR	T-5	R/MR	T	R	R
CP#583	4	S	S	S	B	N/D	N/D	N/D	40-60	MS	50-60	MS	70	S	70	S	S
CP#585	;1	R	MMS	S	A	N/D	N/D	N/D	60	MS	20-30	I	15-30	I	15-30	I	I/S
CP#586	4	S	S	S	B	N/D	N/D	N/D	20-40	I	50-60	S	40-60	I	70	S	I/S
CP#587	;1-	R	R	R	A	N/D	N/D	N/D	T	R	5	MR	T	R	T	R	R
CP#588	1	R	MS	S	A	N/D	N/D	N/D	30	I	20-30	I	15-20	I	15-20	I	I
CP#589	3/4	S	X	X	B	N/D	N/D	N/D	60	S	60	S	DEAD		DEAD		S
CP#590	;1-	R	R	R	A	N/D	N/D	N/D	T-5	R/MR	5	MR	T	R	T-5	R/MR	R
CP#591	3	S	X	X	B	N/D	N/D	N/D	60	MS	15-20	MR/I	DEAD		DEAD		I
CP#592	;1-	R	M	R	A	N/D	N/D	N/D	30-40	I	20-30	I	15-20	I	5-15	MR/I	I
CP#593	2	S	M	R	A	N/D	N/D	N/D	70	S	60	S	15-30	I	15-30	I	I/S
CP#594	;	R	R	R	N/A	N/D	N/D	N/D	5	MR	T	R	5	MR	T	R	R
CP#595	;1	R	M	R	A	N/D	N/D	N/D	60	S	30-40	I	5-15	MR	T-5	R/MR	I
CP#596	3	S	X	X	B	N/D	N/D	N/D	60	S	30	I	DEAD		DEAD		I
CP#597	;1-	R	MMS	S	A	N/D	N/D	N/D	60	S	60	S	20-40	I	10-15	MR/I	I/S
CP#598	;1	R	R	R	A	N/D	N/D	N/D	T	R	T	R	5	R/MR	T	R	R
CP#600	3	S	S	S	B	N/D	N/D	N/D	70	S	70	S	70	S	70	S	S
CP#601	1	R	M	R	A	N/D	N/D	N/D	30	I	30	I	15-30	I	15-30	MR/I	I
CP#602	3	S	M	R	B	N/D	N/D	N/D	60	MS	60	S	70	S	DEAD		S
CP#603	;1--	R	R	R	A	N/D	N/D	N/D	T	R	5	R	T	R	T	R	R
CP#604	;1-	R	M	R	A	N/D	N/D	N/D	40	MS	60	I	5-10	MR	15-20	MR/I	I
CP#605	;1	R	M	R	A	N/D	N/D	N/D	60	S	60	MS	10-15	I	15-40	I	I
CP#606	3	S	X	X	B	N/D	N/D	N/D	60	MS	30-40	I	DEAD		DEAD		I/S

CP#608	;1-	R	M	R	A	N/D	N/D	N/D	40	MS	50-70	MS	20-30	I	10-20	I	I/S
CP#609	1	R	R	R	A	N/D	N/D	N/D	5-10	MR	T	R	T	R/MR	T	R	R
CP#610	1	R	R	R	A	N/D	N/D	N/D	5	MR	T-5	MR	T	R	T	R	R
CP#611	1	R	M	R	A	N/D	N/D	N/D	60	S	40-50	I	15-30	I	15-25	I	I/S
CP#612	3/4	S	X	X	B	N/D	N/D	N/D	30-50	I	20-30	I	DEAD		DEAD		I
CP#614	4	S	S	S	B	N/D	N/D	N/D	70	S	70	S	70	S	70	S	S
CP#615	2	S	M	R	A	N/D	N/D	N/D									NO PLANT
CP#616	3/4	S	X	X	B	N/D	N/D	N/D	10-15	I	15	MR	DEAD		DEAD		R/I
CP#617	4	S	X	X	B	N/D	N/D	N/D	60	MS	50	I	DEAD		DEAD		I/S
CP#618	;1-	R	R	R	A	N/D	N/D	N/D	10	MR	T	R	T	R	T	R	R
CP#619	3/4	S	MS	S	B	N/D	N/D	N/D	60	MS	70	S	DEAD		DEAD		S
CP#620	;1-	R	R	R	A	N/D	N/D	N/D	10	R	T	R	T	R	T-5	R/MR	R
CP#621	;1-	R	R	R	A	N/D	N/D	N/D	T	R	5	MR	T	R	T	R	R
CP#622	3	S	X	X	B	N/D	N/D	N/D	30-40	I	50-60	I	DEAD		DEAD		I
CP#623	4	S	S	S	B	N/D	N/D	N/D	50	MS	70	S	70	S	70	S	S
CP#624	;1	R	R	R	A	N/D	N/D	N/D	15	MR	5	R	T-5	R/MR	T-5	R/MR	R
CP#625	3	S	MMS	S	B	N/D	N/D	N/D	40	S	70	S	70	S	70	S	S
CP#626	3/4	S	M	R	B	N/D	N/D	N/D	60	S	70	S	70	S	70	S	S
CP#627	;1-	R	MR	R	A	N/D	N/D	N/D	70	S	30-40	I	15-20	I	15-30	I	I/S
CP#628	;1	R	R	R	A	N/D	N/D	N/D	10	MR	5-15	MR/I	T-5	R/MR	T-5	R/MR	R
CP#629	2	S	X	X	B	N/D	N/D	N/D	20-40	I	10-15	I	70	S	DEAD		I
CP#632	1	R	M	R	A	N/D	N/D	N/D	50-60	S	30-40	I	15-30	I	15-30	I	I/S
CP#633	4	S	X	X	B	N/D	N/D	N/D	40	I	15-30	I	DEAD		DEAD		I
CP#634	;1--	R	R	R	A	N/D	N/D	N/D	10	R	T	R	T-5	R/MR	T	R	R
CP#635	3/4	S	X	X	B	N/D	N/D	N/D	30-40	MR	30	I	DEAD		DEAD		I
CP#636	;1-	R	R	R	A	N/D	N/D	N/D	5-15	MR	5	R/MR	T-5	R/MR	T	R	R
CP#637	3	S	S	S	B	N/D	N/D	N/D	60	S	70	S	70	S	DEAD		S
CP#638	;1-	R	R	R	A	N/D	N/D	N/D	10	MR	10-15	MR	T	R	T	R	R
CP#639	;1	R	MR	R	A	N/D	N/D	N/D	50	MS	20-40	I	5-10	MR	10-15	MR	I
CP#640	3/4	S	S	S	N/A	N/D	N/D	N/D	70	S	70	S	70	S	70	S	S
CP#641	3	S	X	X	B	N/D	N/D	N/D	20-30	I	10	MR	DEAD		DEAD		R/I

CP#642	1	R	R	R	A	N/D	N/D	N/D	T	R	5	MR	T-5	R/MR	T	R	R
CP#643	1	R	M	R	A	N/D	N/D	N/D	50	I	40-50	MS	5-10	MR	15-30	I	I
CP#644	;1-	R	R	R	A	N/D	N/D	N/D	T	R	T	R	T	R	T-5	R/MR	R
CP#645	;1--	R	R	R	A	N/D	N/D	N/D	15	MR	5	R	T	R	T	R	R
CP#646	3	S	MS	S	B	N/D	N/D	N/D	70	S	70	S	70	S	70	S	S
CP#647	3	S	MMS	S	B	N/D	N/D	N/D	30	I	70	S	70	S	70	S	S
CP#648	4	S	M - MR	R	B	N/D	N/D	N/D	30-40	I	50-60	MS	DEAD		DEAD		I
CP#649	;1--	R	R	R	A	N/D	N/D	N/D	15	MR	5-15	MR	NO DATA		NO DATA		R
CP#650	3	S	S	S	B	N/D	N/D	N/D	60	S	60-70	S	70	S	DEAD		S
CP#651	3	S	MMS	S	A	N/D	N/D	N/D	60	S	60	S	20-40	MS	70	S	S
CP#652	3/4	S	MS	S	B	N/D	N/D	N/D	70	S	20-60	MS	70	S	70	S	S
CP#653	;1	R	MR	R	A	N/D	N/D	N/D	60-70	S	60	MS	15-20	I	15-30	I	I/S
CP#654	3/4	S	S	S	B	N/D	N/D	N/D	70	S	70	S	70	S	70	S	S
CP#655	3	S	S	S	B	N/D	N/D	N/D	70	S	60-70	S	70	S	70	S	S
CP#656	;1-	R	R	R	A	N/D	N/D	N/D	70	S	50	MS	30-40	MS			I/S
CP#657	;1-	R	R	R	A	N/D	N/D	N/D	T-5	R/MR	T	R	T	R	T	R	R
CP#658	4	S	MMS	S	B	N/D	N/D	N/D	70	S	40-60	S	70	S	70	S	S
CP#659	3	S	M	R	B	N/D	N/D	N/D	50	I	20	I	DEAD		DEAD		I
CP#660	;1-	R	MR	R	A	N/D	N/D	N/D	60	S	50-60	MS	30-50	MS	15	MR	I/S
CP#661	;1	R	M	R	A	N/D	N/D	N/D	50	MS	40-50	I	15-20	I	15-30	I	I
CP#662	3	S	X	X	B	N/D	N/D	N/D	40	MS	40-60	I	DEAD		DEAD		I/S
CP#663	3	S	S	S	B	N/D	N/D	N/D	70	S	70	S	70	S	DEAD		S
98-223-27	;1--	R	R	R	A	N/D	N/D	N/D	5	R	T	R	T	R	T-5	R/MR	R
Thatcher	4	S	S	S	B	N/D	N/D	N/D	70	S	70	S	70	S	70	S	S

IT = infection type, Rxn = reaction, R = resistant, MR = moderately resistant, I = intermediate, MS = moderately susceptible, S = susceptible, X = mesothetic reaction, T = trace, DEAD = dead leaves/rating could not be made, N/A = no amplification/ poor resolution, N/D = no data collected, A = allele type corresponding to presence of *Lr34,* B = wild type

DH lines eliminated from dataset (red font): CP#526, 552, 515

Appendix 8.2 Summary of SNP markers converted to KASP markers that demonstrated linkage to *LrCen* in the Illumina Infinium assay

Lab KASP ID	iSelect 90K wheat SNP ID	RF[a]	LG47[b]	C.S.R.[c]	KASP Allele Specific Forward Primer A1 (KASP Allele Specific Forward Primer A2) [KASP Common Reverse Primer C1]
KWH37	BobWhite_c32347_219	0.231		*	GAAGGTGACCAAGTTCATGCTCTTCGCTTGTGTTAATCAAGATGT
					(GAAGGTCGGAGTCAACGGATTCTCTTCGCTTGTGTTAATCAAGATGC)
					[TGAGGATGCTAGGAGGGAAGACAAT]
KWH41	BS00022284_51	0.022		*	GAAGGTGACCAAGTTCATGCTACGCATCTTGCGCAAGTGGTC
					(GAAGGTCGGAGTCAACGGATTACGCATCTTGCGCAAGTGGTT)
					[AGGAGATATTTGGTTGCGGCGGAAA]
KWH42	BS00023003_51	0.110	*	*	GAAGGTGACCAAGTTCATGCTGCATTGCATCATGGGTGCAAAATGTT
					(GAAGGTCGGAGTCAACGGATTCATTGCATCATGGGTGCAAAATGTC)
					[GGAAGAAGGCGCTGCACCGTA]
KWH44	BS00062749_51	0.022		*	GAAGGTGACCAAGTTCATGCTCGTGGGCAATGGAGAAACAAGG
					(GAAGGTCGGAGTCAACGGATTCCGTGGGCAATGGAGAAACAAGT)
					[CCAAACACTTCATGTGTGAATACTTGACTA]
KWH46	BS00065453_51	0.198		*	GAAGGTGACCAAGTTCATGCTAAGAATGCCATTCCTTCCCGTGAA
					(GAAGGTCGGAGTCAACGGATTGAATGCCATTCCTTCCCGTGAG)
					[GTCAATGACAATCACCTTCCCTCCTT]
KWH48	BS00065851_51	0.022			GAAGGTGACCAAGTTCATGCTCATGCCCCAGCATGATCTTTTGC
					(GAAGGTCGGAGTCAACGGATTCATGCCCCAGCATGATCTTTTGA)
					[TTTGGGCGACTGGCATTTCGGAAAA]
KWH49	BS00067682_51	0.176			GAAGGTGACCAAGTTCATGCTGGGGAAACTAATACAAGTCCCCC
					(GAAGGTCGGAGTCAACGGATTGGGGAAACTAATACAAGTCCCCT)
					[ACGCCAAGCAATTTCGGTGCAGTT]
KWH50	BS00068032_51	0.176			GAAGGTGACCAAGTTCATGCTCAAACCCAAAGGACAACTTAGTGTCA
					(GAAGGTCGGAGTCAACGGATTAAACCCAAAGGACAACTTAGTGTCG)
					[CTGCAAGACATACATGACGTGAGGTA]
KWH51	BS00068033_51	0.176			GAAGGTGACCAAGTTCATGCTCTTGCAGGGTATGAGTATGGATCT
					(GAAGGTCGGAGTCAACGGATTGCAGGGTATGAGTATGGATCG)

				[ATGTCTTGCAGCCTCAACACCACAA]
KWH52	BS00076120_51	0.187		GAAGGTGACCAAGTTCATGCTACGGTGGACTATTGCTACTACAAAC
				(GAAGGTCGGAGTCAACGGATTCACGGTGGACTATTGCTACTACAAAT)
				[CCAACGCACGGCCCCAAATGAA]
KWH54	BS00081949_51	0.022		GAAGGTGACCAAGTTCATGCTCCTGCGCCACACCACCTGT
				(GAAGGTCGGAGTCAACGGATTCTGCGCCACACCACCTGC)
				[GGGCAAGGTTTGCGGGCACTA]
KWH55	BS00098482_51	0.352	*	GAAGGTGACCAAGTTCATGCTCAGCAGGGACAGGTGAGATATAG
				(GAAGGTCGGAGTCAACGGATTCAGCAGGGACAGGTGAGATATAA)
				[TACGGTCCAGAGTAGATTGCTGTTTATTT]
KWH61	CAP7_c950_137	0.209		GAAGGTGACCAAGTTCATGCTATTACATGGCATCCTTAAGAAATAACTGA
				(GAAGGTCGGAGTCAACGGATTACATGGCATCCTTAAGAAATAACTGG)
				[ATGCCATGTCATAGTTTTGGATAGGCAT]
KWH64	CAP8_c8600_222	0.198		GAAGGTGACCAAGTTCATGCTTTCTCTGGCCACTGATCTGGC
				(GAAGGTCGGAGTCAACGGATTTTCTCTGGCCACTGATCTGGT)
				[CGAACACCTCGACAAGGGCAGTT]
KWH65	D_GBUVHFX02IBI9T_116	0.022		GAAGGTGACCAAGTTCATGCTAAATTTATACTAGGATATGTTGAGGATACC
				(GAAGGTCGGAGTCAACGGATTGAAATTTATACTAGGATATGTTGAGGATACT)
				[TCAACCATATAGACACTCCCGTTCATATA]
KWH66	Ex_c23405_470	0.187		GAAGGTGACCAAGTTCATGCTAGCACAGACCAAACAGCCG
				(GAAGGTCGGAGTCAACGGATTGCTAGCACAGACCAAACAGCCA)
				[CTCCGCGTAAATATGTTGTTTTGATCCTT]
KWH68	Excalibur_c19225_292	0.022		GAAGGTGACCAAGTTCATGCTGAAGTAGAACATGGAGAGGTAGACA
				(GAAGGTCGGAGTCAACGGATTAAGTAGAACATGGAGAGGTAGACG)
				[ACTTCAACACGATCGTGAAGCCGAT]
KWH69	Excalibur_c20018_214	0.275	*	GAAGGTGACCAAGTTCATGCTGAGCAGGGGTGCTTCAGTGAC
				(GAAGGTCGGAGTCAACGGATTGAGCAGGGGTGCTTCAGTGAT)
				[TCCCTTGCCGATAAGACTTGCCTTT]
KWH71	Excalibur_c33172_60	0.176		GAAGGTGACCAAGTTCATGCTGTGCATATCCCATTGTACTTCAGAAA
				(GAAGGTCGGAGTCAACGGATTGTGCATATCCCATTGTACTTCAGAAC)
				[AAAGCGAGGATTTTCCATGTGCTCATATA]

KWH75	Excalibur_c61603_1052	0.286		*	GAAGGTGACCAAGTTCATGCTCCAGGATTCATCACCTCCCA
					(GAAGGTCGGAGTCAACGGATTCCAGGATTCATCACCTCCCG)
					[GGATGGTAACAGGAAAAGCAAAGAAGTAA]
KWH78	Excalibur_c695_304	0.187			GAAGGTGACCAAGTTCATGCTAAGCTCCAAATCTCCCGGTTCTA
					(GAAGGTCGGAGTCAACGGATTAGCTCCAAATCTCCCGGTTCTG)
					[GATACCAATTGAACTGTTTGACAAGGAATT]
KWH79	Excalibur_c697_1092	0.275			GAAGGTGACCAAGTTCATGCTACCACCACGATCCAAGTGTAATGTA
					(GAAGGTCGGAGTCAACGGATTCACCACGATCCAAGTGTAATGTC)
					[CTTGGCCTGCGGTTGCATTGTTATT]
KWH91	IAAV5550	0.187			GAAGGTGACCAAGTTCATGCTGACCTCTCTGTTGAACTGCTTATCT
					(GAAGGTCGGAGTCAACGGATTACCTCTCTGTTGAACTGCTTATCG)
					[GAAACTTCAGGAGAACGCGCCCAT]
KWH102	Kukri_c65164_1063	0.022			GAAGGTGACCAAGTTCATGCTATGCAATTCTCCTCCTGATTGCTCA
					(GAAGGTCGGAGTCAACGGATTGCAATTCTCCTCCTGATTGCTCC)
					[TGGAGGAGGAGGGTGCAGTCAT]
KWH104	Kukri_c855_1907	0.231			GAAGGTGACCAAGTTCATGCTACTTGTCAAACCCTTGTTCTTCTCG
					(GAAGGTCGGAGTCAACGGATTAAACTTGTCAAACCCTTGTTCTTCTCA)
					[TTTGGGCATGCAGATTGGTGCTCAA]
KWH106	Kukri_c9728_1171	0.187			GAAGGTGACCAAGTTCATGCTAGACACACGAGGGAGCACTCA
					(GAAGGTCGGAGTCAACGGATTGACACACGAGGGAGCACTCG)
					[TCGTCTTGGCCCACTCCACCTT]
KWH116	RAC875_c2532_64	0.198			GAAGGTGACCAAGTTCATGCTCTCCAGCACAAAGTAGCGCG
					(GAAGGTCGGAGTCAACGGATTGTCTCCAGCACAAAGTAGCGCA)
					[CGCAAGATCGGCCGCTCCTTTA]
KWH119	RAC875_c90330_82	0.176			GAAGGTGACCAAGTTCATGCTCCGGTGTTTCCCTGATCACCA
					(GAAGGTCGGAGTCAACGGATTCCGGTGTTTCCCTGATCACCC)
					[GCTGCATGGAGGCATGCATAAACTA]
KWH122	RAC875_rep_c119304_226	0.385			GAAGGTGACCAAGTTCATGCTATCAGGATAATATCATATTGGTGGCG
					(GAAGGTCGGAGTCAACGGATTCATCAGGATAATATCATATTGGTGGCA)
					[GGACTGGAAGCAATCCGTGCAGTA]
KWH125	RFL_Contig6074_1058	0.374	*	*	GAAGGTGACCAAGTTCATGCTCATTACTTCTTGTCGGCGATGACAT

				(GAAGGTCGGAGTCAACGGATTACTTCTTGTCGGCGATGACAC)
				[GGCTTGTCGAGACATACAAGTGGTA]
KWH127	Tdurum_contig12454_585	0.187		GAAGGTGACCAAGTTCATGCTCATGGCTGAAGTTTCTCGAAGACTT
				(GAAGGTCGGAGTCAACGGATTATGGCTGAAGTTTCTCGAAGACTG)
				[AACAGGTCGATTTGCCGCTTCCTTT]
KWH128	Tdurum_contig13276_115	0.022		GAAGGTGACCAAGTTCATGCTGCCACAGTTTGGTGACACTCCA
				(GAAGGTCGGAGTCAACGGATTCCACAGTTTGGTGACACTCCC)
				[ACTCCAAGCTACAAGGACATATTCAGAT]
KWH131	Tdurum_contig20378_260	0.022		GAAGGTGACCAAGTTCATGCTAGCTGATATATCGGAACCTTATAGAGA
				(GAAGGTCGGAGTCAACGGATTGCTGATATATCGGAACCTTATAGAGC)
				[TGCATGCTGCAACTAAGGTGATTTACATA]
KWH134	Tdurum_contig42487_1555	0.176		GAAGGTGACCAAGTTCATGCTCAGAAAGTCATTGTATTATGGAAGGC
				(GAAGGTCGGAGTCAACGGATTAATCAGAAAGTCATTGTATTATGGAAGGT)
				[CACTCTCGGGAACGAAAAACTTATAAGTA]
KWH138	Tdurum_contig59467_534	0.198		GAAGGTGACCAAGTTCATGCTGCACATAGAACTATCATCTGCGG
				(GAAGGTCGGAGTCAACGGATTCGCACATAGAACTATCATCTGCGA)
				[ATTTGGTTAATCCTCAAGCACAGGAACAA]
KWH139	Tdurum_contig59836_250	0.209		GAAGGTGACCAAGTTCATGCTGAAAAGCTTGAAGACCACTCTGTC
				(GAAGGTCGGAGTCAACGGATTCGAAAAGCTTGAAGACCACTCTGTT)
				[GTGCATCTCTGAGTGAATGGCCAAA]
KWH140	Tdurum_contig81947_779	0.022		GAAGGTGACCAAGTTCATGCTATCATGGCGGAGATACACGCAG
				(GAAGGTCGGAGTCAACGGATTAATCATGGCGGAGATACACGCAT)
				[CCCTCCTGCTCATAATAACAAACTCAAAT]
KWH141	Tdurum_contig97505_172	0.187		GAAGGTGACCAAGTTCATGCTCAATACAGCAACAGTCACAGAAACAT
				(GAAGGTCGGAGTCAACGGATTCAATACAGCAACAGTCACAGAAACAG)
				[AGCTGTGGAACCTGCATCTGTACTT]
KWH147	wsnp_Ex_c22547_31738007	0.352	*	GAAGGTGACCAAGTTCATGCTATCCTTGGGAGCTATGTCGGAG
				(GAAGGTCGGAGTCAACGGATTATATCCTTGGGAGCTATGTCGGAA)
				[CTTTCAACAGCCTTCTGGCTTCGTT]
KWH153	wsnp_Ex_c5839_10246915	0.187	*	GAAGGTGACCAAGTTCATGCTGACATAAGCGGAGTGCACTGC
				(GAAGGTCGGAGTCAACGGATTCGACATAAGCGGAGTGCACTGT)

			[AGAAACTCACTCTGAATACCACAACAGTT]
KWH158	wsnp_JD_c1219_1766041	0.022	GAAGGTGACCAAGTTCATGCTCATGAGATAACCTAATTCTGGAGGG
			(GAAGGTCGGAGTCAACGGATTCATGAGATAACCTAATTCTGGAGGA)
			[GGGACAACCAACTCTTTGGACGTAT]
KWH159	wsnp_JD_c1219_1766330	0.022	GAAGGTGACCAAGTTCATGCTTAAGCAATGTACCAGACTTGACGG
			(GAAGGTCGGAGTCAACGGATTCTTAAGCAATGTACCAGACTTGACGA)
			[CTGCATTTGATAATAACCTGAATGGACCTT]
KWH160	wsnp_JD_c1219_1766488	0.022	GAAGGTGACCAAGTTCATGCTCAGTTATCTAACCATTGGAGGGAC
			(GAAGGTCGGAGTCAACGGATTGCAGTTATCTAACCATTGGAGGGAT)
			[GTTGTTTGAGATGTTCAGCTTGGTAAGAT]
KWH161	wsnp_JD_c1219_1768165	0.022	GAAGGTGACCAAGTTCATGCTGAACTGCAACGCTGAGCACGT
			(GAAGGTCGGAGTCAACGGATTGAACTGCAACGCTGAGCACGC)
			[GGCGCTGAAACCGCTAGAGAAATAT]
KWH36	BobWhite_c25527_313	0.187	GAAGGTGACCAAGTTCATGCTGAGATGATTGTTGGAGGTTTTGTGGT
			(GAAGGTCGGAGTCAACGGATTAGATGATTGTTGGAGGTTTTGTGGC)
			[GAAACAGGGTGGTGCTTTCCCAATT]
KWH38	BS00009995_51	0.374	GAAGGTGACCAAGTTCATGCTGCCATATCTATTTGCTCGTCTCGTAA
			(GAAGGTCGGAGTCAACGGATTCCATATCTATTTGCTCGTCTCGTAC)
			[AGATGACAAGACTTTATCCGCAGCATTT]
KWH39	BS00010677_51	0.187	GAAGGTGACCAAGTTCATGCTCGCCGAAGACATGAACGACGAT
			(GAAGGTCGGAGTCAACGGATTGCCGAAGACATGAACGACGAC)
			[GTGCTATGAGGCTGCTACGATCAAT]
KWH40	BS00022169_51	0.374	GAAGGTGACCAAGTTCATGCTCCGAACCCAAGCCCGCTCT
			(GAAGGTCGGAGTCAACGGATTCGAACCCAAGCCCGCTCC)
			[GGGTACCGGGTCGAACTATGGTT]
KWH43	BS00061940_51	0.286	GAAGGTGACCAAGTTCATGCTGAAAGGTACATGTTGATGAAGATGCC
			(GAAGGTCGGAGTCAACGGATTAGAAAGGTACATGTTGATGAAGATGCT)
			[GTAGCAAATGCTCATCACAACTGTCAAAT]
KWH45	BS00064413_51	0.187	GAAGGTGACCAAGTTCATGCTTGGTTGGAACGTCGTGGCTA
			(GAAGGTCGGAGTCAACGGATTGGTTGGAACGTCGTGGCTG)
			[GCACACGCCTTTGCATTGGCGTT]

KWH47	BS00065454_51	0.220		GAAGGTGACCAAGTTCATGCTGGTGATTGTCATTGACATTGTGGTC
				(GAAGGTCGGAGTCAACGGATTAAGGTGATTGTCATTGACATTGTGGTT)
				[CCTCAAACATGACAGGTCCTAGTGAT]
KWH53	BS00081057_51	0.231		GAAGGTGACCAAGTTCATGCTGTAGATGAATCATAAAATGATTTAACAACAC
				(GAAGGTCGGAGTCAACGGATTCTGTAGATGAATCATAAAATGATTTAACAACAA)
				[CCATAGCAGTCCGGGTGAAGCAT]
KWH56	BS00110256_51	0.286	*	GAAGGTGACCAAGTTCATGCTACTAGACAATGATATGGTGCCGAAA
				(GAAGGTCGGAGTCAACGGATTACTAGACAATGATATGGTGCCGAAG)
				[GACTTCTCATCAAGTCTCGATAAACCAAA]
KWH57	BS00110681_51	0.231		GAAGGTGACCAAGTTCATGCTCACTCTGTATGCTGTCTGGCAC
				(GAAGGTCGGAGTCAACGGATTAACACTCTGTATGCTGTCTGGCAT)
				[CCGGACAGCATACAGAGTGTTGAT]
KWH58	BS00110683_51	0.231		GAAGGTGACCAAGTTCATGCTTCCATCAGCTGTATGCCGTCC
				(GAAGGTCGGAGTCAACGGATTGTTCCATCAGCTGTATGCCGTCT)
				[GCCAGACAGCATACAGAGTGTTGAT]
KWH59	BS00110894_51	0.022		GAAGGTGACCAAGTTCATGCTCAACGACCTCGCGTTCGCG
				(GAAGGTCGGAGTCAACGGATTCCAACGACCTCGCGTTCGCA)
				[TATCTCCTGCGTGGGGCTGCAT]
KWH60	CAP11_c31_213	0.209	*	GAAGGTGACCAAGTTCATGCTCAAGACGGATCGAACAGCGCG
				(GAAGGTCGGAGTCAACGGATTCAAGACGGATCGAACAGCGCA)
				[CTCGCCGGAGGAAAAACCGCTT]
KWH62	CAP7_rep_c5949_55	0.209		GAAGGTGACCAAGTTCATGCTCAAATTAATTAGCTAGCTACTGTAAATTAACAT
				(GAAGGTCGGAGTCAACGGATTAAATTAATTAGCTAGCTACTGTAAATTAACAG)
				[CCTTCTATTACCAAAGAGAGCTACCATAT]
KWH63	CAP8_c8600_130	0.198		GAAGGTGACCAAGTTCATGCTACTCTACCCGGAGCTATACG
				(GAAGGTCGGAGTCAACGGATTACTACTCTACCCGGAGCTATACT)
				[CGCTTGATGTGGTGGATGCAGAAT]
KWH67	Excalibur_c1791_819	0.374		GAAGGTGACCAAGTTCATGCTGACCGCCGCAGAACAGCG
				(GAAGGTCGGAGTCAACGGATTCATGACCGCCGCAGAACAGCA)
				[CCCACCGTCATTGTTGACAGTGTT]
KWH70	Excalibur_c24486_947	0.231		GAAGGTGACCAAGTTCATGCTGGTGAAAATAGCAACTCGATAAATCCAT

				(GAAGGTCGGAGTCAACGGATTGTGAAAATAGCAACTCGATAAATCCAC)
				[TCGATGGCTGAAGTGGCTTTCCTTT]
KWH72	Excalibur_c34189_122	0.187	*	GAAGGTGACCAAGTTCATGCTGACATCTGTTTGTTAAGCTGGAAAGA
				(GAAGGTCGGAGTCAACGGATTGACATCTGTTTGTTAAGCTGGAAAGG)
				[GGAATTGAGCCGCTCGAACTACATT]
KWH73	Excalibur_c39817_332	0.374		GAAGGTGACCAAGTTCATGCTGTGGTGGTTGTAGGGCTGGAT
				(GAAGGTCGGAGTCAACGGATTGTGGTGGTTGTAGGGCTGGAC)
				[TTCTAGAGCCTGCTGGTACTTCCTA]
KWH74	Excalibur_c59653_238	0.209		GAAGGTGACCAAGTTCATGCTGGCGTCAATGGAGCTATCCCTAT
				(GAAGGTCGGAGTCAACGGATTGCGTCAATGGAGCTATCCCTAC)
				[CGCAGATCCACGGGAGTTGAGAT]
KWH76	Excalibur_c61603_1138	0.286		GAAGGTGACCAAGTTCATGCTTTGAGAAGAACAGCAGAGATGATGAT
				(GAAGGTCGGAGTCAACGGATTGAGAAGAACAGCAGAGATGATGAC)
				[GCTGGGCTCTCCAGCATACCAA]
KWH77	Excalibur_c61603_1209	0.286		GAAGGTGACCAAGTTCATGCTTATCTTTCTAGGCTCCTGACC
				(GAAGGTCGGAGTCAACGGATTCCTTATCTTTCTAGGCTCCTGACT)
				[TGTAGACCAGCTAAGAGCTATTGAAGTTA]
KWH80	Excalibur_c9083_981	0.275	*	GAAGGTGACCAAGTTCATGCTGCGCTGGATCCAGCACCAAT
				(GAAGGTCGGAGTCAACGGATTGCGCTGGATCCAGCACCAAC)
				[GCACTGAAACAGGATCCAGCAGTT]
KWH81	Excalibur_rep_c102327_102	0.286		GAAGGTGACCAAGTTCATGCTGGTTCTGCCCGCAGAGAAGCA
				(GAAGGTCGGAGTCAACGGATTGTTCTGCCCGCAGAGAAGCG)
				[TGACTCCATGTAATGATCCCATGAAGTA]
KWH82	Excalibur_rep_c66918_307	0.275		GAAGGTGACCAAGTTCATGCTAAGAGGTGTCAGCATGACAAGAGT
				(GAAGGTCGGAGTCAACGGATTGAGGTGTCAGCATGACAAGAGC)
				[CGATGCGTCCATCAAGGAGATGAT]
KWH83	Excalibur_rep_c67229_255	0.187	*	GAAGGTGACCAAGTTCATGCTCTAGTAGTATATTGTCGGGTTTCAG
				(GAAGGTCGGAGTCAACGGATTGTCTAGTAGTATATTGTCGGGTTTCAA)
				[CATCATCTGCACAACGAAAAGCAGATATA]
KWH84	GENE-4528_1252	0.209	*	GAAGGTGACCAAGTTCATGCTGATATGTACTGTCATATAATGTTTCAAATACAA
				(GAAGGTCGGAGTCAACGGATTATATGTACTGTCATATAATGTTTCAAATACAG)

				[TACCTGAAGGTCTCATCAATAYGTCTTT]
KWH85	GENE-4672_55	0.374		GAAGGTGACCAAGTTCATGCTGACTCAAAATGTAAGGAGAGATAGGT
				(GAAGGTCGGAGTCAACGGATTGACTCAAAATGTAAGGAGAGATAGGC)
				[TATCCTCCTCGGCGATGATTCTGAA]
KWH86	GENE-4943_470	0.209		GAAGGTGACCAAGTTCATGCTCAACTCCCAAAGTGAAGCCATG
				(GAAGGTCGGAGTCAACGGATTGTCAACTCCCAAAGTGAAGCCATT)
				[CATATTTAGCYAGCAACTGCCCATA]
KWH87	IAAV14	0.198		GAAGGTGACCAAGTTCATGCTCACTCATCTCCTCTATTTCAGTCTTG
				(GAAGGTCGGAGTCAACGGATTCACTCATCTCCTCTATTTCAGTCTTT)
				[CTGCCTGTATATATGTATACCCTACTGTT]
KWH88	IAAV2968	0.187		GAAGGTGACCAAGTTCATGCTCGACGATGACTCGGACATCTCA
				(GAAGGTCGGAGTCAACGGATTGACGATGACTCGGACATCTCG)
				[GGGGCGCAAAGATAAAATCGTCCAT]
KWH89	IAAV3676	0.209		GAAGGTGACCAAGTTCATGCTTCGTGCGATCCGATCATCATAC
				(GAAGGTCGGAGTCAACGGATTCTTCGTGCGATCCGATCATCATAT)
				[GCTCAACCCATTGCCTTACCAAACTA]
KWH90	IAAV416	0.209	*	GAAGGTGACCAAGTTCATGCTCAATCGAGGCGTTGGACCGC
				(GAAGGTCGGAGTCAACGGATTCAATCGAGGCGTTGGACCGG)
				[CAGTCTCTATGATCTCCATGCCCAA]
KWH92	IAAV6058	0.231		GAAGGTGACCAAGTTCATGCTCCATATATAGATAGGGAAAACATGCCTT
				(GAAGGTCGGAGTCAACGGATTCATATATAGATAGGGAAAACATGCCTG)
				[GTACAAGATGAATGGAAACGAGATGTTAAA]
KWH93	IAAV6961	0.231		GAAGGTGACCAAGTTCATGCTATCAAGATTATGAAGGCCCGCAC
				(GAAGGTCGGAGTCAACGGATTCATCAAGATTATGAAGGCCCGCAT)
				[TGGACTGCGGGGAAGTTCAACATTT]
KWH94	Ku_c19745_892	0.209		GAAGGTGACCAAGTTCATGCTATTGTGTCGTGTAGAGGCACATACT
				(GAAGGTCGGAGTCAACGGATTGTGTCGTGTAGAGGCACATACC)
				[CGTGGAACTTCGGAATCACTCAGAT]
KWH95	Ku_c43151_811	0.187		GAAGGTGACCAAGTTCATGCTTGAAGCTGTGAGACTCGAC
				(GAAGGTCGGAGTCAACGGATTGCTTGAAGCTGTGAGACTCGAT)
				[ATACATCACCATTGATTCTGTTTGGCGAT]

KWH96	Kukri_c10243_475	0.187		GAAGGTGACCAAGTTCATGCTAACGCTGTCCTTGATCAGGAACAT
				(GAAGGTCGGAGTCAACGGATTCGCTGTCCTTGATCAGGAACAG)
				[GAGCTTGGGAGGCAACTTAGAGATA]
KWH97	Kukri_c18055_1740	0.022		GAAGGTGACCAAGTTCATGCTTTGCAACATGCTTTATGTGCCAC
				(GAAGGTCGGAGTCAACGGATTCCTTTGCAACATGCTTTATGTGCCAT)
				[CACGACGACGACCCATGCCATA]
KWH98	Kukri_c28968_130	0.187		GAAGGTGACCAAGTTCATGCTGGAAGGTCTGAGTTTCCGATCAATT
				(GAAGGTCGGAGTCAACGGATTGAAGGTCTGAGTTTCCGATCAATC)
				[CATGCATCAATATTGGGCTGAAATGTGTT]
KWH99	Kukri_c33620_129	0.220		GAAGGTGACCAAGTTCATGCTACTCCAACATGAATGTGACGTCAAG
				(GAAGGTCGGAGTCAACGGATTCACTCCAACATGAATGTGACGTCAAT)
				[AAGTGGAAGACTGGGATGGGCAAA]
KWH100	Kukri_c5693_1983	0.231		GAAGGTGACCAAGTTCATGCTCGGGAGACAGATGACCTCTA
				(GAAGGTCGGAGTCAACGGATTCGGGAGACAGATGACCTCTG)
				[CAGCTCAGGTAGCAGAACTGATCAA]
KWH101	Kukri_c57593_79	0.275		GAAGGTGACCAAGTTCATGCTATGTCCACAGTAGGCTAAATGGACA
				(GAAGGTCGGAGTCAACGGATTGTCCACAGTAGGCTAAATGGACG)
				[ACGCTCACTGCTGAGGCTCCAT]
KWH103	Kukri_c7284_1859	0.187	*	GAAGGTGACCAAGTTCATGCTGACATAAGCGGAGTGCACTGC
				(GAAGGTCGGAGTCAACGGATTCGACATAAGCGGAGTGCACTGT)
				[AGAAACTCACTCTGAATACCACAACAGTT]
KWH105	Kukri_c855_2107	0.231		GAAGGTGACCAAGTTCATGCTATCTCGTTTCCATTCATCTTGTACTTATA
				(GAAGGTCGGAGTCAACGGATTCTCGTTTCCATTCATCTTGTACTTATG)
				[CCACTCTATGTTTGAGACAATGTAACCTA]
KWH107	Kukri_rep_c70481_509	0.220	*	GAAGGTGACCAAGTTCATGCTGGATGGCGTCCACATATGGTG
				(GAAGGTCGGAGTCAACGGATTGGGATGGCGTCCACATATGGTA)
				[GCGTTCAAACAGTATCTCGAGGAGTA]
KWH108	Kukri_rep_c98227_230	0.275		GAAGGTGACCAAGTTCATGCTAAGTTCAACATGATGGTGAAAGACCA
				(GAAGGTCGGAGTCAACGGATTAGTTCAACATGATGGTGAAAGACCG)
				[GGCCCTCTTGTGTTTTACATTCCGTT]
KWH109	Kukri_rep_c98227_390	0.275		GAAGGTGACCAAGTTCATGCTGCGGTCGAGTCCCTCGTGTTT

				(GAAGGTCGGAGTCAACGGATTCGGTCGAGTCCCTCGTGTTC)
				[GGATGCCTCCATAGGTGGCGAA]
KWH110	Ra_c114158_328	0.374	*	GAAGGTGACCAAGTTCATGCTGAGAGCTGACCAATTTGGCAATTC
				(GAAGGTCGGAGTCAACGGATTATGAGAGCTGACCAATTTGGCAATTA)
				[CAGAGGAATTAACTGAAGAGGATTCACAT]
KWH111	RAC875_c11283_379	0.198		GAAGGTGACCAAGTTCATGCTCCAGGCCTACGTCTGCGTAT
				(GAAGGTCGGAGTCAACGGATTCCAGGCCTACGTCTGCGTAC)
				[CACACTCGCCAGCGTCACCAT]
KWH112	RAC875_c15215_417	0.022		GAAGGTGACCAAGTTCATGCTGGCATTCAAACTTATTGACAAGGGC
				(GAAGGTCGGAGTCAACGGATTGGCATTCAAACTTATTGACAAGGGT)
				[CAATGCCGTGCACATGCCGGTT]
KWH113	RAC875_c18550_228	0.022		GAAGGTGACCAAGTTCATGCTGAAACCGCTAGAGAAATATACAG
				(GAAGGTCGGAGTCAACGGATTGCTGAAACCGCTAGAGAAATATACAA)
				[GGCTCTAGGCACCGAACTGCAA]
KWH114	RAC875_c18550_65	0.022		GAAGGTGACCAAGTTCATGCTACGATACCAATTCATCATCCGTAGC
				(GAAGGTCGGAGTCAACGGATTAAACGATACCAATTCATCATCCGTAGT)
				[CACCATTCCTACCTAGTAGTGCCTA]
KWH115	RAC875_c18668_513	0.022		GAAGGTGACCAAGTTCATGCTGGTGTAATTGACAGTGAGTATGTCG
				(GAAGGTCGGAGTCAACGGATTCGGTGTAATTGACAGTGAGTATGTCA)
				[GGTCCTCCTAATGATGGTATCCCTT]
KWH117	RAC875_c38483_151	0.022		GAAGGTGACCAAGTTCATGCTGGCGTTTCTAGTCTGCTCTTGG
				(GAAGGTCGGAGTCAACGGATTATGGCGTTTCTAGTCTGCTCTTGA)
				[GACGCTCAGATTGTACGATCAAGGAT]
KWH118	RAC875_c6911_412	0.022		GAAGGTGACCAAGTTCATGCTACACTTGATTTCCACACCTTCCTTG
				(GAAGGTCGGAGTCAACGGATTGACACTTGATTTCCACACCTTCCTTA)
				[GAATACCCATCCATTATCAAGGAGAAGAA]
KWH120	RAC875_rep_c105769_150	0.385		GAAGGTGACCAAGTTCATGCTTTCTCTCCAGAACGAGGC
				(GAAGGTCGGAGTCAACGGATTCTGCTTTCTCTCCAGAACGAGGA)
				[TCTTCCCACGTGGTTTCCTTGCAAA]
KWH121	RAC875_rep_c115178_103	0.231		GAAGGTGACCAAGTTCATGCTATCAAACTCTTTACCCTCATGATCCA
				(GAAGGTCGGAGTCAACGGATTCAAACTCTTTACCCTCATGATCCG)

			[TCTTGATGCACAAGCAAAGCCGCTT]
KWH123	RAC875_rep_c83934_91	0.209	GAAGGTGACCAAGTTCATGCTAAAGCAGCATTTTCTGTGTTCTCCATT
			(GAAGGTCGGAGTCAACGGATTAGCAGCATTTTCTGTGTTCTCCATC)
			[CGACAGTGCTGCTCAAGTGTGATTA]
KWH124	RFL_Contig4980_884	0.022	GAAGGTGACCAAGTTCATGCTGGTTTCCGAAGGCTTAGAAATCACA
			(GAAGGTCGGAGTCAACGGATTGTTTCCGAAGGCTTAGAAATCACG)
			[TTGATAGATCAGGATCCAATGCAGGTT]
KWH126	TA002896-0591	0.231	**CONVERSION TO KASP UNSUCCESSFUL**
KWH129	Tdurum_contig14983_299	0.022	GAAGGTGACCAAGTTCATGCTACCTCGTGCCTCGCCTCCA
			(GAAGGTCGGAGTCAACGGATTCCTCGTGCCTCGCCTCCG)
			[CCCGGTGCGAACCAAAATGGAATA]
KWH130	Tdurum_contig17062_221	0.286	GAAGGTGACCAAGTTCATGCTGTCCACCGTCTGCCTCAGC
			(GAAGGTCGGAGTCAACGGATTGAGTCCACCGTCTGCCTCAGT)
			[TCGTCTTCCCGGTGGTCCACTT]
KWH132	Tdurum_contig27856_230	0.209	GAAGGTGACCAAGTTCATGCTGTCTGGAGTATCTGTTGCTTAGTTTT
			(GAAGGTCGGAGTCAACGGATTGTCTGGAGTATCTGTTGCTTAGTTTC)
			[CATTTCATCGACAGACCGAGCACAT]
KWH133	Tdurum_contig29240_206	0.275	GAAGGTGACCAAGTTCATGCTCCAACATACTCTACACTAAATTAACGTTAA
			(GAAGGTCGGAGTCAACGGATTCAACATACTCTACACTAAATTAACGTTAG)
			[AAGAAATTCAGTGTATGTTGGTTACACGTT]
KWH134	Tdurum_contig42487_1555	0.176	GAAGGTGACCAAGTTCATGCTCAGAAAGTCATTGTATTATGGAAGGC
			(GAAGGTCGGAGTCAACGGATTAATCAGAAAGTCATTGTATTATGGAAGGT)
			[CACTCTCGGGAACGAAAAACTTATAAGTA]
KWH135	Tdurum_contig48870_249	0.022	GAAGGTGACCAAGTTCATGCTCGTGCCCCAGCATGATCTTTTGA
			(GAAGGTCGGAGTCAACGGATTGTGCCCCAGCATGATCTTTTGC)
			[TTTGGGCGACTGGCATTTCGGAAAA]
KWH136	Tdurum_contig54832_139	0.286	GAAGGTGACCAAGTTCATGCTGTTGTAGATGATCTAGCTGTGTTTTTG
			(GAAGGTCGGAGTCAACGGATTCTGTTGTAGATGATCTAGCTGTGTTTTTA)
			[CGGGCAACTACACAGGAAGAAAGAA]
KWH137	Tdurum_contig59467_433	0.198	GAAGGTGACCAAGTTCATGCTCATCCATCTCCTCTATTTCAGTCTTT
			(GAAGGTCGGAGTCAACGGATTCATCCATCTCCTCTATTTCAGTCTTG)

				[CCTGTATATATGTATACCCTACTGTGTCTT]
KWH142	tplb0024d01_1740	0.022		GAAGGTGACCAAGTTCATGCTATAATGACTTCGTTTGCAAATTGCTCG
				(GAAGGTCGGAGTCAACGGATTGAATAATGACTTCGTTTGCAAATTGCTCA)
				[CTGTTACCGACAGTGTGGCACATAA]
KWH143	tplb0032g12_1241	0.275		GAAGGTGACCAAGTTCATGCTGCCCTCCATAAAATGCGCATCG
				(GAAGGTCGGAGTCAACGGATTCGCCCTCCATAAAATGCGCATCA)
				[TGCATGCTCCCATGTACAACAGCAA]
KWH144	tplb0051f21_452	0.286	*	GAAGGTGACCAAGTTCATGCTAGAGGTCGTGGCGCACGGT
				(GAAGGTCGGAGTCAACGGATTGAGGTCGTGGCGCACGGC)
				[CCGCCAACATCTCCACCGAGAA]
KWH145	wsnp_CAP11_rep_c4346_2050918	0.209		GAAGGTGACCAAGTTCATGCTGAGCAGCACAATGGTCGAGAG
				(GAAGGTCGGAGTCAACGGATTGGAGCAGCACAATGGTCGAGAA)
				[CAGGCACGGCGCTGGAGAGAA]
KWH146	wsnp_CAP11_rep_c4346_2051052	0.209		GAAGGTGACCAAGTTCATGCTCCGGCCAGCAAGATGCAAATAC
				(GAAGGTCGGAGTCAACGGATTCCCGGCCAGCAAGATGCAAATAT)
				[CTTGGCCGTTAATTTACAGTATATGGGTA]
KWH148	wsnp_Ex_c24486_33732900	0.231		GAAGGTGACCAAGTTCATGCTCTTCGCTTGTGTTAATCAAGATGC
				(GAAGGTCGGAGTCAACGGATTCTCTTCGCTTGTGTTAATCAAGATGT)
				[TGAGGATGCTAGGAGGGAAGACAAT]
KWH149	wsnp_Ex_c48558_53427000	0.209		GAAGGTGACCAAGTTCATGCTGTTCTTTGGATCATCCACATATTTTGCA
				(GAAGGTCGGAGTCAACGGATTCTTTGGATCATCCACATATTTTGCG)
				[CCAAAATGAAGCCATGTATGGGCAGTT]
KWH150	wsnp_Ex_c53387_56639949	0.187	*	GAAGGTGACCAAGTTCATGCTAACGTGTCATCAGCTGGAAGTGTT
				(GAAGGTCGGAGTCAACGGATTCGTGTCATCAGCTGGAAGTGTC)
				[GTATCTACACTAAATGTTTGCTTGAAGCTA]
KWH151	wsnp_Ex_c53387_56641291	0.187	*	GAAGGTGACCAAGTTCATGCTAGCAGCAACAGACTTCCCAGAG
				(GAAGGTCGGAGTCAACGGATTCAGCAGCAACAGACTTCCCAGAA)
				[GATTAGTATGACTATCATGCTGCTGCAAA]
KWH152	wsnp_Ex_c5839_10246812	0.187	*	GAAGGTGACCAAGTTCATGCTGTGTTGGTACACAATACGTATGCG
				(GAAGGTCGGAGTCAACGGATTCGTGTTGGTACACAATACGTATGCA)
				[GTGGGTAGCATTCTTTTTTCTTGTGACTT]

KWH154	wsnp_Ex_c6142_10746442	0.187	*	GAAGGTGACCAAGTTCATGCTGCAGCAAGAAGAGAAAGAAAGGATC
				(GAAGGTCGGAGTCAACGGATTGGCAGCAAGAAGAGAAAGAAAGGATT)
				[TGCAGCAGCTTCAGCGGCCTT]
KWH155	wsnp_Ex_c6961_11998812	0.231		GAAGGTGACCAAGTTCATGCTGCAATTTGTACATTACTCTCACCTGAAT
				(GAAGGTCGGAGTCAACGGATTCAATTTGTACATTACTCTCACCTGAAG)
				[GAGATTGCACCTGCTGAGAGCTTAA]
KWH156	wsnp_Ex_c6961_12000176	0.231		GAAGGTGACCAAGTTCATGCTAACACTCTGTATGCTGTCTGGCAT
				(GAAGGTCGGAGTCAACGGATTCACTCTGTATGCTGTCTGGCAC)
				[CCGGACAGCATACAGAGTGTTGAT]
KWH157	wsnp_Ex_c916_1767286	0.187	*	GAAGGTGACCAAGTTCATGCTATAGAAGAAATTGTTGACTCGCTCAAC
				(GAAGGTCGGAGTCAACGGATTCATAGAAGAAATTGTTGACTCGCTCAAT)
				[GGAGACCTCGGTAACTTTTTGGCTT]
KWH162	wsnp_Ku_c4035_7363089	0.385		GAAGGTGACCAAGTTCATGCTCCCGATTAGTAAGATCACATAGCC
				(GAAGGTCGGAGTCAACGGATTGCCCGATTAGTAAGATCACATAGCT)
				[GTCTCATACAATCAATGAGGAACTAAAGTT]
KWH163	wsnp_Ku_c44760_51961180	0.385		GAAGGTGACCAAGTTCATGCTGAATTAACTGAAGAGGATTCACATGCAT
				(GAAGGTCGGAGTCAACGGATTAATTAACTGAAGAGGATTCACATGCAG)
				[CCATGAGAGCTGACCAATTTGGCAA]
KWH164	wsnp_Ku_c5693_10079278	0.231		GAAGGTGACCAAGTTCATGCTTTGAACTTACACGAGGAGGCTCTA
				(GAAGGTCGGAGTCAACGGATTGAACTTACACGAGGAGGCTCTC)
				[CCTAAACAGGCGAATTTGCCCAGTT]
KWH165	wsnp_Ra_c19741_28965647	0.385		GAAGGTGACCAAGTTCATGCTCACTATCAATGATATAGAAGGATCTTTCA
				(GAAGGTCGGAGTCAACGGATTCACTATCAATGATATAGAAGGATCTTTCG)
				[AAGGGCGCACTTTCGGCTTGAAAA]
KWH166	wsnp_Ra_c389_826890	0.022		GAAGGTGACCAAGTTCATGCTCGAGAACTAGGTTACCTCGTGA
				(GAAGGTCGGAGTCAACGGATTCGAGAACTAGGTTACCTCGTGG)
				[CCCATGAGTTTGTTGTGTTGAAGTTCTAA]
KWH167	wsnp_Ra_c7112_12318340	0.187	*	GAAGGTGACCAAGTTCATGCTCAGCCTTGTTGTTAGAAGATGGTC
				(GAAGGTCGGAGTCAACGGATTCCAGCCTTGTTGTTAGAAGATGGTT)
				[AACGACGTCAATTTCTTCATCTGAGCAT]
KWH168	wsnp_RFL_Contig2805_2579582	0.286		GAAGGTGACCAAGTTCATGCTAGAAACAGACGGCAGCACTACC

(GAAGGTCGGAGTCAACGGATTAAAGAAACAGACGGCAGCACTACT)

[AGAGATCGGCCATCTTTTTATGACCTAT]

[a] iSelect recombination fraction

[b] LG47 = marker present in Cabral et al. (2014) consensus map in linkage group 47, representing chromosome 7AL

[c] Representative marker selected for a co-segregating group of SNPs

Appendix 8.3 Summary of infection types collected for *P. triticina* race TDBG, BBBD, MBDS, and MGBJ and alleles amplified for SSR molecular marker *cfa2240* on a diverse set of wheat germplasm.

Genotypes	TDBG IT	TDBG rxn	cfa 2240	BBBD	12-3 MBD S	74-2 MGBJ	Wheat class	Year	Locality	Pedigree	Reference
8829(BW8312)	;	R	A	0	1^{-}	$;1^{=}$				No data available	
Knudson (N96-0144)	$;1^{=}$	R	A	0	$;1^{=}$	$;1^{=}$	HRSW	2003	US	KARL/KRONA/3/BERGEN//ERIK/MN-73167	A
ND643	$;1^{-}3^{-}$ X	R	A	0	0	;				No data available	
Cunningham	$;1^{-}$	R	A	0	;	0;		1999	AUS	3-AG-3/4*CONDOR//COOK	A
93464	X	R	A	0	3^{+}	3^{+}	CWRS		CAN	CIANO-67/BLUEBIRD//GALLO/3/GAINES/OROFEN(SIB)//BEZOSTAYA-1/GABO//MAGNIF-27/3/NE-75437	A
BW591	X^{-}	R	A	3	3^{+}	3^{-}	CWRS	1984	CAN	Fortuna/Chris/Chester/Canuck	A
BW717	X^{+}	R	A	3	3^{+}	$1^{+}3^{-}$		1996		ND643/BW621//BW591	B
LEADER (BW535)	$;1^{-}3^{-}$ X-	R	A	3	3^{+}	3	CWRS	1981	CAN	Fortuna/Chris	C
BW710	$;1^{=}3^{-}$ X	R	A	;	3^{+}	3^{+}		1995		CDC_Teal/BW608//BW621	B
BW807 = 9505-LP03A	X^{+}	R	A	;	;	$;1^{=}$		1984	CAN	L.S.3.1./PITIC-62//INIA-66/3/CORRECAMINOS/INIA-66	A
DM5637*B8	$;1^{-}X^{-}$	R	A	;	;	;				No data available	
Hamer	X	R	A	;	1^{+}	3^{-}		1995	US	BERGEN/N-86-0111	A
Janz	3^{+}	S	A	;	0	0		1989	AUS	3-AG-3/4*CONDOR//COOK	A
8802-EB3C	$;1^{=}$	R	A	$;1^{=}$	3^{+}	$1^{+}3^{-}$				No data available	
8901-DL1C	$;1^{-}3^{-}$ X	R	A	$;1^{=}$	;	;				No data available	
BW608	$;1^{=}X$	R	A	$;1^{=}$	3^{+}	3^{-}	CWRS	1986	CAN	7504-78/Columbus	A
BW621	$;1^{=}3^{-}$ X	R	A	$;1^{=}$	3^{+}	3	CWRS	1987	CAN	LEADER/COLUMBUS//7504-78	A
MN93413=McVey	$;1^{=}$	R	A	$;1^{=}$	1^{+}	3^{-}				No data available	
Lillian = BW776	;	R	A	$1^{-}2^{-}$	$;1^{=}$	$;1^{-}2^{-}$	CWRS	2003	CAN	BW-621*3/90-B-07-AU-2-B	C
Ceres	X	R	A	$1^{-}2^{-}$	3^{+}	3^{+}	CWRS	1926	US	Marquis / Kota	D
Chester	$;^{-};1^{=}$	R	A	3^{-}	12	$2^{+}3^{-}$	CWRS	1976	CAN	RENOWN/S-615//RESCUE/3/KENDEE/4/MIDA/CADET	C
Lancer	X	R	A	3^{-}	3	3^{+}	CWRS	1985	CAN	(Turkey*Cheyenne)*(Hope*(Cheyenne)2)	E

AC ABBEY(BW691)	X+	R	A	3+	3+	3-	CWRS	1998	CAN	(93464/BW608)/BW591	A
AC EATONIA (BW642)	;1-3- X	R	A	3+	3+	3	CWRS	1993	CAN	Lancer/Leader	A
CANUCK (BW004)	;1=3- X	R	A	3+	3+	3+	CWRS	1974	CAN	Canthatch*((Mida*Cadet)*Rescue)	C
Rescue	X	R	A	3+	3+	1+3-	CWRS	1946	CAN	Apex*S 615	E
ND2827 (2)	;	R	A	3+	3+	3+			US	SUMAI-3/STOA	A
Garnet	;1	R	A	3+	3+	3+		1926	CAN	Preston A*Riga	E
BW666	X	R	A	3+	3+	3-		1992	CAN	BW-591/7902-GK-3-C	A
Cypress	X	R	A	3+	3+	3	CWRS	1962	CAN	Rescue*Chinook	C
Burnside	;	R	A	NA	NA	NA	CWES	2004	CAN	(Pasqua*2/ND643/RL4452)/Glenlea	C
Fortuna	;1=	R	A	NA	NA	NA	CWRS	1966	US	Rescue / Chinook / (Frontana / Kenya 58 / Newthatch)	D
McMurachy-Exchange	;	R	A	NA	NA	NA	CWRS		CAN	McMurachy/Exchange	A
Parula	;1=	R	A	NA	NA	NA	CWRS			FKN/3/2*Frontana//Kenya 350 AD.9C.2/Gabo 55/4/Bluebird/Chante	F
Kitt	;1=	R	A	NA	NA	NA	CWES	1975	US	MNII-55-14*MNII-60-15	E
McMurachy	X	R	A	NA	NA	NA	CWRS	1937	CAN	Garnet selection	A
BW688	;1=	R	B	0	0	0					
ND721	; -;1=	R	B	0	;	0;					
ND744	; -;1=	R	B	0	0;	0;					
Benito	3	S	B	0	0;	;					
Stanley	;1=	R	B	3	3+	3+					
Chinook	3	S	B	3	3+	3					
Canthatch	3+	S	B	3	3+	3+					
BW828	;1=3- X	R	B	;	;	;					
Grandin	1+	R	B	;	;	;					
Lee	X	R	B	;	3	3-					
McKenzie	;1-	R	B	;	;1=	;1=					
Pasqua	;1=	R	B	;	;	;1=					
AC Cora	;1=	R	B	;	0;	0;					
Stoa	1+	R	B	;	0	;					

AC Intrepid	$2^{+}3$	R	B	;	$;1^{-}$	$;1^{=}$					
Pembina	X	R	B	;	1^{+}	3^{+}					
AC Cadillac	1^{+}	R	B	;	3^{-}	3					
AC Domain	3	S	B	;	$1^{-}2^{-}$	3					
Invader	3	S	B	;	1^{+}	3^{-}					
Roblin	3	S	B	;	3^{-}	$;1^{-}2^{-}$					
Sunstate	3	S	B	;	3^{-}	$;1^{=}1^{-}$ n					
CDC Merlin	3	S	B	;	1^{+}	$1^{+}3^{-}$					
Hartog	3^{+}	S	B	;	3^{+}	3^{-}					
Harvest	3^{+}	S	B	;	1^{+-}	$1^{+}3^{-}$					
94B30-C6D	3^{+}	S	B	;	3^{+}	3^{-}					
AC Elsa	3	S	B	;	1^{-}	$1^{-}2^{-}$					
CDC Teal	3^{+}	S	B	;	3^{-}	1^{+}					
AC Minto	$1^{+}3^{-}$	MR	B	$;1^{-}$	$;1^{-}$	$;1^{=}$					
Columbus	1	R	B	$;1^{-}$	1^{+}	$1^{+}3^{-}$					
RL 4137	1^{+}	R	B	$;1^{-}$	$;1^{-}$	3^{+}					
Prodigy	3	R	B	$;1^{-}$	1^{+}	3^{-}					
Kanata	1^{+}	R	B	$;1^{-}$	1^{+}	3^{+}					
Kenyon	1^{+}	R	B	$;1^{-}$	1^{+}	1^{+}					
BW303	3	S	B	$;1^{-}$	2^{+}	3^{+}					
AC Splendor	3^{+}	S	B	$;1^{-}$	1^{-}	1^{-}					
Code 15	;	R	B	$;1^{=}$	3^{-}	$;1^{-}$					
AC Barrie	X	R	B	$;1^{=}$	1^{-}	3					
Selkirk	X	R	B	$;1^{=}$	1+	3^{+}					
AC Majestic	1^{+}	R	B	$;1^{=}$	1^{-}n	1^{-}					
8021-V2	3^{+}	S	B	$;1^{=}$	3^{+}	3^{+}					
MILAN 13	3^{+}	S	B	0;	3^{+}	3					
Norseman	3^{+}	S	B	0;	0	;					
Pacific	3	S	B	0;	3^{+}	3^{+}					

Canus	3?	S	B	1-2-	3+	3+					
AC Vista	X?	S	B	22+	3+	3-2+					
Redman	X?	R	B	2-3-	3+	3+					
AC Michael	3	S	B	2-3X	3+	3-					
Park	1+/3	MR	B	3-	3-	3					
Manitou	3	S	B	3-	3+	3+					
Katepwa	3+	S	B	3-	3+	3+					
Alikat	3+	S	B	3-	3-	3					
Neepawa	3+	S	B	3-	3-	3-					
Marquis	;1=	R	B	3+	3	3+					
Huron	; -;1=	R	B	3+	3+	3+					
Red Fife	; -;1=	R	B	3+	3+	3+					
Coronation	;1-	R	B	3+	3+	3					
Broatch's Whitehead	;1=	R	B	3+	3+	3+					
Percy	;1=	R	B	3+	3+	3+					
Preston	;1=	R	B	3+	3+	3+					
Renfrew	;1=	R	B	3+	3+	3+					
White Fife	;1=	R	B	3+	3+	3+					
Lake	X	R	B	3+	3+	3-					
Suprime	X	R	B	3+	3+	3+					
Conway	3	S	B	3+	3+	3+					
Kota	3	S	B	3+	3+	3+					
Ruby	3	S	B	3+	3+	3+					
Thatcher	3	S	B	3+	3+	3+					
Weebill-1	3	S	B	3+	3-	3+					
Ladoga	3+	S	B	3+	3+	3+					
Prelude	3+	S	B	3+	3+	3					
Saunders	3+	S	B	3+	3+	3+					
CDC Makwa	3	S	B	33-	3+	3-					

Frontana	3^{+}	S	B	X^{-}	3^{-}	X^{+}					
Parshall	$1^{+}3$	MR	B	NA	NA	NA					
Chinese Spring	$X/3^{+}$	MR	B	NA	NA	NA					
Helios	$1^{+}3^{-}$	MR	B	NA	NA	NA					
Goodeve	$1^{+}3^{-}$	MR	B	NA	NA	NA					
Genesis	$;1^{-}$	R	B	NA	NA	NA					
Glenn	$;1^{-}$	R	B	NA	NA	NA					
KANE	$;1^{-}$	R	B	NA	NA	NA					
Romany	$;1^{=}$	R	B	NA	NA	NA					
Sonora64	$;1^{=}$	R	B	NA	NA	NA					
CDC Rama	$;\,^{-};1$	R	B	NA	NA	NA					
Field Star	$;\,^{-};1^{-}$	R	B	NA	NA	NA					
5700PR	$;\,^{-};1^{=}$	R	B	NA	NA	NA					
Lovitt	1^{-}	R	B	NA	NA	NA					
Bluesky	$;1\,^{-}X$	R	B	NA	NA	NA					
5600HR	$;1^{=}$	R	B	NA	NA	NA					
Norpro	$;1^{=}$	R	B	NA	NA	NA					
Oslo	$;1^{=}$	R	B	NA	NA	NA					
Tobari66	1^{+}	R	B	NA	NA	NA					
Infinity	1^{-}	R	B	NA	NA	NA					
Selkirk-8-Exchange	1^{+}	R	B	NA	NA	NA					
Journey	1^{+}	R	B	NA	NA	NA					
Snowbird	1^{+}	R	B	NA	NA	NA					
Cutler	1^{-}	R	B	NA	NA	NA					
Red Bobs	;X	R	B	NA	NA	NA					
5500HR	1^{-}	R	B	NA	NA	NA					
Carberry	1^{+}	R	B	NA	NA	NA					
HY344	X	R	B	NA	NA	NA					
Stettler	1^{+}	R	B	NA	NA	NA					

AC Taber	3	S	B	NA	NA	NA					
AC2000	3	S	B	NA	NA	NA					
CT262	3	S	B	NA	NA	NA					
Glenlea	3	S	B	NA	NA	NA					
Muchmore	3	S	B	NA	NA	NA					
Salamouni	3	S	B	NA	NA	NA					
Shaw	3	S	B	NA	NA	NA					
Sumai 3	3	S	B	NA	NA	NA					
Waskada	3	S	B	NA	NA	NA					
Caranzinho	3	S	B	NA	NA	NA					
Synthetic	3+	S	B	NA	NA	NA					
Peace	3+	S	B	NA	NA	NA					
Norquay	3+	S	B	NA	NA	NA					
AC Superb	3+	S	B	NA	NA	NA					
Alvena	3+	S	B	NA	NA	NA					
Amazon	3+	S	B	NA	NA	NA					
CDC Abound	3+	S	B	NA	NA	NA					
CDC Go	3+	S	B	NA	NA	NA					
Chris	3+	S	B	NA	NA	NA					
Foremost	3+	S	B	NA	NA	NA					
Glenavon	3+	S	B	NA	NA	NA					
Kenya Farmer	3+	S	B	NA	NA	NA					
Maringa	3+	S	B	NA	NA	NA					
Wildcat	3+	S	B	NA	NA	NA					
CDC Imagine	3+	S	B	NA	NA	NA					
CDC Walrus	3+	S	B	NA	NA	NA					
McNeal	3+	S	B	NA	NA	NA					
Fasan	;-;1-	R	NA	NA	NA	NA					
Macon	;-;1=	R	NA	NA	NA	NA					
Lang	;1=	R	NA	NA	NA	NA					

Lolo	;1-X	R	NA	NA	NA	NA					
AC Crystal	X	R	NA	NA	NA	NA					
AC Karma	X	R	NA	NA	NA	NA					
Diamondbird	3	S	NA	NA	NA	NA					
AC Corinne	3+	S	NA	NA	NA	NA					
Biggar	3+	S	NA	NA	NA	NA					
Laser	3+	S	NA	NA	NA	NA					
HW320	3+	S	NA	NA	NA	NA					
Laura	3	S	NA	;	;1-	;1=					
Sinton	3	S	NA	;	3+	1+3-					
Alsen	3+	S	NA	;	0	;					
Reward	3/X	MR	NA	3+	3+	3+					
Regent	X-3?	MR	NA	3+	3+	3+					
Reliance	3	R	NA	3+	3+	3+					
Nandu	;	R	NA	3+	3+	3+					
Apex	X	R	NA	3+	3+	3+					
Renown	;1=	R	NA	3+	3+	3+					
RL6058	3	S	NA	NA	NA	NA					

A = GIRS, B = McCallum unpublished, C = McCallum and DePauw 2008, D = NDSU Variety Development, E = BBSRC, F = Herrera-Foessel et al. 2012, Year = year of registration, R = resistance, S = susceptible, IT = infection type, rxn = reaction, NA = data not available, HRSW = hard red spring wheat, CWRS = Canada western red spring, CWES = Canada western extra strong, CAN = Canada, US = United States of America, AUS = Australia

Appendix 8.4 Microsatellite markers used to genotype chromosome 7AL in the *LrCen*/*Lr20* genomic region of lines from Table 5.2 that carried the *Lr20*-positive allele of STS638.

SSR	Annealing Tm (°C)	Forward Sequence	Reverse Sequence	Reference
barc1166	58	GCGATGCTCACATGGAAGTCTTGAACTC	GCGTGTACTCATTCTGCCCCCTCA	A
barc121	51	ACTGATCAGCAATGTCAACTGAA	CCGGTGTCTTTCCTAACGCTATG	A
barc195	51	CCCACATGTCATTGGCTGTTTAA	GCCCGGCCCAGAACGATTTAAATG	A
barc231	58	GCGATCAATAACCGTGCCACCA	GCACTTGCGATGTCACTAAAATG	A
barc253	50	GGGAAGACACGACACGACTC	TCGTAAGATTACCTCGGATGAAGAA	A
barc292	51	GCGTGTGAGTCAATCCGTGCTTTAT	GCGTTGGTTTTAAGAGGTGCCTGAA	A
barc49	51	GTCCCACCAAATTAACAGCTCCTA	AGGCGCAGTGCTCGAAGAATATTAT	A
cfa2019	60	GACGAGCTAACTGCAGACCC	CTCAATCCTGATGCGGAGAT	B
cfa2040	51	TCAAATGATTTCAGGTAACCACTA	TTCCTGATCCCACCAAACAT	B
cfa2240	61	TGCAGCATGCATTTTAGCTT	TGCCGCACTTATTTGTTCAC	B
cfa2257	61	GATACAATAGGTGCCTCCGC	CCATTATGTAAATGCTTCTGTTTGA	B
gpw1100	60	AGGAATAGTCCTTCTCCCGC	GCCACCTTCTTATTCATGGC	B
gpw1164	60	TTCATGAAGGGTGTTGTCCA	TCTTCTCCCACCTGTGCTCT	B
gpw2083	60	AGATGGTAACTGGGCAATGG	GAATTGGGGGTCAGCATCTA	B
gpw209	60	TGTTGGGGAAGGATCTGAAG	CTTCCTCGGGAAGAAAAAGG	B
gpw2092	60	GTGAGAAACTAGCGCAACCC	AATTACACCCCTGTCATCGC	B
gpw2103	60	CGTATGCAGCATGGCATC	GCTATGTTGTGTGGCATTGG	B
gpw2118	60	ACCTTGCTCTTGTCGTCCTG	TTGTCGCTGCTCTTCAACTG	B
gpw2203	60	ACCACAACGTCACAAGTGGA	AACTCCGTCTTGTTGATGCC	B
gpw2252	60	GTGGTGCTATTGTGTGGGTG	CCAGATCGAGGTGAATCCAT	B
gpw2264	60	TTGCTTTCCCAAATTGTGCT	GGCATTGAGAATCCAAGCAT	B
gpw2269	60	CACATCAACAGGTCCTCTTCTA	CTAGCTGGTGGTGGTCTTGG	B
gpw2308	60	GGAGGAACCGAATCCAGAGT	GAGGCCGATCACATAAAGGA	B
gpw2338	60	TAGGCAATTCGACAGGGATG	AAGAGTTGGCGGAAGTAGCA	B
gpw3038	60	ACACTTACACGCCCATCTCC	AAGTCAAGGAGGAGGCCATT	B
gpw3127	60	TGTTAGCAGAATAGACCCACCC	CATCATCCTCAATGCCACAG	B
gpw3213	60	CGGAGATAATGGCTGGAAAA	TGATGAACAACACAAGGGACA	B

gpw3278	60	ATCACCATGATCTTGAGTGT	ATGTACCAGTAGCTATGTGTT	B
gpw356	60	GCTCTTGGACCTGTGTTTTC	TGGCAATCATGGCTTATCAA	B
gpw4050	60	CTAAGAAAATGCGAGCACCC	GAGTGGACTCGTTGGTTGGT	B
gpw4066	60	CGCACGTTGTTCTCTATGCC	TGTGTGGGAGAGGGGTGT	B
gpw4092	60	TGAAAATGATTGCACACTTGC	ATCCAAGCCTTTGTTTGCTG	B
gpw4100	60	AGCGACTTCTTAACTGTTAC	CTATCAAGGCGACTAGATT	B
gpw4124	60	TGTGTGACAAGCGCCAGTTT	GTTCTGCTTGGAACTAGGATGG	B
gpw4386	60	AGGGTCAGCGCAACAATC	CGGGAAGCAATCTTACAAGTG	B
gpw7386	60	GAATCTTGGTTATGATCCGGG	TTCTCGATCCGCGTCTTC	B
gpw7486	60	ACGTGACGAGCAGTTGGC	ATTCCTCCCTCTCGGCTTC	B
gwm260	61	GCCCCCTTGCACAATC	CGCAGCTACAGGCC	C
gwm276	51	ATTTGCCTGAAGAAAATATT	AATTTCACTGCATACACAAG	C
gwm282	61	TTGGCCGTGTAAGGCAG	TCTCATTCACACACAACACTAGC	C
gwm332	60	AGCCAGCAAGTCACCAAAAC	AGTGCTGGAAAGAGTAGTGAAGC	C
gwm344	61	CAAGGAAATAGGCGGTAACT	ATTTGAGTCTGAAGTTTGCA	C
gwm4	61	GCTGATGCATATAATGCTGT	CACTGTCTGTATCACTCTGCT	C
gwm554	61	TGCCCACAACGGAACTTG	GCAACCACCAAGCACAAAGT	C
wmc139	61	TGTAACTGAGGGCCATGAAT	CATCGACTCACAACTAGGGT	D
wmc273	61	AGTTATGTATTCTCTCGAGCCTG	GGTAACCACTAGAGTATGTCCTT	D
wmc422	61	GGACTACTGAACTGGAGAGTGTG	GCATTAGAATTTGGAGTTTGGAG	D
wmc488	61	AAAGCACAACCAGTTATGCCAC	GAACCATAGTCACATATCACGAGG	D
wmc525	61	GTTTGACGTGTTTGCTGCTTAC	CTACGGATAATGATTGCTGGCT	D
wmc596	61	TCAGCAACAAACATGCTCGG	CCCGTGTAGGCGGTAGCTCTT	D
wmc603	61	ACAAACGGTGACAATGCAAGGA	CGCCTCTCTCGTAAGCCTCAAC	D
wmc607	61	ATATATGCCCATGAAGCTCAAG	GATCGAGCTAAAGCTGATACCA	D
wmc633	61	ACACCAGCGGGGATATTTTGTTAC	GTGCACAAGACATGAGGTGGATT	D
wmc65	61	TGGATGGGAAGGAGAATAAGTG	ATCCAACCGGAACTACCGTCAG	D
wmc790	60	AATTAAGATAGACCGTCCATATCATCCA	CGACAACGTACGCGCC	D

A = Song et al. 2005, B = Soudille et al. 2004, C = Roder et al. 1998, D = Somers and Isaac 2004, Tm = temperature

Appendix 8.5 Table of abbreviations

AFLP	Amplified Fragment Length Polymorphism
AI	Active Ingredient
APR	Adult Plant Resistance
BLAST	Basic Local Alignment Search Tool
BYDV	Barley Yellow Dwarf Virus
CRC-AAFC	Cereal Research Centre – Agriculture and Agri-Food Canada
DH	Double Haploid
DNA	Deoxyribonucleic Acid
DPI	Days Post Inoculation
IT	Infection Type
ITMI	International Triticeae Mapping Initiative
KASP	Kompetitive Allele Specific PCR
MAS	Marker Assisted Selection
MS	Moderately Susceptible
MR	Moderately Resistant
OP	Open Pollinated
PCR	Polymerase Chain Reaction
R	Resistant
RAPD	Random Amplified Polymorphic DNA

RCBD	Random Complete Block Design
RF	Recombination Fraction
RFLP	Restriction Fragment Length Polymorphism
S	Susceptible
SNP	Single Nucleotide Polymophism
SP	Self Pollinated
SSR	Simple Sequence Repeats
STS	Sequence-Tagged Site