Characterization and Genetic Mapping of Leaf Rust (*Puccinia triticina*) Resistance Genes *Lr2a* and *Lr46* in Canadian Spring Wheat (*Triticum aestivum*) Germplasm

By

Mallorie Kim Lewarne

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ABSTRACT

Of the fungal diseases that can infect bread wheat (*Triticum aestivum* L.), leaf rust, caused by *Puccinia triticina* Eriks. is the most common and widespread. Development of wheat cultivars with genetic resistance is a desirable control method, as it is cost effective and environmentally friendly. Pyramiding multiple resistance genes in a cultivar using conventional breeding techniques is often expensive and time consuming. Alternatively, marker assisted selection (MAS) allows for accelerated and accurate selection of resistance gene combinations.

The objectives of this study were to characterize two leaf rust resistance genes: an adult plant resistance (APR) gene, hypothesized to be *Lr46,* from wheat line BW278, and a seedling resistance gene, *Lr2a*, from wheat cultivar Superb. To characterize the APR, two mapping populations derived from BW278 were genotyped with the iSelect 90K wheat SNP array. Both populations were evaluated for leaf rust in inoculated field nurseries for five years. Quantitative trait locus (QTL) analysis revealed two QTL controlling resistance in the BW278/AC Foremost population, one in the region of interest, chromosome 1B and another on chromosome 5A. Two QTL were detected in Superb/BW278, on chromosomes 4B and 5B, however no QTL were detected in the region of interest on 1B. The QTL on 1B in BW278/AC Foremost, designated *QLr.mrdc-1B,* was tightly linked to both *csLV46G22* and *DK0900,* two markers previously described as tightly linked to the *Lr46* locus. Ten SNPs in the *QLr.mrdc-1B* region were selected for kompetitive allele-specific PCR (KASP) assay design.

To characterize *Lr2a*, two mapping populations derived from Superb (Superb/BW278 & Superb/86ISMN 2137) were genotyped with the iSelect 90 K wheat SNP array, and evaluated with a single race of *P. triticina* under greenhouse conditions. Two-point linkage analysis between the marker data and phenotypic infection type ratings revealed that the gene mapped to

chromosome 2DS in both mapping populations. The linkage maps generated for the two mapping populations had 11 SNP markers in common and displayed collinearity. Seven SNPs that either flanked or co-segregated with *Lr2a* in Superb/BW278 were selected for KASP assay design. Of the seven markers, kwh740 (*Excalibur_c1944_1017*) was polymorphic in both populations and displayed clear clusters, making it the most applicable for use in MAS.

CHAPTER 1

GENERAL INTRODUCTION

Common bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, providing 20% of human caloric intake, and is the main source of protein in developing nations (Curtis et al. 2002). Wheat leaf rust is caused by fungal pathogen *Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex. Desmaz. f. sp. *tritici*) and is an important and widespread disease threatening wheat production world-wide (Khan et al. 2013; Figueroa et al. 2018). Yield loss resulting from leaf rust infection can range from 5-25% (Oelke and Kolmer 2005) depending on genetics of the host cultivar, crop stage at the time of initial infection and environmental conditions.

Currently, there are over 81 characterized leaf rust resistance genes (Aktar-Uz-Zaman et al. 2017). Most of these genes are effective from the seedling stage to the adult plant stage but tend to break down relatively fast in the field due to the ability of *P. triticina* to evolve rapidly (Herrera-Foessel et al. 2012; Kolmer 2013). Seedling resistance genes that were deployed in several Canadian wheat cultivars, that are no longer effective against common pathogen races include *Lr1, Lr10, Lr13* and *Lr14a* (Randhawa et al. 2013). While chemical control of leaf rust is possible with synthetic fungicides, breeding for resistance tends to be the most desirable disease control method due to its economic and environmental consideration. The goal of breeding for resistance is to incorporate durable resistance genes into agronomically desirable cultivars. For resistance to be durable, it must remain effective over a long period of time when used widely in wheat growing regions (Aktar-Uz-Zaman et al. 2017). Race non-specific adult plant resistance genes are more durable than race-specific resistance genes due to their ability to confer multi-race and multi-pathogen resistance (Aktar-Uz-Zaman et al. 2017). To date, four of these genes

have been characterized in wheat and designated *Lr34* (Dyck 1987), *Lr46* (Singh et al. 1998), *Lr67* (Hiebert et al. 2010), and *Lr68* (Herrara-Foessel et al. 2012). An effective strategy to combat virulent *P. triticina* isolates is to either pyramid multiple race non-specific APR genes (Herrera-Foessel et al. 2012), or combine a single APR with one or more seedling resistance genes (Roelfs 1988; Dyck 1991; German and Kolmer 1992; Singh 1992)

Marker assisted selection (MAS) is a technique that utilizes the theory that segments of DNA located close together on a chromosome are likely to be inherited together in subsequent generations (Singh and Singh 2015). Markers are the segments of DNA located near the gene of interest and can be used to determine if an individual carries the desired gene (Dubcovsky 2004). The wheat genome is hexaploid, making genomic study challenging. However, the iSelect wheat 90k array developed by Wang et al. (2014) provides many molecular markers across the whole wheat genome.

The objectives of this study were to (i) identify and confirm the location of an adult plant resistance (APR) gene, thought to be *Lr46* in BW278 using two populations, BW278/AC Foremost, a recombinant inbred line (RIL) population and Superb/BW278, a doubled haploid (DH) population (ii) identify tightly linked markers for the APR gene for use in breeding programs, (iii) genetically map and identify markers for seedling resistance gene *Lr2a* using two doubled haploid populations, Superb/BW278, and Superb/86ISMN 2137, and (iv) characterize and evaluate the interaction between *Lr46* and *Lr2a*.

CHAPTER 2

LITERATURE REVIEW

2.1 Wheat

2.1.1 Importance and Production Statistics

Wheat (*Triticum aestivum* L.) is one of the most important crops worldwide, providing approximately one fifth of the calories consumed by humans (Dubcovsky and Dvorak 2007). Worldwide, over 220 M ha of wheat are planted annually, with average annual production reaching 670 Mt (Shiferaw et al. 2013). In Canada, an estimated 32 Mt of wheat were produced during the 2020 season, making it a top agricultural export earner and one of the largest cultivated crops (Government of Canada 2019). The prairie provinces of Alberta, Manitoba and Saskatchewan accounted for over 92% of the nation's wheat production for the 2020 growing season (Government of Canada 2020).

Wheat is segregated into 11 classes in Canada, based on varying end use quality parameters including kernel colour, gluten strength and protein concentration (McCallum and DePauw 2008; DePauw et al. 2011). Canada Western Red Spring (CWRS) is a hard red spring wheat with high protein content, typically used in the production of bread and pasta. The most widely produced class of wheat grown in Canada is CWRS, which accounts for over 60% of annual wheat production (Canadian Cereals 2020).

2.1.2 Domestication and Genetics

Common bread wheat was domesticated approximately 10 000 years ago in the fertile crescent (Dubcovsky and Dvorak 2007, Krasileva et al. 2016). Common wheat is an allohexaploid (2*n* = 6*x* = 42, AABBDD) and has a very large genome of 16×10^9 base pairs (Gupta et al. 2002). The genome of common wheat is composed of three sub-genomes resulting from the interspecific hybridization between three diploid species *Triticum urartu* Thumanian *ex* Gandilyan (2*n* = 14, AA), an *Aegilops speltoides* L. -related species (2*n* = 14, BB) and *Aegilops tauschii* Coss. (2*n* = 14, DD) (El Baidouri et al. 2017). While the donor species of the A and D genomes are widely accepted, the donor species of the B genome is more controversial. The B genome displays the highest level of variability among the three wheat sub-genomes which could explain the difficulty in identifying the donor species (Petersen et al. 2006). While the identity of the B genome donor is still relatively unclear, literature on the subject suggests that *Aegilops speltoides* is either the most closely related species to the donor, or the actual donor of the B genome (Haider 2012, El Baidouri et al. 2017).

The hybridization between the three progenitor species, each of which was specifically adapted to certain environmental conditions allowed for success of the resulting species (*T. aestivum*) in a wide range of environments (Dubcovsky and Dvorak 2007). Advantages of cultivated hexaploid wheat over primitive forms of hexaploid wheat include elimination of spike shattering and tough glumes, increased seed size, decreased number of tillers, improved tolerance to pH and salinity, erect growth habit, reduced seed dormancy and improved resistance to pests (Jantasuriyarat et al. 2004; Dubcovsky and Dvorak 2007). There are also negative aspects to polyploidy, one of which is known as the polyploidy bottleneck effect and is observed when gene diversity in a new polyploidy species is limited, due to a small number of individuals

contributing to the initial formation of the species (Dubcovsky and Dvorak 2007). In common wheat, this is observed in the D genome, where only a few *Ae. tauschii* individuals contributed to the formation of the new species resulting in low genetic diversity within the D genome (Dubcovsky and Dvorak 2007).

2.1.3 Wheat Production Constraints

There are many biotic and abiotic constraints to wheat production that result in yield loss. Among the most common abiotic stressors affecting wheat production are extreme temperature, drought, flooding, and nutrient stress. Breeding for resistance to abiotic stresses tends to be challenging due to the complexity of the genotype by environment interaction, the tendency of most improvement efforts to be focused on yield, and that desired traits are most easily transferred from close relatives (Tester and Bacic 2005). Tester and Bacic (2005) suggested that a more robust understanding of physiological processes behind abiotic stress tolerance is needed for breeding and transgenic programs to be successful in developing tolerance to abiotic stressors. However, there has been some success in transfer of important agronomic traits from wild ancestors into high performing wheat cultivars via marker assisted selection (MAS), and these tools will become even more important as we deal with changing environmental conditions in the future (Dubcovsky 2004).

In addition to abiotic stresses, wheat production is affected by many biotic pests including fungi, bacteria, viruses, nematodes, insects, and mites. Annual wheat yield loss attributed to plant pathogens worldwide is estimated to be 10% (Oerke 2006). There are many economically important diseases that affect wheat production in Canada, including but not

limited to, obligate parasites such as powdery mildew (*Blumeria graminis* DC f. sp. *tritici*) and leaf rust (*Puccinia triticina* Eriks.) and non-obligate pathogens such as fusarium head blight (main species in Canada: *Fusarium graminearium* Schwabe) and tan spot (*Pyrenophora tritici-repentis* Drechsler) (Pest Management Program-Agriculture and Agri-Food Canada [PMP-AAFC] 2019). The main control methods for wheat diseases in Canada include chemical pesticide application and use of resistant cultivars.

Major insect and mite pests of wheat include grasshopper, cutworm, wheat midge, and wheat stem sawfly, all of which are established in Canada and result in high pest pressure (PMP-AAFC 2019). Other insect pests that are established in Canada, but do not currently result in high pest pressure, include aphid and wireworm (PMP-AAFC 2019). Insect pests of wheat in Canada are typically controlled with a combination of methods, including chemical insecticide application, early seeding, crop rotation and use of resistant cultivars.

2.2 The Pathogen

2.2.1 Leaf Rust of Wheat

Wheat leaf rust is caused by basidiomycete fungal pathogen *Puccinia triticina* Eriks. (= *P. recondita* Rob. Ex. Desmaz. f. sp. *tritici*) and is the most common rust disease of wheat. In addition to the leaf blade, *P. triticina* can infect the glumes and leaf sheath of highly susceptible cultivars (Huerta-Espino et al. 2011). Infection results in yield loss due to low kernel weight and fewer kernels per spike (Bolton et al. 2008; Khan et al. 2013; Liu et al. 2014). Infection early in the growing season tends to result in higher yield loss. For example, Huerta-Espino et al. (2011)

reported that infection of the flag leaf at head emergence can result in up to 70% yield loss while infection at the soft dough growth stage resulted in less than 10% yield loss. In Canada, *P. triticina* can cause annual yield loss of up to 25%, depending on time of inoculum arrival, genetics of the host, and growth stage of the crop (Kolmer 2005). Leaf rust continues to be problematic worldwide as high pathogen diversity has resulted in constant emergence of new races and adaptation to many growing environments (Kolmer 2005; Huerta-Espino et al. 2011; McCallum et al. 2016; Figueroa et al. 2018).

2.2.2 Biology of *Puccina triticina*

The primary host of *P. triticina* is bread wheat (*Triticum aestivum*), but the pathogen can also infect tetraploid durum wheat (*T. turgidum* ssp. *Durum*), cultivated and wild emmer wheat (*T. dicoccum* and *T. dicoccoides*), diploid *Ae. speltoides*, common goatgrass (*A. cylindrica*) triticale (*X Triticosecale*) and other species (Liu et al. 2014). *P. triticina* uredinia are small, round to oval in shape, brown to orange in colour, and tend to be distributed along the upper and lower surfaces of the wheat leaf blade (Bolton et al. 2008). Each pustule produces thousands of urediniospores which are dispersed by wind. Infection typically occurs first on the upper leaves of the wheat plant from urediniospores that were deposited by wind or rain. During favourable environmental conditions, pustules increase in number on the leaf surface. A minimum dew period of three hours at around 20°C is required to initiate infection (Kolmer 1996).

P. triticina is heteroecious, meaning it completes its lifecycle on two unrelated hosts (Kolmer 2013). It is also macrocyclic, indicating that the pathogen has five distinct spore stages (Kolmer 2013). The primary host is most commonly hexaploid wheat, and the alternate hosts

include species of *Thalictrum* and *Isopyrum.* Dikaryotic teliospores develop in uredinial infections on wheat. They diploid muclei then undergo meosis, and each haploid nucleus migrates to an expanding basidiospore. Upon maturation, basidiospores are ejected into the air and carried via wind to alternate hosts located nearby. The basidiospores infect the epidermal cells of the alternate host, producing pycinal structures on the upper and lower leaf surfaces. When a haploid pycniospore of one mating type fuses with the flexuous hyphae of the opposite mating type, the dikaryotic nuclear state is restored in the resulting mycelium. The mycelium moved through the leaf, ultimately forming an aecium on the lower surface of the leaf. Dikaryotic aeciospores produced in the aecium are dispersed by wind, and the lifecycle is complete when dikaryotic asexual urediniospores are produced once again on the primary host (Bolton et al. 2008).

While alternate host species are present in North America, the sexual lifecycle of *P. triticina* is not a significant source of disease spread. This could be due to the rarity of alternative hosts (Ordonez and Kolmer 2009; Kolmer 2013), or that species of *Thalictrum* and *Isopyrum* in North America are resistant to infection (Bolton et al. 2008). As a result, the emergence of new *P. triticina* genotypes can be attributed primarily to mutation and migration (Goyeau et al. 2007; Pinto da Silva et al. 2018). The development of leaf rust from year to year in North America is the result of uredinia overwintering on volunteer wheat and fall seeded winter wheat crops in the Southern United States and Mexico (Kolmer 2013). The uredia can survive in the same conditions as the wheat plant, and under favourable spring conditions the urediniospores are released and able to land on a suitable host plant. Upon landing on a host plant, the urediniospores germinate and produce germ tubes which extend until a stomatal opening is located. For successful germ tube development, free water must be available on the leaf surface.

The stomatal opening becomes covered by an appressorium, and the leaf surface is then invaded by a penetration peg (Roelfs et al. 1992; Bolton et al. 2008). If the susceptible host in penetrated, the pathogen produces haustoria that infect the host plant and spread locally throughout the leaf tissue (Roelfs et al. 1992). If the urediniospores land on a resistant host, haustoria development is inhibited or slowed depending on the resistance genes carried by the host plant (Roelfs et al. 1992). The pathogen can cycle continuously on the host plant if living tissue is available and conditions are favourable, allowing for production of inoculum quantities. Urediniospores can then travel long distances by wind. Leaf rust infections in Canada are usually first observed in June, with peak disease pressure in August.

2.2.3 Population Genetics and Race Classification

Virulence surveys for leaf rust have been conducted annually in Canada since 1931 to characterize new races and determine which races are prevalent in particular regions (McCallum et al. 2016). Traditionally, *P. triticina* isolates were classified based on their infection type on a set of wheat cultivars containing undefined resistance genes, however, this method was complicated by the presence of multiple resistance genes in a single cultivar, and influence of genetic background on gene expression (McCallum et al. 2016). Currently, classification in North America is performed by inoculating leaf rust isolates onto a differential set composed of near isogenic lines (NILs), each of which contains a single *Lr* gene in a Thatcher (susceptible) background (developed by Dr. Peter Dyck, AAFC-CRC, Winnipeg). The 16 NILs are in sets of four and the infection type for each line is recorded. The virulence (high infection type)/avirulence (low infection type) combination within each set of four can be coded using a

letter, resulting in each leaf rust isolate being classified as a four-letter code (Table 2.1). In Canada, the leaf rust differential set is as follows: set 1- *Lr1, Lr2a, Lr2c, Lr3*; set 2- *Lr9, Lr16, Lr24, Lr26*; set 3- *Lr3ka, Lr11, Lr17, Lr30* and set 4- *LrB, Lr10, Lr14a, Lr18* (Long and Kolmer 1989; McCallum and Seto-Goh 2003). Virulence surveys in North America detect between 22-72 races of *P. triticina* each year (Kolmer 2013; McCallum et al. 2016). In North America, a wide variety of wheat cultivars are grown, each containing unique sets of resistance genes. The introduction of virulent phenotypes of *P. triticina* into new geographical regions can be attributed to the long-distance transport of spores via wind to locations where certain resistance genes have not yet been utilized (Kolmer 2013). Dominant *P. triticina* races in Canada based on the 2017 and 2018 virulence surveys include MNPS (37.2% and 29.4% of virulence phenotypes in 2017 and 2018, respectively), TBBG (15.3% and 28.5% of virulence phenotypes in 2017 and 2018, respectively) (McCallum, unpublished). Race MBDS was the third most dominant race in 2017, accounting for 6.9% of observed virulence phenotypes, however in 2018, a shift in virulence was observed resulting in TNBG (4.8% of virulence phenotypes) being the third most dominant race (McCallum, unpublished).

Table 2.1. Nomenclature code for the 16 North American differential hosts for *Puccinia triticina*, separated into four sets.

		Infection type[b] on near isogenic *Lr* lines			
	Host set 1	*Lr1*	*Lr2a*	*Lr2c*	*Lr3*
	Host set 2	*Lr9*	*Lr16*	*Lr24*	*Lr26*
	Host set 3	*Lr3ka*	*Lr11*	*Lr17*	*Lr30*
Letter code[a]	Host set 4	*LrB*	*Lr10*	*Lr14a*	*Lr18*
B		L	L	L	L
C		L	L	L	H
D		L	L	H	L
F		L	L	H	H
G		L	H	L	L
H		L	H	L	H
J		L	H	H	L
K		L	H	H	H
L		H	L	L	L
M		H	L	L	H
N		H	L	H	L
P		H	L	H	H
Q		H	H	L	L
R		H	H	L	H
S		H	H	H	L
T		H	H	H	H

[a]Letter code indicates designation for set 1, followed by sets 2, 3 and 4.

[b]Infection type where L= low (pathogen is avirulent) and H=high (pathogen is virulent).

Molecular marker technologies such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) have also been used to study *P. triticina* populations. A study by Kolmer et al. (1995) utilized RAPD marker technology to evaluate population biology of P. *triticina* isolates in Canada and found that the markers were successful in detecting differences between isolates belonging to different groups. However, levels of polymorphism within the groups were low (Kolmer et al. 1995). A similar study using AFLP marker technology was performed, and differences between the groups

were distinguished without difficulty (Kolmer 2001). However, both RAPD and AFLP marker technologies produce dominant markers meaning that heterozygous individuals cannot be distinguished from homozygous individuals, in turn limiting their use in studies of *P. triticina* population biology (Ordonez and Kolmer 2009).

Simple sequence repeat (SSR) markers were able to provide a more robust understanding of *P. triticina* population biology due to the relatively high levels of detectable polymorphism, capacity to distinguish between multiple alleles at a single locus and repeatability among projects (Kolmer 2013). In North America there are six SSR genotype groups of *P. tritcina* races (NA-1 through NA-6) (Kolmer 2013). Groups NA-3 and NA-5 account for approximately 95% of *P. triticina* isolates found throughout the Great Plains region, eastern and southern states of the USA (Kolmer 2013). High levels of heterozygosity, high levels of linkage disequilibrium of SSR markers, and correlation between virulence and SSR genotypes provide further evidence that *P. triticina* populations are highly clonal, and the sexual cycle is not a significant contributor to pathogen diversity (Kolmer 2013). Ordonez et al. (2010) also used SSR markers to study migration patterns of the pathogen, and found that *P. triticina* isolates from Uruguay, Argentina, Chile, and Brazil were very similar or identical to North American isolates, indicating common ancestry.

2.2.4 Leaf Rust Disease Rating Scales

Indoor phenotypic evaluation of leaf rust resistance can be performed on both seedling and adult wheat plants. Indoor evaluations concentrate on infection type (IT) rather than disease severity. The scale used to evaluate IT ranges from 0 to 4, where “0” represents an immune

response with no uredia or signs of infection present, “1” represents a very resistant response with small uredia and necrosis, “2” represents a moderately resistant response with small/medium uredia with chlorosis/necrosis, “3” represents a moderately susceptible response with medium uredia and potentially some chlorosis, “4” represents a very susceptible response with large uredia and no chlorosis or necrosis. In between “0” and “1” there is another infection type known as “;” which represents a nearly immune response with necrotic flecks but no sporulation (Long and Kolmer 1989; McCallum et al. 2010). There are also “Y” and “Z” responses, which represent variably sized uredia near the leaf tip, and leaf base, respectively (McIntosh et al. 1995). Plus (+) and minus (-) characters can be added to the initial rating to represent individuals that fall in the upper (+) or lower (-) range of each category (Roelfs et al. 1992)

Phenotypic evaluations in the field are performed on the flag leaves of adult plants, usually near the end of the growing season, or when severity of susceptible check varieties reaches 70% (Kolmer 2013). Disease severity is evaluated using the modified Cobb scale, previously described by Peterson et al. (1948), and measures the percentage of the flag leaf covered by leaf rust pustules. In addition to disease severity, infection type is also evaluated, where (S) represents a susceptible response with large uredia and little to no chlorosis/necrosis, (MS) represents an moderately susceptible response with medium sized uredia and some chlorosis, (M or I) represents an intermediate response with variable sized uredia and some necrosis/chlorosis, (MR), represents a moderately resistant response with small uredia that are surrounded by necrotic or chlorotic areas, and (R) represents a resistant response where no uredia are present but some necrosis or chlorosis may be visible (Roelfs et al. 1992).

2.2.5 Disease Control

Cultural control practices provide farmers with methods to partially control leaf rust, however no single method is completely effective. Elimination of the stem rust (*Puccinia graminis*) alternate host, common barberry (*Berberis vulgaris* L.), was successful in eliminating the sexual lifecycle, however, this has not been the case with leaf rust. The alternate host of *P. triticina* is *Thalictrum speciosissimum* L., a species that is not native to North America, and the close relative *Thalictrum flavum* subsp. *glaucum* is resistant to infection by sexual basidiospores, resulting in strictly asexual uredinial infections (Kolmer 2013). Early seeding of wheat crops can reduce *P. triticina* infection as the crop is more mature and less susceptible to infection when inoculum arrives via wind transport from the southern United States in June (Samborski 1985, McCallum et al. 2007). Similarly, early maturing cultivars are less likely to be infected by inoculum arriving late in the growing season (Roelfs et al. 1992).

Leaf rust can also be effectively controlled using chemical fungicides. The earliest form of chemical control of *P. triticina* was with sulphur dusts (Bailey and Greaney 1928). Alternatively, wheat leaf rust can be controlled using synthetic chemical fungicides. There are many foliar fungicides currently registered for control of wheat leaf rust, including but not limited to: Acapela (active ingredient (AI): picoxystrobin), Caramba (AI: metconazole), Folicur (AI: tebuconazole), Headline (AI: pyraclostrobin), Nexicor (AI: pyraclostrobin, fluxapyroxad, propiconazol), Proline (AI: prothioconazole), Prosaro (AI: prothioconazole, tebuconazole), Tilt (AI: propiconazole) and Twinline (AI: metconazole, pyraclostrobin). However, chemical control can be challenging due to small and varying application windows for different diseases (Lopez et al. 2015). Multiple chemical applications also increase the risk of the pathogen developing resistance, in turn reducing fungicide efficacy. Chemicals may also have negative impacts on the

environment and may not be financially feasible for smaller farming operations (Figueroa et al. 2018). The most efficient, economic, and environmentally friendly method to control leaf rust is genetic host resistance.

2.2.6 Leaf Rust Resistance

Breeding for resistance is an important control strategy for many plant pathogens including leaf rust of wheat, as the harmful effects of chemicals on the environment and additional costs to producers are minimized. To date, there are more than 81 characterized leaf rust (*Lr*) resistance genes (Aktar-Uz-Zaman et al. 2017). The most widely used *Lr* genes in Canadian wheat cultivars include *Lr1, Lr10, Lr13, Lr14a, Lr16, Lr21* and *Lr34* (McCallum et al. 2016), however pathogen virulence has evolved to *Lr1, Lr10, Lr13*, and *Lr14a,* making these genes ineffective against *P. triticina* isolates in Canada (Randhawa et al. 2013).

Lr genes can be subdivided into two main categories. First are seedling resistance genes, which are effective from the seedling stage all the way until plant maturity. The majority of *Lr* genes belong to the seedling resistance category, and tend to be race-specific, meaning they provide effective resistance to a subset of *P. triticina* isolates (Lagudah 2011). These genes usually manifest as a hypersensitive response and result in host cell death around the site of infection. Constant shifting of virulence in *P. triticina* populations means that these race specific seedling genes can quickly become ineffective in the field. Pathogen virulence has developed to many seedling *Lr* genes including *Lr14a* and *Lr16* which have been extensively deployed in Canadian wheat breeding programs (McCallum et al. 2016). There are some exceptions to rapid breakdown of seedling resistance, for example, virulence to *Lr32* has been reported, but at low frequency (Kerber 1987; McIntosh et al. 1995; Thomas et al. 2010) and virulence to *Lr21* was

only reported in 2011 after many years of deployment in North America (McCallum et al. 2011). As with many other plant disease resistance genes, many of these race-specific seedling resistance genes encode nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins (Figueroa et al. 2018).

Alternatively, there are adult plant resistance (APR) genes, which become more effective after the seedling stage. Of the fifteen APR genes that have been given an *Lr* gene designation, seven are race-specific including *Lr12, Lr13, Lr22a, Lr22b, Lr35, Lr37, Lr48* and *Lr49* (Pinto da Silva et al. 2018). The race-specific APR genes *Lr12, Lr13,* and *Lr22a* have been shown to exhibit a hypersensitive response, resulting in a low infection type on the wheat leaf (Kolmer 2013). The remaining APR genes, *Lr34, Lr46, Lr67, Lr68, Lr74, Lr75, Lr77* and *Lr78* are considered non-race specific (Pinto da Silva et al. 2018). Of these, *Lr34, Lr46* and *Lr67* demonstrate 'slow-rusting' resistance characterized by fewer and smaller uredinia on the leaf surface, as well as longer latent periods (Lagudah 2011). These APR genes are inherited quantitatively, provide partial resistance to all races of *P. triticina*, and are associated with improved resistance to stem rust, stripe rust and powdery mildew (Lagudah 2011).

The pleiotrophic leaf rust, stripe rust, powdery mildew resistance gene *Lr34/Yr18/Pm38/Sr57* originated from cultivar Frontana and landraces of Chinese origin (Dyck 1987) and has remained effective in the field for many years (Lagudah 2011). *Lr34* is located on chromosome 7D of the wheat genome and is closely associated with leaf tip necrosis (*Ltn1*, Rinaldo et al. 2017) as well as improved resistance to barley yellow dwarf virus (*Bdvl1*, Singh 1993). When deployed alone *Lr34* provides only partial leaf rust resistance, however, the efficacy and durability of race specific *Lr* genes are improved when in combination with *Lr34* (German and Kolmer 1992; Kolmer 1996). Examples of durable leaf rust resistance

combinations include *Lr34+Lr12* and *Lr34+Lr13* (German and Kolmer 1992). *Lr34* encodes an ATP-binding cassette (ABC) transporter protein, which are known to be involved in the transport of substances across membranes (Krattinger et al. 2009). *Lr34* is more effective at cooler temperatures (McIntosh et al. 1995). This important APR gene has been cloned, allowing for development of gene-based DNA markers (Krattinger et al. 2009; Lagudah et al. 2009; Dakouri et al. 2010).

Singh *et al.* (1998) discovered APR gene *Lr46* on chromosome 1B in the wheat cultivar Pavon76 which has maintained effective leaf rust resistance in Mexico for over 40 years. Similarly to the multi-pest adult plant resistance associated with *Lr34*, *Lr46* co-segregates with stripe rust resistance (*Yr29,* Singh et al. 1998), stem rust resistance (*Sr58,* Singh et al. 2013), powdery mildew resistance (*Pm39,* Rosewarne et al. 2006), as well as leaf tip necrosis (*Ltn2,* Lillemo et al. 2008). *Lr46* is also more effective at cooler temperatures (Lagudah 2011). Preliminary studies by CSIRO Plant Industry, Australia, suggest that the molecular mechanisms of *Lr34* and *Lr46* differ, and that *Lr46* does not encode an ABC transporter protein (Lagudah 2011). Previous mapping studies have mapped the APR in cultivar Oligoculm, between simple sequence repeat (SSR) markers *Xwmc44* and *Xgwm259* (Suenaga et al. 2003) and in cultivar Saar, between *Xwmc719* and *Xhbe248* (Lillemo et al. 2008). Two cleaved amplified polymorphic sequence (CAPS) markers, *csLV46* and *csLV46G22* are also being used for MAS in breeding programs (Lagudah, personal communication).

Pleiotrophic APR gene *Lr67/Yr46/Sr55/Pm46* was first discovered in the Pakistan wheat line PI250413 and was transferred into the susceptible cultivar Thatcher to produce RL6077, a near isogenic line (Dyck and Samborski 1979). Initially the gene was thought to be *Lr34*, carried on a reciprocal translocation (Dyck et al. 1994). However, further investigation of this region

revealed that the translocation was not associated with the resistance carried in RL6077, and that the resistance was a novel gene, later given the designation *Lr67* (Hiebert et al. 2010). *Lr67* has been cloned, determining that the gene encodes a protein resembling a hexose transporter, and allowing for development of gene-specific molecular markers (Moore et al. 2015).

Other leaf rust APR genes include *Lr68*, *Lr74*, *Lr75*, *Lr77* and *Lr78* located on wheat chromosomes 7B (Herrera-Foessel et al. 2012), 3B (Kolmer et al. 2018c), 1B (Singla et al. 2017), 3B (Kolmer et al. 2018b) and 5D (Kolmer et al. 2018a), respectively.

2.2.7 Host-pathogen interaction

To develop durable resistance to *P. triticina*, knowledge and understanding of the genetic relationship between the host and the pathogen is required. Within *P. triticina*, avirulence/virulence is controlled by many loci widely distributed throughout the genome that function both independently and in combination (Dyck and Samborski 1974; Leonard and Szabo 2005).

The gene-for-gene theory of host-pathogen interaction was first shown using flax rust (*Melamspora lini* Desm.) and flax (*Linum usitatissimum* L.) (Flor 1956). The theory states that avirulence genes in the pathogen have corresponding resistance genes in the host. An incompatible (resistant) reaction will occur if the host is either homozygous or heterozygous for a dominant resistance gene (RR or Rr) and the pathogen has the corresponding avirulence gene (AvAv or Avav). Alternatively, a compatible (susceptible) reaction will occur if the pathogen has a corresponding virulence gene (avav), regardless of the presence of a resistance gene in the host.

This model is evidence that pathogen evolution is not random but guided by the genetics of the host.

The gene-for-gene theory is accurate the majority of the time for the wheat-*P.triticina* pathosystem. Genes that function in this manner tend to be very effective in the short term, but may be easily overcome. While the majority of *Lr* genes function in a gene-for-gene manner, there are some exceptions including, race non-specific genes, presence of regulatory loci, and gene/allele dosage (Bolton et al. 2008). It should also be noted that the expression of resistance genes can vary because of genetic background, temperature, gene interactions and resistance gene suppressors (McCallum et al. 2016).

In addition to the examples mentioned above, allelic sets also differ from the classical gene-for-gene model. Seedling resistance gene *Lr2* has three alleles, designated as *Lr2a*, *Lr2b* and *Lr2c*. A single gene in the pathogen controls avirulence to all three alleles, however, a dominant gene at another locus differentially prevents expression of avirulence to the three alleles (Dyck and Samborski 1974; Kolmer 1996). Races that are virulent to *Lr2a* are always virulent to both *Lr2b* and *Lr2c*, races that show intermediate infection types on *Lr2a* (IT = 2) are generally higher (IT = 2-3) on *Lr2b* and *Lr2c*, and races that show a low infection type (IT = 0) on *Lr2a* also show low infection types (IT = 0;) on *Lr2b* and *Lr2c* (Dyck and Samborski 1974; Kolmer 1996).

2.3 Molecular Analysis and Genetic Mapping of Traits in Wheat

2.3.1 Mapping Populations

There are several different types of mapping populations utilized by researchers in genetic mapping studies. The most common are backcross (BC), recombinant inbred line (RIL), doubled haploid (DH) and F_2 populations. When considering a type of mapping population, factors such as time and labour requirements, plant species, budget, population size requirements and pollination system must be considered (Collard et al. 2005). F_2 populations are derived directly from the F_1 generation, through selfing or sib-mating, where as BC populations are derived from backcrossing the F_1 to one of the parental lines. Advantages of both F_2 and BC populations are that they are relatively easy and fast to produce (Collard et al. 2005). The main disadvantage of both F_2 and BCF_1 populations is that they cannot be reproduced to maintain a homozygous genetic background for replicated testing.

RIL populations are developed through repeated inbreeding of F_2 plants through single seed descent (Singh and Singh 2015). At any given locus, approximately 94% homozygosity is achieved in F_5-derived RILs, and approximately 97% homozygosity is achieved in F_6-derived RILs. This allows RILs to be tested over several seasons and locations which is critical for quantitive traits. The major disadvantage of using RIL populations in mapping studies is the development time, as 6-8 generations are often required (Collard et al. 2005).

In wheat, DH lines are developed from haploid tissue through chromosome elimination and embryo rescue techniques (Laurie and Bennett 1988). The resulting lines are homozygous, making selection via marker assisted selection more efficient, as heterozygosity is not a concern (Baenziger and DePauw 2009). However, potential disadvantages of DH population

development include intensive labour, cost, and space requirements. In the case of RIL and DH populations, both dominant and co-dominant marker types can be used, making them highly useful for studies involving replicated trials and quantitative trait mapping (Collard et al. 2005).

2.3.2 Molecular Markers

Molecular markers are sites of variation within DNA sequence that result from different mutations such as substitutions, insertions, deletions, or errors in replication (Paterson 1996). A variety of molecular marker systems have been developed for use in crop species, however, not all systems are suitable for all research projects. Choice of molecular marker technology depends on a variety of factors including: the goal of the project, availability of financial resources, convenience of the technology, and the genetic trait of the species being studied (Caixeta et al. 2014). Characteristics of the marker technology, such as efficiency of data interpretation and analysis, reproducibility, availability, frequency of occurrence in the genome, and codominance vs. dominance should also be considered (Caixeta et al. 2014).

The first molecular marker system to be developed was restriction fragment length polymorphism (RFLP) and was initially used for human genome mapping (Botstein et al. 1980). This marker type is hybridization-based, using restriction enzymes to cut DNA at recognition sites, followed by gel electrophoresis to separate the fragments (Caixeta et al. 2014; Nadeem et al. 2018). While the system is reliable and transferable between populations, it has limited use in wheat studies as the frequency of polymorphic RFLPs in the hexaploid wheat genome is low (Tanksley et al. 1989; Gupta et al. 1999). Other drawbacks of the RFLP marker technology include time and labour costs, and the large amounts of DNA required (Tanksley et al. 1989; Gupta et al. 1999; Caixeta et al. 2014) Due to these challenges, RFLP markers are rarely, if at all, used in for marker assisted selection in wheat (Gupta et al. 1999).

Both random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) marker systems are polymerase chain reaction (PCR) based, allowing for amplification of small amounts of DNA (Nadeem et al. 2018). RAPDs are a dominant marker type, developed independently by Williams et al. (1990) and Welsh and McClelland (1990). The RAPD technology uses a single, short (10 nucleotide) primer for DNA amplification via PCR (Gupta et al. 1999; Nadeem et al. 2018). PCR products are visualized on agarose gel, and polymorphism is characterized by presence or absence of specific bands (Nadeem et al. 2018). RAPD use has been limited in wheat studies, primarily due to the low levels of detectable polymorphism in the large wheat genome, issues with reproducibility, as well as high cost and time requirements associated with primer testing (Gupta et al. 1999).

Many of the issues with RFLP and RAPD technologies were overcome with the introduction of AFLP markers. With AFLP marker technology, first described by Vos et al. (1995), quantity of DNA is not a concern, prior sequence information is not required, and cost per marker is relatively low (Nadeem et al. 2018). The AFLP marker technology uses two steps, first is digestion of DNA with specific restriction enzymes, followed by ligation of sequence adapters which act as primer annealing sites for PCR amplification. A subset of fragments are amplified via the specific primer binding sites, and the bands are scored for polymorphism using gel electrophoresis (Gupta et al. 1999; Caixeta et al. 2014; Nadeem et al. 2018). Even though AFLP is a dominant marker type, the technology has been successfully used in many studies of hexaploid wheat including mapping disease resistance genes (Anderson et al. 1998; Bai et al. 1998; Goodwin et al. 1998; Hartl et al. 1998), mapping agronomic traits (Parker et al. 1997; Torp et al. 1998), genome mapping (Penner et al. 1998), and genetic diversity analysis (Barrett et al. 1998; Barrett and Kidwell 1998)

Simple sequence repeats (SSRs), also known as microsatellites, are small (1-6 base pairs) tandem motifs that are abundant in many plant genomes (Gupta et al. 1999; Caixeta et al. 2014; Nadeem et al. 2018). These changes in DNA sequence are the result of mutagenic events including retrotransposition, DNA polymerase slippage, unequal crossing over, and co-dominant expression (Caixeta et al. 2014). The technology, first reported in plants by Condit and Hubbel (1991), is co-dominant and highly polymorphic compared to RFLP and RAPD markers, allowing for widespread use in studies of hexaploid wheat (Gupta et al. 1999). Development of species-specific primers can be long and expensive process; however, DNA sequencing of many species has opened the door for the widespread use of SSRs (Caixeta et al. 2014). After the development of the first wheat genome SSR map (Roder et al. 1998), the use of SSR markers became popular in wheat genetics and mapping studies.

Cleaved amplified polymorphic sequence (CAPS) markers are a co-dominant marker technology based on the combination of RFLP and PCR (Maeda et al. 1990). Primers used in CAPS marker technology are designed from sequence information from either cloned RAPD bands or cDNA sequences (Nadeem et al. 2018). Sample DNA is first amplified using PCR, and the amplified product is then digested with restriction enzymes. The digested products are visualized using gel electrophoresis. CAPS markers are versatile, allowing for widespread use in map-based cloning and genotyping studies (Nadeem et al. 2018). Other advantages include the simple and affordable equipment requirements and uncomplicated protocols (Shavrukov 2016). Like all marker technologies, there are some drawbacks to CAPS markers, including high cost of rare restriction enzymes, length of the screening process, and that the technology is less suitable for high-throughput systems (Shavrukov 2016). Like RAPDs and RFLPs, development of CAPS markers in wheat is challenging due to low levels of detectable polymorphism (Shavrukov 2016).

Despite the challenges, CAPS markers have been used successfully in wheat research, including studies of grain weight (Jiang et al. 2015) and disease resistance derived from wild ancestors (Raats et al. 2014).

Single nucleotide polymorphisms (SNPs) are changes in a single base pair in the DNA sequence of an individual (Caixeta et al. 2014; Nadeem et al. 2018). Markers based on SNPs are generally bi-allelic, making them less informative than SSRs which tend to be multi-allelic (Gupta et al. 1999; Caixeta et al. 2014). However, this is compensated for by the abundance of SNPs in the genome (1 SNP in every 100-300 base pairs) (Gupta et al. 1999; Xu 2010).

There are many different SNP genotyping technologies available, each with different chemistries, detection methods, and reaction formats (Semagn et al. 2013). Currently, multiplexed chip-based technologies are the highest throughput genotyping systems available and are the most suitable for studies that require thousands of SNPs for individual samples (Semagn et al. 2013). The two multiplex chip based technologies in wide use currently are BeadArray technology from Illumina (San Diego, CA, USA; www.illumina.com) and GeneChip microarray from Affymetrix (Santa Clara, CA, USA; www.affimetrix.com).

An important advance in wheat genotyping technology was the development of the 90 K SNP array by Illumina, which has become an important tool for many studies of wheat, including diversity and haplotype analyses, and genetics of trait variation (Wang et al. 2014). In some cases, where fewer SNPs are required for many samples, (ie. QTL mapping, marker assisted backcrossing, and marker assisted recurrent selection), uniplex SNP genotyping platforms may be more suitable (Neelam et al. 2013; Semagn et al. 2013). Kompetitive Allele Specific PCR (KASP) is a genotyping technology based on fluorescence resonance energy transfer (FRET) and allele-specific oligo extension (Kumpatla et al. 2012; Semagn et al. 2013). KASP technology

was reported by Semagn et al. (2013) to be highly accurate, with the average genotyping error of positive controls being 0.7-1.6%. The technology is also cost and time effective, making it an attractive tool for plant breeders. The value of KASP markers has been demonstrated in the development of predictive markers for presence of leaf rust resistance genes in wheat (Neelam et al. 2012; Che et al. 2019).

2.3.3 Marker Assisted Selection (MAS)

Marker assisted selection (MAS) is a technique that utilizes molecular markers to indirectly select for genes of interest. The molecular markers used in MAS are either derived from the gene sequence or, more commonly, are tightly linked to the gene of interest. The use of MAS in breeding programs can aid in selecting for traits that are difficult to manage through conventional selection, traits where selection depends on environmental conditions or plant growth stage, pyramiding multiple traits, and acceleration of backcross breeding (Xu and Crouch 2008). MAS is an application of biotechnology that is not contentious because it is used to select desirable alleles in the crop gene pool and does not involve transgenics (Dubcovsky 2004). However, the application of MAS in plant breeding is still limited. Linkage drag, limited availability of highly predictive molecular markers, and fixation of large genomic regions are all genetic constraints to MAS (Miedaner and Korzun 2012). MAS is still quite expensive; however, this may change with improvements in technology such as the chip-based, high throughput genotyping platforms that have been developed for many important crop species (Miedaner and Korzun 2012). It is also worth noting that in many crops, the most expensive part of implementing MAS is the time and labour requirements to collect samples from plants.

In the case of wheat leaf rust (*Puccinia triticina*) resistance, MAS is an important alternative to conventional breeding methods that are challenging and time consuming (Khan et al. 2013). Many *Lr* genes are deployed in gene pyramids, or express similar low infection types, making differentiation between single genes and pyramids challenging. MAS has been successfully used to incorporate *Lr* genes including *Lr21, Lr22a, Lr32, Lr34* and *Lr46* into wheat cultivars in Canada (Randhawa et al. 2013). *P. triticina* is a quickly evolving pathogen, thus deployment of new genes and pyramiding of genes in a timely manner is important for long term control of the disease (Khan et al. 2013).

CHAPTER 3

CHARACTERIZATION AND GENETIC MAPPING OF ADULT PLANT LEAF RUST (*Puccina triticina*) RESISTANCE IN SPRING WHEAT (*Triticum aestivum*) BREEDING LINE BW278

3.1 Abstract

Wheat leaf rust caused by the fungal pathogen *Puccinia triticina* Eriks. (=*P. recondita* Rob. Ex Desmaz. f. sp. *tritici*), is a common and widespread disease of wheat. Utilizing wheat varieties with host genetic resistance is an effective method of disease control. The spring wheat line BW278 carries APR to leaf rust that has not been characterized to date. Leaf rust resistance was studied in a recombinant inbred line (RIL) population derived from the cross BW278/AC Foremost (n = 183), and a doubled haploid (DH) population derived from the cross Superb/BW278 (n = 142). To characterize the APR observed in BW278, both mapping populations were evaluated for adult plant leaf rust resistance in replicated field trials. To characterize the APR under controlled environmental conditions, BW278/AC Foremost was evaluated with *P. triticina* isolates 77-2 TJBJ and 12-3 MBDS in the greenhouse. These populations and their parents were genotyped with the iSelect 90K wheat Infunium SNP array. QTL analysis identified a QTL, designated *QLr.mrdc-1B*, conferring adult plant leaf rust resistance in the field that mapped to the long arm of wheat chromosome 1B in the BW278/AC Foremost population, which is the expected location of the APR gene *Lr46*. No significant QTL were detected on chromosome 1B in the Superb/BW278 DH population. Ten SNPs linked to

QLr.mrdc-1B were selected to develop KASP markers for marker assisted selection in wheat breeding programs.

3.2 Introduction

Common wheat (*Triticum aestivum* L.) is one of the most important crops worldwide, providing approximately one fifth of calories consumed by humans (Dubcovsky and Dvorak 2007). Wheat crops around the world have been threatened by wheat leaf rust, caused by the fungal pathogen *Puccinia triticina* Eriks. (=*P. recondita* Rob. Ex Desmaz. f. sp. *tritici*) for thousands of years. Leaf rust is the most widespread disease of wheat and causes significant yield reduction in wheat growing regions nearly every year (Huerta-Espino et al. 2011). While the pathogen can be controlled with chemical fungicides, breeding for resistance is the preferred control method as it reduces costs to producers and is also environmentally friendly, as fungicide application can be reduced or eliminated. However, breeding for resistance is challenging as the pathogen displays high diversity and can adapt to many climates (Figueroa et al. 2018). To date, 81 leaf rust resistance genes have been designated in wheat (Aktar-Uz-Zaman et al. 2017).

Puccinia triticina evolves primarily through mutation and new pathogen races have rendered many resistance genes ineffective. While some of these genes still provide resistance when deployed in combination, the characterization of durable sources of resistance is important for long term control of the disease. One of the most important resistance genes currently in use is *Lr34*, a race-nonspecific, multi-pest, adult plant resistance (APR) gene located on wheat chromosome 7D (Dyck 1987). In addition to leaf rust, *Lr34* provides improved resistance to stem rust (Dyck 1987; Kerber and Aung 1999), stripe rust (McIntosh 1992; Singh 1992), and powdery mildew (Spielmeyer et al. 2005; Lillemo et al. 2008). Leaf rust resistance provided by *Lr34* has

remained durable, despite being widely deployed by wheat breeding programs around the world for many years (Singh et al. 2000). Singh et al. (1998) discovered a similar gene on the long arm of chromosome 1B, in the durably resistant cultivar 'Pavon 76' and designated it *Lr46*. A third multi-pest, APR gene, was discovered on chromosome 4D, and was designated *Lr67* (Hiebert et al. 2010; Herrera-Foessel et al. 2011).

To characterize the leaf rust resistance in spring wheat line BW278, two populations were studied that exhibited a slow-rusting, non-hypersensitive response to leaf rust at the adult plant stage. We hypothesized that the APR in BW278 could be conferred by *Lr46*, as its resistance was not as effective as observed with *Lr34* in previous tests.

The objectives of this study included (i) to determine if the leaf rust APR gene in BW278 is *Lr46*, and (ii) genetically map and develop markers for this gene that are well-suited for marker-assisted breeding.

3.3 Materials and Methods

3.3.1 Plant Materials

BW278 is a backcross derived doubled haploid (DH) line (AC Domain*2/Sumai 3), and carries seedling leaf rust resistance gene *Lr16*, as well as an APR gene, hypothesized to be *Lr46* (McCallum, unpublished). A recombinant inbred line (RIL) population of 2,300 $F_{5:7}$ lines was developed from the cross BW278/AC Foremost by Dr. Jim Menzies at the Cereal Research Center in Winnipeg, Manitoba. AC Foremost (HY321*5/BW553//HY320*6/7424-BW5B4) is a spring wheat cultivar that carries an unidentified seedling leaf rust resistance gene, temporarily designated *LrF* (McCallum, unpublished). A subset of 305 RILs were evaluated under field

conditions and in greenhouse assays for adult plant leaf rust resistance. Lines that carried seedling resistance gene *Lr16* were excluded from this study. Due to time and cost contraints, 186 RILs were randomly selected to be genotyped with the wheat 90K iSelect SNP array (Wang et al. 2014). Three RILs were removed from further analysis due to large amounts of missing data (>10%), leaving a final mapping population of 183 RILs.

Superb is a backcross derived DH line (Grandin*2/AC Domain) developed at the Cereal Research Center in Winnipeg, MB (Townley-Smith et al. 2010). Superb carries seedling leaf rust resistance genes *Lr2a* and *Lr10* (McCallum and Seto-Goh 2010), but is moderately susceptible in the field in Canada. A DH population of 402 lines was developed from the cross Superb/BW278 using the maize pollination method (Thomas et al. 1997), of which 142 were genotyped with the wheat 90K iSelect SNP array. Due to low seed quantity, 115 DH lines were selected for evaluation of adult plant leaf rust resistance under field conditions.

3.3.2 *P. triticina* Indoor Inoculations on the BW278/AC Foremost population

Two plants per line of the BW278/AC Foremost RIL population were germinated in 4x8 root trainers containing a 3:2 mixture of bulk soil to Sun Gro Horticulture Sunshine Professional Growing Mixture No. 5 (Agawam, MA, USA; www.sungro.com). Parental lines, Thatcher and near-isogenic line Tc-*Lr16* were included in the experiment as checks. Plants were placed in a greenhouse (20 ± 4ºC), grown for 10 days and watered as needed. Inoculum was developed using methods described by McCallum and Seto-Goh (2003). To ensure that the race of the inoculum was pure and validate its race designation, a differential set of 16 NILs (developed by the late Dr. Peter Dyck, Agriculture and Agri-Food Canada) was inoculated, and the virulence pattern was observed (Long and Kolmer 1989).

The seedlings were then inoculated with isolate 77-2 TJBJ (virulent to *Lr16*/avirulent to *LrF*) using the oil suspension method (McCallum and Seto-Goh 2003). The vacuum dried urediniospores were removed from refrigeration and left to rehydrate at room temperature for approximately six hours. They were then heat shocked at 40°C in a water bath for six minutes. Based on the North American nomenclature system (Long and Kolmer 1989), the isolate 77-2 was determined to be race TJBJ (77-2 TJBJ), and spore viability was tested by spreading a thin layer of spores over a 2% water agar petri plate. The spores were left on the plate for approximately four hours and observed under a microscope to ensure that a high proportion of the urediniospores germinated. Spores were suspended in light mineral oil, Soltrol 170 (Chevron Phillips Chemical Company, TX, USA), and sprayed onto the wheat leaves using compressed air.

After inoculation, the seedlings were left to dry at room temperature for approximately 30 minutes. They were then placed in a dew chamber set to 20 °C under dark conditions and 100% relative humidity for approximately 24 hours. The next day, plants were removed from the dew chamber and allowed to air dry before being placed back in the greenhouse. A pustule type rating was performed 12 days after inoculation. Seedling leaf rust reactions were classified according to Long and Kolmer (1989) using an infection type (IT) scale, where ITs of '0' (completely immune response), to '2' (moderately resistant response) were considered as resistant responses. Infection types of '3' (moderately susceptible response) and '4' (highly susceptible response) were considered susceptible responses (Appendix 7.1). Lines that displayed a resistant response were removed from further analysis to eliminate *Lr16* and *LrF* from the population.

After the seedling rating was performed, a single plant per line was transplanted into a one-liter pot containing the same soil mixture described above. Plants were watered as needed and treated with water soluble fertilizer (20-20-20 NPK) once weekly. The three healthiest tillers were selected, and the remaining tillers were trimmed. The same inoculation process outlined above was repeated when plants reached the boot/head emergence stage (Zadoks et al. 1974). Inoculum was applied directly to the flag leaves. Indoor adult plant ratings were performed approximately 16 days post inoculation using the infection type rating scale outlined above.

Due to low levels of infection achieved in the greenhouse, as well as the observation that *P. triticina* isolate 77-2 TJBJ was avirulent on AC Foremost (susceptible parent) at the adult plant stage, an additional adult plant indoor screening of the BW278/AC Foremost population in a growth cabinet (set to 16 light and 8 dark hours at 16°C and 14°C respectively) with isolate 12-3 MBDS (avirulent to *Lr16*, *LrF*) was performed using the same procedures described above (Appendix 7.1).

3.3.3 Leaf Rust Field Trials

Phenotypic leaf rust evaluations were performed during the 2016-2020 growing seasons at the Morden Research and Development Center (MRDC) in Morden, Manitoba. Trials were organized in a randomized complete block design (RCBD) with three replicates of Superb/BW278 and two replicates of BW278/AC Foremost, except for 2016 and 2017 where a single replicate of BW278/AC Foremost was performed. Plots were single 1 m rows, in groups of six rows, with a seeding density of approximately 65 seeds/m. Spreader rows were included in the experimental design and alternated between the third and fourth rows in attempt to achieve

even inoculum spread. The spreader rows contained 70% Morocco, 20% Thatcher (both susceptible to leaf rust) and 10% Wolfe barley. The barley was included as a marker so spreader rows could be easily identified. Rows of susceptible check Thatcher, intermediate check McKenzie, parental lines, and positional Bethune flax checks were incorporated into the experimental design. Spreader rows were inoculated three times throughout each growing season, except for 2016 where two inoculations were performed. Timing of inoculation was chosen based on predicted ideal relative humidity to ensure presence of free moisture for successful spore germination. Spreader rows were inoculated with an epidemic mix of leaf rust races, increased from all isolates from western Canada in the previous year's wheat leaf rust field survey. The inoculum consisted of 1.5 gm of urediniospores per L of light mineral oil (Soltrol 170; Chevron Phillips Chemical Company, Woodlands, TC, USA). Spreader rows were checked frequently for onset of disease symptoms and disease ratings were performed and susceptible checks reached approximately 80% disease severity.

Phenotypic ratings were classified according to Peterson et al. (1948) using the Modified Cobb scale (% of flag leaf infected) to evaluate disease severity and the infection type (IT) scale described by Roelfs et al. (1992), ranging from highly susceptible (S) to entirely resistant (R) (Appendix 7.2-7.4). All IT ratings were converted into a numerical value where '0' was resistant, '0.25' was moderately resistant, '0.5' was intermediate, '0.75' was moderately susceptible and '1' was susceptible. Following conversion to a numerical value, the coefficient of infection (CI) was calculated for each line by multiplying the disease severity by the infection type value. The field trials were hand harvested and threshed, with all replicates kept separate.

3.3.4 Statistical Analysis

A combined analysis of variance (ANOVA) for disease severity (SEV), infection type (IT) and coefficient of infection (CI) was performed for both mapping populations across all five site years using the "PROC MIXED" procedure in SAS University Edition software package (SAS Institute Inc., Cary, NC, USA). The model statement for the combined analysis was "variables= year rep(year) genotype genotype*year. All variables in the statement were considered random except for genotype. F-test values were considered approximate, as the measures of leaf rust infection were not normally distributed.

3.3.5 DNA Extraction

The following protocol was used for DNA extraction for kompetitive allele specific PCR (KASP), cleaved amplified polymorphic sequence (CAPS) and 90K SNP array marker analysis. Wheat seedlings from the initial inoculation with 77-2 TJBJ were used as the tissue source for DNA isolation in the BW278/AC Foremost population. The tissue source for DNA isolation for Superb/BW278 were wheat seedlings from a previous experiment performed in Dr. Curt McCartney's lab. Six pieces of leaf tissue (approximately 1-2 cm in length) were collected from the youngest uninfected leaves of each plant and placed directly into 96-well collection microtube extraction plates. Each well contained a tungsten carbide bead to crush tissue after being lyophilized. Tissue was lyophilized for 24 hours and then stored at -20°C. Immediately prior to extraction, tissue was brought to room temperature and crushed into a fine powder using the Qiagen TissueLyser II (Qiagen, Missassauga, ON; www.qiagen.com) for 10 minutes, changing plate orientation every 2.5 minutes. DNA extraction was performed using a modified

ammonium acetate extraction protocol based on the procedures of Pollotta et al. (2003). DNA was quantified using a fluorometer and Hoechst 33258 stain and diluted to a 15 ng/µl working solution for both KASP and CAPS marker analysis. DNA for the 90K SNP array was diluted to a 60 ng/µl working solution.

3.3.6 Genotyping

Both mapping populations were genotyped with the Illumina Infinium assay (San Diego, CA, USA; www.illumina.com) using the iSelect 90K wheat SNP array (Wang et al. 2014). Superb/BW278 was also genotyped with a set of 270 microsatellite (SSR) markers. SNP marker filtering and genotype calling was performed in GenomeStudio software (Illumina Inc; http://bioinformatics.illumina.com/informatics/sequencing-microarray-data-analysis/genomestudio.html).

In addition to the 90K SNP array (Wang et al. 2014), both populations and parental lines were screened with two molecular markers described as tightly linked to the *Lr46* allele (Table 3.1). Molecular marker *csLV46G22* (Lagudah, personal communication) is a cleaved amplified polymorphism sequence (CAPS) marker, and *DK0900* (Konkin and Cuthbert, personal communication) is a SNP-derived KASP marker.

Table 3.1. Parental molecular marker scores for two markers described as tightly linked to the gene of interest, *Lr46*.

Gene	Marker	Parental Line			Reference
		BW278	AC Foremost	Superb	
Lr46	*csLV46G22*	+	-	+	E. Lagudah Pers. Comm. 2019
	DK0900	+	-	+	D. Konkin and R. Cuthbert Pers. Comm. 2019

Ten SNP markers from the 90K Infinium assay that demonstrated linkage to resistance on chromosome 1B in BW278/AC Foremost were selected to design KASP markers (Table 3.2). Primer sequences were designed using Primer Picker (Appendix 7.8). PCR reactions for all KASP assays were performed in 384-well plates. Each reaction contained 2 µl of template DNA, diluted to 15 ng/µl (30 ng of DNA per reaction), 0.5 µl ddH_2O, 2.5 µl KASP 2X Mastermix (contains universal FRET cassettes, ROX passive reference dye, Taq polymerase, free nucleotides, $MgCl_2$, and buffer), and 0.07 µl primer mix (containing 12 µM both allele-specific forward primers, and 30 µM of reverse primer), for a total volume of 5 µl per reaction, as per the manufacturer's guidelines (LGC Genomics LLC, Beverly, MA, USA). PCR was performed under the following conditions: 1. denaturation at 94°C for 15 min, 2. 94°C for 20 sec followed by 65°C for 1 min (10 cycles) and 3. 94°C for 20 sec, 57°C for 01 min (26 cycles) and an infinite hold at 4°C. Fluorescence profiling of SNPs was complete using the FLUOstar Omega microplate reader (BMG Labtech- Ortenberg, Germany; www.bmglabtech.com). Data were visualized using KlusterCaller software (LGC Genomics- Kbioscience, Teddington, Middlesex, UK; www.lgcgenomics.com).

PCR reactions for the *csLV46G22* CAPS marker were performed in 96-well plates. Each reaction contained 8.0 µl of template DNA diluted to 15ng/µl (120 ng/reaction), 2.0 µl 10× PCR buffer (Invitrogen, Waltham, MA, USA; https://www.thermofisher.com/ca/en/home.html) 2.0 µl dNTPs (0.2 mM), 4.8 µl ddH_2O, 2.0 mM $MgCl_2$, 5.0 pmols forward primer, 5.0 pmols reverse primer and 1U *Taq* DNA polymerase, resulting in a final volume of 20 µl per reaction. PCR conditions were as follows: denaturation at 95°C for 2 min, 35 cycles of 95°C for 30 sec, 64°C for 30 sec, 72° for 1 min followed by a final extension step of 72°C for 8 min and an infinite hold at 4°C (Lagudah, personal communication). The PCR product was then digested with *Bsp*EI restriction enzyme. The total volume of each reaction was brought up to 30.5 µl by adding 8.0 µl ddH_2O, 2.0 µl 10X restriction buffer (manufacturer) and 0.5 µl *Bsp*EI restriction enzyme. Plates were incubated at 37 °C for 1 h. Following incubation, 5.0 µl of the digested PCR product and 1.0 µl of FlashGel™ Loading Dye (Lonza, Rockland, ME, USA; www.bioscience.lonza.com) were loaded onto 2.2% agarose, 16+1 well double tier FlashGel™DNA Cassettes (Lonza) and were allowed to run for 10 min at 80V using the FlashGel™ system (Lonza).

Table 3.2. SNP-derived KASP markers that displayed linkage to *QLr.mrdc-1B* in BW278/AC Foremost RIL population using the 90 K Illumina Infinium assay.

Lab KASP ID	iSelect 90K wheat SNP ID	Chrm.[a]	Pos.[b]	Wang et al. 2014 Pos.[c]	RefSeq v1.0[d]
kwh742	Tdurum_contig4997_974	1B	124.413	141.82	668.5
kwh743	IAAV4844	1B	124.413	141.82	668.1
kwh744	CAP7_rep_c6352_375	1B	124.689	141.82	668.9
kwh745	GENE-0223_239	1B	125.527	141.82	669.0
kwh746	Excalibur_c35888_208	1B	125.527	141.82	669.0
kwh747	BS00000010_51	1B	126.940	143.98	670.8
kwh748	Tdurum_contig10354_170	1B	128.532	148.40	673.8
kwh749	BS00021877_51	1B	128.532	154.58	673.7
kwh750	Tdurum_contig10354_270	1B	128.532	148.40	673.8
kwh751	BS00084990_51	1B	128.532	154.58	673.7

[a]Chrm., chromosome

[b]Pos., position in the BW278/AC Foremost RIL population (cM).

[c]Position in the Wang et al. (2014) consensus map (cM).

[d]Physical location (Mb) in the Chinese Spring reference genome, RefSeq v1.0; International Wheat Genome Sequencing Consortium 2018.

3.3.7 Genetic map construction

The SNP markers that were polymorphic between the parents of each mapping populations were sorted into clusters, AA, AB, and BB, corresponding to the three expected genotypes for bi-allelic SNPs. Raw data was exported to MS Excel (2016), and genotypes were

converted into numeric values, where AA = 0, AB = 1 and BB = 2. Parental variance scores were calculated, and numeric values were then converted into A, B, or missing data (-) based on sorting by parental variance values. Missing data (-) values were also recorded for markers that could not be accurately scored. Lines with more than 10% missing data were removed from further analysis. Markers that were monomorphic, significantly distorted ($p < 0.01$), had more than 10% missing data, or displayed more than two clusters were removed from further analysis. In both mapping populations, the female parent was coded as 'A' and the male parent was coded as 'B'. Redundant, co-segregating markers were sorted into preliminary linkage bins using the BIN module in QTL IciMapping v.4.2 (Meng et al. 2015). A single marker with the least amount of missing data was chosen to represent each bin in linkage mapping. The resulting set of markers was used to generate a linkage map in MapDisto v. 1.7.7 (Lorieux 2012). The Kosambi mapping function (Kosambi 1943), with a minimum logarithm of odds (LOD) threshold of 3.0 and a maximum recombination fraction of 0.3, was used to calculate genetic distances (cM) from recombination fractions. The "AutoMap" function was used to order loci. Marker data were checked using an error detection threshold of 0.005, and error candidates were replaced with missing data. Linkage groups were assigned to chromosomes based on the published consensus map by Wang et al. (2014). Genetic maps were drawn using MapChart v. 2.32 (Voorrips 2002).

3.3.8 QTL Analysis

QTL analysis was performed using interval mapping (IM) and inclusive composite interval mapping (ICIM) in QTL IciMapping version 4.1 (Meng et al. 2015). Least Squares means (LSmeans) were generated using SAS University Edition software package (SAS Institute Inc., Cary, NC, USA). In years where more than one replicate was performed and for the pooled analysis, LSmeans were used for QTL analysis. In 2016 and 2017, a single replicate of

BW278/AC Foremost was evaluated in the field, and raw measures of SEV, IT and CI were used for QTL analysis. Analysis for additive effect QTL was conducted using 0.1 cM steps, and the 5% LOD significance threshold was calculated with 1,000 permutations.

3.4 Results

3.4.1 Adult Plant Responses of BW278/AC Foremost to *P. triticina*

Adult plant leaf rust reactions of parental lines BW278 and AC Foremost are displayed in Table 3.3. BW278 showed a reaction type of "3-/few/Z" to both races of *P. triticina* (77-2 TJBJ and 12-3 MBDS), which was observed as large uredia near the leaf base, with smaller and fewer uredia along the leaf tip (Figure 3.1). AC Foremost was rated as ";3-/few" in response to 77-2 TJBJ which was observed as a few large uredia and some necrotic flecks. The observed flecking indicated that AC Foremost (susceptible parent) was resistant to 77-2 TJBJ, and a second inoculation with 12-3 MBDS was completed. In response to 12-3 MBDS, AC Foremost displayed a susceptible reaction type of "3-", characterized by large uredia along the entire leaf blade (Figure 3.1)

The adult plant phenotypic rating of the BW278/AC Foremost population with *P. triticina* isolate 77-2 TJBJ showed 135 resistant and 167 susceptible lines which fit the expected single gene ratio ($p = 0.066$; Table 3.4). Three RILs either did not germinate or died prior to reaching the adult plant stage. When inoculated in a growth cabinet with isolate 12-3 MBDS, BW278/AC Foremost showed a 135:131 resistant to susceptible segregation ratio, which also fit a single gene segregation ratio ($p = 0.806$; Table 3.4). Thirty-nine lines either did not germinate

or died prior to reaching the adult plant stage. Phenotypic ratings were the same in 66.5% of the RILs that received a rating for both 77-2 TJBJ and 12-3 MBDS. Ratings for 33.5% of the RILs did not agree, meaning that the lines were rated as resistant to one isolate and susceptible to the other.

During the controlled environment inoculations, very little disease development was observed. Problems with spore viability were ruled out, as the spores successfully germinated on the water agar plate prior to inoculation. The adult plants did not display any symptoms of nutrient or moisture deficiency, however high levels of powdery mildew contamination, thrip infestation, and high greenhouse temperatures were observed and were suspected as the most likely reason for limited disease development. Additional controlled environment adult plant screenings were not attempted as five years of field leaf rust evaluations were available for QTL analysis.

Table 3.3. Adult plant infection type responses of parental lines when inoculated with two *Puccinia triticina* isolates under greenhouse conditions.

Parental Line	**12-3 MBDS[a]**	**77-2 TJBJ[a]**
BW278	3-/few/Z	3-/few/Z
AC Foremost	3-	;3-/few

[a]Reaction types (0-4) where 0-2 are resistant and 3-4 are susceptible. “Few” refers to a small number of uredia on the leaf surface. “;” indicates a resistant hypersensitive response. “Z” indicates variable sized uredia, with larger uridinia towards the leaf base.

Table 3.4. Phenotypic segregation ratios for resistance to two *Puccinia triticina* isolates in the recombinant inbred line (RIL) population BW278/AC Foremost, performed under greenhouse conditions at the adult plant stage.

Isolate	**R[a]**	**S[b]**	**-[c]**	**X^2 (p-value)**	**Expected ratio**
77-2 TJBJ	135	167	3	0.066	1:1
12-3 MBDS	135	131	39	0.806	1:1

[a]Resistant lines

[b]Susceptible lines

[c]Missing data

Figure 3.1. Flag leaves from the BW278/AC Foremost RIL population when inoculated with *Puccinia triticina* isolate 12-3 MBDS in the greenhouse. From left to right: BW278 (resistant parent), AC Foremost (susceptible parent), Thatcher (susceptible check), qe J 415 (resistant RIL), pb E 266 (resistant RIL), pb E 103 (susceptible RIL), pb K 391 (susceptible RIL).

3.4.2 Field Trials

In all five years (2016-2020) that the BW278/AC Foremost and Superb/BW278 populations were tested in the field, high levels of leaf rust infection were achieved, resulting in a variable distribution of phenotypes (Appendices 7.2-7.4).

BW278/AC Foremost

The ANOVA for the combined site-years indicated that all sources of variation for all measures of leaf rust, (SEV, PT, CI) in the BW278/AC Foremost population were statistically significant (Table 3.5). Correlation analysis revealed that SEV, IT and CI were all strongly correlated (Table 3.6). Distributions of mean (LSmeans) SEV, IT and CI are displayed in Figure 3.2. BW278 displayed a resistant response to leaf rust, with mean SEV and IT values of 14.0 and 0.13, respectively. AC Foremost also displayed a resistant response to leaf rust, with mean SEV and IT values of 7.5 and 0, respectively.

Table 3.5. Analysis of variance for three phenotypic field variables, leaf rust severity (SEV), infection type (IT) and coefficient of infection (CI) for the pooled BW278/AC Foremost dataset, grown in Morden from 2016-2020.

Source	df	Type III SS	MS	F-value	P-value
Variable: SEV					
Genotype	304	1433112	4714.2	16.2	<0.0001
Year	4	185328	46332	53.3	0.0001
Rep(Year)	3	1920.3	640.1	7.9	<0.0001
Year*Genotype	1204	373782	310.4	3.9	<0.0001
Residual	896	72273	80.7		
Variable: IT					
Genotype	304	255.0	0.84	13.5	<0.0001
Year	4	30.9	7.73	35.0	0.0009
Rep(Year)	3	0.52	0.17	9.01	<0.0001
Year*Genotype	1204	79.7	0.07	3.4	<0.0001
Residual	896	17.4	0.02		
Variable: CI					
Genotype	304	1785671	5873.9	13.54	<0.0001
Year	4	268070	67018	50.52	0.0002
Rep(Year)	3	2978.5	992.8	7.8	<0.0001
Year*Genotype	1204	556753	462.4	3.6	<0.0001
Residual	896	114079	127.3		

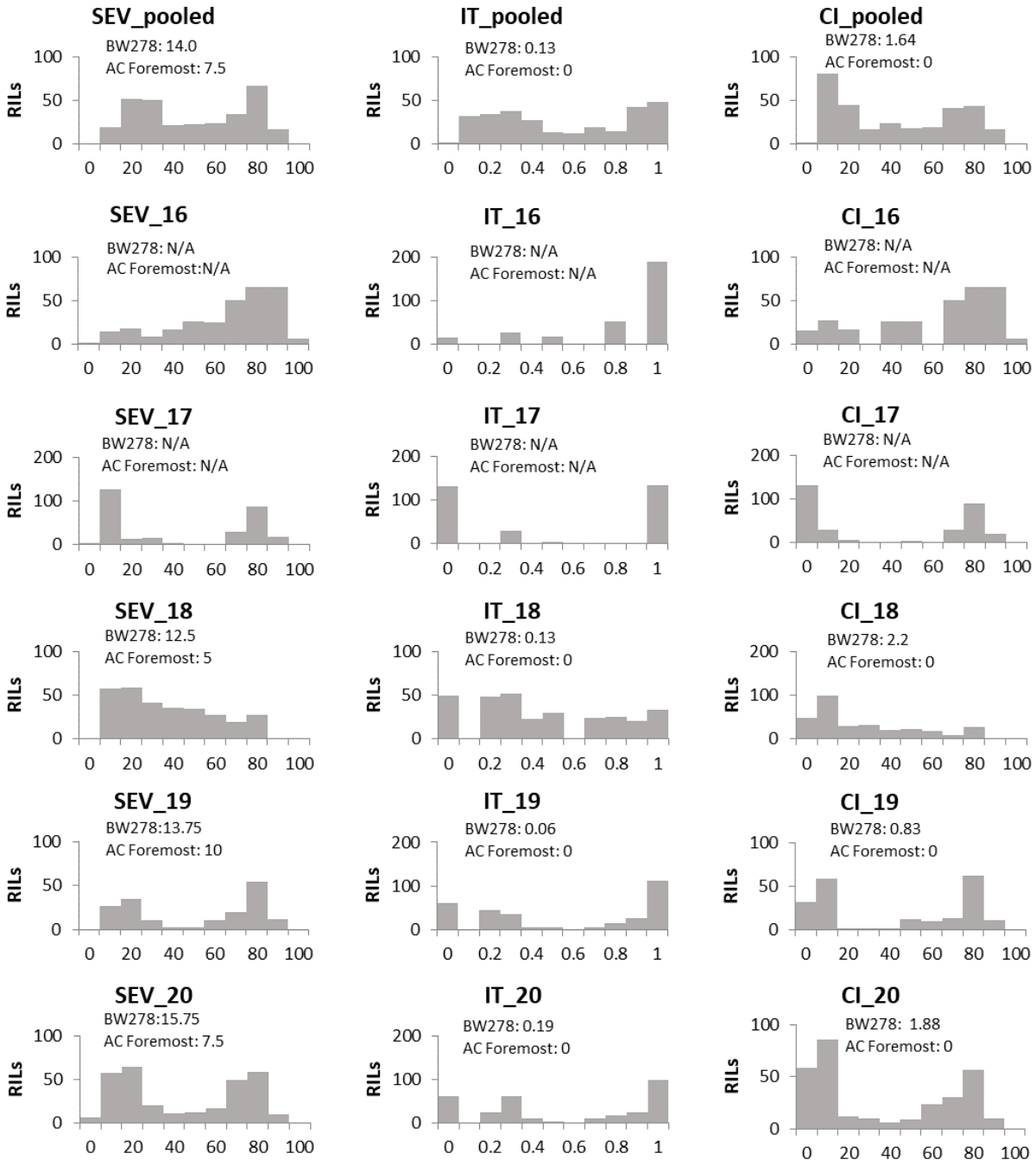

Figure 3.2. Histograms of mean leaf rust severity (SEV), infection type (IT), and coefficient of infection (CI) for the BW278/AC Foremost RIL population in 5 field site-years, and the pooled dataset.

Table 3.6. Pearson's correlation coefficients between pooled disease severity (SEV), infection type (IT) and coefficient of infection (CI) values from the BW278/AC Foremost population (n = 305).

	SEV	IT
IT	0.99[a]	
	<0.0001[b]	
CI	0.99[a]	0.99[a]
	<0.0001[b]	<0.0001[b]

[a]r2

[b]p-value

Superb/BW278

Combined ANOVA for Superb/BW278 across all site-years also indicated that all variables were statistically significant for all measures of leaf rust (Table 3.7). Correlation analysis revealed that the three measures of leaf rust were strongly correlated to each other (Table 3.8). Distributions of mean (LSmeans) SEV, IT and CI are displayed in Figure 3.3. Superb displayed an intermediate response to leaf rust across the five site-years, with average SEV and IT ratings of 46.8 and 0.66, respectively. BW278 displayed a moderately resistant response, with average SEV and IT ratings of 29.7 and 0.36, respectively. The Superb/BW278 population was skewed towards resistance in all five site-years and all measures of leaf rust, a result that suggests Superb also carries a source of leaf rust resistance.

Table 3.7. Analysis of variance for three phenotypic field variables, leaf rust severity (SEV), infection type (IT) and coefficient of infection (CI) for the Superb/BW278 pooled dataset, grown in Morden from 2016 to 2020.

Source	df	Type III SS	MS	F-value	P-value
Variable: SEV					
Genotype	114	450993	3956.1	16.0	<0.0001
Year	4	52975	13244	32.0	<0.0001
Rep(Year)	10	2577.0	257.7	2.8	0.0017
Year*Genotype	456	112773	247.3	2.7	<0.0001
Residual	1140	103336	90.6		
Variable: IT					
Genotype	114	100.0	0.88	14.4	<0.0001
Year	4	13.1	3.26	28.7	<0.0001
Rep(Year)	10	0.79	0.08	3.0	0.0009
Year*Genotype	456	27.8	0.06	2.3	<0.0001
Residual	1140	30.0	0.03		
Variable: CI					
Genotype	114	614923	5394.1	14.0	<0.0001
Year	4	67852	16963	24.9	<0.0001
Rep(Year)	10	4327.2	432.7	3.2	0.0005
Year*Genotype	456	175908	385.8	2.8	<0.0001
Residual	1140	154916	135.9		

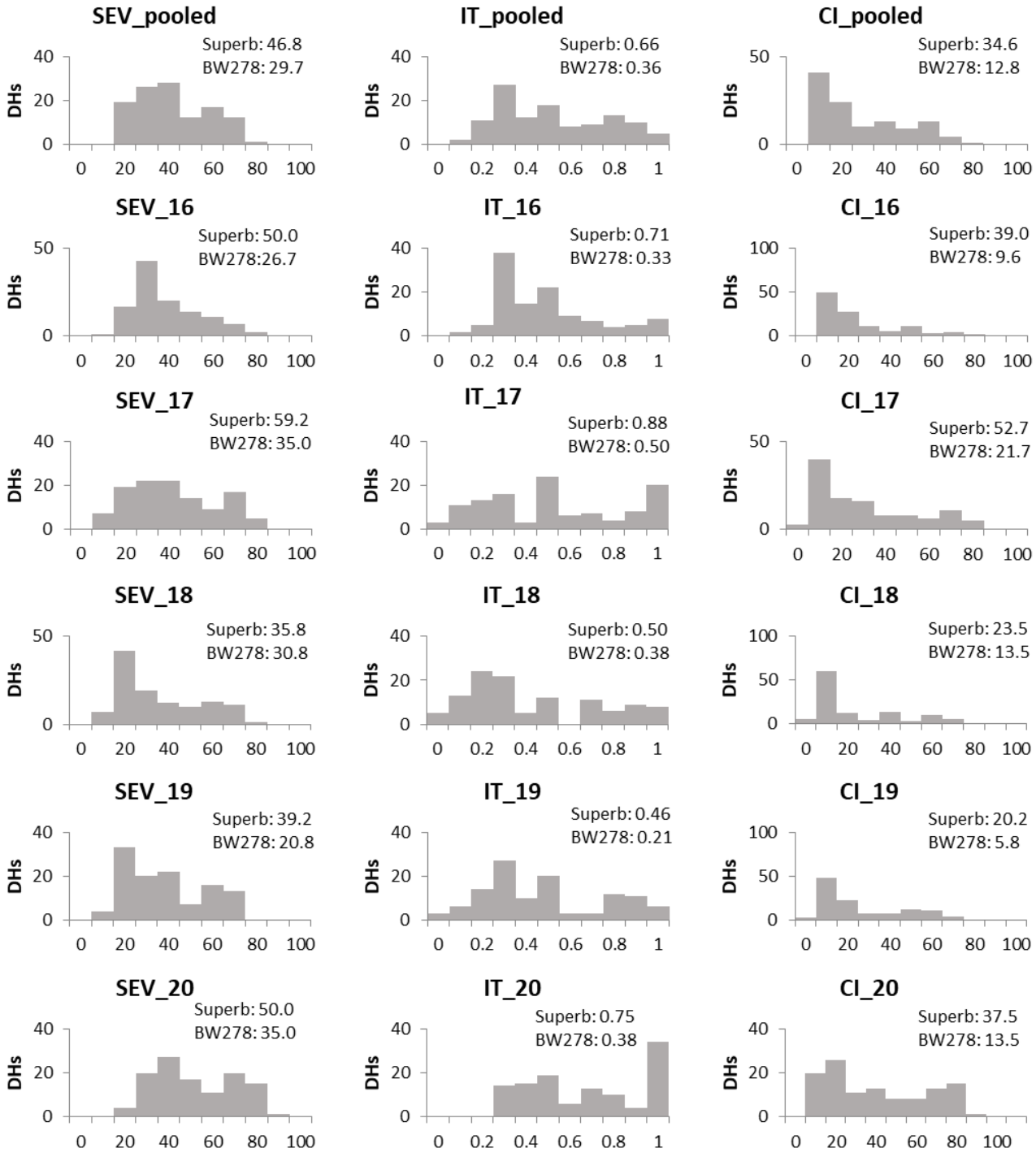

Figure 3.3. Histograms of mean leaf rust severity (SEV), infection type (IT), and coefficient of infection (CI) for the Superb/BW278 DH population in 5 site-years, and the pooled dataset.

Table 3.8. Pearson's correlation coefficients between pooled disease severity (SEV), infection type (IT) and coefficient of infection (CI) values from the Superb/BW278 DH population (n = 115).

	SEV	IT
IT	0.97[a]	
	<0.0001[b]	
CI	0.99[a]	0.95[a]
	<0.0001[b]	<0.0001[b]

[a]r2

[b]p-value

3.4.3 High-Density Genetic Map

BW278/AC Foremost

The BW278/AC Foremost RIL population was genotyped with the 90K wheat Infinium SNP array. Three RILs displayed greater than 10% missing data and were removed from further analysis, leaving a mapping population of 183 RILs. Of the 81,000 markers tested, 13,624 were polymorphic between the two parental lines, however 4,145 markers were removed due to large amounts of missing data or deviation from expected segregation ratios. The remaining 9,470 SNPs were sorted into linkage bins using the BIN function in QTL IciMapping, resulting in 1772 preliminary linkage bins. Marker data were then inputted into MapDisto, where 39 linkage groups (LGs) corresponding to the 21 chromosomes of hexaploid wheat were identified. The length of the whole genome map was 2,243.38 cM (Appendix 7.5). The population was also screened with two makers described as being tightly linked to *Lr46*, *csLV46G22* (CAPS;

Lagudah, personal communication) and *DK0900* (KASP; Konkin and Cuthbert, personal communication), and it was observed that both were polymorphic (Appendix 7.9).

Superb/BW278

One hundred and forty-two DH lines from the Superb/BW278 population were also genotyped with the 90K SNP array (Wang et al. 2014) and a set of microsatellite markers. A total of 7,298 markers were determined to be polymorphic between the parental lines. Mapping software MSTMap (Meng et al. 2015) was used to assist in sorting the markers into 70 linkage bins. A linkage map was generated in MapDisto using 1,015 non-redundant markers and resulted in 31 LGs spanning 1,880.01 cM (Appendix 7.6). This population was also screened with two *Lr46*-predictive markers described above, *DK0900* and *csLV46G22* (Appendix 7.9). However, both markers were monomorphic, as both parental lines and all the progeny tested positive for the allele associated with resistance.

3.4.4 QTL Analysis

BW278/AC Foremost

The LOD significance threshold for declaring a QTL was determined to be 3.5 for both IM and ICIM. Analysis for epistatic QTL was performed using 2.0 cM steps and a default LOD significance threshold of 5.0. QTL analyses were conducted for each individual site-year/leaf rust measure combination and the pooled data set for all five site years. Analysis revealed two QTL for leaf rust resistance in the BW278/AC Foremost RIL population, one on chromosome 1B and another on chromosome 5A (Table 3.9). These QTL were temporarily designated as

QLr.mrdc-1B and *QLr.mrdc-5A*, respectively. The resistance *QLr.mrdc-1B* was contributed by BW278 and was detected in all site-years and all measures of leaf rust (SEV, IT, CI; Figure 3.3). The highest LOD score for *QLr.mrdc-1B* was 27.42, for disease severity (SEV) in 2017 (Table 3.9). For *QLr.mrdc-5A,* the resistance allele was contributed by AC Foremost and was detected in one site-year (2018) and two measures of leaf rust (SEV, IT). The highest LOD score for *QLr.mrdc-5A* was 4.10 (Table 3.9). There are currently no leaf rust resistance genes catalogued on chromosome 5A (McIntosh et al. 2013).

Analysis for epistatic QTL was performed, and a significant interaction was detected between the *QLr.mrdc-1B* and a region on 5B that explained 1.87 and 6.98% of phenotypic variation for infection type (IT) and coefficient of infection (CI) respectively, in 2016 (Table 3.10). The interaction was detected with IM and was not present in the ICIM analysis.

Table 3.9. Additive effect QTL detected in the BW278/AC Foremost RIL population for leaf rust severity (SEV), infection type (IT) and coefficient of infection (CI). LOD threshold for declaring significant QTL was 3.5.

			IM[a]	IM	IM	IM	ICIM	ICIM	ICIM	ICIM
QTL	**Trait_ Year**	**LG[b]**	**Pos[c]**	**LOD[d]**	**PVE[e]**	**Add[f]**	**Pos**	**LOD**	**PVE (%)**	**Add**
QLr.mrdc-1B	SEV_16	1B	126.9	14.23	15.5	-12.84	126.9	17.20	28.6	-12.67
	IT_16	1B	126.9	9.30	10.9	-0.13	126.9	9.30	21.6	-0.13
	CI_16	1B	126.9	15.36	16.5	-16.74	126.9	15.36	32.8	-16.74
	SEV_17	1B	127.3	25.19	18.5	-24.88	126.9	27.42	46.5	-24.13
	IT_17	1B	127.3	24.85	14.3	-0.33	126.9	25.62	47.1	-0.32
	CI_17	1B	127.3	21.70	13.1	-26.04	126.9	22.04	42.6	-25.07
	SEV_18	1B	126.9	10.67	13.4	-10.97	126.9	12.73	24.5	-11.06
	IT_18	1B	126.9	10.17	14.0	-0.16	126.9	11.38	23.8	-0.15
	CI_18	1B	126.9	8.61	7.3	-11.16	126.9	9.46	19.6	-10.86
	SEV_19	1B	126.9	16.17	15.0	-17.69	126.9	18.00	34.1	-17.46
	IT_19	1B	126.9	20.45	24.2	-0.26	126.9	22.11	40.6	-0.26
	CI_19	1B	126.9	20.00	23.8	-21.95	126.9	20.00	39.9	-21.95
	SEV_20	1B	134.7	4.83	12.0	-9.74	134.7	5.60	12.0	-10.21
	IT_20	1B	134.7	4.28	10.6	-0.13	134.7	4.78	10.6	-0.13
	CI_20	1B	134.7	5.70	14.0	-11.99	134.7	6.68	14.0	-12.25
	SEV_pooled	1B	126.9	22.58	16.3	-16.53	126.9	25.03	43.4	-16.42
	IT_pooled	1B	126.9	22.12	16.2	-0.22	126.9	24.49	42.9	-0.21
	CI_pooled	1B	126.9	21.90	16.0	-18.35	126.9	24.03	42.5	-18.14
QLr.mrdc-5A	SEV_18	5A.1	88.0	3.73	4.2	6.04	88.0	4.10	7.0	5.98
	IT_18	5A.1	87.5	3.61	4.0	0.08	88.0	3.59	6.8	0.08

[a]IM, interval mapping; ICIM, inclusive composite interval mapping

[b]LG, linkage group

[c]Pos, position on linkage group (cM)

[d]LOD, peak LOD score, LOD significance threshold (IM)=3.5; LOD significant threshold (ICIM)=3.5

[e]PVE, phenotypic variation explained (r^2, %)

[f]Add, additive effect of allele substitution. The units are those of the respective trait. Negative in sign indicated the BW278 allele improved disease resistance, and vice-versa.

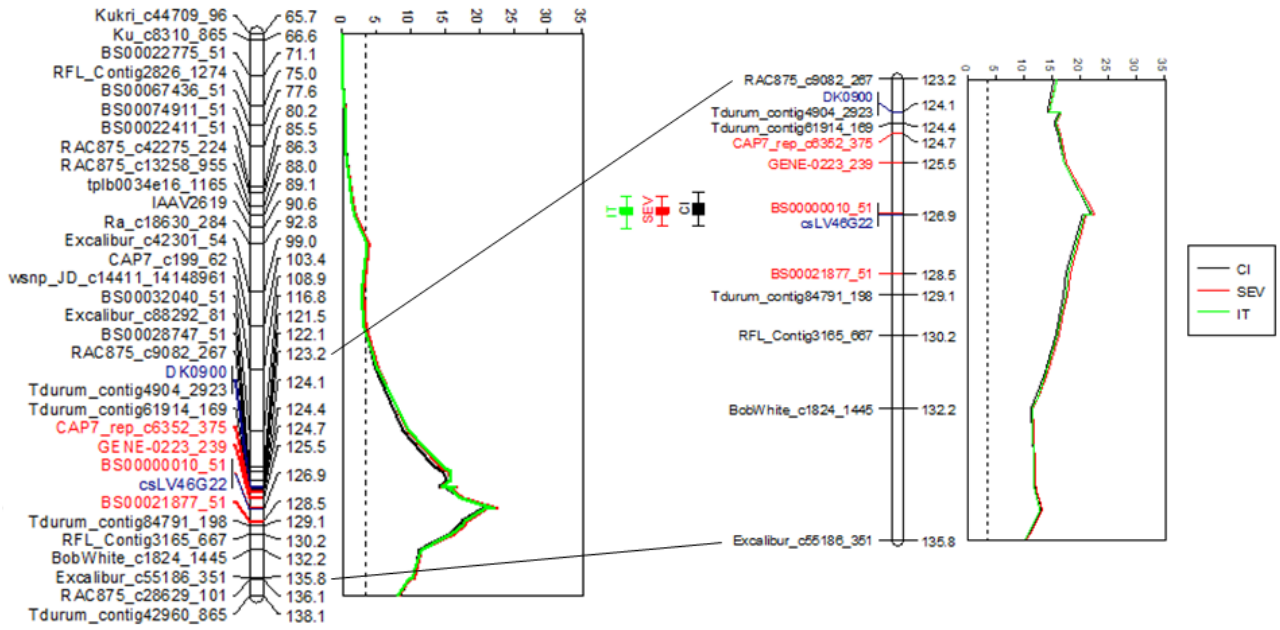

Figure 3.4. Partial linkage map of chromosome 1B from the BW278/AC Foremost RIL population. Plot of inclusive composite interval mapping (ICIM) LOD statistic from the pooled dataset identifies *QLr.mrdc-1B*. Tightly linked CAPS and KASP markers are indicated in blue. Markers selected for KASP development are indicated in red. One and two drop LOD support intervals are also indicated.

Table 3.10. Epistatic effect QTL detected in the BW278/AC Foremost RIL population for leaf rust severity (SEV), infection type (IT) and coefficient of infection (CI), using interval mapping (IM). The LOD threshold for declaring a significant epistatic interaction was 5.0.

Trait_ Year	Chrm_1[a]	Pos_1[b]	Chrm_2	Pos_2	LOD[c]	PVE(%)[d]	Add1[e]	Add2	AddbyAdd[f]
IT_16	1B	128.0	5B	144	6.34	1.87	-0.19	0.14	0.15
CI_16	1B	128.0	5B	144	5.03	6.98	-17.73	12.74	13.48

[a]Chrm, chromosome

[b]Pos, position on linkage group (cM)

[c]LOD, peak LOD score

[d]PVE, phenotypic variation explained (r^2, %)

[e]Add, additive effect of allele substitution. The units are those of the respective trait. Negative in sign indicated the BW278 allele increased disease resistance, and vice-versa.

[f]AddbyAdd, interaction between additive effects

Superb/BW278

Based on the permutation analysis, the LOD significance threshold for declaring a QTL was determined to be 3.2 for both IM and ICIM. Analysis for epistatic QTL was performed using 2.0 cM steps and a default LOD significance threshold of 5.0. QTL analysis was performed on the Superb/BW278 DH population and two significant leaf rust QTL were identified, one on chromosome 4B (*QLr.mrdc-4B*) and on on chromsome 5B (*QLr.mrdc-5B*). For both QTL the Superb allele decreased disease and thus contributed to resistance. *QLr.mrdc-4B* was detected in a single site-year (2017), for disease severity (SEV), and the highest LOD score was 3.30 (Table 3.11). *QLr.mrdc-5B* was detected in a single site year (2016) for all three measures of leaf rust

(SEV, IT, CI), and the pooled analysis for one measure of leaf rust (IT). The highest LOD score was 11.82 for disease severity (SEV) in 2016 (Table 3.11).

No significant QTL were detected on chromosome 1B. The linkage map generated for chromosome 1B spanned 15.74 cM, and did not cover the long arm, where *Lr46* has previously been mapped, as all markers in this region were monomorphic (Appendix 7.7). Analysis for epistatic QTL was performed, and no significant interaction between QTL was detected.

Table 3.11. Additive effect QTL detected in the Superb/BW278 DH population for leaf rust severity (SEV), infection type (IT) and coefficient of infection (CI). LOD threshold for declaring significant QTL was 3.1.

			IM[a]	IM	IM	IM	ICIM	ICIM	ICIM	ICIM
QTL	**Trait_ Year**	**LG[b]**	**Pos[c]**	**LOD[d]**	**PVE (%)[e]**	**Add[f]**	**Pos**	**LOD**	**PVE (%)**	**ADD**
QLr.mrdc-4B	SEV_17	4B	60.2	3.30	9.41	-7.04	60.1	3.30	9.4	-7.04
QLr.mrdc-5B	SEV_16	5B.1	31.0	9.80	27.3	-8.70	30.5	11.82	30.1	-8.02
	IT_16	5B.1	31.1	9.11	25.8	-0.13	30.5	10.97	28.2	-0.12
	CI_16	5B.1	30.6	8.47	24.4	-9.77	30.5	10.24	26.9	-9.12
	IT_ pooled	5B.1	30.5	4.69	15.5	-0.10	30.5	6.61	15.7	-0.09

[a]IM, interval mapping; ICIM, inclusive composite interval mapping

[b]LG, linkage group

[c]Pos, position on linkage group (cM)

[d]LOD, peak LOD score, LOD significance threshold (IM)= 3.2 LOD significant threshold (ICIM)= 3.2

[e]PVE, phenotypic variation explained (r^2, %)

[f]Add, additive effect of allele substitution. The units are those of the respective trait. Negative in sign indicated the allele increased disease resistance, and vice-versa.

3.4.5 Comparison of Parental Line Haplotypes on Chromosome 1BL

SNP marker data revealed that AC Domain and BW278 are identical for the entire length of chromosome 1B. Further inspection of the interval containing the 1B QTL in BW278/AC Foremost revealed that 64 markers within the interval were identical in BW278, Superb, and AC Domain (Table 3.12).

Table 3.12. Comparison of the parental lines with AC Domain in the 1B interval predicted to carry *Lr46*, based on the BW278/AC Foremost RIL population.

SNP Name	Wang et al. 2014[a]	RefSeq v1.0[b]	BW278	AC Domain	Superb	AC Foremost
Kukri_rep_c103550_213	1B_137	667,822,404	A	A	A	B
BobWhite_c8293_236	1B_137	667,931,309	A	A	A	B
wsnp_CAP11_rep_c4138_1957291	1B_137	667,965,756	A	A	A	B
wsnp_CAP11_rep_c4138_1957470	1B_137	667,966,065	A	A	A	B
Ex_c4436_1947	1B_141.82	668,124,283	A	A	A	B
wsnp_Ex_c4436_7981037	1B_141.82	668,124,497	A	A	A	B
BS00037246_51	1B_141.82	668,200,606	A	A	A	B
Tdurum_contig4997_166	1B_141.82	668,468,123	-	-	-	B
Tdurum_contig4997_178	1B_141.82	668,468,135	-	-	-	B
Tdurum_contig4997_816	1B_141.82	668,468,846	-	-	-	B
Tdurum_contig4997_974	1B_141.82	668,469,004	A	A	A	B
Tdurum_contig4838_349	1B_141.82	668,469,717	-	-	-	B
Tdurum_contig4838_718	1B_141.82	668,470,086	-	-	-	B
Kukri_c8395_1352	1B_141.82	668,654,432	A	A	A	B
Kukri_c56333_106	.	670,176,131	A	A	A	B
BS00000010_51	1B_143.98	670,783,574	A	A	A	B
Excalibur_c14706_421	1B_144.94	671,505,564	A	A	A	B
BS00066052_51	1B_145.76	671,607,056	A	A	A	B
RAC875_c16292_499	1B_144.94	671,965,231	-	B	B	B
IAAV1913	1B_145.8	671,995,928	A	A	A	-
BS00089959_51	1B_145.8	671,996,127	A	A	A	-
BS00036439_51	1B_145.8	671,997,689	A	A	A	-
BS00023105_51	1B_145.8	672,684,826	A	A	A	-
BobWhite_rep_c50057_164	1B_145.8	672,779,761	A	A	A	B
Tdurum_contig93330_263	1B_148.4	673,176,270	A	A	A	B
wsnp_Ex_c48407_53323801	1B_145.76	673,176,399	A	-	-	B
Kukri_c1259_864	1B_148.4	673,176,471	A	A	A	B

GENE-0514_210	1B_148.4	673,177,310	A	A	A	B
BS00021877_51	1B_154.58	673,742,894	A	A	A	B
IACX17310	1B_148.4	673,743,193	A	A	A	B
RAC875_c3001_1236	1B_148.4	673,743,425	A	A	A	B
BS00084990_51	1B_154.58	673,745,662	A	A	A	B
Tdurum_contig62905_530	1B_148.4	673,784,676	A	A	A	B
Tdurum_contig10354_270	1B_148.4	673,786,365	A	A	A	B
Tdurum_contig10354_170	1B_148.4	673,786,465	A	A	A	B
Excalibur_c11190_617	1B_152.85	673,849,271	A	A	A	B
Kukri_c15757_1117	1B_152.85	673,851,296	A	A	A	B

[a]Position (cM) in the Wang et al. 2014 consensus map.

[b]Physical location (bp) in the Chinese Spring reference genome sequence, RefSeq v1.0 (International Wheat Genome Sequencing Consortium 2018).

[c]Letters ‘A’ and ‘B’ represent the BW278 allele, and alternate allele, respectively.

3.4.6. Marker Validation

Ten SNP markers that were linked to *QLr.mrdc-1B* in the BW278/AC Foremost RIL population were converted to KASP markers to evaluate their potential utility in breeding programs. Due to the limited timeframe of a M.Sc. project, and the ongoing novel coronavirus pandemic, testing of these markers has temporarily been put on hold.

3.5 Discussion

Spring wheat line BW278 is derived from AC Domain, a cultivar that has played an important role as a parent in Canadian wheat breeding programs, as it is high-yielding, well adapted, and carries good resistance to pre-harvest sprouting (Townley-Smith and Czarnecki 2008). BW278 displayed a 'slow-rusting' reaction or quantitative resistance response to leaf rust in preliminary field trials. Initially, the gene responsible for the resistance was hypothesized to be *Lr34*, a slow-rusting, multi-pest, adult plant resistance gene (APR) present on wheat chromosome 7D (Kolmer et al. 2008). However, prior to this study, marker testing and further characterization of BW278/AC Foremost revealed that the gene responsible was likely present on chromosome 1B and could be *Lr46* which confers a similar phenotype as *Lr34* (Hiebert, personal communication). Other leaf rust resistance genes present on 1B include *Lr26, Lr33, Lr44, Lr51, LrZH84, Lr71* and *Lr75.* None of these genes are thought to be present in BW278 as they are either derived from alien species (*Lr51*), are seedling resistance genes (*Lr33, Lr44*), display a different reaction to *P. triticina* than *Lr46* (*LrZH84, Lr26*), or have been mapped near the centromere (*Lr71*) or short arm (*Lr75*) of chromosome 1B (Singh et al. 2013, Zhao et al. 2008).

In the present study, two populations derived from BW278 were evaluated for leaf rust resistance over five years in inoculated field trials, indicating that the line provides resistance to prevalent *P. triticina* isolates in Western Canada. A QTL on chromosome 1B, *QLr.mrdc-1B*, derived with the resistant allele from BW278 was detected in the BW278/AC Foremost RIL population in all five site-years and all three measures of leaf rust resistance. The QTL was tightly linked to CAPS marker *csLV46G22* (Lagudah, personal communication) and KASP marker *DK0900* (Konkin and Cuthbert, personal communication), both described as closely

linked to the *Lr46* locus. A second QTL was detected in the BW278/AC Foremost population, on chromosome 5A (*QLr.mrdc-5A*) with the resistant allele from parental line AC Foremost. No known *Lr* genes have been catalogued on 5A (McIntosh et al. 2013). *QLr.mrdc-5A* was only significant in one environment, so further investigation is required to confirm the QTL.

While not in the region of interest, two leaf rust QTL were detected in Superb/BW278, on chromosomes 4B and 5B, designated *QLr.mrdc-4B* and *QLr.mrdc-5B*, respectively. The resistant allele for both QTL was contributed from parental line Superb. Superb carries *Lr2a* on 2D and *Lr10* on 1A (McCallum and Seto-Goh 2010). Four *Lr* genes have been catalogued to chromosome 4B (*Lr12, Lr31, Lr49*) and two to 5B (*Lr18, Lr52*). Further investigation is required to characterize these QTL. No significant QTL were detected on chromosome 1B in Superb/BW278. When parental lines BW278 and Superb were compared with AC Domain (Table 3.12), it was revealed that all three lines had the same haplotype in the 1B region. This was an unexpected result because this indicates that Superb also has *Lr46*, however, it is moderately susceptible to leaf rust under field conditions (McCallum, personal communication). It is possible that the long arm of 1B was selected for throughout the breeding process, as it seems to have remained completely intact from AC Domain to Superb. Results from this study indicate that the Superb/BW278 population is fixed for the 1B QTL and both parents carry *Lr46*. While our attempt to map *Lr46* in Superb/BW278 was unsuccessful, this result still provides insight into the genetics of Canadian wheat germplasm. The interactions between *Lr46* and other resistance genes could be critical since Superb is much more susceptible than BW278, although both have *Lr46*. This population could be used in future studies to investigate the interaction of *Lr46* with other resistance genes.

Lan et al. (2015) reported that efficacy of the *Lr46* locus is highly dependent on the genetic background of the host and environmental conditions. It is also possible that the gene interacts with other resistance genes, as is the case with *Lr34* (German and Kolmer 1992; Kolmer 1996). The APR could be interacting with both effective (pathogen virulence has not yet evolved, or viulence is not prevalent in pathogen population) and defeated (pathogen virulence has evolved and is common in the pathogen population) resistance genes. In previous QTL analyses, the *Lr46* locus explained between 2.9 and 74.5% of total phenotypic variance (PVE) (Pinto da Silva et al. 2018). A recent study of Toropi, a Brazilian cultivar that is durably resistant to leaf rust, showed that a QTL at the *Lr46* locus explained between 1.76 and 16.61% of total PVE under Canadian growing conditions (Rosa et al. 2019). Our study, performed under the same environmental conditions as the study of Toropi, revealed that the QTL present on 1B in BW278/AC Foremost explained between 12.0 and 46.5% of total PVE in disease severity. This suggests that the APR gene in BW278 has a larger effect than the gene in Toropi under similar growing conditions.

In Toropi, the QTL associated with *Lr46* did not provide high levels of leaf rust resistance alone, but resistance improved when in combination with other QTL on chromosomes 5A and 5D (Rosa et al. 2019). Kolmer et al. (2015) showed that the effect of the *Lr46* locus was enhanced when combined with a QTL on chromosome 5B, derived from cultivar Americano 25e. We observed epistatic interaction between the leaf rust QTL on 1B and a region on chromosome 5B (Table 3.10). However, the interaction was only significant in a single site-year and no significant additive effect QTL were detected in the 5B region in the present study.

Other characterized leaf rust APR genes provide similar levels of resistance as the QTL on 1B in our study. In wheat cultivar Sujata, *Lr67,* located on chromosome 4D, contributed

between 33.6 and 57.9% of the total PVE (Lan et al. 2015). Similarly, *Lr67* contributed between 20 and 44.5% of total PVE in wheat line W195 (Chherti et al. 2016). To date, *Lr34*, located on chromosome 7D has been reported in 20 different studies, where the total PVE contributed by the QTL ranged from 8.0 to 75.2% (Pinto da Silva et al. 2018).

Lr46 has the potential to be highly useful in developing disease resistant wheat cultivars, due to its ability to provide improved resistance to four fungal pathogens including *P. triticina* (*Lr46*), *P. graminis* f. sp. *tritici* (*Sr58*), *P. striiformis* f. sp. *tritici* (*Yr29*) (Singh et al. 2013) and *Blumeria graminis* f. sp. *tritici* (*Pm39*) (Lillemo et al. 2008). The *Lr46* locus has been mapped in several studies of wheat (Herrera-Foessel et al. 2008; Kolmer et al. 2015; Qi et al. 2015; Aktar-Uz-Zaman et al. 2017; Ren et al. 2017) and has been implemented into breeding programs (Rosewarne et al. 2008; Lan et al. 2017). However, the gene has been difficult to map, which may be due to challenges with phenotyping, or it could be the result of more than a single gene in the region with similar effects.

This study has provided insight into leaf rust resistance genetics in Canadian wheat breeding lines. To further characterize the adult plant resistance carried in BW278, additional mapping populations should be developed with that specific goal in mind. In addition, the interaction between the adult plant resistance and other resistance genes should be investigated. Markers developed in this project should be used to screen Canadian wheat germplasm to determine the distribution of the gene. The information generated here will provide a foundation for development of breeder-friendly diagnostic markers for incorporation into wheat breeding programs.

CHAPTER 4

GENETIC MAPPING OF LEAF RUST RESISTANCE GENE *Lr2a* IN COMMON WHEAT (*Triticum aestivum)*

4.1 Abstract

Leaf rust, caused by the fungal pathogen *Puccina triticina* Eriks., is a threat to wheat production worldwide. Incorporation of effective leaf rust resistance (*Lr*) genes into high yielding wheat cultivars is an efficient method of disease control. *Lr2* is a multiallelic resistance gene, with alleles *Lr2a, Lr2b* and *Lr2c*. This study was performed to map and develop diagnostic markers for *Lr2a*. Superb was a widely grown cultivar in Canada from 2003-2012 due to its yield potential and lodging resistance and carries *Lr2a* and *Lr10*. In this study, two doubled haploid (DH) mapping populations developed from the crosses Superb/BW278 and Superb/86ISMN 2137 were genotyped with the wheat 90K iSelect SNP array and microsatellite markers and evaluated for leaf rust resistance in greenhouse assays. Two-point linkage analysis was performed between the SNP markers and infection type ratings using isolates 74-2 MGBJ (Superb/BW278) and 12-3 MBDS (Superb/86ISMN 2137). A KASP marker, kwh740, was designed from iSelect SNP marker *Excalibur_c1944_1017* that was closely linked to seedling resistance gene *Lr2a* on wheat chromosome 2DS in both mapping populations.

4.2 Introduction

Wheat is a staple food crop worldwide, providing the main source of protein for more than four billion people (Shiferaw et al. 2013). In Canada, over 34,000 t of wheat were produced in 2020 (Canadian Cereals 2020). Despite its successful cultivation around the world, wheat yield is limited by various diseases. Of these diseases, wheat leaf rust caused by the fungal pathogen *Puccinia triticina* Eriks. is the most common and widespread (Kolmer 1996; Oelke and Kolmer 2005). Depending on environmental conditions and genetics of the host cultivar, yield reduction due to leaf rust infection can range from 5-20% (McCallum et al. 2010). While application of synthetic chemical fungicides can control the pathogen, genetic host resistance is the most desirable control method as it provides resistance throughout the growing season with little additional cost to producers and the environment.

Due to the rapid evolution of *P. triticina*, virulence surveys are performed annually in North America to monitor pathogen populations and provide important information to breeding programs. In recent years, pathogen virulence patterns in North America have shifted, with the most prevalent race in 2018 being MNPS, accounting for 35.3% of isolates in the United States (Kolmer, unpublished) and 29.4% of isolates in Canada (McCallum, unpublished). This shift in virulence suggests that many *Lr* genes previously described as ineffective could once again be useful in breeding programs if released in combination with other *Lr* genes.

Seedling resistance gene *Lr2a* has been widely used in Canadian wheat cultivars, and results in a low infection type on wheat plants in response to infection. Despite its widespread use in Canada, *Lr2a* has only been mapped using cytogenetic techniques, where it was reported to be approximately 38 cM distal to the centromere (McIntosh and Baker 1968) on the short arm

of wheat chromosome 2D (Luig and McIntosh 1968). Researchers at the Morden Research and Development Center- Agriculture and Agri-Food Canada (MRDC-AAFC) developed two doubled haploid (DH) populations from Superb, a spring wheat cultivar that carries *Lr2a*.

The objectives of this study were to: (i) genetically map seedling leaf rust resistance gene *Lr2a* carried in spring wheat cultivar 'Superb' and (ii) identify tightly linked flanking markers to *Lr2a* that could be useful in breeding programs.

4.3 Materials and Methods

4.3.1 Population Development

Two doubled haploid (DH) mapping populations were produced from the cultivar Superb for use in this study using the maize pollination method (Thomas et al. 1997). Superb is a backcross derived DH line (Grandin*2/AC Domain) that carries the seedling resistance gene *Lr10* in addition to the gene of interest, *Lr2a* (Townley-Smith et al. 2010). The Superb/BW278 DH population was initially used in QTL analysis studies of yield and yield components. BW278 is a backcross derived DH line (AC Domain*2/Sumai 3) that carries seedling resistance gene *Lr16* as well as an adult plant resistance (APR) gene, hypothesized to be *Lr46*. The population consists of 402 DH lines, but due to time and space limitations, a subset of 142 lines were used to map *Lr2a* in this study.

A second DH mapping population derived from the cross Superb/86ISMN 2137 was also used in this study to generate a linkage map for *Lr2a*. This population consists of 198 DH lines, but due to high amounts of missing data and inconsistencies with genotyping, 167 DH lines were

used for mapping. 86ISMN 2137 is a spring wheat line of unknown pedigree that carries an unnamed leaf rust resistance gene, either *LrCen* or *LrMar* (McCallum, personal communication).

4.3.2 Indoor Seedling Inoculations

Approximately five seeds per line from the DH mapping populations were germinated in clusters in large fiber trays containing a 3:2 mixture of bulk soil to Sun Gro Horticulture Sunshine Professional Growing Mix 5 (Agawam, MA, USA; www.sungro.com). Two replicates of each parental line, Thatcher, and Thatcher near isogenic lines (Tc-*Lr16*, Tc-*LrCen*, Tc-*Lr10*, Tc-*Lr2a*, Tc-*Lr2b*, Tc-*Lr2c*) were included as checks in the Superb/BW278 single race inoculations. For single race inoculations of the Superb/86ISMN 2137 mapping population, parental lines, Thatcher, Neepawa, CDC Stanley, Trident, Little Club, and Thatcher Near Isogenic lines (Tc-*Lr16*, Tc-*Lr22a*, Tc-*Lr37*, Tc-*Lr32*, Tc-*Lr60* and Tc-*LrCen*) were included as checks.

Seedlings were grown for ten days in a growth chamber under 16 light and 8 dark hours, set to 20°C and 18°C, respectively. Ten-day old seedlings were inoculated with single race inoculum developed via methods previously described by McCallum and Seto-Goh (2003). A differential set of 16 NILs, was inoculated at the same time as the DH populations to confirm the race designation for each isolate. Prior to inoculation, spore viability was tested by spreading a single layer of spores across a petri plate containing 2% water agar. The plate was left on the work bench, out of direct sunlight for approximately four hours and was then viewed under a microscope to verify successful urediniospore germination.

Four isolates (06-1-1 TDBG, 12-3 MBDS, 74-2 MGBJ, and 77-2 TJBJ) were used to inoculate the Superb/86ISMN 2137 population in separate experiments (Appendix 7.11). Isolates

used are listed with their isolate number along with their virulence phenotypes that were coded using an alphabetic system described by (Long and Kolmer 1989). Six isolates (1-1 BBBD, 12-3 MBDS, 128-1 MBRJ, 74-2 MGBJ, 11-180-1 TDBJ, and 77-2 TJBJ) were used to inoculate the Superb/BW278 population in separate experiments (Appendix 7.10). Additional single race inoculations using 74-2 MGBJ (Superb/BW278) and 12-3 MBDS (Superb/86ISMN 2137) were performed as confirmation of phenotypic infection type ratings for two-point linkage analysis, and to evaluate DH lines that were missing from the original data sets.

To prepare inoculum, vacuum dried urediniospores were removed from refrigeration and heat shocked for six minutes in a water bath set to 40°C. The urediniospores were suspended in light mineral oil (Soltrol 170; Chevron Phillips Chemical Company, TX, USA) and sprayed onto leaves using compressed air. Immediately after inoculation, plants were air dried for 30 minutes to allow the oil to volatize. The seedlings were then placed in a dew chamber set to 100% relative humidity under dark conditions and left overnight. Seedlings were removed from the dew chamber the following day and allowed to air dry before being placed in the previously described growth cabinet.

4.3.3 Disease Assessment

Seedling ratings were performed on the first and second leaves approximately 12 days post inoculation (DPI). Infection types were evaluated using the leaf rust rating scale described by Long and Kolmer (1989) with modifications described by Roelfs et al. (1992). Infection types (ITs) “;”(hypersensitive flecking), “1” (necrosis and small uredinia), and “2” (chlorosis with small to medium sized uredinia) were considered resistant. Plants with an IT of “3” (no necrosis or chlorosis, medium sized uredinia) or “4” (no necrosis or chlorosis, large uredinia) were

considered as susceptible (Long and Kolmer 1989). Resulting phenotypic ratios were tested for goodness of fit using Chi-Square analysis.

4.3.4 DNA Extraction

The following protocol was used for DNA extraction for 90K Illumina Infinium assay analysis. Approximately 4-6 pieces (1-2 cm in length) of leaf tissue from young, uninfected leaves were collected after seedling ratings were performed. Tissue was collected directly into 96-well microtube extraction plates. Each well contained a tungsten carbide bead to macerate the tissue. Tissue was lyophilized for 24 hours and stored at -20ºC. Prior to extraction, tissue was brought to room temperature and ground into a fine powder using a TissueLyser II (Quiagen, Mississauga, ON; www.qiagen.com). Orientation of the plates was reversed every 2.5 minutes for a total of 10 minutes.

The Qiagen DNeasy 96 Plant Kit (Quiagen, Mississauga, ON; www.qiagen.com) was used to extract DNA from the Superb/BW278 DH mapping population. DNA was quantified using PicoGreen stain (Life Technologies Inc, Burlington, ON; www.lifetechnologies.com) on a fluorometer, and stock DNA was then diluted to a 50 ng/µl working solution. DNA from the Superb/86ISMN 2137 DH population was extracted using the modified ammonium acetate protocol (Pallotta et al. 2003). DNA was quantified on a fluorometer using Hoechst 33258 stain, and stock DNA was diluted to a 50 ng/µl working solution.

4.3.5 Genotyping and genetic map construction

The Illumina Infinium (San Diego, CA, USA; www.illumina.com) iSelect 90K wheat SNP array (Wang et al. 2014) was used to genotype both DH populations. The Superb/BW278

population was also genotyped with a set of 270 microsatellite (SSR) markers distributed across the wheat genome (Cuthbert et al. 2008). Marker filtering and genotype calling for Infinium markers was performed using GenomeStudio (Illumina Inc; http://bioinformatics.illumina.com/informatics/sequencing-microarray-data-analysis/genomestudio.html). Assays were sorted into three distinct clusters, AA, AB, and BB, which corresponded to the expected genotypes for bi-allelic SNPs. Genotypes were then converted into numeric scores, where AA = 0, AB = 1 and BB = 2. Numeric values were then converted into A, B or missing data (-) based on sorting of SNP markers by both parental lines. Missing data (-) values were recorded for markers than could not be accurately scored for a line, and lines with more than 10% missing data were removed from analysis. No lines were removed from linkage analysis of the Superb/BW278 mapping population (n = 142), and 31 lines were removed from Superb/86ISMN 2137 (n = 198), leaving a final mapping population of 167 DH lines. The female parent in both crosses, Superb, was coded as 'A'. The male parents, BW278 and 86ISMN 2137, were coded as 'B'. Redundant, co-segregating markers from the Superb/86ISMN 2137 mapping population were sorted into linkage bins using the BIN function in mapping software QTL IciMapping v. 4.2 (Meng et al. 2015). Minimum Spanning Tree Map (MSTMap) (Wu et al. 2008) was used to sort redundant, co-segregating markers from the Superb/BW278 mapping population into linkage bins.

The selected markers from MSTMap and QTL IciMapping were used for linkage mapping in MapDisto v. 1.7.7 (Lorieux, 2012). The Kosambi mapping function (Kosambi 1943), with a minimum logarithm of odds (LOD) threshold of 3.0 and a maximum recombination fraction of 0.3, was used to calculate genetic distances (cM) from recombination fractions. Loci were ordered using the AutoMap function. Marker data was checked, and error candidates were

replaced with missing data (-). Markers that displayed significant segregation distortion ($p<0.01$) were removed from further analysis. Linkage groups were assigned to chromosomes based on the published consensus map location of SNP markers (Wang et al. 2014). Phenotypic leaf rust reactions were mapped as Mendelian factors. Phenotypic ratings from the seedling inoculation with 74-2 MGBJ were re-coded to allow for two-point linkage with the generated marker data in the Superb/86ISMN 2137 mapping population. Infection types of "3-3+" and "1-1+" were scored as B (susceptible or resistant non *Lr2a* infection type), and infection types of "0" were scored as A (resistant or typical of *Lr2a*). Phenotypic results from the seedling inoculation with 12-3 MBDS were re-coded to allow for two-point linkage analysis with the generated marker data for the Superb/BW278 population. DH lines with ITs of "3-3+" and "1+" were coded as B (susceptible or resistance not typical of *Lr2a*) and DH lines with IT of "0-0;" were coded as A (resistant- typical of *Lr2a*). MapChart version. 2.3 (Voorrips 2002) was used to draw and compare genetic maps. Linkage maps were compared to the consensus map generated by Wang et al. (2014) as well as the Chinese Spring reference genome (RefSeq v1.0; International Wheat Genome Sequencing Consortium 2018).

Seven 90K Infinium SNP markers that either flanked or co-segregated with *Lr2a* in the Superb/BW278 mapping population were selected for development of KASP assays (Table 4.1), with primers selected with Primer Picker software (Appendix 7.12). KASP conditions used in the present study were the same as described in Chapter 3 of this thesis. Two of the seven selected SNPs, kwh375 and kwh740 were also polymorphic and flanked *Lr2a* in the Superb/86ISMN 2137 mapping population (Table 4.1). The KASP assays were re-tested on both mapping populations. The Omega Fluorostar plate reader (BMG LABTECH GmbH, Ortenberg, Germany) was used to detect fluorescence, and KlusterCaller (LGC Genomics, Beverly, USA) was used to

call the genotypes. For each marker, individual lines that did not fit in either parental cluster were scored as missing data.

Table 4.1. SNP-derived KASP markers that displayed linkage to *Lr2a* in the Illumina Infinium assay. Missing data are reported as ".", and occurred as a result of the markers being monomorphic, or un-mapped.

			Position			
KASP ID	**90K wheat SNP ID**	**Chrm.[a]**	**Superb/ BW278[b]**	**Superb/ 86ISMN2137[c]**	**Wang et al. 2014. Pos.[d]**	**RefSeq v1.0[e]**
kwh735	wsnp_CAP12_c1503_764765	2D	49.9	24.7	28.1	35.7
kwh736	Kukri_c25843_669	2D	65.1	.	.	56.2
kwh737	D_GBUVHFX02GV41H_67	2D	65.1	.	36.5	57.1
kwh738	Kukri_c20972_618	2D	65.1	.	36.5	59.7
kwh739	Excalibur_c20175_370	2D	65.1	.	40.1	60.9
kwh740	Excalibur_c1944_1017	2D	65.1	37.2	.	61.2
kwh741	RAC875_c65419_229	2D	65.1	.	36.5	61.2

[a]Chrm, chromosome

[b]Position in the Superb/BW278 DH population.

[c]Position in the Superb/86ISMN 2137 DH population.

[d]Position in the consensus map developed by Wang et al. 2014.

[e]Physical location (Mb) in the Chinese Spring reference genome RefSeq v1.0; International Wheat Genome Sequencing Consortium 2018.

4.4 Results

4.4.1 Seedling Leaf Rust Resistance Inoculations

Superb/BW278 seedling inoculations

The Superb/BW278 mapping population (n = 142), plus the parental lines were phenotypically evaluated using six *P. triticina* isolates to characterize seedling resistance within the population. Certain DH lines had low seed quantity, or did not germinate in seedling assays, resulting in missing data. A second replicate of the inoculation with 74-2 MGBJ was performed to complete the data set for linkage mapping. Table 4.2. summarizes the parental infection types (ITs). Superb carries seedling leaf rust resistance genes *Lr2a* and *Lr10* (McCallum and Seto-Goh 2010). BW278 carries seedling resistance gene *Lr16* (McCartney et al. 2005). Superb displayed an infection type of "00;" in response to four isolates of *P. triticina* (1-1 BBBD, 12-3 MBDS, 128-1 MBRJ, 74-2 MGBJ), indicated by no pustule development or small hypersensitive flecks on the leaf surface. When inoculated with isolates 11-180-1 TDBJ and 77-2 TJBJ, which are virulent to both *Lr2a* and *Lr10*, Superb displayed a susceptible IT of "3+", indicated by large uredinia and absence of necrosis or chlorosis. BW278 displayed an IT of ";1-1+" in response to all six *P. triticina* isolates (1-1 BBBD, 12-3 MBDS, 128-1 MBRJ, 74-2 MGBJ, 11-180-1 TDBJ and 77-2 TJBJ), indicated by small uredinia, hypersensitive flecking and necrosis on the leaf surface, characteristic of *Lr16* and distinct from the *Lr2a* resistance response.

Table 4.2. Seedling reactions of parental wheat lines (Superb and BW278) to six isolates of *Puccinia triticina.*

Parental Line	**1-1 BBBD[a]**	**12-3 MBDS[a]**	**128-1 MBRJ[a]**	**74-2 MGBJ[a]**	**11-180-1 TDBJ[a]**	**77-2 TJBJ[a]**
Superb	0;	0;	0	0	3+	3+
BW278	;1-	1+	1+	1+	1+	1+

[a]Reaction types 0-4, where 0-2 represent a resistant response and 3-4 represent a susceptible response.

Both parental lines in the Superb/BW278 population displayed resistance to 1-1 BBBD (Table 4.2), indicated by hypersensitive flecking, necrosis and small/few uredinia. A segregation ratio of 102 resistant to 13 susceptible was observed in the progeny at the seedling stage, which fit a three-gene (7:1) segregation ratio (p = 0.693; Table 4.3). The 13 lines observed to be susceptible to 1-1 BBBD were also susceptible to the other five *P. triticina* isolates, an observation that is consistent with three genes controlling resistance to 1-1 BBBD.

Parental line BW278 showed a resistant response of '1+' to isolates 11-180-1 TDBG and 77-1 TJBJ, which is not typical of *Lr2a*, but is typical of *Lr16.* Both TDBG and TJBJ are virulent to *Lr2a* and avirulent to *Lr16*. Parental line Superb displayed a susceptible response of '3+' (Table 4.2). These inoculations resulted in 51:63 and 50:65 resistant to susceptible segregation ratios, respectively (Table 4.3). Both ratios fit an expected single gene (1:1) segregation ratio (p = 0.261; p = 0.162; Table 4.3). The 50 resistant lines in the TJBJ inoculation were also resistant to TDBG, indicating that a single gene contributed from parental line BW278, likely *Lr16*, is responsible for resistance to both isolates.

The Superb/BW278 population segregated 86 resistant to 29 susceptible segregation ratios when inoculated with either 12-3 MBDS or 128-1 MBRJ. Both isolates are avirulent on *Lr2a* and *Lr16*, and virulent on *Lr10*. This result fit a two-gene (3:1) segregation ratio (p = 0.957;

Table 4.3), and lines that were resistant to 12-3 MBDS were also resistant to 128-1 MBRJ. This indicates that both isolates were detecting the same two genes, *Lr2a* from Superb and *Lr16* from BW278.

Based on the North American race nomenclature system (Long and Kolmer 1989), *P. triticina* isolate 74-2 MGBJ is avirulent on *Lr2a* and virulent on *Lr16* and *Lr10*, which would result in a predicted single gene (1:1) segregation ratio in the Superb/BW278 DH population. However, the inoculation resulted in 70 resistant and 41 susceptible DH lines, which did not fit the expected single gene ratio ($p = 0.006$; Table 4.3). The resistant lines in this inoculation were also resistant to 12-3 MBDS and 128-1 MBRJ, indicating that 74-2 MGBJ is either avirulent or partially avirulent to *Lr16*, the seedling resistance gene contributed by parental line BW278. While MGBJ is classified as virulent on *Lr16*, it is often rated as avirulent when in combination with other genes or when in backgrounds other than Thatcher (McCallum, personal communication). However, IT ratings for this inoculation were still able to be used in linkage mapping, as the IT typical of *Lr16* (1-1+) was easily distinguished from the IT of *Lr2a* (0-0;). Therefore, all lines could be scored unambiguously for the presence or absence of *Lr2a*.

Table 4.3. Segregation of seedling resistance to seven isolates of *Puccinia triticina* in the Superb/BW278 DH population under controlled environmental conditions.

Isolate	R[a]	S[b]	-[c]	Expected R[a]	Expected S[b]	Expected Ratio	χ^2 (p-value)
1-1 BBBD	102	13	27	100.6	14.4	7:1	0.693
77-2 TJBJ	50	65	27	57.5	57.5	1:1	0.162
11-180-1 TDBG	51	63	28	57	57	1:1	0.261
128-1 MBRJ	86	29	27	86.25	28.75	3:1	0.957
12-3 MBDS	86	29	27	86.25	28.75	3:1	0.957
74-2 MGBJ_1	70	41	31	57.5	57.5	1:1	0.006
74-2 MGBJ_2	81	58	3	69.5	69.5	1:1	0.051

[a]Resistant DH lines

[b]Susceptible DH lines

[c]Missing data

Superb/86ISMN 2137 seedling inoculations

The Superb/86ISMN 2137 DH mapping population (n = 198), plus the parental lines were phenotypically evaluated using four isolates of *P. triticina* to characterize the seedling leaf rust resistance present in the population. Table 4.4 summarizes the parental infection types (ITs). Superb displayed a low IT of '0' in response to two isolates (74-1 MGBJ and 12-3 MBDS) indicated by no uredinia development and absence of necrosis and chlorosis, and high ITs of '3+' and '3' in response to isolates 06-1-1 TDBG and 77-2 TJBJ.

Parental line 86ISMN 2137 displayed high ITs of '3+' to 74-1 MGBJ and 12-3 MBDS, and '3-' to isolate 77-2 TJBJ. In response to 06-1-1 TDBG, parental line 86ISMN 2137 displayed an 'X' IT, indicated by variably sized uredinia and necrotic flecking on the leaf.

Table 4.4. Seedling reactions of parental lines (Superb and 86ISMN 2137) to four races of *Puccinia triticina.*

Parental line	74-2 MGBJ[a]	12-3 MBDS[a]	77-2 TJBJ[a]	06-1-1 TDBG[a]
Superb	0	0	3-	3+
86ISMN 2137	3+	3+	3-	X

[a]Infection types 0-4 where 0-2 represent resistant responses and 3-4 represent susceptible responses. An infection type of "X" is a mesothetic response and is classified as resistant.

Superb displayed an IT of '0', a response typical of *Lr2a,* when inoculated with both 74-2 MGBJ and 12-3 MBDS, and the progeny displayed 79:60 and 97:82 resistant to susceptible segregation ratios, respectively. The observed segregation ratios for both 74-2 MGBJ and 12-3 MBDS fit the expected single gene (1:1) ratio ($p = 107$; $p = 0.262$; Table 4.5), indicating that a single gene contributed by Superb is responsible for resistance to both races. Most of the DH lines were either resistant to both isolates or susceptible to both isolates, however, 19 DH lines that displayed resistance to 74-2 MGBJ were rated as susceptible to 12-3 MBDS.

P. triticina isolate 06-1-1 TDBG is virulent on both *Lr2a* and *Lr10*, the two seedling genes contributed by Superb in this population. The mesothetic 'X' response displayed by parental line 86ISMN 2137 and the observed 80 resistant to 89 susceptible segregation ratio of the progeny indicates that 86ISMN 2137 is contributing a seedling resistance gene, likely *LrCen* (McCallum, personal communication). The observed ratio fit a single gene segregation ratio ($p = 0.489$; Table 4.5).

When inoculated with 77-2 TJBJ, both parental lines displayed a susceptible IT of '3-', indicating that this isolate is virulent on all seedling resistance genes in this population. However, an observed segregation ratio of 42 resistant DHs to 133 susceptible DHs was observed (Table 4.5), a result that did not fit the expectation that none of the DH lines would display a resistant response. Of the 42 DHs rated as resistant, 15 displayed an IT of "2/3", 19 were rated between "2-2+" and could be attributed to borderline infection types.

Table 4.5. Segregation of seedling resistance to four races of *Puccina triticina* in the Superb/86ISMN 2137 DH mapping population performed under controlled environmental conditions.

Isolate	**R[a]**	**S[b]**	**-[c]**	**Expected R[a]**	**Expected S[b]**	**Expected Ratio**	**χ^2 (p-value)**
74-2 MGBJ	79	60	59	69.5	69.5	1:1	0.107
12-3 MBDS	97	82	19	89.5	89.5	1:1	0.262
77-2 TJBJ	42	133	23	0	175	0:1	N/A
06-1-1 TDBG	80	89	29	84.5	84.5	1:1	0.489

[a]Resistant DH lines

[b]Susceptible DH lines

[c]Missing data

4.4.2 Genetic Map Construction

Genetic mapping of Superb/BW278

The Superb/BW278 mapping population (n = 142) was genotyped with the iSelect 90K wheat SNP array (Wang et al. 2014), and of the 81,000 SNPs tested, 7,298 SNPs were detected as polymorphic between parental lines Superb and BW278 using GenomeStudio software (Illumina Inc; http://bioinformatics.illumina.com/informatics/sequencing-microarray-data-analysis/genomestudio.html). MSTmap (Wu et al. 2008) software was used to bin co-segregating markers, resulting in 71 linkage bins. A representative marker with the least amount of missing data was chosen from each linkage bin for linkage analysis in MapDisto, resulting in 1,015 markers assembled into 32 linkage groups. A total of 125 markers in two linkage groups, 2D.1 and 2D.2, were associated with wheat chromosome 2D based on the Wang et al. (2014) consensus map. 2D.1 and 2D.2 were extracted from the whole genome map for use in two-point linkage analysis with the phenotypic data generated with *P. triticina* isolate 74-2 MGBJ.

A representative marker from each group of redundant markers (mapped to the same location in MapDisto) was selected for genetic map construction, resulting in 27 markers distributed along the chromosome. The final map lengths for linkage groups 2D.1 and 2D.2 were 95.11 cM and 40.39 cM, respectively. Seedling resistance gene *Lr2a* co-segregated with a group of 31 molecular markers consisting of two SSRs and 29 SNPs, at a location of 67.20 cM on 2D.1. The gene was flanked proximally by SNP marker *wsnp_CAP12_c1503_764765* at 50.8 cM and distally by SNP marker *TA012840-0369* at 86.6 cM (Figure 4.1). Marker order agreed well with the Wang et al. (2014) consensus map and Chinese Spring reference genome sequence (RefSeq v1.0; International Wheat Genome Sequencing Consortium 2018).

Genetic mapping of Superb/86ISMN 2137

Of the 81,000 SNP markers screened via the iSelect 90K wheat SNP chip (Wang et al. 2014) in the Superb/86ISMN 2137 mapping population (n = 198), 14,727 SNPs were determined to by polymorphic between parental lines Superb and 86ISMN 2137 using GenomeStudio software (Illumina Inc; http://bioinformatics.illumina.com/informatics/sequencing-microarray-data-analysis/genomestudio.html). Due large amounts of missing data (>10%), 31 DH lines were removed from further analysis, leaving a mapping population of 167 DH lines. The BIN function in QTL IciMapping was used to group co-segregating SNPs into 1299 preliminary linkage bins. A representative marker with the least amount of missing data was selected from each linkage bin for use in linkage analysis. Using MapDisto, 3,808 SNP markers were assembled into 25 linkage groups. Of the 3,808 polymorphic markers 142 were associated with wheat chromosome 2D based on the Wang et al. (2014) consensus map and were extracted from the whole genome map for two-point linkage analysis with phenotypic ratings using *P. triticina* isolate 12-3 MBDS. A representative marker from each group of redundant markers (mapped to the same location in MapDisto) was selected for mapping. A total of 42 non-redundant markers were distributed along the linkage group. The complete linkage map for chromosome 2D spanned 89.3 cM. Seedling resistance gene *Lr2a* mapped to a location of 38.4 cM, and co-segregated with a single SNP marker. The gene was flanked proximally by SNP marker *wsnp_CAP12_c1503_764765* at a location of 25.6 cM, and distally by SNP marker *Excalibur_c1944_1017* at a location of 39.0 cM (Figure 4.1). Mapping order was consistent with the consensus map by Wang et al. (2014) as well as the Chinese Spring reference genome sequence (RefSeq v1.0; International Wheat Genome Sequencing Consortium 2018). The two maps of *Lr2a* had 11 markers in common and showed collinearity (Figure 4.1).

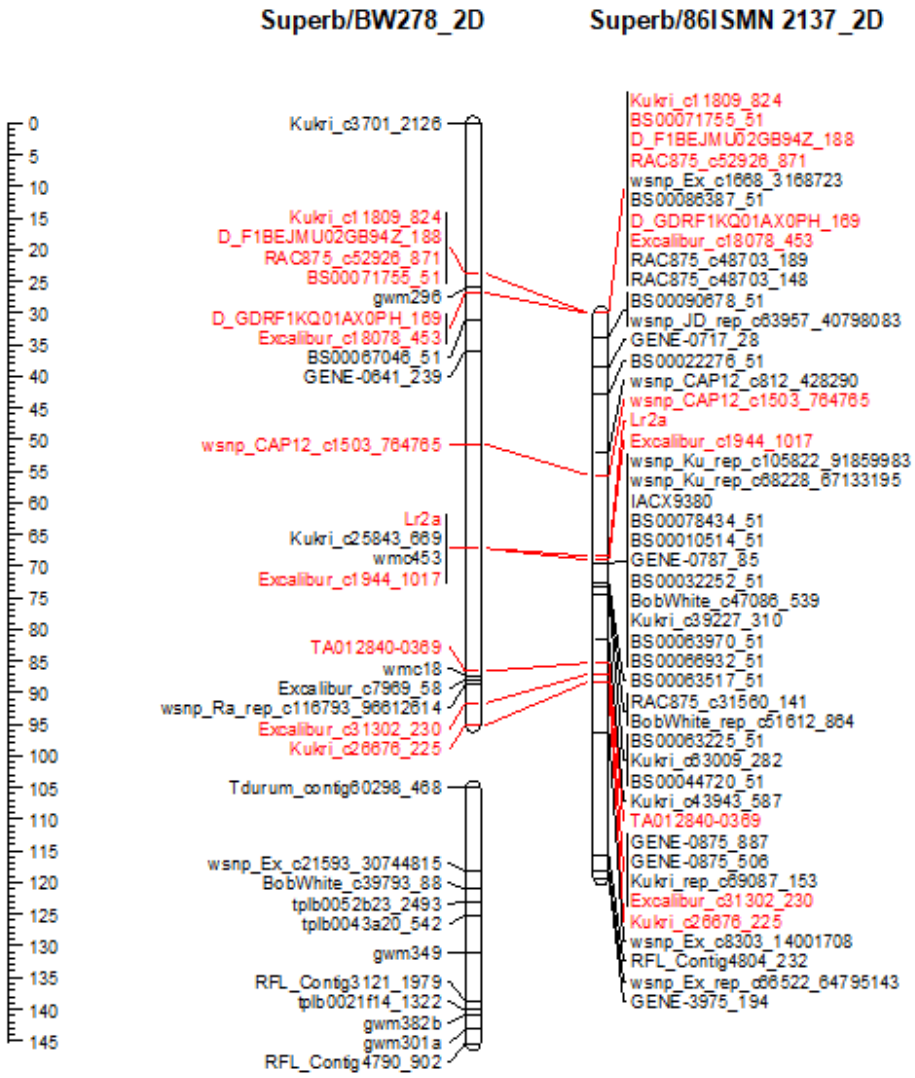

Figure 4.1. Comparison of the Superb/BW278 and Superb/86ISMN 2137 linkage maps. Markers in common between the two maps and the *Lr2a* locus are indicated in red. Ruler indicates genetic distance in centimorgans (cM).

4.4.3 Marker Validation

Seven Infinium SNPs that either co-segregated with, or flanked *Lr2a* in the Superb/BW278 DH population were chosen for KASP assay design. Two of the selected SNPs were also polymorphic and were linked to *Lr2a* in the Superb/86ISMN 2137 DH population. The KASP markers were re-tested on both mapping populations to verify that genotypes obtained by the Infinium assay and KASP analysis matched (Appendix 7.13-7.14). Of the two SNPs that were polymorphic in both mapping populations, kwh740 (*Excalibur_c1944_1017*) worked better as a KASP marker than kwh735 (*wsnp_CAP12_c1503_764765*), as it displayed clear clusters for both populations (Figure 4.2).

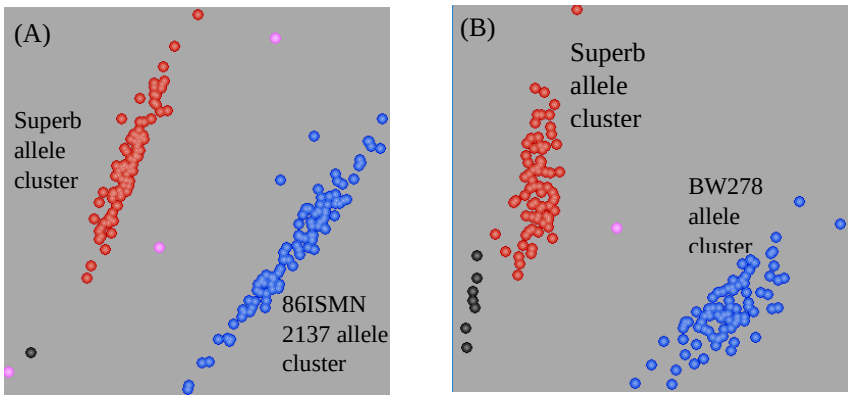

Figure 4.2. Cartesian cluster plots of KASP marker kwh740 [Excalibur_c1944_1017] on (A) Superb/86ISMN 2137 (B) Superb/BW278 tested to evaluate the usefulness of kwh740 for high-throughput genotyping. Black dots indicate no template DNA controls. Pink dots represent failed PCR reactions. X-axis represents fluorescence intensity of FAM, and y-axis represents fluorescence intensity of HEX.

4.5 Discussion

Single race-specific resistance genes have broken down quickly in the past due to the constantly shifting virulence in pathogen populations (McCallum et al. 2016). Nevertheless, combining multiple resistance genes into a single cultivar is a promising approach to achieve durable and effective disease resistance. In a study of the interaction between APR gene *Lr34* and other seedling *Lr* genes, it was observed that the *Lr34+Lr2a* combination resulted in higher levels of resistance than the seedling gene alone, but only to isolates avirulent to that seedling gene (German and Kolmer 1992).

Knowledge of gene locations is an important step in pyramiding genes to develop cultivars with multi-gene resistance. In previous cytogenetic mapping efforts, *Lr2a* mapped 38 cM distal to the centromere (McIntosh and Baker 1968) on wheat chromosome arm 2DS (Luig and McIntosh 1968). In the present study, leaf rust infection type (IT) ratings were performed at the seedling stage on two doubled haploid (DH) populations derived from spring wheat cultivar Superb, in an effort to map seedling resistance gene *Lr2a*, which displays a very low infection type (“0-0;) in response to certain races of *P. triticina.* Our study revealed that the gene was located on chromosome arm 2DS, at a location of 67.2 cM in Superb/BW278 and 38.4 cM in Superb/86ISMN 2137 within the respective linkage maps. In both maps, there were common markers that were associated with *Lr2a* and the maps showed good collinearity (Fig. 4.1).

Other resistance genes known to be present on chromosome 2DS include *Lr15, Lr22, and Lr39. Lr15* is a seedling resistance gene derived from common wheat and could be allelic with *Lr2* (McIntosh and Baker 1968); *Lr22a* is a race-specific APR gene derived from *Ae. squarrosa* L. var *strangulate* (Rowland and Kerber 1974); *Lr22b* is a race-specific APR gene derived from

common wheat (Dyck 1979); *Lr39* provides seedling resistance and was derived from *Aegilops taischii* (Raupp at al. 2001), *Lr2a* is distinguished from *Lr39* as the latter mapped near the telomere of 2DS (Raupp et al. 2001, Sun et al. 2009), while *Lr2a* mapped closer to the centromere in our study. The gene can also be distinguished from the *Lr22* locus, as *Lr2a* is reported as linked to the centromere, whereas *Lr22* is not (McIntosh 1995).

Isolates that are avirulent to *Lr2a* represented 50.5% of all isolates collected in Canada (McCallum, unpublished) and 89.5% of all isolates collected in the US (Kolmer, unpublished) in 2018, indicating that the resistance conferred by *Lr2a* could be useful in providing effective leaf rust resistance. Thus, stacking *Lr2a* with other seedling *Lr* genes as well as race non-specific APR genes such as *Lr34, Lr46,* or *Lr67* could provide effective leaf rust resistance while improving resistance to other fungal pathogens simultaneously (Hiebert et al. 2010; Herrera-Foessel et al. 2011; McCallum et al. 2011).

Expression of resistance is influenced by many things including crop growth stage, temperature, epistasis and infection type variability, making gene pyramids using conventional breeding techniques very challenging. Additional challenges include time, cost and space requirements for identifying genes through traditional infection type ratings. Predictive molecular markers that are tightly linked to the gene of interest can be used to overcome these challenges and allow for efficient incorporation or retention of resistance into high yielding cultivars. Improved molecular marker technologies have made mapping resistance genes, as well as incorporating resistance genes into cultivars using MAS easier than ever before. Until now, no DNA markers for *Lr2a* had been developed. In our study, a set of seven KASP markers for *Lr2a* were designed and tested on both mapping populations. A single marker, kwh740, designed from SNP marker *Excalibur_c1944_1017* was polymorphic in both mapping populations, co-

segregated with *Lr2a* in Superb/BW278, and was tightly linked to *Lr2a* in Superb/86ISMN 2137. The marker also displayed clear clusters in in KASP assays and could be useful in situations requiring high-throughput analysis.

In summary, the gene detected in Superb was confirmed as seedling resistance gene *Lr2a*, located on the short arm of chromosome 2D. While virulence to *Lr2a* has been detected in North American virulence surveys, many currently predominant leaf rust isolates are avirulent to the gene, indicating that it could be useful if deployed in combination with other resistance genes, particularly the race-nonspecific APR genes that form the basis of durable leaf rust resistance. Genetic host resistance is a highly desirable pathogen control method as it reduces input costs and potentially harmful environmental impacts of synthetic fungicides. KASP marker kwh740 has the potential be used to select *Lr2a* in marker-assisted breeding projects. We suggest that this research is continued by screening a panel of Canadian wheat lines to assess the usefulness of kwh740 in MAS programs. This study provides a strong foundation for map-based cloning research to further characterize the *Lr2a* and other alleles at the *Lr2* locus.

CHAPTER 5

GENERAL DISCUSSION

Growing enough food to feed the global population in a sustainable manner is no small challenge. In addition to high input costs, changing environmental conditions, and shifting consumer preferences, farmers must also deal with a wide range of pest pressures. Wheat is one of the most important food crops worldwide, however, its successful cultivation is challenged by many abiotic and biotic constraints. Wheat leaf rust caused by fungal pathogen *Puccinia triticina* has long been described as the most widespread and common disease of wheat (Roelfs 1988). Incorporation of genetic host resistance into high yielding cultivars is viewed as the most economic and environmentally friendly control method. Traditionally, resistance has been transferred via conventional breeding techniques which are often time-consuming and expensive. Marker assisted selection (MAS) has enabled breeders to select individuals for traits that are difficult to phenotypically evaluate, as well as pyramid multiple resistance genes into a desirable genetic background. Development of breeder-friendly, predictive, and reliable molecular markers is important for the continued release of cultivars with effective disease resistance packages.

Researchers at the Morden Research and Development Center (MRDC) noticed spring wheat line ‘BW278’ displayed moderate to high levels of leaf rust resistance under field conditions, and cultivar ‘Superb’ displayed a very low infection type (IT) in response to certain leaf rust races under controlled environmental conditions. Three mapping populations developed by researchers at AAFC were selected to further characterize the resistance present in these two spring wheat lines.

A recombinant inbred line (RIL) population developed from the cross BW278/AC Foremost was selected to characterize the APR present in 'BW278'. Spring wheat line 'BW278' is derived from cultivar 'Domain' which has been an important parent in Canadian wheat breeding programs. In the field, BW278 displayed a 'slow-rusting' resistance in response to *P. triticina*. Efforts to identify the location of the APR responsible for the resistance have been difficult due to the presence of other leaf rust resistance genes in the developed mapping populations, as well as observations that the gene is highly affected by environmental conditions (Kolmer et al. 2015). Quantitative trait loci (QTL) analysis detected a leaf rust resistance QTL, *QLr.mrdc-1B* on the long arm of chromosome 1B in the BW278/AC Foremost. Previous study of leaf rust resistance in this population hypothesized that the APR gene carried by BW278 was *Lr34*, an important non-race specific, multi-pest APR. However, the results of our study indicate that the QTL is not *Lr34* and is most likely APR gene *Lr46.* The QTL was detected in all site-years and all disease evaluation methods (SEV, IT and CI). Based on the generated linkage map and QTL analysis, 10 SNP markers that displayed linkage to *QLr.mrdc-1B* were selected for KASP assay design. Efforts to determine the utility of these markers for MAS is ongoing. If these markers are robust and broadly cross applicable for marker assisted selection of *Lr46* they would be very useful for incorporating this important resistance gene, since it is difficult to select phenotypically.

To confirm the presence of *Lr46* in BW278, QTL analysis was performed on a second mapping population, Superb/BW278. The generated linkage map for chromosome 1B in this cross spanned just over 15 cM and did not cover the long arm. Further investigation revealed that both Superb and BW278 had the same SNP marker haplotype for the majority of the chromosome. This suggests that the Superb/BW278 population is fixed for the APR. While this

result was unexpected, it will allow for investigation of the interaction between *Lr46* and seedling resistance genes, specifically *Lr2a* from Superb. The pooled field severity data for Superb/BW278 discussed in Chapter 3 was compared to the phenotypic pustule type ratings with 74-2 MGBJ. Presence of the *Lr2a* allele lowered average disease severity from 38.5% to 35.9%. This also demonstrates the variable expression of *Lr46* in different genetic backgrounds since it is much more effective in BW278 than in Superb.

To genetically map the seedling resistance gene *Lr2a*, present in spring wheat cultivar 'Superb', two doubled haploid (DH) populations were developed from crosses Superb/BW278 and Superb/86ISMN 2137. As discussed in Chapter 4, this study used the 90K wheat SNP array (Wang et al. 2014) to identify a putative mapping location for the seedling resistance gene in 'Superb' by identifying tightly linked markers through two-point linkage analysis with phenotypic data from inoculation with 12-3 MBDS (Superb/86ISMN 2137) and 74-1 MGBJ (Superb/BW278) and creation of linkage maps of chromosome 2D. Results from linkage mapping revealed 142 SNPs in Superb/86ISMN 2137, spanning 89.3 cM. In the Superb/BW278 population, 125 SNPs were associated with chromosome 2D. The chromosome was split into two linkage groups, 2D.1 and 2D.2, which spanned 95.11 and 40.39 cM, respectively.

Results from the research completed here suggest that combining durable sources of leaf rust resistance, specifically multi-pest APR genes, with other effective or defeated genes can result in effective resistance to *P. triticina* over time. We have demonstrated that the *Lr46+Lr2a* combination slightly reduced overall disease severity under field conditions in Manitoba.

In conclusion, this study provides a foundation for new genetic research to develop a more complete understanding of seedling and adult plant resistance present in Canadian spring wheat germplasm. This study confirmed the presence of *Lr46* in AC Domain and Superb. The

gene may therefore be broadly distributed in Canadian wheat germplasm since both these cultivars were used extensively as parental lines. The maps generated for *Lr2a* provide a foundation for map-based cloning efforts. The KASP markers developed in this study will facilitate the determination of the presence or absence of this important resistance gene in Canadian wheat germplasm. To further characterize the APR present in 'BW278' new mapping populations should be developed. The genetic maps and marker information generated in this study will facilitate future fine mapping and development of predictive markers for use in MAS and breeding programs. The continued effort to map and characterize resistance genes provides hope that sustainable wheat production will continue in the future.

CHAPTER 6

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CHAPTER SEVEN

APPENDICES

Appendix 7.1. Adult plant test on the BW278/AC Foremost RIL population to two *Puccinia triticina* isolates.

		77-2 TJBJ		12-3 MBDS	
Line ID	**Entry #**	**Adult PT**	**Rxn**	**Adult PT**	**Rxn**
pb E 9	6	3-/few/Z	R	-	-
pb E 20	12	3+	S	-	-
pb E 25	15	3+	S	3+	S
pb E 39	27	3-	S	-	-
pb E 51	32	-	-	-	-
pb E 65	39	3-/few/Z	R	3-/few/Z	R
pb E 76	45	3-	S	-	-
pb E 77	46	;2-	R	-	-
pb E 79	47	3-	S	3+	S
pb E 83	49	3-	S	-	-
pb E 84	50	3-/few/Z	R	-	-
pb E 85	51	3-	S	-	-
pb E 103	59	3-	S	3-	S
pb E 111	62	3-	S	-	-
pb E 122	69	3-	S	-	-
pb E 127	71	3-	S	3-/few/Z	R
pb E 128	72	2-/few	R	3-/few/Z	R
pb E 163	86	;2-	R	-	-
pb E 204	109	3-	S	-	-
pb E 206	110	3+	S	-	-
pb E 210	111	3-/few/Z	R	-	-
pb E 218	116	3-	S	3+	S
pb E 220	118	3-/few/Z	R	3-	S
pb E 228	124	3/few/Z	R	3-/few/Z	R
pb E 250	140	3-	S	3-/few/Z	R
pb E 253	141	;2-	R	-	-
pb E 262	148	;2-	R	;2-/few	R
pb E 266	150	3+/few/Z	R	3+/few/Z	R
pb E 275	159	;2-	R	;3-/few/Z	R
pb E 276	160	3-	S	3+/few/Z	R
pb E 280	164	3-/few/Z	R	-	-
pb E 290	173	3-	S	3-/few/Z	R
pb E 300	182	3+	S	3+/few/Z	R
pb E 320	194	;2-	R	;3-/few/Z	R
pb E 346	209	3+	S	3-/few/Z	R

pb E 349	212	3+	S	3+	S
pb E 354	217	;2-	R	3+/few/Z	R
pb E 363	224	3-	S	3+	R
pb E 399	242	3-	S	3+	S
pb E 400	243	3-	S	-	-
pb E 407	248	;2-	R	3-	S
pb E 423	253	3+	S	3+	S
pb E 435	257	3-/few/Z	R	3-/few/Z	R
pb E 452	265	3-	S	3+/few/Z	R
pb E 453	266	3+	S	3+	S
pb E 455	267	3-/few/Z	R	3-/few/Z	R
pb E 469	274	;2-	R	3+/few/Z	R
pb E 477	277	3-	S	3+/few/Z	R
pb E 490	281	;	R	3-/few/Z	R
pb I 8	285	3+	S	3+	S
pb I 18	290	3-	S	3+	S
pb I 23	295	3-/few/Z	R	3+	S
pb I 30	298	3-	S	3-/few/Z	R
pb I 37	301	;2-	R	3-/few/Z	R
pb I 61	312	3-	S	3-/few/Z	R
pb I 72	317	3-/few/Z	R	;3+/few/Z	R
pb I 79	321	;2-	R	;3-/few/Z	R
pb I 88	328	;2-/few	R	3-/few/Z	R
pb I 100	340	3+	S	3+/few/Z	R
pb I 103	343	3+	S	3+	S
pb I 104	344	3-/few/Z	R	3-/few/Z	R
pb I 106	346	3-/few/Z	R	3+	S
pb I 164	382	3-/few/Z	R	3-/few/Z	R
pb I 168	385	3-	S	3-	S
pb I 174	389	3-/few/Z	R	3-/few/Z	R
pb I 196	397	3-/few/Z	R	-	-
pb I 201	398	3+	S	3+	S
pb I 203	399	2-/few	R	;3-/few/Z	R
pb I 219	408	2-	R	3-/few/Z	R
pb I 232	415	3-	S	3+	S
pb I 237	418	2-	R	3+	S
pb I 252	428	3-	S	-	-
pb I 257	431	3-/few/Z	R	3+	S
pb I 259	432	2-	R	-	-
pb I 286	442	-	-	3-/few/Z	R
pb I 293	445	3-	S	3+	S
pb I 308	455	3-/few/Z	R	3+	S
pb I 313	458	3-/few/Z	R	3+	S
pb I 315	459	3-	S	3+	S

pb I 317	460	3-	S	3-	S
pb I 319	461	;2-	R	X/3-	S
pb I 322	462	3-	S	3+/few/Z	R
pb I 324	464	3-	S	3+	S
pb I 332	469	3-/few/Z	R	3-/few/Z	R
pb I 338	474	3-	S	-	-
pb I 356	486	3-	S	3-/few/Z	R
pb I 361	489	2-/few	R	3-/few/Z	R
pb I 372	500	3-	S	3+/few/Z	R
pb I 388	514	3-	S	3+	S
pb K 22	531	3+	S	3+	S
pb K 29	535	;2-	R	3-/few/Z	R
pb K 30	536	3+/few/Z	R	3+/few/Z	R
pb K 39	541	3-/few/Z	R	3-/few/Z	R
pb K 89	584	3-	S	3-	S
pb K 91	586	3+	S	3+	S
pb K 92	587	3+	S	3-	S
pb K 95	590	3-	S	3+	S
pb K 107	601	;3-/few/Z	R	3-/few/Z	R
pb K 114	607	3-	S	3-	S
pb K 118	611	3+	S	3+/few/Z	R
pb K 122	614	3-	S	3+	S
pb K 130	621	3-	S	3+	S
pb K 137	625	3-	S	3+	S
pb K 144	631	3+	S	3+	S
pb K 146	632	3+	S	3+	S
pb K 153	637	3+	S	3-	S
pb K 161	641	;3-/few/Z	R	3-/few/Z	R
pb K 205	670	3-	S	3+	S
pb K 210	672	3-	S	-	-
pb K 212	674	3-	S	3-/few/Z	R
pb K 214	675	;2+	R	3-/few/Z	R
pb K 280	707	3-	S	3-/few/Z	R
pb K 320	732	3-	S	3+/few/Z	S
pb K 337	743	3+	S	3+	S
pb K 344	749	;2-	R	-	-
pb K 351	755	3-	S	-	-
pb K 362	765	3-/few/Z	R	3+/few/Z	R
pb K 381	782	3-	S	3+/few/Z	R
pb K 391	792	3-	S	3+	S
pb U 13	806	3+	S	3+	R
pb U 14	807	3-	S	3+	S
pb U 24	816	3-	S	3+	S
pb U 25	817	3-	S	3+/few/Z	R

pb U 32	822	3-	S	3+	S
pb U 35	824	3-/few/Z	R	3+	S
pb U 39	828	3-	S	3+/few/Z	R
pb U 40	829	;2-	R	3+	S
pb U 66	844	3-/few/Z	R	3+/few/Z	R
pb U 69	846	;2-	R	3-/few/Z	R
pb U 70	847	3-/few/Z	R	-	-
pb U 73	850	3-	S	3+/few/Z	R
pb U 78	852	3-	S	3-/few/Z	R
pb U 81	854	3-/few/Z	R	3-/few/Z	R
pb U 95	863	;2-	R	-	-
pb U 100	866	3-	S	3-/few/Z	R
pb U 102	868	;2-	R	3-/few/Z	R
pb U 125	879	;2-	R	3-/few/Z	R
pb U 128	881	;2-	R	3-	S
pb U 183	907	3-	S	3-	S
pb U 187	910	3-	S	3+	S
pb U 189	912	3-	S	3-/few/Z	R
pb U 200	918	;2+	R	3-	S
pb U 205	921	;2-	R	3-/few/Z	R
pb U 222	928	-	-	3-/few/Z	R
pb U 235	937	;2-	R	3+/few/Z	R
pb U 236	938	3+	S	3+/few/Z	R
pb U 253	949	;2-	R	-	-
pb U 254	950	3-	S	3+	S
pb U 285	975	3-	S	3+	S
pb U 289	979	3-	S	-	-
pb U 291	981	3-	S	-	-
pb U 294	984	3-	S	3+	S
pb U 297	987	;2-	R	3-/few/Z	R
pb U 298	988	3-	S	3+	R
pb U 299	989	;2-	R	3-	R
pb U 304	994	3-	S	3-/few/Z	R
pb U 309	998	3-	S	3-/few/Z	R
pb U 310	999	3-/few/Z	R	3-/few/Z	R
pb U 312	1001	3-	S	3+	S
pb U 317	1002	3-	S	3-	S
pb U 319	1003	3-/few/Z	R	3-/few/Z	R
pb U 321	1005	3-	S	3+	S
pb U 323	1006	3-	S	3+	S
pb U 364	1026	3-	S	3+	S
pb U 411	1044	2-	R	3-/few/Z	R
pb U 427	1047	2-	R	3-/few/Z	R
pb U 443	1053	3-	S	-	-

pb U 449	1057	2-	R	3-/few/Z	R
pb U 461	1061	3-	S	3+/few/Z	R
pb U 475	1064	;3-	S	3-/few/Z	R
qe J 1	1073	3-	S	3+	S
qe J 3	1075	3-	S	3+/few/Z	R
qe J 22	1087	3-	S	3+	S
qe J 25	1089	3-	S	3-	S
qe J 64	1108	;3-	R	3+/few/Z	R
qe J 70	1110	;3-	R	3-	S
qe J 74	1113	2-/few	R	3-/few/Z	R
qe J 136	1130	3-	S	3-/few/Z	R
qe J 141	1133	;2-	R	3+/few/Z	R
qe J 168	1143	;2-	R	3-/few/Z	R
qe J 170	1145	;3-/few/Z	R	3+/few/Z	R
qe J 182	1150	3-	S	3-/few/Z	R
qe J 189	1152	;3-	R	3-/few/Z	R
qe J 199	1156	;2-	R	3+	S
qe J 218	1166	3+	S	3-	S
qe J 228	1172	;3-	R	3-/few/Z	R
qe J 279	1201	3-	S	3+	S
qe J 295	1216	;3-	R	3-/few/Z	R
qe J 298	1219	;3-/few/Z	R	3-/few/Z	R
qe J 303	1223	3-	S	3-/few/Z	R
qe J 304	1224	3+	S	3+	S
qe J 324	1240	2-	R	-	-
qe J 334	1244	3-	S	3-	S
qe J 367	1258	;2-	R	3-	S
qe J 383	1263	;2-	R	3+	S
qe J 413	1276	;2-	R	3-	S
qe J 415	1278	3-	S	;2-	S
qe J 420	1281	3-	S	3+	S
qe J 433	1286	;2-	R	3-/few/Z	R
qe J 512	1320	3-	S	3+/few/Z	R
qe J 513	1321	3-	S	3+/few/Z	R
qe J 528	1329	;2-	R	3-/few/Z	R
qe J 531	1331	3-	S	3-/few/Z	R
qe J 534	1333	3-	S	3+	S
qe J 536	1334	3-	S	3+	S
qe J 544	1341	3-	S	3+	S
qe J 549	1343	;2-	R	3-	S
qe J 550	1344	;2-	R	3-/few/Z	R
qe J 552	1345	3+	S	3-	S
qe J 555	1347	;2-	R	;3-/few/Z	R
qe J 557	1349	3-/few/Z	R	3-/few/Z	R

qe J 565	1357	2-	R	3+/few/Z	R
qe J 567	1359	3-/few/Z	R	3+	S
qe J 570	1362	2-/few	R	3+/few/Z	R
qe J 582	1369	3+	S	3-/few/Z	R
qe J 583	1370	;2-	R	3-	S
qe J 586	1373	;2-/few	R	-	-
qe M 2	1377	3-	S	3+	S
qe M 14	1388	2-	R	3+	S
qe M 19	1392	;2-	R	3-	S
qe M 35	1404	3-	S	3-	S
qe M 72	1424	2-	R	3-	S
qe M 123	1441	3-	S	3+	S
qe M 152	1456	3-/few/Z	R	3-/few/Z	R
qe M 170	1463	;2-	R	3-/few/Z	R
qe M 178	1465	;2-	R	;3-	S
qe M 180	1467	;2-	R	3-/few/Z	R
qe M 192	1473	3-	S	3-/few/Z	R
qe M 235	1495	3+	S	3+	S
qe M 241	1499	;2-	R	3-/few/Z	R
qe M 249	1502	3-	S	3+	S
qe M 252	1505	;2-	R	;3-/few/Z	R
qe M 270	1520	2-	R	-	-
qe M 284	1530	;3-/few/Z	R	3+	S
qe M 286	1532	;2-	R	-	-
qe M 289	1535	;2-	R	3-/few/Z	R
qe M 290	1536	3+	S	3+/few/Z	R
qe M 298	1542	3+	S	3-/few/Z	R
qe M 299	1543	;2-	R	3-/few/Z	R
qe M 302	1546	3-	S	3-	S
qe M 308	1552	3-	S	3-	S
qe M 312	1554	3-	S	-	-
qe M 336	1569	3-	S	3+	S
qe M 339	1572	3-	S	3+	S
qe M 344	1575	;2-	R	3-/few/Z	R
qe M 350	1577	3+	S	3+/few/Z	R
qe M 372	1589	;2-	R	3-/few/Z	R
qe M 401	1605	3-	S	3-	S
qe M 402	1606	;2-	R	3-	S
qe M 419	1615	3+	S	3-	S
qe M 425	1619	;2-	R	3+/few/Z	R
qe M 436	1623	3-	S	3+	S
qe M 437	1624	3-/few/Z	R	3-/few/Z	R
qe M 469	1642	;2-	R	3-	S
qe M 478	1647	3-	S	3+/few/Z	R

qe M 490	1652	3-	S	3+	S
qe M 506	1662	;3-/few/Z	R	3-	S
qe M 534	1678	3-	S	3+/few/Z	R
qe M 541	1684	;2-	R	;3-/few/Z	R
qe M 542	1685	3-/few/Z	R	3+/few/Z	R
qe M 551	1692	;2-	R	3-/few/Z	R
qe M 559	1700	3-	S	3-/few/Z	R
qe M 568	1709	3-	S	3+	S
qe M 578	1717	3-	S	3-/few/Z	R
qe M 588	1727	3+	S	3+	S
qe Z 15	1739	3-	S	3+	S
qe Z 16	1740	;2-	R	3-	S
qe Z 57	1761	3-	S	3-	S
qe Z 61	1764	3-/few/Z	R	3-/few/Z	R
qe Z 63	1765	2-	R	3-	S
qe Z 85	1769	3-	S	3+	S
qe Z 86	1770	3-	S	3-	S
qe Z 97	1773	3-	S	3-/few/Z	R
qe Z 99	1774	3+	S	3-/few/Z	R
qe Z 107	1780	3-	S	-	-
qe Z 115	1784	;2-	R	3-/few/Z	R
qe Z 188	1807	3-/few/Z	R	3+	S
qe Z 192	1809	3-	S	3-	S
qe Z 201	1812	;2+	R	3+	S
qe Z 208	1816	3-	S	3-	S
qe Z 209	1817	3-/few/Z	R	3-/few/Z	R
qe Z 211	1819	3+	S	3-	S
qe Z 217	1822	2-	R	3+/few/Z	R
qe Z 226	1827	3-	S	3+	S
qe Z 240	1832	3-	S	3+	S
qe Z 242	1833	3-	S	-	-
qe Z 251	1837	3-	S	3+	S
qe Z 257	1841	3-/few/Z	R	3-/few/Z	R
qe Z 261	1843	3-	S	3-	S
qe Z 266	1845	3-/few/Z	R	3+/few/Z	R
qe Z 274	1849	3-	S	3-	S
qe Z 279	1852	3-	S	3-	S
qe Z 290	1863	3-/few/Z	R	3-/few/Z	R
qe Z 311	1882	3-/few/Z	R	3-	S
qe Z 317	1886	3-	S	3+	S
qe Z 359	1902	3-	S	3+	S
qe Z 380	1909	;2-	R	3-	S
qe Z 383	1910	3-	S	3-	S
qe Z 482	1942	3-	S	3-	S

qe Z 520	1955	3-	S	3-/few/Z	R
qe Z 522	1957	3-	S	3+	S
qe Z 530	1961	3-/few/Z	R	-	-
qe Z 543	1966	3-	S	3+	S
qe Z 567	1981	2-	R	3-	S
qe Z 580	1988	3+	S	3-/few/Z	R
BW278 #1		3-/few/Z	R	3-/few/Z	R
BW278#2		3-/few/Z	R	3-/few/Z	R
AC Foremost		;3-/few	R	3-	S
TcLr16		1+	R	3-	S
Thatcher		3+	S	3+	S

Appendix 7.2. Summary of field phenotypic data for BW278/AC Foremost, inoculated with an epidemic mixture of *Puccinia triticina* isolates.

		2016		2017		2018_1		2018_2		2019_1		2019_2		2020_1		2020_2	
Line ID	**Entry #**	**SEV**	**IT**	**SEV**	**IT**	**SEV**	**IT**	**SEV**	**IT**	**SEV**	**IT**	**SEV**	**IT**	**SEV**	**IT**	**SEV**	**IT**
pb E 9	6	60	MS	10	R	20	MR	40	I	20	MR	-	-	15	MR	20	MR
pb E 20	12	40	I	5	R	15	MR	30	MR	15	MR	20	MR	20	MR	20	MR
pb E 25	15	80	S	90	S	70	S	50	MS	80	S	80	S	85	S	80	S
pb E 39	27	80	S	70	S	10	R	20	MR	15	MR	15	MR	30	MR	15	MR
pb E 51	32	30	MR	20	MR	10	R	5	R	25	MR	10	R	5	R	20	MR
pb E 65	39	80	S	70	S	50	MS	40	I	70	S	65	S	70	S	60	MS
pb E 76	45	70	S	90	S	40	I	30	MR	60	MS	80	S	65	S	70	S
pb E 77	46	10	R	10	R	10	R	10	R	10	R	10	R	10	R	20	MR
pb E 79	47	80	S	70	S	40	I	15	MR	70	S	75	S	25	MR	15	MR
pb E 83	49	80	S	70	S	60	MS	30	MR	60	MS	65	S	65	S	60	MS
pb E 84	50	10	R	0	R	15	MR	10	R	5	R	10	R	10	R	5	R
pb E 85	51	15	MR	10	R	5	R	15	MR	5	R	10	R	10	R	0	R
pb E 103	59	90	S	90	S	60	MS	70	S	80	S	80	S	70	S	80	S
pb E 111	62	15	MR	10	R	5	R	5	R	3	R	15	MR	10	R	10	R
pb E 122	69	70	S	80	S	40	I	15	MR	30	MR	30	MR	25	MR	40	I
pb E 127	71	75	S	80	S	60	MS	50	MS	80	S	70	S	70	S	80	S
pb E 128	72	80	S	70	S	40	I	40	I	60	MS	40	I	20	MR	15	MR
pb E 163	86	40	I	-	-	-	-	-	-	10	R	-	-	10	R	10	R
pb E 204	109	50	MS	10	R	10	R	10	R	10	R	20	MR	20	MR	10	R
pb E 206	110	80	S	90	S	80	S	70	S	80	S	80	S	40	I	45	MS
pb E 210	111	80	S	90	S	70	S	60	MS	80	S	80	S	70	S	70	S
pb E 218	116	90	S	90	S	70	S	40	I	80	S	80	S	80	S	80	S
pb E 220	118	80	S	90	S	80	S	80	S	80	S	80	S	70	S	75	S
pb E 228	124	90	S	80	S	40	I	40	I	70	S	80	S	85	S	70	S
pb E 250	140	90	S	80	S	70	S	50	MS	80	S	75	S	70	S	80	S
pb E 253	141	55	MS	5	R	5	R	10	R	30	MR	15	MR	15	MR	20	MR
pb E 262	148	5	R	0	R	5	R	15	MR	1	R	5	R	5	R	1	R
pb E 266	150	70	S	30	MR	20	MR	20	MR	30	MR	35	MR	20	MR	15	MR

pb E 275	159	30	MR	5	R	50	MS	30	MR	80	S	-	-	70	S	80	S
pb E 276	160	80	S	80	S	40	I	15	MR	15	MR	50	MS	40	I	50	MS
pb E 280	164	90	S	80	S	40	I	30	MR	80	S	80	S	80	S	80	S
pb E 290	173	80	S	90	S	40	I	60	MS	65	S	70	S	70	S	60	MS
pb E 300	182	90	S	80	S	80	S	60	MS	80	S	80	S	65	S	65	S
pb E 320	194	60	MS	5	R	10	R	5	R	10	R	15	MR	10	R	1	R
pb E 346	209	80	S	10	R	20	MR	15	MR	25	MR	10	R	40	I	35	I
pb E 349	212	90	S	90	S	60	MS	40	I	80	S	-	-	80	I	80	S
pb E 354	217	20	MR	5	R	5	R	5	R	3	R	5	R	1	R	1	R
pb E 363	224	70	S	5	R	5	R	20	MR	20	MR	15	MR	15	MR	10	R
pb E 399	242	90	S	90	S	30	MR	30	MR	80	S	75	S	80	S	70	S
pb E 400	243	90	S	80	S	70	S	30	MR	60	MS	75	S	50	MS	65	S
pb E 407	248	90	S	60	MS	15	MR	20	MR	5	R	40	I	15	MR	20	MR
pb E 423	253	35	I	5	R	5	R	10	R	3	R	10	R	5	R	5	R
pb E 435	257	40	I	5	R	5	R	15	MR	5	R	20	MR	10	R	0	R
pb E 452	265	90	S	90	S	70	S	70	S	80	S	80	S	80	S	80	S
pb E 453	266	90	S	90	S	60	MS	60	MS	80	S	85	S	70	S	70	S
pb E 455	267	70	S	10	R	5	R	20	MR	15	MR	20	MR	25	MR	35	I
pb E 469	274	60	MS	10	R	25	MR	25	MR	10	R	5	R	10	R	15	MR
pb E 477	277	90	S	80	S	40	I	30	MR	60	MS	80	S	55	MS	65	S
pb E 490	281	5	R	5	R	5	R	10	R	1	R	1	R	0	R	0	R
pb I 8	285	80	S	80	S	50	MS	30	MR	80	S	80	S	65	S	60	MS
pb I 18	290	80	S	80	S	80	S	70	S	70	S	80	S	65	S	60	MS
pb I 23	295	50	MS	5	R	15	MR	10	R	5	R	10	R	5	R	5	R
pb I 30	298	60	MS	5	R	25	MR	30	MR	15	MR	20	MR	20	MR	15	MR
pb I 37	301	90	S	80	S	15	MR	50	MS	80	S	75	S	60	MS	70	S
pb I 61	312	90	S	80	S	40	I	25	MR	80	S	70	S	60	MS	65	S
pb I 72	317	50	MS	5	R	10	R	5	R	10	R	15	MR	5	R	10	R
pb I 79	321	70	S	15	MR	15	MR	30	MR	50	MS	60	MS	10	R	15	MR
pb I 88	328	45	MS	5	R	10	R	5	R	5	R	15	MR	10	R	15	MR
pb I 100	340	90	S	80	S	60	MS	40	I	80	S	80	S	85	S	70	S
pb I 103	343	15	MR	80	S	80	S	50	MS	50	MS	80	S	90	S	80	S

pb I 104	344	65	MS	5	R	20	MR	20	MR	10	R	15	MR	5	R	10	R
pb I 106	346	45	MS	5	R	10	R	10	R	15	MR	15	MR	15	MR	20	MR
pb I 164	382	20	MR	5	R	5	R	10	R	10	R	5	R	1	R	5	R
pb I 168	385	90	S	80	S	70	S	40	I	80	S	75	S	80	S	70	S
pb I 174	389	80	S	20	MR	15	MR	15	MR	20	MR	20	MR	20	MR	20	MR
pb I 196	397	40	I	5	R	5	R	5	R	5	R	10	R	5	R	5	R
pb I 201	398	90	S	90	S	30	MR	30	MR	80	S	80	S	70	S	85	S
pb I 203	399	80	S	10	R	10	R	20	MR	30	MR	30	MR	30	MR	20	MR
pb I 219	408	50	MS	5	R	5	R	5	R	15	MR	10	R	5	R	10	R
pb I 232	415	90	S	80	S	50	MS	50	MS	70	S	75	S	70	S	70	S
pb I 237	418	90	S	80	S	70	S	50	MS	90	S	75	S	80	S	80	S
pb I 252	428	90	S	-	-	60	MS	50	MS	40	I	70	S	35	I	50	MS
pb I 257	431	80	S	5	R	30	MR	60	MS	-	-	10	R	-	-	-	-
pb I 259	432	90	S	20	MR	15	MR	40	I	70	S	75	S	30	MR	35	I
pb I 286	442	90	S	5	R	15	MR	20	MR	10	R	5	R	10	R	5	R
pb I 293	445	90	S	80	S	70	S	80	S	80	S	80	S	80	S	80	S
pb I 308	455	50	MS	10	R	10	R	15	MR	10	R	15	MR	20	MR	20	MR
pb I 313	458	90	S	80	S	60	MS	70	S	90	S	80	S	80	S	70	S
pb I 315	459	90	S	90	S	70	S	60	MS	90	S	80	S	90	S	80	S
pb I 317	460	70	S	80	S	60	MS	30	MR	40	I	80	S	40	I	40	I
pb I 319	461	30	MR	5	R	10	R	15	MR	5	R	10	R	1	R	0	R
pb I 322	462	65	S	5	R	25	MR	30	MR	10	R	15	MR	15	MR	15	MR
pb I 324	464	90	S	80	S	80	S	80	S	90	S	80	S	90	S	90	S
pb I 332	469	60	MS	5	R	40	I	15	MR	15	MR	15	MR	15	MR	20	MR
pb I 338	474	90	S	80	S	40	I	20	MR	80	S	80	S	80	S	70	S
pb I 356	486	90	S	80	S	30	MR	60	MS	80	S	80	S	70	S	65	S
pb I 361	489	50	MS	5	R	5	R	10	R	5	R	10	R	5	R	10	R
pb I 372	500	60	MS	20	MR	70	S	70	S	70	S	65	S	20	MR	35	I
pb I 388	514	90	S	5	R	60	MS	60	MS	10	R	30	MR	30	MR	30	MR
pb K 22	531	90	S	80	S	60	MS	20	MR	80	S	80	S	80	S	70	S
pb K 29	535	90	S	80	S	20	MR	25	MR	60	MS	70	S	70	S	70	S
pb K 30	536	80	S	5	R	5	R	10	R	40	I	80	S	20	MR	30	MR

pb K 39	541	70	S	5	R	50	MS	70	S	10	R	30	MR	25	MR	15	MR
pb K 89	584	85	S	80	S	70	S	25	MR	60	MS	80	S	60	MS	70	S
pb K 91	586	90	S	80	S	70	S	40	I	80	S	80	S	80	S	70	S
pb K 92	587	80	S	80	S	60	MS	30	MR	70	S	80	S	80	S	80	S
pb K 95	590	90	S	80	S	30	MR	40	I	70	S	65	S	80	S	80	S
pb K 107	601	90	S	20	MR	20	MR	30	MR	70	S	80	S	65	S	60	MS
pb K 114	607	80	S	20	MR	40	I	70	S	80	S	75	S	30	MR	30	MR
pb K 118	611	50	MS	10	R	20	MR	10	R	10	R	10	R	5	R	1	R
pb K 122	614	80	S	90	S	80	S	70	S	90	S	80	S	80	S	70	S
pb K 130	621	85	S	10	R	20	MR	40	I	60	MS	50	MS	20	MR	15	MR
pb K 137	625	90	S	90	S	70	S	50	MS	80	S	75	S	60	MS	70	S
pb K 144	631	90	S	90	S	30	MR	25	MR	75	S	85	S	70	S	70	S
pb K 146	632	80	S	90	S	80	S	80	S	80	S	80	S	80	S	85	S
pb K 153	637	20	MR	5	R	15	MR	10	R	5	R	20	MR	1	R	5	R
pb K 161	641	40	I	10	R	10	R	25	MR	20	MR	20	MR	15	MR	20	MR
pb K 205	670	90	S	80	S	80	S	60	MS	80	S	80	S	80	S	70	S
pb K 210	672	75	S	30	MR	10	R	5	R	75	S	80	S	30	MR	15	MR
pb K 212	674	70	S	80	S	40	I	50	MS	80	S	80	S	70	S	60	MS
pb K 214	675	70	S	30	MR	25	MR	40	I	90	S	40	I	40	I	50	MS
pb K 280	707	90	S	80	S	70	S	25	MR	80	S	85	S	80	S	70	S
pb K 320	732	80	S	80	S	40	I	50	MS	80	S	80	S	70	S	80	S
pb K 337	743	90	S	80	S	30	MR	50	MS	80	S	80	S	60	MS	70	S
pb K 344	749	90	S	70	S	40	I	40	I	30	MR	20	MR	45	MS	50	MS
pb K 351	755	80	S	80	S	40	I	70	S	50	MS	80	S	50	MS	40	I
pb K 362	765	90	S	80	S	40	I	40	I	60	MS	80	S	70	S	80	S
pb K 381	782	70	S	5	R	40	I	30	MR	40	I	75	S	60	MS	70	S
pb K 391	792	90	S	80	M	80	S	80	S	80	S	80	S	90	S	90	S
pb U 13	806	70	S	5	R	25	MR	30	MR	40	I	80	S	40	I	25	MR
pb U 14	807	75	S	5	R	10	R	15	MR	15	MR	15	MR	20	MR	30	MR
pb U 24	816	90	S	80	S	80	S	80	S	80	S	80	S	65	S	80	S
pb U 25	817	90	S	80	S	70	S	80	S	90	S	80	S	70	S	70	S
pb U 32	822	90	S	80	S	70	S	70	S	80	S	80	S	80	S	70	S

pb U 35	824	90	S	10	R	70	S	70	S	60	MS	70	MS	50	MS	60	MS
pb U 39	828	80	S	5	R	5	R	5	R	5	R	10	R	10	R	10	R
pb U 40	829	90	S	80	S	70	S	80	S	70	S	80	S	65	S	65	S
pb U 66	844	75	S	30	MR	30	MR	20	MR	20	MR	30	MR	20	MR	20	MR
pb U 69	846	90	S	20	MR	10	R	20	MR	10	R	5	R	15	MR	15	MR
pb U 70	847	65	S	5	R	50	MS	40	I	3	R	25	MR	10	R	10	R
pb U 73	850	90	S	80	S	80	S	80	S	80	S	80	S	65	S	60	MS
pb U 78	852	45	MS	5	R	1	R	5	R	1	R	10	R	1	R	5	R
pb U 81	854	40	I	5	R	5	R	15	MR	20	MR	10	R	5	R	5	R
pb U 95	863	60	MS	5	R	15	MR	30	MR	5	R	20	MR	15	MR	10	R
pb U 100	866	70	S	30	MR	30	MR	25	MR	30	MR	20	MR	30	MR	30	MR
pb U 102	868	90	S	20	MR	30	MR	40	I	70	S	60	MS	50	MS	65	S
pb U 125	879	10	R	5	R	10	R	10	R	1	R	10	R	5	R	5	R
pb U 128	881	20	MR	10	R	10	R	20	MR	5	R	10	R	5	R	10	R
pb U 183	907	50	MS	5	R	15	MR	25	MR	20	MR	10	R	10	R	15	MR
pb U 187	910	90	S	80	S	50	MS	60	MS	70	S	80	S	80	S	70	S
pb U 189	912	90	S	80	S	70	S	80	S	90	S	80	S	80	S	80	S
pb U 200	918	90	S	80	S	50	MS	50	MS	80	S	75	S	70	S	65	S
pb U 205	921	30	MR	5	R	30	MR	25	MR	10	R	20	MR	1	R	0	R
pb U 222	928	60	MS	5	R	10	R	10	R	15	MR	10	R	30	MR	30	MR
pb U 235	937	50	MS	5	R	5	R	15	MR	3	R	20	MR	1	R	0	R
pb U 236	938	70	S	10	R	10	R	25	MR	20	MR	-	-	5	R	15	MR
pb U 253	949	55	MS	5	R	5	R	15	MR	10	R	5	R	10	R	5	R
pb U 254	950	95	S	80	S	60	MS	40	I	80	S	80	S	70	S	70	S
pb U 285	975	95	S	70	S	40	I	40	I	40	I	80	S	70	S	70	S
pb U 289	979	65	S	5	R	5	R	10	R	10	R	10	R	50	MS	50	MS
pb U 291	981	80	S	80	S	50	MS	70	S	70	S	75	S	60	MS	60	MS
pb U 294	984	70	S	5	R	5	R	30	MR	80	S	60	MS	60	MS	50	MS
pb U 297	987	40	I	5	R	30	MR	20	MR	60	MS	80	S	80	S	80	S
pb U 298	988	80	S	30	MR	30	MR	60	MS	40	I	45	MS	40	I	50	MS
pb U 299	989	80	S	25	MR	60	MS	70	S	80	S	65	S	45	MS	60	MS
pb U 304	994	80	S	80	S	60	MS	60	MS	70	S	80	S	70	S	85	S

pb U 309	998	85	S	80	S	80	S	80	S	80	S	80	S	80	S	80	S
pb U 310	999	60	MS	20	MR	15	MR	10	R	25	MR	15	MR	10	R	15	MR
pb U 312	1001	80	S	80	S	80	S	80	S	80	S	75	S	70	S	70	S
pb U 317	1002	80	S	80	S	70	S	80	S	85	S	80	S	80	S	70	S
pb U 319	1003	80	S	40	I	50	MS	30	MR	40	I	75	S	40	I	45	MS
pb U 321	1005	70	S	80	S	30	MR	30	MR	80	S	70	S	60	MS	70	S
pb U 323	1006	40	I	5	R	20	MR	30	MR	20	MR	15	MR	20	MR	15	MR
pb U 364	1026	80	S	70	S	70	S	80	S	70	S	75	S	70	S	70	S
pb U 411	1044	45	MS	5	R	10	R	10	R	20	MR	10	R	20	MR	15	MR
pb U 427	1047	40	I	5	R	60	MS	60	MS	70	S	70	S	30	MR	40	I
pb U 443	1053	80	S	70	S	60	MS	30	MR	70	S	50	MS	70	S	60	MS
pb U 449	1057	40	I	5	R	20	MR	20	MR	10	R	15	MR	20	MR	15	MR
pb U 461	1061	65	S	30	MR	60	MS	60	MS	90	S	65	S	45	MS	30	MR
pb U 475	1064	60	MS	5	R	70	S	15	MR	10	R	40	I	10	R	15	MR
qe J 1	1073	75	S	80	S	70	S	40	I	70	S	70	S	60	MS	70	S
qe J 3	1075	30	MR	10	R	30	MR	30	MR	20	MR	25	MR	20	MR	15	MR
qe J 22	1087	90	S	70	S	40	I	10	R	3	R	15	MR	20	MR	15	MR
qe J 25	1089	50	MS	5	R	5	R	10	R	5	R	10	R	10	R	10	R
qe J 64	1108	80	S	70	S	15	MR	15	MR	60	MS	80	S	65	S	65	S
qe J 70	1110	40	I	5	R	5	R	5	R	3	R	10	R	5	R	5	R
qe J 74	1113	25	MR	5	R	40	I	25	MR	15	MR	30	MR	15	MR	20	MR
qe J 136	1130	50	MS	30	MR	10	R	15	MR	20	MR	30	MR	20	MR	15	MR
qe J 141	1133	60	MS	5	R	10	R	25	MR	15	MR	35	I	20	MR	20	MR
qe J 168	1143	1	R	5	R	1	R	10	R	3	R	1	R	0	R	0	R
qe J 170	1145	70	S	5	R	15	MR	10	R	10	R	10	R	10	R	15	MR
qe J 182	1150	20	MR	5	R	10	R	10	R	5	R	10	R	5	R	10	R
qe J 189	1152	70	S	5	R	20	MR	15	MR	10	R	20	MR	10	R	25	MR
qe J 199	1156	80	S	70	S	50	MS	15	MR	60	MS	60	MS	20	MR	20	MR
qe J 218	1166	95	S	80	S	80	S	70	S	70	S	80	S	90	S	90	S
qe J 228	1172	80	S	40	I	40	I	40	I	80	S	50	MS	50	MS	65	S
qe J 279	1201	75	S	60	MS	30	MR	30	MR	40	I	65	S	40	I	25	MR
qe J 295	1216	60	MS	30	MR	60	MS	50	MS	10	R	30	MR	30	MR	20	MR

qe J 298	1219	95	S	80	S	40	I	70	S	80	S	80	S	80	S	80	S
qe J 303	1223	80	S	80	S	70	S	80	S	90	S	80	S	75	S	70	S
qe J 304	1224	70	S	70	S	50	MS	20	MR	60	MS	75	S	70	S	70	S
qe J 324	1240	60	MS	10	R	5	R	20	MR	10	R	15	MR	15	MR	15	MR
qe J 334	1244	80	S	80	S	40	I	50	MS	70	S	75	S	80	S	70	S
qe J 367	1258	70	S	5	R	10	R	15	MR	5	R	15	MR	15	MR	20	MR
qe J 383	1263	50	MS	5	R	10	R	25	MR	10	R	30	MR	5	R	5	R
qe J 413	1276	30	MR	5	R	5	R	5	R	1	R	10	R	10	R	10	R
qe J 415	1278	-	-	80	S	50	MS	30	MR	30	MR	-	-	70	S	70	S
qe J 420	1281	80	S	80	S	70	S	80	S	70	S	75	S	70	S	60	MS
qe J 433	1286	15	MR	10	R	10	R	10	R	5	R	10	R	10	R	1	R
qe J 512	1320	20	MR	5	R	5	R	5	R	5	R	5	R	1	R	10	R
qe J 513	1321	70	S	-	-	50	MS	50	MS	80	S	80	S	65	S	70	S
qe J 528	1329	55	MS	20	MR	5	R	20	MR	20	MR	10	R	10	R	10	R
qe J 531	1331	-	-	70	S	15	MR	25	MR	-	-	-	-	60	MS	50	MS
qe J 534	1333	60	MS	70	S	5	R	5	R	10	R	70	S	15	MR	20	MR
qe J 536	1334	70	S	80	S	70	S	80	S	70	S	75	S	80	S	80	S
qe J 544	1341	70	S	70	S	25	MR	30	MR	80	S	70	S	70	S	80	S
qe J 549	1343	80	S	70	S	50	MS	40	I	50	MS	75	S	45	MS	55	MS
qe J 550	1344	5	R	5	R	10	R	25	MR	5	R	20	MR	5	R	0	R
qe J 552	1345	65	S	5	R	10	R	10	R	15	MR	10	R	20	MR	10	R
qe J 555	1347	1	R	5	R	15	MR	25	MR	10	R	20	MR	15	MR	20	MR
qe J 557	1349	70	S	70	S	15	MR	25	MR	70	S	70	S	70	S	70	S
qe J 565	1357	45	MS	5	R	5	R	10	R	10	R	10	R	1	R	10	R
qe J 567	1359	60	MS	5	R	10	R	5	R	10	R	10	R	5	R	5	R
qe J 570	1362	60	MS	5	R	15	MR	15	MR	10	R	15	MR	5	R	10	R
qe J 582	1369	70	S	5	R	5	R	10	R	10	R	10	R	15	MR	15	MR
qe J 583	1370	80	S	70	S	50	MS	80	S	60	MS	70	S	65	S	40	I
qe J 586	1373	60	MS	5	R	20	MR	40	I	20	MR	5	R	15	MR	10	R
qe M 2	1377	80	S	80	S	70	S	40	I	80	S	70	S	70	S	70	S
qe M 14	1388	20	MR	5	R	10	R	5	R	1	R	5	R	5	R	5	R
qe M 19	1392	80	S	70	S	50	MS	30	MR	80	S	70	S	70	S	70	S

qe M 35	1404	70	S	30	MR	10	R	20	MR	10	R	10	R	20	MR	15	MR
qe M 72	1424	90	S	70	S	15	MR	30	MR	80	S	75	S	65	S	65	S
qe M 123	1441	95	S	80	S	60	MS	40	I	75	S	80	S	70	S	90	S
qe M 152	1456	65	S	40	I	60	MS	70	S	30	MR	60	MS	30	MR	30	MR
qe M 170	1463	40	I	30	MR	15	MR	60	MS	30	MR	40	I	30	MR	20	MR
qe M 178	1465	80	S	80	S	70	S	25	MR	80	S	80	S	70	S	80	S
qe M 180	1467	10	R	5	R	40	I	5	R	10	R	10	R	0	R	5	R
qe M 192	1473	80	S	80	S	60	MS	70	S	70	S	80	S	60	MS	60	MS
qe M 235	1495	80	S	80	S	80	S	80	S	90	S	80	S	85	S	80	S
qe M 241	1499	5	R	5	R	1	R	10	R	5	R	10	R	0	R	0	R
qe M 249	1502	90	S	80	S	60	MS	70	S	70	S	75	S	80	S	80	S
qe M 252	1505	1	R	5	R	25	MR	10	R	10	R	10	R	80	S	80	S
qe M 270	1520	95	S	80	S	40	I	40	I	80	S	80	S	80	S	80	S
qe M 284	1530	30	MR	5	R	5	R	5	R	10	R	10	R	10	R	5	R
qe M 286	1532	15	MR	5	R	3	R	1	R	5	R	5	R	0	R	0	R
qe M 289	1535	35	I	5	R	10	R	15	MR	20	MR	55	MS	25	MR	20	MR
qe M 290	1536	10	R	0	R	1	R	15	MR	1	R	1	R	0	R	0	R
qe M 298	1542	40	I	0	R	25	MR	20	MR	5	R	15	MR	10	R	10	R
qe M 299	1543	65	S	10	R	15	MR	10	R	10	R	10	R	15	MR	25	MR
qe M 302	1546	70	S	5	R	15	MR	20	MR	15	MR	20	MR	20	MR	15	MR
qe M 308	1552	50	MS	70	S	5	R	15	MR	60	MS	70	S	30	MR	20	MR
qe M 312	1554	40	I	30	MR	40	I	50	MS	80	S	30	MR	20	MR	20	MR
qe M 336	1569	90	S	80	S	60	MS	70	S	70	S	75	S	70	S	70	S
qe M 339	1572	70	S	80	S	60	MS	40	I	80	S	75	S	70	S	70	S
qe M 344	1575	0	R	5	R	1	R	5	R	5	R	1	R	1	R	0	R
qe M 350	1577	90	S	80	S	15	MR	30	MR	75	S	75	S	60	MS	60	MS
qe M 372	1589	15	MR	5	R	10	R	15	MR	5	R	10	R	1	R	1	R
qe M 401	1605	80	S	80	S	60	MS	40	I	70	S	80	S	80	S	70	S
qe M 402	1606	3	R	5	R	5	R	-	-	1	R	3	R	0	R	0	R
qe M 419	1615	80	S	80	S	40	I	40	I	70	S	70	S	60	MS	60	MS
qe M 425	1619	65	S	70	S	10	R	15	MR	35	I	40	I	40	I	30	MR
qe M 436	1623	90	S	80	S	70	S	80	S	75	S	80	S	80	S	70	S

qe M 437	1624	15	MR	5	R	40	I	60	MS	10	R	5	R	15	MR	15	MR
qe M 469	1642	20	MR	5	R	15	MR	10	R	15	MR	5	R	5	R	1	R
qe M 478	1647	45	MS	20	MR	60	MS	60	MS	80	S	50	MS	30	MR	40	I
qe M 490	1652	15	MR	5	R	15	MR	30	MR	10	R	5	R	15	MR	15	MR
qe M 506	1662	70	S	5	R	60	MS	10	R	30	MR	10	R	30	MR	20	MR
qe M 534	1678	70	S	70	S	50	MS	20	MR	60	MS	65	S	70	S	80	S
qe M 541	1684	45	MS	5	R	10	R	15	MR	10	R	3	R	10	R	15	MR
qe M 542	1685	10	R	5	R	5	R	15	MR	10	R	10	R	15	MR	15	MR
qe M 551	1692	35	I	5	R	40	I	25	MR	10	R	20	MR	15	MR	15	MR
qe M 559	1700	60	MS	5	R	60	MS	20	MR	70	S	60	MS	25	MR	40	I
qe M 568	1709	90	S	80	S	70	S	40	I	60	MS	80	S	70	S	80	S
qe M 578	1717	70	S	70	S	50	MS	30	MR	60	MS	70	S	40	I	45	MS
qe M 588	1727	90	S	80	S	60	MS	50	MS	80	S	80	S	80	S	70	S
qe Z 15	1739	80	S	80	S	80	S	80	S	80	S	80	S	90	S	90	S
qe Z 16	1740	85	S	80	S	80	S	60	MS	80	S	75	S	70	S	80	S
qe Z 57	1761	55	MS	5	R	10	R	15	MR	10	R	15	MR	10	R	10	R
qe Z 61	1764	70	S	5	R	10	R	30	MR	5	R	30	MR	20	MR	10	R
qe Z 63	1765	20	MR	5	R	15	MR	15	MR	5	R	10	R	15	MR	5	R
qe Z 85	1769	70	S	40	I	40	I	20	MR	30	MR	40	I	80	S	80	S
qe Z 86	1770	80	S	80	S	80	S	80	S	80	S	80	S	80	S	70	S
qe Z 97	1773	80	S	80	S	60	MS	50	MS	80	S	75	S	70	S	70	S
qe Z 99	1774	75	S	30	MR	60	MS	40	I	70	S	50	MS	55	MS	50	MS
qe Z 107	1780	60	MS	70	S	15	MR	25	MR	-	-	50	MS	70	S	70	S
qe Z 115	1784	-	-	5	R	25	MR	25	MR	30	MR	10	R	5	R	15	MR
qe Z 188	1807	45	MS	5	R	10	R	10	R	10	R	10	R	15	MR	10	R
qe Z 192	1809	70	S	80	S	60	MS	70	S	80	S	80	S	65	S	70	S
qe Z 201	1812	60	MS	5	R	10	R	5	R	15	MR	15	MR	15	MR	10	R
qe Z 208	1816	80	S	80	S	80	S	80	S	80	S	80	S	80	S	80	S
qe Z 209	1817	20	MR	5	R	25	MR	20	MR	5	R	10	R	5	R	5	R
qe Z 211	1819	80	S	80	S	70	S	80	S	70	S	85	S	70	S	70	S
qe Z 217	1822	-	-	5	R	10	R	5	R	3	R	10	R	20	MR	5	R
qe Z 226	1827	80	S	80	S	30	MR	15	MR	80	S	80	S	80	S	70	S

qe Z 240	1832	70	S	70	S	60	MS	40	I	80	S	70	R	30	MR	15	MR
qe Z 242	1833	70	S	80	S	30	MR	15	MR	30	MR	35	I	20	MR	20	MR
qe Z 251	1837	45	MS	5	R	5	R	5	R	5	R	10	R	1	R	5	R
qe Z 257	1841	30	MR	5	R	15	MR	15	MR	15	MR	20	MR	0	R	1	R
qe Z 261	1843	80	S	5	R	5	R	10	R	5	R	-	-	80	S	90	S
qe Z 266	1845	65	S	20	MR	25	MR	10	R	10	R	20	MR	20	MR	30	MR
qe Z 274	1849	80	S	70	S	70	S	70	S	70	S	70	S	60	MS	50	MS
qe Z 279	1852	65	S	5	R	30	MR	5	R	3	R	-	-	-	-	-	-
qe Z 290	1863	70	S	10	R	10	R	15	MR	15	MR	30	MR	20	MR	15	MR
qe Z 311	1882	50	MS	20	MR	50	MS	40	I	25	MR	20	MR	80	S	65	S
qe Z 317	1886	80	S	80	S	40	I	60	MS	80	S	80	S	70	S	70	S
qe Z 359	1902	70	S	80	S	80	S	40	I	80	S	80	S	80	S	70	S
qe Z 380	1909	-	-	5	R	20	MR	20	MR	10	R	40	I	15	MR	25	MR
qe Z 383	1910	50	MS	30	MR	50	MS	30	MR	30	MR	40	I	40	I	50	MS
qe Z 482	1942	55	MS	5	R	10	R	60	MS	30	MR	30	MR	10	R	15	MR
qe Z 520	1955	50	MS	70	S	40	I	30	MR	60	MS	-	-	70	S	55	MS
qe Z 522	1957	80	S	80	S	80	S	80	S	80	S	80	S	80	S	80	S
qe Z 530	1961	45	MS	5	R	15	MR	10	R	5	R	10	R	5	R	10	R
qe Z 543	1966	70	S	40	I	30	MR	15	MR	20	MR	75	S	60	MS	45	MS
qe Z 567	1981	10	R	5	R	1	R	5	R	1	R	1	R	5	R	5	R
qe Z 580	1988	70	S	5	R	15	MR	20	MR	90	S	70	S	30	MR	30	MR
BW278 #1		-	-	-	-	-	-	-	-	-	-	-	-	20	MR	20	MR
BW278#2		-	-	-	-	-	-	-	-	30	MR	10	R	15	MR	8	R
AC Foremost		-	-	-	-	-	-	-	-	-	-	-	-	5	R	10	R
Thatcher		-	-	-	-	-	-	-	-	80	S	80	S	-	-	-	-

SEV=severity, % infection of the flag leaf; IT=infection type, R=resistant, MR=moderately resistant, I=intermediate, MS=moderately susceptible, S=susceptible, - indicates missing data.

Appendix 7.3. Summary of field phenotypic data from 2016-2018 for Superb/BW278, inoculated with an epidemic mixture of *Puccinia triticina* isolates.

	2016_1		2016_2		2016_3		2017_1		2017_2		2017_3		2018_1		2018_2		2018_3	
DH	**SEV**	**PT**	**SEV**	**PT**	**SEV**	**PT**	**SEV**	**PT**	**SEV**	**PT**	**SEV**	**PT**	**SEV**	**PT**	**SEV**	**PT**	**SEV**	**PT**
98B08*A002	60	MS	60	MS	60	MS	70	S	45	MS	50	MS	50	MS	50	MS	40	I
98B08*A006	10	R	20	MR	20	MR	5	R	5	R	10	R	5	R	5	R	5	R
98B08*A007	35	I	70	S	25	MR	70	S	70	S	30	MR	60	MS	70	S	50	MS
98B08*A009	15	MR	30	MR	20	MR	30	MR	30	MR	25	MR	25	MR	15	MR	15	MR
98B08*A018	30	MR	40	I	40	I	30	MR	50	MS	50	MS	40	I	25	MR	40	I
98B08*A019	15	MR	15	MR	15	MR	30	MR	25	MR	30	MR	25	MR	15	MR	25	MR
98B08*A022	25	MR	25	MR	25	MR	10	R	15	MR	10	R	15	MR	5	R	10	R
98B08*A026	55	MS	50	MS	40	I	70	S	80	S	70	S	60	MS	60	MS	60	MS
98B08*A029	55	MS	40	I	35	I	20	MR	55	MS	35	I	15	MR	30	MR	25	MR
98B08*A038	65	S	70	S	60	MS	70	S	65	S	60	MS	60	MS	40	I	50	MS
98B08*A039	35	I	20	MR	35	I	30	MR	40	I	35	I	30	MR	30	MR	25	MR
98B08*A042	30	MR	25	MR	25	MR	30	MR	30	MR	55	MS	30	MR	25	MR	40	I
98B08*A044	30	MR	15	MR	20	MR	40	I	15	MR	35	I	10	R	15	MR	20	MR
98B08*A046	35	I	40	I	30	MR	70	S	70	S	70	S	50	MS	60	MS	70	S
98B08*A048	25	MR	25	MR	25	MR	35	I	20	MR	35	I	25	MR	30	MR	15	MR
98B08*A049	20	MR	20	MR	25	MR	30	MR	50	MS	50	MS	30	MR	60	MS	30	MR
98B08*A050	50	MS	35	I	15	MR	30	MR	20	MR	30	MR	5	R	25	MR	30	MR
98B08*A051	65	S	55	MS	30	MR	50	MS	50	MS	70	S	30	MR	25	MR	25	MR
98B08*A052	10	R	10	R	15	MR	5	R	5	R	5	R	20	MR	3	R	10	R
98B08*A054	15	MR	20	MR	30	MR	20	MR	20	MR	10	R	5	R	20	MR	10	R
98B08*A055	15	MR	15	MR	15	MR	10	R	15	MR	5	R	40	I	15	MR	50	MS
98B08*A056	55	MS	40	I	30	MR	30	MR	45	MS	55	MS	40	I	60	MS	50	MS
98B08*A059	15	MR	35	I	20	MR	15	MR	25	MR	15	MR	15	MR	15	MR	10	R
98B08*A061	65	S	40	I	65	S	50	MS	55	MS	50	MS	5	R	20	MR	30	MR
98B08*A064	65	S	75	S	75	S	70	S	60	MS	65	S	40	I	70	S	70	S
98B08*A067	55	MS	65	S	65	S	65	S	60	MS	50	MS	40	I	40	I	70	S

98B08*A075	35	I	25	MR	30	MR	40	I	40	I	20	MR	40	I	25	MR	25	MR
98B08*A076	35	I	40	I	30	MR	50	MS	65	S	45	MS	50	MS	20	MR	15	MR
98B08*A077	15	MR	25	MR	30	MR	10	R	30	MR	20	MR	10	R	10	R	15	MR
98B08*A079	40	I	70	S	50	MS	70	S	60	MS	70	S	50	MS	60	MS	70	S
98B08*A081	20	MR	25	MR	25	MR	10	R	25	MR	20	MR	15	MR	15	MR	15	MR
98B08*A084	30	MR	25	MR	10	R	10	R	15	MR	50	MS	15	MR	10	R	25	MR
98B08*A086	45	MS	25	MR	15	MR	20	MR	5	R	10	R	15	MR	5	R	15	MR
98B08*A090	40	I	20	MR	15	MR	30	MR	25	MR	10	R	30	MR	15	MR	10	R
98B08*A091	15	MR	45	MS	30	MR	5	R	5	R	15	MR	15	MR	10	R	15	MR
98B08*A092	65	S	40	I	40	I	70	S	70	S	70	S	60	MS	50	MS	50	MS
98B08*A102	40	I	20	MR	25	MR	10	R	60	MS	45	MS	80	S	60	MS	60	MS
98B08*A108	30	MR	25	MR	15	MR	20	MR	50	MS	30	MR	10	R	10	R	15	MR
98B08*A109	35	I	50	MS	30	MR	70	S	65	S	60	MS	40	I	60	MS	60	MS
98B08*A110	80	S	80	S	50	MS	65	S	45	MS	60	MS	40	I	60	MS	50	MS
98B08*A111	25	MR	15	MR	25	MR	50	MS	25	MR	30	MR	20	MR	20	MR	30	MR
98B08*A113	45	MS	25	MR	55	MS	10	R	30	MR	35	I	40	I	50	MS	60	MS
98B08*A117	15	MR	20	MR	10	R	70	S	50	MS	35	I	25	MR	20	MR	15	MR
98B08*A119	30	MR	60	MS	15	MR	60	MS	35	I	55	MS	20	MR	20	MR	30	MR
98B08*A125	15	MR	25	MR	25	MR	10	R	10	R	25	MR	10	R	15	MR	25	MR
98B08*A126	20	MR	30	MR	25	MR	10	R	20	MR	5	R	20	MR	30	MR	25	MR
98B08*A127	40	I	65	S	65	S	40	I	35	I	20	MR	15	MR	15	MR	25	MR
98B08*A128	20	MR	15	MR	25	MR	10	R	20	MR	20	MR	10	R	5	R	10	R
98B08*A135	30	MR	55	MS	30	MR	30	MR	30	MR	50	MS	15	MR	30	MR	30	MR
98B08*A141	30	MR	20	MR	25	MR	40	I	45	MS	30	MR	60	MS	60	MS	50	MS
98B08*A143	30	MR	35	I	20	MR	70	S	60	MS	60	MS	25	MR	40	I	40	I
98B08*A145	20	MR	25	MR	20	MR	5	R	5	R	5	R	10	R	5	R	15	MR
98B08*A146	20	MR	30	MR	30	MR	10	R	15	MR	50	MS	15	MR	5	R	25	MR
98B08*A149	25	MR	20	MR	30	MR	80	S	70	S	70	S	50	MS	60	MS	50	MS
98B08*A150	30	MR	45	MS	35	I	20	MR	40	I	45	MS	25	MR	5	R	25	MR
98B08*B003	35	I	15	MR	20	MR	10	R	20	MR	15	MR	10	R	10	R	10	R
98B08*B005	40	I	80	S	45	MS	50	MS	30	MR	70	S	10	R	40	I	40	I
98B08*B008	75	S	70	S	60	MS	70	S	60	MS	55	MS	40	I	60	MS	60	MS

98B08*B011	60	MS	75	S	75	S	60	MS	70	S	70	S	10	R	25	MR	20	MR
98B08*B013	30	MR	25	MR	30	MR	50	MS	60	MS	20	MR	20	MR	30	MR	20	MR
98B08*B014	20	MR	25	MR	15	MR	40	I	30	MR	10	R	25	MR	70	S	20	MR
98B08*B016	30	MR	35	I	25	MR	20	MR	30	MR	15	MR	70	S	60	MS	60	MS
98B08*B021	10	R	25	MR	30	MR	20	MR	20	MR	30	MR	10	R	10	R	20	MR
98B08*B022	55	MS	55	MS	20	MR	70	S	30	MR	20	MR	70	S	70	S	70	S
98B08*B024	5	R	15	MR	10	R	5	R	5	R	20	MR	5	R	15	MR	20	MR
98B08*B025	65	S	60	MS	75	S	70	S	65	S	65	S	50	MS	70	S	70	S
98B08*B026	30	MR	55	MS	50	MS	30	MR	35	I	50	MS	50	MS	40	I	50	MS
98B08*B027	15	MR	15	MR	30	MR	10	R	40	I	15	MR	10	R	15	MR	10	R
98B08*B028	20	MR	15	MR	20	MR	50	MS	5	R	15	MR	15	MR	10	R	30	MR
98B08*B029	75	S	55	MS	25	MR	40	I	50	MS	60	MS	15	MR	5	R	20	MR
98B08*B030	45	MS	30	MR	30	MR	65	S	45	MS	60	MS	80	S	60	MS	70	S
98B08*B031	20	MR	25	MR	40	I	20	MR	20	MR	10	R	15	MR	10	R	15	MR
98B08*B034	15	MR	20	MR	25	MR	5	R	5	R	15	MR	15	MR	5	R	15	MR
98B08*B035	60	MS	70	S	60	MS	65	S	70	S	70	S	50	MS	70	S	60	MS
98B08*B036	65	S	50	MS	50	MS	5	R	10	R	25	MR	30	MR	15	MR	25	MR
98B08*B037	25	MR	40	I	40	I	20	MR	25	MR	20	MR	25	MR	25	MR	20	MR
98B08*B044	30	MR	35	I	55	MS	50	MS	40	I	35	I	15	MR	5	R	15	MR
98B08*B048	25	MR	30	MR	30	MR	10	R	25	MR	35	I	20	MR	15	MR	20	MR
98B08*B050	15	MR	15	MR	25	MR	30	MR	5	R	30	MR	10	R	30	MR	10	R
98B08*B052	40	I	40	I	35	I	50	MS	45	MS	50	MS	40	I	50	MS	60	MS
98B08*B053	40	I	45	MS	40	I	70	S	30	MR	50	MS	30	MR	5	R	15	MR
98B08*B055	30	MR	30	MR	20	MR	50	MS	30	MR	55	MS	30	MR	10	R	15	MR
98B08*B058	25	MR	30	MR	40	I	70	S	70	S	60	MS	30	MR	40	I	60	MS
98B08*B062	20	MR	25	MR	20	MR	30	MR	10	R	15	MR	30	MR	15	MR	10	R
98B08*B063	25	MR	65	S	35	I	70	S	65	S	55	MS	60	MS	40	I	50	MS
98B08*B065	15	MR	25	MR	15	MR	5	R	10	R	25	MR	10	R	5	R	20	MR
98B08*B069	35	I	30	MR	30	MR	20	MR	5	R	20	MR	20	MR	20	MR	25	MR
98B08*B070	40	I	25	MR	30	MR	30	MR	30	MR	30	MR	40	I	40	I	15	MR
98B08*B071	60	MS	25	MR	30	MR	20	MR	25	MR	25	MR	15	MR	30	MR	25	MR
98B08*B077	35	I	30	MR	25	MR	40	I	45	MS	30	MR	30	MR	25	MR	20	MR

98B08*B080	10	R	20	MR	15	MR	5	R	20	MR	30	MR	10	R	10	R	5	R
98B08*B085	25	MR	20	MR	15	MR	10	R	30	MR	15	MR	10	R	15	MR	10	R
98B08*B096	35	I	25	MR	20	MR	30	MR	60	MS	30	MR	15	MR	40	I	25	MR
98B08*B098	20	MR	30	MR	15	MR	10	R	30	MR	10	R	5	R	25	MR	20	MR
98B08*B099	30	MR	20	MR	30	MR	35	I	10	R	25	MR	5	R	10	R	30	MR
98B08*B100	65	S	40	I	45	I	40	I	20	MR	40	I	50	MS	30	MR	20	MR
98B08*B101	60	MS	70	S	30	MR	50	MS	10	R	60	MS	60	MS	60	MS	50	MS
98B08*B102	40	I	55	MS	25	MR	45	MS	35	I	50	MS	30	MR	40	I	40	I
98B08*B104	15	MR	20	MR	15	MR	20	MR	15	MR	30	MR	10	R	10	R	20	MR
98B08*B105	60	MS	80	S	30	MR	80	S	70	S	80	S	60	MS	60	MS	70	S
98B08*B109	70	S	50	MS	55	MS	70	S	70	S	70	S	60	MS	70	S	70	S
98B08*B110	25	MS	20	MR	30	MR	40	I	50	MS	30	MR	30	MR	30	MR	40	I
98B08*B111	35	I	30	MR	25	MR	50	MS	40	I	20	MR	30	MR	40	I	40	I
98B08*B119	20	MR	50	MS	30	MR	80	S	80	S	70	S	70	S	70	S	60	MS
98B08*B123	30	MR	20	MR	25	MR	40	I	5	R	10	R	15	MR	25	MR	15	MR
98B08*B124	25	MR	45	MS	40	I	70	S	70	S	70	S	80	S	50	MS	60	MS
98B08*B126	15	MR	30	MR	20	MR	20	MR	35	I	40	I	5	R	10	R	10	R
98B08*B129	25	MR	50	MS	55	MS	70	S	70	S	70	S	30	MR	50	MS	40	I
98B08*B137	75	S	80	S	70	S	80	S	80	S	80	S	70	S	80	S	70	S
98B08*B138	35	I	70	S	25	MR	70	S	70	S	70	S	80	S	70	S	60	MS
98B08*B139	75	S	45	MS	40	I	50	MS	50	MS	40	I	50	MS	50	MS	60	MS
98B08*B140	25	MR	30	MR	35	I	50	MS	65	S	30	MR	25	MR	15	MR	20	MR
98B08*B141	20	MR	50	MS	35	I	45	MS	20	MR	40	I	60	MS	80	S	70	S
98B08*B142	15	MR	15	MR	25	MR	30	MR	35	I	45	MS	25	MR	25	MR	10	R
98B08*B143	15	MR	20	MR	15	MR	35	I	10	R	25	MR	5	R	15	MR	25	MR
Superb-1	35	I	40	I	30	MR	70	S	65	S	50	MS	15	MR	25	MR	15	MR
Superb-2	55	MS	70	S	70	S	50	MS	55	MS	65	S	40	I	70	S	50	MS
BW278-1	25	MR	35	I	20	MR	40	I	50	MS	10	R	20	MR	25	MR	25	MR
BW278-2	35	I	25	MR	20	MR	20	MR	45	MS	45	MS	25	MR	40	I	50	MS
Thatcher	85	S	80	S	80	S	80	S	80	S	-	-	90	S	80	S	80	S
McKenzie	20	MR	10	R	20	MR	5	R	50	MS	-	-	15	MR	10	R	10	R

Appendix 7.4. Summary of field phenotypic data from 2019-2020 for Superb/BW278, inoculated with an epidemic mixture of *Puccinia triticina* isolates.

	2019_1		2019_2		2019_3		2020_1		2020_2		2020_3	
DH	**SEV**	**PT**	**SEV**	**PT**	**SEV**	**PT**	**SEV**	**PT**	**SEV**	**PT**	**SEV**	**PT**
98B08*A002	55	MS	60	MS	60	MS	70	S	80	S	75	S
98B08*A006	5	R	10	R	5	R	35	I	20	MR	30	MR
98B08*A007	60	MS	60	MS	65	S	70	S	70	S	80	S
98B08*A009	25	MR	15	MR	35	I	30	MR	30	MR	35	I
98B08*A018	45	MS	25	MR	25	MR	25	MR	40	I	35	I
98B08*A019	25	MR	20	MR	10	R	30	MR	20	MR	40	I
98B08*A022	10	R	15	MR	10	R	30	MR	30	MR	20	MR
98B08*A026	70	S	60	MS	60	MS	90	S	70	S	70	S
98B08*A029	20	MR	40	I	30	MR	70	S	70	S	60	MS
98B08*A038	50	MS	60	MS	60	MS	60	MS	55	MS	70	S
98B08*A039	40	I	30	MR	30	MR	55	MS	50	MS	40	I
98B08*A042	30	MR	30	MR	30	MR	40	I	40	I	45	MS
98B08*A044	10	R	25	MR	30	MR	30	MR	30	MR	30	MR
98B08*A046	60	MS	55	MS	60	MS	60	MS	50	MS	55	MS
98B08*A048	30	MR	20	MR	20	MR	25	MR	25	MR	30	MR
98B08*A049	30	MR	30	MR	45	MS	40	I	40	I	30	MR
98B08*A050	40	I	25	MR	30	MR	40	I	55	MS	40	I
98B08*A051	40	I	50	MS	45	MS	70	S	70	S	65	S
98B08*A052	10	R	10	R	10	R	15	MR	15	MR	15	MR
98B08*A054	60	MS	20	MR	15	MR	25	MR	35	I	35	I
98B08*A055	15	MR	10	R	20	MR	25	MR	15	MR	30	MR
98B08*A056	40	I	50	MS	40	I	55	MS	60	MS	60	MS
98B08*A059	20	MR	15	MR	15	MR	35	I	45	MS	30	MR
98B08*A061	70	S	15	MR	40	I	70	S	70	S	80	S
98B08*A064	60	MS	70	S	70	S	85	S	90	S	70	S
98B08*A067	60	MS	60	MS	55	MS	80	S	65	S	75	S
98B08*A075	30	MR	25	MR	30	MR	25	MR	35	I	30	MR

98B08*A076	35	MR	40	I	40	I	55	MS	55	MS	40	I
98B08*A077	15	MR	15	MR	10	R	40	I	40	I	35	I
98B08*A079	60	MS	65	S	65	S	70	S	85	S	80	S
98B08*A081	10	R	10	R	25	MR	35	I	35	I	35	I
98B08*A084	15	MR	20	MR	20	MR	20	MR	35	I	25	MR
98B08*A086	10	R	20	MR	15	MR	35	I	30	MR	30	MR
98B08*A090	20	MR	20	MR	15	MR	30	MR	30	MR	35	I
98B08*A091	15	MR	10	R	5	R	35	I	30	MR	40	I
98B08*A092	60	MS	60	MS	60	MS	60	MS	70	S	70	S
98B08*A102	15	MR	30	MR	40	I	60	MS	60	MS	40	I
98B08*A108	20	MR	15	MR	20	MR	35	I	40	I	40	I
98B08*A109	70	S	60	MS	55	MS	70	S	80	S	80	S
98B08*A110	60	MS	60	MS	60	MS	80	S	80	S	70	S
98B08*A111	20	MR	20	MR	20	MR	30	MR	35	I	35	I
98B08*A113	50	MS	25	MR	40	I	60	MS	70	S	70	S
98B08*A117	25	MR	30	MR	40	I	45	MS	40	I	55	MS
98B08*A119	30	MR	25	MR	40	I	60	MS	60	MS	45	MS
98B08*A125	15	MR	15	MR	25	MR	25	MR	35	I	30	MR
98B08*A126	20	MR	15	MR	30	MR	45	MS	45	MS	35	I
98B08*A127	30	MR	20	MR	25	MR	30	MR	45	MS	40	I
98B08*A128	15	MR	15	MR	10	R	20	MR	35	I	35	I
98B08*A135	30	MR	30	MR	35	I	35	I	45	MS	40	I
98B08*A141	20	MR	30	MR	45	MS	35	I	50	MS	45	MS
98B08*A143	50	MS	25	MR	30	MR	30	MR	30	MR	35	I
98B08*A145	40	I	10	R	10	R	35	I	30	MR	40	I
98B08*A146	25	MR	15	MR	20	MR	60	MS	60	MS	70	S
98B08*A149	60	MS	65	S	65	S	80	S	70	S	70	S
98B08*A150	20	MR	30	MR	30	MR	55	MS	55	MS	60	MS
98B08*B003	10	R	15	MR	15	MR	15	MR	30	MR	20	MR
98B08*B005	30	MR	40	I	55	MS	70	S	65	S	70	S
98B08*B008	60	MS	70	S	60	MS	75	S	65	S	85	S
98B08*B011	60	MS	65	S	55	MS	75	S	65	S	80	S

98B08*B013	25	MR	30	MR	30	MR	45	MS	55	MS	45	MS
98B08*B014	40	I	30	MR	30	MR	40	I	40	I	30	MR
98B08*B016	40	I	15	MR	60	MS	70	S	60	MS	65	S
98B08*B021	20	MR	15	MR	20	MR	30	MR	40	I	30	MR
98B08*B022	60	MS	60	MS	60	MS	55	MS	70	S	70	S
98B08*B024	10	R	10	R	10	R	25	MR	20	MR	20	MR
98B08*B025	70	S	60	MS	60	MS	65	S	65	S	65	S
98B08*B026	70	S	50	MS	60	MS	60	MS	70	S	70	S
98B08*B027	10	R	10	R	15	MR	20	MR	20	MR	30	MR
98B08*B028	10	R	10	R	15	MR	20	MR	15	MR	30	MR
98B08*B029	50	MS	60	MS	45	MS	80	S	65	S	80	S
98B08*B030	50	MS	60	MS	40	I	40	I	65	S	80	S
98B08*B031	25	MR	25	MR	15	MR	35	I	40	I	40	I
98B08*B034	15	MR	15	MR	25	MR	25	MR	20	MR	35	I
98B08*B035	60	MS	70	S	70	S	70	S	70	S	80	S
98B08*B036	15	MR	15	MR	25	MR	60	MS	55	MS	45	MS
98B08*B037	15	MR	15	MR	20	MR	20	MR	35	I	30	MR
98B08*B044	20	MR	30	MR	70	S	40	I	55	MS	55	MS
98B08*B048	20	MR	15	MR	25	MR	35	I	30	MR	45	MS
98B08*B050	10	R	15	MR	15	MR	35	I	20	MR	25	MR
98B08*B052	60	MS	55	MS	55	MS	65	S	60	MS	65	S
98B08*B053	30	MR	15	MR	45	MS	45	MS	30	MR	35	I
98B08*B055	20	MR	20	MR	30	MR	35	I	40	I	45	MS
98B08*B058	60	MS	50	MS	40	I	65	S	65	S	60	MS
98B08*B062	15	MR	10	R	20	MR	15	MR	20	MR	30	MR
98B08*B063	50	MS	60	MS	50	MS	50	MS	60	MS	65	S
98B08*B065	30	MR	10	R	10	R	30	MR	25	MR	25	MR
98B08*B069	15	MR	10	R	20	MR	40	I	30	MR	35	I
98B08*B070	50	MS	30	MR	30	MR	45	MS	55	MS	40	I
98B08*B071	35	I	15	MR	35	I	50	MS	60	MS	55	MS
98B08*B077	30	MR	40	I	35	I	65	S	55	MS	65	S
98B08*B080	15	MR	20	MR	15	MR	25	MR	15	MR	20	MR

98B08*B085	60	MS	15	MR	15	MR	45	MS	50	MS	40	I
98B08*B096	30	MR	30	MR	30	MR	45	MS	60	MS	45	MS
98B08*B098	20	MR	20	MR	10	R	15	MR	25	MR	20	MR
98B08*B099	20	MR	20	MR	10	R	30	MR	30	MR	35	I
98B08*B100	40	I	25	MR	55	MS	70	S	70	S	60	MS
98B08*B101	50	MS	60	MS	60	MS	35	I	45	MS	45	MS
98B08*B102	25	MR	35	I	40	I	40	I	45	MS	40	I
98B08*B104	20	MR	15	MR	15	MR	40	I	45	MS	30	MR
98B08*B105	60	MS	60	MS	65	S	65	S	65	S	70	S
98B08*B109	70	S	70	S	60	MS	80	S	70	S	70	S
98B08*B110	30	MR	20	MR	25	MR	40	I	40	I	40	I
98B08*B111	40	I	40	I	40	I	35	I	45	MS	35	I
98B08*B119	60	MS	70	S	65	MS	60	MS	60	MS	45	MS
98B08*B123	20	MR	20	MR	20	MR	45	MS	35	I	55	MS
98B08*B124	65	S	50	MS	60	MS	80	S	65	S	80	S
98B08*B126	15	MR	20	MR	70	S	40	I	55	MS	45	MS
98B08*B129	50	MS	65	S	55	MS	45	MS	60	MS	60	MS
98B08*B137	60	MS	70	S	70	S	70	S	70	S	60	MS
98B08*B138	70	S	70	S	30	MR	70	S	55	MS	70	S
98B08*B139	30	MR	60	MS	55	MS	70	S	65	S	70	S
98B08*B140	40	I	30	MR	15	MR	50	MS	40	I	55	MS
98B08*B141	30	MR	35	I	55	MS	50	MS	65	S	65	S
98B08*B142	20	MR	20	MR	25	MR	15	MR	15	MR	20	MR
98B08*B143	15	MR	20	MR	10	R	35	I	15	MR	30	MR
Superb-1	30	MR	30	MR	45	MS	45	MS	55	MS	45	MS
Superb-2	60	MS	30	MR	40	I	55	MS	55	MR	45	MS
BW278-1	20	MR	10	R	10	R	45	MS	30	MR	40	I
BW278-2	35	I	20	MR	30	MR	30	MR	35	I	30	MR
Thatcher	90	S	80	S	80	S	80	S	80	S	80	S
McKenzie	20	MR	25	MR	20	MR	20	MR	20	MR	25	MR

SEV=severity, % infection of the flag leaf; IT=infection type, R=resistant, MR=moderately resistant, I=intermediate, MS=moderately susceptible, S=susceptible, - indicates missing data

Appendix 7.5. Size (cM) of linkage groups in the BW278/AC Foremost RIL population.

Linkage Group	Size (cM)	# of SNPs (unique loci/bins)
1A.1	10.53	23 (6)
1A.2	92.66	672 (106)
1B	138.66	1189 (133)
1D.1	73.44	156 (33)
1D.2	23.12	254 (9)
2A	133.63	748 (102)
2B	113.49	525 (74)
2D.1	31.60	31 (7)
2D.2	86.19	211 (33)
3A	137.70	461 (74)
3B.1	57.20	265 (44)
3B.2	73.87	390 (66)
3D.1	40.19	17 (11)
3D.2	0.82	93 (6)
4A.1	72.60	225 (47)
4A.2	27.76	271 (30)
4B	125.44	324 (71)
4D	20.71	11 (6)
5A.1	96.26	348 (72)
5A.2	40.69	76 (26)
5B	193.89	757 (140)

5D.1	5.16	3 (3)
5D.2	13.99	32 (8)
5D.3	14.17	5 (5)
5D.4	27.55	7 (7)
5D.5	16.64	162 (15)
6A	87.84	673 (60)
6B.1	48.59	209 (23)
6B.2	24.03	76 (22)
6D.1	38.35	52 (8)
6D.2	21.23	8 (7)
6D.3	33.78	102 (15)
7A.1	64.64	314 (46)
7A.2	94.90	105 (36)
7B.1	34.15	33 (12)
7B.2	54.54	521 (66)
7D.1	18.03	49 (10)
7D.2	54.79	51 (20)
7D.3	0.55	21 (3)

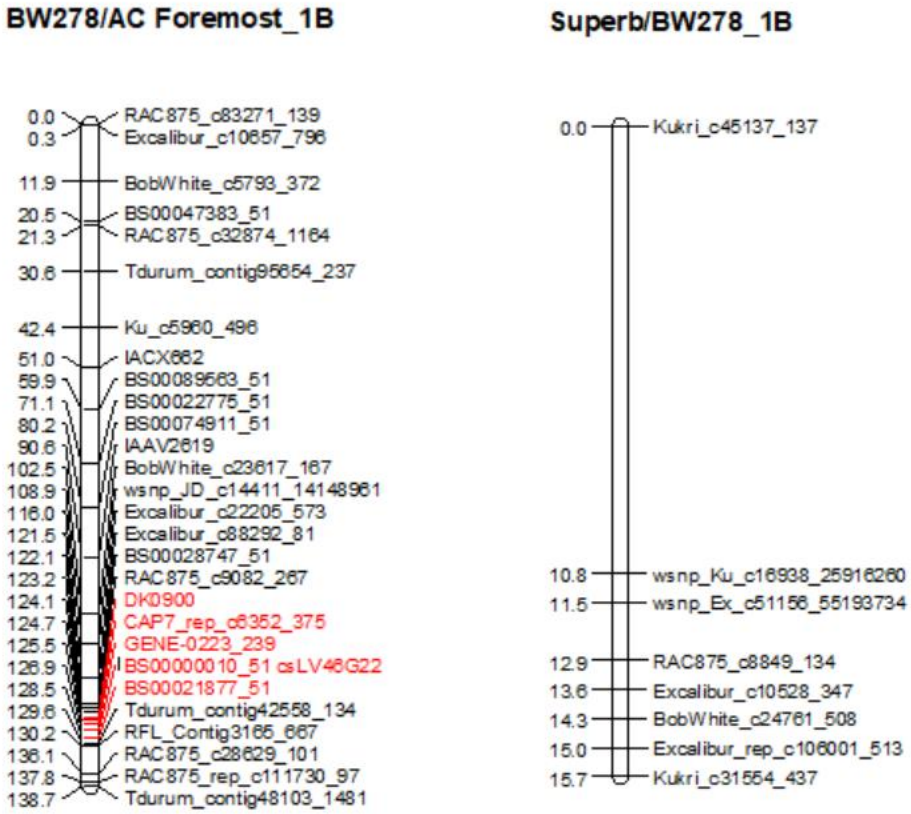
Appendix 7.6. Size (cM) of linkage groups in the Superb/BW278 DH mapping population.

Linkage Group	Size (cM)	# of SNPs and SSRs (unique loci/bins)
1A	131.97	617 (59)
1B	15.74	93 (9)
1D	67.44	39 (11)
2A.1	5.04	63 (6)
2A.2	100.45	289 (46)
2B	33.28	309 (22)
2D.1	92.84	59 (12)
2D.2	40.13	66 (10)
3A	12.45	174 (11)
3B.1	8.73	106 (6)
3B.2	112.55	894 (55)
3D	47.69	17 (13)
4A	17.37	8 (3)
4B	105.90	400 (47)
4D.1	35.84	31 (9)
4D.2	14.65	4 (3)
5A.1	108.24	258 (41)
5A.2	27.83	111 (17)
5B.1	85.13	482 (42)
5B.2	32.28	32 (4)
5D	142.27	50 (19)
6A.1	7.14	156 (7)
6A.2	19.79	138 (11)

6A.3	13.51	239 (12)
6B	130.61	1013 (70)
6D.1	33.78	21 (9)
6D.2	6.45	11 (5)
6D.3	0.70	7 (2)
7A	174.35	750 (55)
7B	92.42	664 (59)
7D.1	124.35	53 (21)
7D.2	39.09	4 (4)

Appendix 7.7. Simplified linkage maps of chromosome 1B in (A)BW278/AC Foremost and (B) Superb/BW278. Region of interest is indicated in red font.

(A) (B)



Appendix 7.8. SNP markers selected to design KASP markers that displayed linkage to *QLr.mrdc-1B* in the BW278/AC Foremost population in the Illumina Infinium Assay.

LAB KASP ID	90 K iSelect SNP ID	Allele Specific Forward Primer A1 Allele Specific Forward Primer A2 Common Reverse Primer C1
kwh742	Tdurum_contig4997_974	GAAGGTGACCAAGTTCATGCTGAAAGACTTGCATCTGGAGAGGT GAAGGTCGGAGTCAACGGATTGAAAGACTTGCATCTGGAGAGGC TCTCCAACCGAAGCTCCCGCTT
kwh743	IAAV4844	GAAGGTGACCAAGTTCATGCTCTTCAGAACGTGCGTGTGGAT GAAGGTCGGAGTCAACGGATTCTTCAGAACGTGCGTGTGGAC GCTAATCTCCGAAGTAGAGAAAGTTGAA
kwh744	CAP7_rep_c6352_375	GAAGGTGACCAAGTTCATGCTACCATTGATGCGGAACCCTTCG GAAGGTCGGAGTCAACGGATTGACCATTGATGCGGAACCCTTCT AGGTCCTTCTCATGGGCCTGGT
kwh745	GENE-0223_239	GAAGGTGACCAAGTTCATGCTTGTCCTTTGTTATCTCAAATTAATCTAGTATA GAAGGTCGGAGTCAACGGATTGTCCTTTGTTATCTCAAATTAATCTAGTATG CCATCCATCTTCCACCTGTTCAGTA
kwh746	Excalibur_c35888_208	GAAGGTGACCAAGTTCATGCTGGGAGTTGTAACAGCTGTCAG GAAGGTCGGAGTCAACGGATTCTGGGAGTTGTAACAGCTGTCAA CATGTTACAAAACAACAATGTGCACACCAA
kwh747	BS00000010_51	GAAGGTGACCAAGTTCATGCTGCGGGAAGAGAGCAAGGGG GAAGGTCGGAGTCAACGGATTGAGCGGGAAGAGAGCAAGGGT CGAACCCCTTGGCCTTGTCCAA
kwh748	Tdurum_contig10354_170	GAAGGTGACCAAGTTCATGCTAGCTGATGACATGGTCACTGAACAA GAAGGTCGGAGTCAACGGATTCTGATGACATGGTCACTGAACAG CATTGCGTCCGTGCGGAAGCTT
kwh749	BS00021877_51	GAAGGTGACCAAGTTCATGCTGGCTGGAAAAGGGATCAACAAGTTTT GAAGGTCGGAGTCAACGGATTGCTGGAAAAGGGATCAACAAGTTTC TTCAGTTATTCGCACAATCAGTCCACAA
kwh750	Tdurum_contig10354_270	GAAGGTGACCAAGTTCATGCTCCTAAGAGGTCGGCGGTGTAT GAAGGTCGGAGTCAACGGATTCCTAAGAGGTCGGCGGTGTAC AACACCGGTGAACAAGGCCATGAT
kwh751	BS00084990_51	GAAGGTGACCAAGTTCATGCTGATATATCTCAAGTAAAGGAATGGAGTGT GAAGGTCGGAGTCAACGGATTATATCTCAAGTAAAGGAATGGAGTGC GGCAGCATCAATAATACTCCCAGGAA

Appendix 7.9. Results from screening diagnostic markers *csLV46G22* and *DK0900* on the BW278/AC Foremost RIL population.

Line	Entry #	*DK0900*	*csLV46G22*
pb E 9	6	A	A
pb E 20	12	A	A
pb E 25	15	-	B
pb E 39	27	-	B
pb E 51	32	B	B
pb E 65	39	B	-
pb E 76	45	B	-
pb E 77	46	A	A
pb E 79	47	B	B
pb E 83	49	A	A
pb E 84	50	-	A
pb E 85	51	A	A
pb E 103	59	-	B
pb E 111	62	B	A
pb E 122	69	B	B
pb E 127	71	B	B
pb E 128	72	B	B
pb E 204	109	A	A
pb E 206	110	B	B
pb E 210	111	B	B
pb E 218	116	B	B
pb E 220	118	A	B
pb E 228	124	B	B
pb E 253	141	-	A
pb E 262	148	A	A
pb E 266	150	-	A
pb E 275	159	A	A
pb E 276	160	B	B
pb E 280	164	A	A
pb E 290	173	B	B
pb E 300	182	B	-
pb E 320	194	A	B
pb E 346	209	A	A
pb E 349	212	B	B
pb E 354	217	A	A
pb E 363	224	A	A
pb E 399	242	B	B
pb E 400	243	A	A
pb E 407	248	B	B
pb E 423	253	A	A

pb E 435	257	A	A
pb E 452	265	B	B
pb E 455	267	A	A
pb E 469	274	A	A
pb E 477	277	B	B
pb E 490	281	A	A
pb I 8	285	-	B
pb I 18	290	B	B
pb I 23	295	A	A
pb I 30	298	A	A
pb I 37	301	A	-
pb I 61	312	B	B
pb I 72	317	A	-
pb I 79	321	B	-
pb I 88	328	A	A
pb I 100	340	B	B
pb I 103	343	B	B
pb I 104	344	A	A
pb I 106	346	A	A
pb I 164	382	A	A
pb I 168	385	B	B
pb I 174	389	A	A
pb I 196	397	A	A
pb I 201	398	A	-
pb I 203	399	A	A
pb I 232	415	B	B
pb I 237	418	B	B
pb I 252	428	A	-
pb I 257	431	A	A
pb I 259	432	B	A
pb I 286	442	A	A
pb I 293	445	A	A
pb I 308	455	A	A
pb I 313	458	-	B
pb I 315	459	B	B
pb I 317	460	A	B
pb I 319	461	A	-
pb I 322	462	A	A
pb I 324	464	A	A
pb I 332	469	A	A
pb I 338	474	A	B
pb I 356	486	B	-
pb I 361	489	B	A
pb I 372	500	A	-

pb I 388	514	B	B
pb K 22	531	B	B
pb K 29	535	A	-
pb K 30	536	B	B
pb K 39	541	A	A
pb K 89	584	B	-
pb k 91	586	A	A
pb K 92	587	B	-
pb K 95	590	B	B
pb K 107	601	B	-
pb K 114	607	B	B
pb K 122	614	A	A
pb K 130	621	A	-
pb K 144	631	B	B
pb K 153	637	A	A
pb K 161	641	A	A
pb K 205	670	A	A
pb K 210	672	B	B
pb K 212	674	A	A
pb K 214	675	A	-
pb K 280	707	B	B
pb K 320	732	B	B
pb K 337	743	A	A
pb K 344	749	A	-
pb K 351	755	A	B
pb K 362	765	B	B
pb K 381	782	A	A
pb K 391	792	B	-
pb U 13	806	A	A
pb U 14	807	A	A
pb U 24	816	B	B
pb U 25	817	A	B
pb U 32	822	A	B
pb U 35	824	A	A
pb U 39	828	A	A
pb U 40	829	B	B
pb U 66	844	A	A
pb U 69	846	A	B
pb U 70	847	A	A
pb U 73	850	B	B
pb U 78	852	A	A
pb U 81	854	A	A
pb U 95	863	A	A
pb U 100	866	B	B

pb U 102	868	B	B
pb U 125	879	A	A
pb U 128	881	B	B
pb U 183	907	A	A
pb U 187	910	B	-
pb U 189	912	B	B
pb U 200	918	B	B
pb U 205	921	A	A
pb U 222	928	A	A
pb U 235	937	A	A
pb U 236	938	B	B
pb U 253	949	A	A
pb U 254	950	A	B
pb U 285	975	A	A
pb U 289	979	A	A
pb U 291	981	B	B
pb U 294	984	A	A
pb U 297	987	A	A
pb U 298	988	B	B
pb U 299	989	B	B
pb U 304	994	B	B
pb U 309	998	B	B
pb U 312	1001	B	B
pb U 319	1003	B	B
qe J 383	1263	A	A
pb U 323	1006	A	A
pb U 364	1026	B	B
pb U 411	1044	A	A
pb U 427	1047	A	A
pb U 443	1053	B	B
pb U 449	1057	A	A
pb U 461	1061	A	A
pb U 475	1064	A	A
qe J 1	1073	B	B
qe J 3	1075	A	A
qe J 22	1087	B	B
qe J 25	1089	A	A
qe J 413	1276	A	A
qe J 64	1108	B	B
qe J 70	1110	A	A
qe J 74	1113	A	A
qe J 136	1130	A	B
qe J 141	1133	A	A
qe J 168	1143	A	A

qe J 170	1145	A	A
qe J 415	1278	B	A
qe J 189	1152	A	A
qe J 199	1156	B	B
qe J 218	1166	B	B
qe J 228	1172	B	B
qe J 279	1201	B	B
qe J 295	1216	A	A
qe J 298	1219	-	B
qe J 303	1223	B	B
qe J 304	1224	A	A
BW278		A	A
AC Foremost		B	B

Appendix 7.10. Summary of greenhouse seedling infection type (IT) ratings of the Superb/BW278 DH population to various races of *Puccina triticina.*

	1-1 BBBD		77-2 TJBJ		11-180-1 TDBG		128-1 MBRJ		12-3 MBDS		74-2 MGBJ_ 1		74-2 MGBJ_ 2	
DH Line	**IT**	**Rxn**	**IT**	**Rxn**	**IT**	**Rxn**	**IT**	**Rxn**	**IT**	**Rxn**	**IT**	**Rxn**	**IT**	**Rxn**
98B08*A002	0	R	1+	R	1+	R	;1=	R	0	R	0	R	0	R
98B08*A005	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*A006	;	R	3	S	3	S	1+	R	1+	R	3+	S	3+	S
98B08*A007	;1-	R	1+	R	1+	R	1+	R	1+	R	3	S	3	S
98B08*A009	;	R	3+	S	3	S	3	S	3	S	3	S	3	S
98B08*A014	-	-	-	-	-	-	-	-	-	-	-	-	0	R
98B08*A018	0	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*A019	0	R	3+	S	3	S	0	R	0	R	0	R	0	R
98B08*A022	0	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*A026	;1=	R	3+	S	3+	S	3	S	3	S	3	S	3	S
98B08*A029	;	R	3	S	3+	S	;	R	;	R	0	R	0	R
98B08*A033	-	-	-	-	-	-	-	-	-	-	-	-	0	R
98B08*A037	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*A038	;	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
98B08*A039	;	R	3	S	3	S	3	S	3	S	3+	S	3+	S
98B08*A042	;	R	3	S	1+	R	1+	R	1+	R	3+	S	3+	S
98B08*A044	;	R	1+	R	1+	R	;	R	;	R	0	R	0	R
98B08*A046	3-	S	3	S	3	S	3	S	3	S	3	S	3	S
98B08*A048	;1=	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
98B08*A049	;	R	1+	R	1+	R	1+	R	1+	R	3+	S	3+	S
98B08*A050	0;	R	3	S	3	S	0	R	0	R	0	R	0	R
98B08*A051	0	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*A052	;1=	R	3+	S	3+	S	3+	S	3-	S	3	S	3	S
98B08*A054	0;	R	3+	S	3	S	0;	R	0;	R	0	R	0	R
98B08*A055	;	R	1+	R	3+	S	1+	R	1+	R	3+	S	3+	S
98B08*A056	3	S	3	S	3	S	3	S	3	S	3+	S	3+	S
98B08*A059	;	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*A061	;	R	3+	S	3+	S	0	R	0	R	0	R	0	R
98B08*A064	0;	R	3+	S	3	S	0	R	0	R	0	R	0	R
98B08*A067	0;	R	3	S	3	S	0	R	0	R	0	R	0	R
98B08*A075	;	R	3+	S	3	S	;	R	;	R	0	R	0	R
98B08*A076	;	R	3	S	3+	S	0	R	0	R	0	R	0	R
98B08*A077	;	R	3	S	3	S	3+	S	3+	S	3	S	3	S
98B08*A079	;1=	R	3+	S	3	S	1+	R	1+	R	3+	S	3+	S
98B08*A081	;	R	1+	R	1+	R	1+	R	1+	R	3+	S	3+	S
98B08*A082	-	-	-	-	-	-	-	-	-	-	-	-	0	R
98B08*A083	-	-	-	-	-	-	-	-	-	-	-	-	0	R
98B08*A084	;	R	1+	R	1+	R	0	R	0	R	0	R	0	R

98B08*A086	0;	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*A090	0;	R	1+	R	1+	R	;	R	;	R	0	R	0	R
98B08*A091	;	R	3	S	3	S	3	S	3	S	3	S	3	S
98B08*A092	0	R	3+	S	3	S	0	R	0	R	1+/0	R	1+/0	R
98B08*A093	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*A094	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*A095	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*A102	;	R	1+	R	1+	R	1+	R	1+	R	1+/0	R	1+	S
98B08*A103	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*A104	-	-	-	-	-	-	-	-	-	-	-	-	0	R
98B08*A108	0	R	1+	R	1+	R	;	R	;	R	0	R	0	R
98B08*A109	;1=	R	1+	R	1+	R	1+	R	1+	R	1+/0	R	1+/0	S
98B08*A110	0	R	3	S	3	S	0	R	0	R	0	R	0	R
98B08*A111	0	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*A113	;	R	3	S	3+	S	3+	S	3+	S	3	S	3	S
98B08*A117	3+	S	3	S	3+	S	3+	S	3+	S	3+	S	3+	S
98B08*A119	0	R	3	S	3+	S	0	R	0	R	0	R	0	R
98B08*A121	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*A122	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*A125	;	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
98B08*A126	0	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*A127	;	R	3	S	3+	S	0	R	0	R	0	R	0	R
98B08*A128	0;	R	3+	S	3+	S	0	R	0	R	0	R	0	R
98B08*A132	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*A135	1=	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
98B08*A136	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*A141	;	R	1+	R	1+	R	1+	R	1+	R	-	-	1+	R
98B08*A143	;	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
98B08*A144	-	-	-	-	-	-	-	-	-	-	-	-	0	R
98B08*A145	3-	S	3+	S	3+	S	3	S	3	S	3	S	3	S
98B08*A146	X	R	3	S	3+	S	3+	S	3	S	3	S	3	S
98B08*A149	3+	S	3+	S	3+	S	3+	S	3+	S	3	S	3	S
98B08*A150	;1=	R	1+	R	1+	R	1+	R	1+	R	-	-	1+	R
98B08*B003	X	R	3	S	3+	S	3+	S	3+	S	3+	S	3+	S
98B08*B005	0	R	3+	S	3+	S	0	R	1+	R	0	R	0	R
98B08*B008	;	R	3+	S	3+	S	0	R	0	R	0	R	0	R
98B08*B011	0	R	3	S	3	S	0	R	0	R	0	R	0	R
98B08*B013	;	R	3	S	3	S	0;	R	0;	R	0	R	0	R
98B08*B014	0	R	3	S	3	S	3+	S	3+	S	3+	S	3+	S
98B08*B016	3	S	3+	S	3+	S	3+	S	3+	S	3	S	3	S
98B08*B021	0	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*B022	3+	S	3+	S	3+	S	3+	S	3+	S	3+	S	3+	S
98B08*B024	;	R	1+	R	1+	R	1+	R	1+	R	3	S	3	S
98B08*B025	3+	S	3	S	3	S	3+	S	3+	S	3+	S	3+	S

98B08*B026	;	R	3+	S	3+	S	3+	S	3+	S	3+	S	3+	S
98B08*B027	0	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*B028	0;	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*B029	;1=	R	3	S	3	S	0	R	0	R	0	R	0	R
98B08*B030	0	R	3	S	3+	S	0	R	0	R	0	R	0	R
98B08*B031	0	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*B034	3+	S	3	S	3+	S	3+	S	3+	S	3+	S	3+	S
98B08*B035	0	R	3+	S	3+	S	0	R	0	R	0	R	0	R
98B08*B036	;	R	3	S	3+	S	3+	S	3+	S	3+	S	3+	S
98B08*B037	;	R	3	S	3+	S	0	R	0	R	0	R	0	R
98B08*B044	0	R	3	S	-	-	0	R	0	R	-	-	0	R
98B08*B048	0	R	1+	R	1+	R	1+	R	1+	R	0	R	0	R
98B08*B050	;1=	R	1+	R	1+	R	1+	R	1+/0	R	3+	S	3	S
98B08*B052	0	R	3+	S	3+	S	0	R	0	R	0	R	0	R
98B08*B053	0	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*B055	;1=	R	1+	R	1+	R	1+	R	1+	R	3+	S	3+	S
98B08*B058	3+	S	3	S	3	S	3+	S	3+	S	3+	S	3+	S
98B08*B062	;	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
98B08*B063	;	R	1+	R	1+	R	0	R	;	R	0	R	0	R
98B08*B065	;	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*B066	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*B069	;	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
98B08*B070	;	R	3+	S	3	S	0	R	0	R	0	R	0	R
98B08*B071	;1=	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
98B08*B073	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*B077	;1=	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
98B08*B078	-	-	-	-	-	-	-	-	-	-	-	-	0	R
98B08*B080	;1=	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
98B08*B081	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*B082	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*B084	-	-	-	-	-	-	-	-	-	-	-	-	0	R
98B08*B085	0	R	3+	S	3	S	0;	R	0;	R	0	R	0	R
98B08*B088	-	-	-	-	-	-	-	-	-	-	-	-	0	R
98B08*B093	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*B096	0	R	3+	S	3+	S	0	R	0	R	0	R	0	R
98B08*B098	0	R	1+	R	1+	R	0	R	;	R	0	R	0	R
98B08*B099	0	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*B100	0	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*B101	3-	S	3+	S	3	S	3+	S	3	S	3	S	3	S
98B08*B102	0	R	3	S	3	S	0	R	0	R	0	R	0	R
98B08*B104	0	R	3	S	3+	S	0	R	0	R	-	-	-	-
98B08*B105	;1=	R	3+	S	3	S	3+	S	3	S	3+	S	3+	S
98B08*B109	3+	S	3	S	3+	S	3+	S	3+	S	3+	S	3+	S
98B08*B110	;	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R

98B08*B111	0	R	1+	R	1+	R	0	R	0	R	0	R	0	R
98B08*B119	;	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
98B08*B122	-	-	-	-	-	-	-	-	-	-	-	-	3	S
98B08*B123	;1=	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
98B08*B124	3+	S	3	S	3	S	3+	S	3+	S	3+	S	3+	S
98B08*B126	0	R	3+	S	3	S	0	R	0	R	0	R	0	R
98B08*B129	;1=	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
98B08*B134	-	-	-	-	-	-	-	-	-	-	-	-	-	-
98B08*B135	-	-	-	-	-	-	-	-	-	-	-	-	-	-
98B08*B137	;	R	3	S	3+	S	3+	S	3+	S	3	S	3	S
98B08*B138	;	R	3	S	3+	S	3+	S	3+	S	3+	S	3+	S
98B08*B139	0	R	3	S	3	S	0	R	0	R	0	R	0	R
98B08*B140	0	R	3	S	3	S	0	R	0	R	0	R	0	R
98B08*B141	;1=	R	3	S	3+	S	3+	S	3+	S	3	S	3	S
98B08*B142	;1=	R	3	S	1+	R	1+	R	1+	R	3	S	0	R
98B08*B143	;	R	1+	R	1+	R	1+	R	1+	R	3	S	3	S
Superb-1	0	R	3+	S	3+	S	0	R	0;	R	0	R	0	R
Superb-2	0;	R	3+	S	3+	S	0	R	0;	R	0	R	0	R
BW278-1	;1-	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
BW278-2	;1-	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
Thatcher	3+	S	3+	S	3	S	3+	S	3+	S	3+	S	3+	S
Tc-Lr16	;1-	R	1+	R	1+	R	1+	R	1+	R	1+	R	1+	R
Tc-LrCen	3	S	3	S	X	R	3+	S	3+	S	3	S	3	S
Tc-Lr10	;1=	R	3	S	3	S	3+	S	3+	S	3	S	3	S
Tc-Lr2a	-	-	-	-	-	-	-	-	-	-	0	R	0	R
Tc-Lr2b	-	-	-	-	-	-	-	-	-	-	0	R	0	R
Tc-Lr2c	-	-	-	-	-	-	-	-	-	-	0	R	0	R

Appendix 7.11. Summary of greenhouse seedling infection type (IT) ratings of the Superb/86ISMN 2137 DH population to various races of *Puccina triticina.* DH lines in red font were removed prior to linkage mapping.

	06-1-1 TDBG		77-2 TJBJ		12-3 MBDS				74-2 MGBJ	
DH Line	**IT**	**Rxn**	**IT**	**Rxn**	**IT**	**Rxn**	**IT**	**Rxn**	**IT**	**Rxn**
09WP01*A0023	3+	S	3-	S	3+	S	3+	S	1+	R
09WP01*A0025	X-	R	3	S	3+	S	3+	S	3+	S
09WP01*A0026	3+	S	3-	S	0;	R	-	-	-	-
09WP01*A0036	3+	S	X	R	0;	R	-	-	-	-
09WP01*A0037	X	R	3+	S	X	R	3+	S	3+	S
09WP01*A0040	-	-	-	-	-	-	3+	S	-	-
09WP01*A0041	-	-	-	-	-	-	3+	S	-	-
09WP01*A0042	-	-	-	-	-	-	3+	S	-	-
09WP01*A0043	-	-	-	-	-	-	-	-	-	-
09WP01*A0057	-	-	-	-	-	-	-	-	-	-
09WP01*A0058	X	R	2-	R	3+	S	3+	S	23	R
09WP01*A0059	-	-	3	S	0	R	-	-	-	-
09WP01*A0061	X-	R	3-	S	0	R	0	R	0	R
09WP01*A0062	3+	S	X-	R	0	R	0	R	0	R
09WP01*A0064	3	S	2/3	R	0	R	0	R	0;	R
09WP01*A0065	3	S	3	S	3+	S	3+	S	-	-
09WP01*A0066	3+	S	2-	R	0	R	3	S	-	-
09WP01*A0067	3+	S	3+	S	3+	S	3	S	3+	S
09WP01*A0183	X-	R	2+	R	X	R	X	R	X	R
09WP01*A0185	3+	S	3+	S	3+/X	S	3+/X	S	3+	S
09WP01*A0186	X-	R	3+	S	0	R	0	R	0	R
09WP01*A0187	3+	S	2-	R	0	R	0	R	-	-
09WP01*A0188	3+	S	3-	S	3+	S	3+	S	-	-
09WP01*A0190	X	R	3+	S	0	R	-	-	-	-
09WP01*A0192	3+	S	-	-	3+	S	3	S	-	-
09WP01*A0195	X-	R	2/2+	R	3+	S	3+	S	-	-
09WP01*A0198	-	-	-	-	-	-	3+	S	-	-
09WP01*A0199	X-	R	3+	S	3+	S	3+	S	3+	S
09WP01*A0200	-	-	-	-	-	-	3+	S	-	-
09WP01*A0243	3+	S	3+	S	0	R	-	-	-	-
09WP01*A0244	;1-	R	2+/3-	R	X	R	3+	S	2+/3-;	R
09WP01*A0245	X-	R	3+	S	3+	S	3+/X	S	3+	S
09WP01*A0247	3+	S	1-	R	0	R	0	R	-	-
09WP01*A0248	-	-	-	-	;	R	-	-	-	-
09WP01*A0249	3	S	3	S	X	R	3+/X	S	3+	S
09WP01*A0272	X-	R	2+/3-	R	3+	S	3+	S	2+/3-	R
09WP01*A0273	X-	R	3+	S	3+	S	3+	S	3+	S

09WP01*A0275	3+	S	2/3	R	3+	S	3+	S	-	-
09WP01*A0291	3+	S	3	S	;	R	0	R	-	-
09WP01*A0292	X-	R	3	S	3+	S	3+	S	3+	S
09WP01*A0293	X-	R	3+	S	;	R	;	R	0	R
09WP01*A0294	3+	S	-	-	0	R	0	R	-	-
09WP01*A0297	3+	S	3	S	3+	S	3+	S	3+	S
09WP01*A0298	3+	S	3	S	0	R	0	R	-	-
09WP01*A0300	3+	S	3+	S	0	R	-	-	-	-
09WP01*A0458	3+	S	3+	S	3+	S	3+	S	3+	S
09WP01*B0070	3+	S	3+	S	3+	S	3+	S	3+	S
09WP01*B0074	X	R	3-	S	0	R	-	-	-	-
09WP01*B0076	3+	S	2-	R	0	R	-	-	-	-
09WP01*B0077	3+	S	2-	R	0	R	0	R	0	R
09WP01*B0079	X-	R	2-	R	0	R	0	R	-	-
09WP01*B0080	X-	R	2-	R	0;	R	0;	R	0	R
09WP01*B0083	3+	S	3	S	3+	S	3+	S	3+	S
09WP01*B0084	X	R	3	S	3+	S	3+	S	3+	S
09WP01*B0085	X	R	2-	R	0;	R	0	R	-	-
09WP01*B0088	3+	S	3	S	0	R	0	R	0	R
09WP01*B0090	3+	S	3+	S	3+	S	3+	S	3+	S
09WP01*B0125	3+	S	2-	R	0;	R	0	R	0	R
09WP01*B0130	3+	S	3+	S	3+	S	3+	S	-	-
09WP01*B0133	X-	R	2-	R	0	R	0;	R	-	-
09WP01*B0134	3+	S	3	S	0	R	0	R	0	R
09WP01*B0136	X-	R	3+	S	3+	S	3+	S	3+	S
09WP01*B0201	X	R	2/3	R	3+	S	3+	S	X	R
09WP01*B0204	X-	R	3+	S	3+	S	3+	S	3+	S
09WP01*B0205	3+	S	3+	S	0;	R	0;	R	0	R
09WP01*B0207	X-	R	3	S	0;	R	0	R	0	R
09WP01*B0208	X-	R	3+	S	0;	R	0	R	0	R
09WP01*B0210	X-	R	3+	S	3+	S	3+	S	2+/3-	R
09WP01*B0211	-	-	-	-	-	-	-	-	-	-
09WP01*B0212	3+	S	3	S	3+	S	3+	S	1+	R
09WP01*B0213	X-	R	3+	S	0	R	0	R	0	R
09WP01*B0214	3+	S	3+	S	0	R	-	-	0	R
09WP01*B0215	3+	S	3	S	0	R	-	-	-	-
09WP01*B0220	3+	S	3	S	0	R	0;	R	0	R
09WP01*B0225	3+	S	2-	R	0	R	0	R	-	-
09WP01*B0227	3+	S	3	S	3+	S	3+	S	3+	S
09WP01*B0303	X-	R	3+	S	3+	S	3+	S	3+	S
09WP01*B0307	3+	S	3	S	3+	S	3+	S	2+/3-	R
09WP01*B0311	-	-	-	-	-	-	-	-	-	-
09WP01*B0312	-	-	-	-	-	-	-	-	-	-
09WP01*B0315	3+	S	3+	S	3+	S	3+	S	3+	S

09WP01*B0316	X-	R	3	S	0	R	0	R	0	R
09WP01*B0318	-	-	3	S	3+	S	3+	S	3+	S
09WP01*B0320	-	-	-	-	-	-	-	-	-	-
09WP01*B0323	3+	S	3+	S	3+	S	3+	S	3+	S
09WP01*B0324	X-	R	3	S	-	-	3+	S	3+	S
09WP01*B0325	X-	R	2/3	R	3+	S	3+	S	2+/3-	R
09WP01*B0326	3+	S	3	S	0	R	0	R	0	R
09WP01*B0328	-	-	3	S	3+	S	-	-	-	-
09WP01*B0329	3+	S	3	S	0	R	0	R	-	-
09WP01*B0330	-	-	-	-	-	-	0	R	-	-
09WP01*B0331	X-	R	3+	S	0	R	0;	R	-	-
09WP01*B0333	3+	S	3+	S	3+	S	3+	S	3+	S
09WP01*B0334	3+	S	3	S	3+	S	3+	S	3+	S
09WP01*B0337	X-	R	3	S	0;	R	0	R	0	R
09WP01*B0341	-	-	3	S	3+	S	3+	S	-	-
09WP01*B0343	X-	R	3	S	3+	S	3+	S	2+/3-	R
09WP01*B0344	-	-	3	S	3+	S	3+	S	3+	S
09WP01*B0345	X-	R	3+	S	3+	S	3+	S	3+	S
09WP01*B0354	3+	S	3-	S	3+	S	3+	S	3+	S
09WP01*B0355	3+	S	3	S	3+	S	3+	S	3+	S
09WP01*B0356	3+	S	3-	S	3+	S	3+	S	3+	S
09WP01*B0360	-	-	-	-	-	-	3	S	-	-
09WP01*B0361	-	-	-	-	-	-	0	R	-	-
09WP01*B0364	X-	R	3+	S	0	R	0	R	0	R
09WP01*B0365	X-	R	3+	S	3+	S	3+	S	3+	S
09WP01*B0368	3	S	3	S	0;	R	0;	R	0	R
09WP01*B0369	3/X	S	3-/;	S	1+	R	1+	R	3+	S
09WP01*B0370	3+	S	3	S	0	R	0	R	0	R
09WP01*B0373	X	R	2-?	R	0	R	0	R	0	R
09WP01*B0374	-	-	3	S	0	R	3	S	0	R
09WP01*B0403	-	-	-	-	-	-	0	R	-	-
09WP01*B0404	3+	S	3	S	3+	S	3+	S	3+	S
09WP01*B0405	X-	R	3	S	0	R	0	R	0	R
09WP01*B0407	3+	S	2/3	R	3+	S	3+	S	X	R
09WP01*B0408	-	-	-	-	-	-	0	R	-	-
09WP01*B0413	3+	S	3	S	0	R	0	R	0	R
09WP01*C0027	3+	S	3	S	3+	S	3+	S	1+/2-	R
09WP01*C0028	X	R	3	S	0	R	0	R	-	-
09WP01*C0030	X	R	2?	R	0	R	0	R	0	R
09WP01*C0031	X	R	2/3	R	0	R	0	R	0	R
09WP01*C0033	X	R	3	S	3+	S	3+	S	3+	S
09WP01*C0094	-	-	2-	R	3+	S	3+	S	3+	S
09WP01*C0096	X	R	3	S	-	-	0	R	-	-
09WP01*C0097	3+	S	3	S	0	R	-	-	-	-

09WP01*C0098	X	R	3	S	0	R	0	R	0	R
09WP01*C0103	-	-	3+	S	3+	S	3+	S	3+	S
09WP01*C0104	3+	S	2/3	R	3+	S	3+	S	1+/X	R
09WP01*C0109	X	R	3+	S	0	R	0	R	-	-
09WP01*C0137	3+	S	2-?	R	0	R	-	-	0	R
09WP01*C0139	-	-	-	-	0	R	-	-	-	-
09WP01*C0140	X	R	3+	S	3+	S	3	S	-	-
09WP01*C0141	X	R	3+	S	3+	S	3+	S	3+	S
09WP01*C0142	X	R	;1-	R	0	R	0	R	0	R
09WP01*C0145	X	R	3	S	3+	S	3+	S	3+	S
09WP01*C0146	3+	S	3+	S	3+	S	3+	S	3+	S
09WP01*C0148	X	R	3	S	0	R	0	R	0	R
09WP01*C0150	X	R	3	S	X	R	3+	S	3+	S
09WP01*C0151	X	R	2/3	R	0	R	0	R	0	R
09WP01*C0152	X	R	3	S	0	R	0	R	0	R
09WP01*C0157	X	R	3+	S	0	R	0	R	0	R
09WP01*C0158	3	S	2/3	R	3+	S	3+	S	2+/3-	R
09WP01*C0159	X-	R	3+	S	0	R	0	R	0	R
09WP01*C0160	X-	R	1/2	R	X	R	X	R	1+/2-	R
09WP01*C0162	3	S	3+	S	0	R	0	R	0	R
09WP01*C0261	3	S	2/3	R	0	R	0	R	0	R
09WP01*C0266	-	-	3-	S	;	R	0;	R	0	R
09WP01*C0267	3+	S	3	S	X	R	3+	S	;1	R
09WP01*C0349	3+	S	3	S	3+	S	3+	S	3+	S
09WP01*C0352	3+	S	3	S	0	R	0	R	0	R
09WP01*C0376	3/X	S	3+	S	3+	S	3+	S	3+	S
09WP01*C0378	-	-	2+	R	3+	S	-	-	-	-
09WP01*C0380	X	R	3-	S	3+	S	3+	S	3+	S
09WP01*C0382	1+	R	1/2	R	0	R	0	R	0;	R
09WP01*C0383	X	R	3	S	3+	S	3+	S	1;	R
09WP01*C0384	3+	S	3	S	0;	R	0;	R	0	R
09WP01*C0385	-	-	3	S	0	R	0	R	0	R
09WP01*C0429	X	R	3+	S	3+	S	3+	S	3+	S
09WP01*C0433	X	R	3	S	3+	S	3+	S	2/3	R
09WP01*C0437	3+	S	3	S	0	R	0	R	0	R
09WP01*D0253	3+	S	3+	S	3+	S	3+	S	3+	S
09WP01*D0390	3+	S	3+	S	3+	S	-	-	3+	S
09WP01*D0391	X	R	3-	S	3+	S	-	-	3+	S
09WP01*D0392	3+	S	3	S	3+	S	3+	S	3+	S
09WP01*D0393	3+	S	3	S	3+	S	3+	S	3+	S
09WP01*E0002	X	R	3+	S	3+	S	3+	S	3+	S
09WP01*E0004	;1-	R	3+	S	0;	R	0	R	0	R
09WP01*E0009	3+	S	3+	S	3+	S	3+	S	3+	S
09WP01*E0010	X	R	3+	S	0	R	0	R	0	R

09WP01*E0020	X	R	3+	S	0	R	0	R	0	R
09WP01*E0022	3+	S	-	-	3+	S	-	-	3+	S
09WP01*E0051	3+	S	3	S	0	R	0	R	0	R
09WP01*E0053	3+	S	2-	R	0	R	0	R	-	-
09WP01*E0120	3+	S	3	S	3+	S	3+	S	3-	S
09WP01*E0123	3+	S	3+	S	0	R	0	R	0	R
09WP01*E0167	3+	S	3	S	0	R	0	R	0	R
09WP01*E0168	X	R	3+	S	3+	S	3+	S	3+	S
09WP01*E0170	X	R	3+	S	0	R	0	R	0	R
09WP01*E0172	3+	S	3	S	;	R	0	R	0	R
09WP01*E0174	3+	S	2/3-	R	0	R	0	R	0;	R
09WP01*E0175	X	R	3+	S	3+	S	3+	S	3+	S
09WP01*E0178	3+	S	3	S	0	R	-	-	-	-
09WP01*E0229	X	R	3	S	-	-	0	R	0	R
09WP01*E0233	X	R	3+	S	0	R	0	R	0	R
09WP01*E0234	X	R	3+	S	0	R	-	-	-	-
09WP01*E0235	X	R	3+	S	0	R	0	R	0	R
09WP01*E0238	3+	S	-	-	0	R	0	R	-	-
09WP01*E0279	3+	S	3+	S	3+/X	S	3+/X	S	3+	S
09WP01*E0282	X	R	2/2+	R	3+	S	3+	S	X	R
09WP01*E0415	X	R	2/3	R	3+	S	3+	S	2+/3-	R
09WP01*E0417	3+	S	3	S	3+	S	3+	S	3+	S
09WP01*E0419	3+	S	-	-	3+	S	-	-	3+	S
09WP01*E0440	3+	S	3	S	3+	S	3+	S	3+	S
09WP01*E0442	3+	S	3+	S	3+	S	3+	S	3+	S
09WP01*E0446	X	R	3+	S	X	R	-	-	-	-
09WP01*E0447	3+	S	3+	S	0	R	0	R	0	R
09WP01*E0452	X	R	;1/2	R	0	R	0	R	0	R
09WP01*E0454	X	R	2/3	R	0	R	-	-	-	-
Superb	3+	S	3-	S	0	R	-	-	0	R
86ISMN 2137	X	R	3-	S	3+	S	3+	S	3-	S
Thatcher	3+	S	3-	S	3+	S	3+	S	3+	S
Thatcher	3+	S	3+	S	3+	S	3+	S	3+	S
Neepawa	3+	S	3+	S	3+	S	-	-	-	-
CDC Stanley (BW880)	-	-	-	-	3+	S	-	-	3+	S
Trident	X	R	-	-	3+	S	3+	S	3+	S
Little Club	X	R	3+	S	3+	S	3+	S	3+	S
Tc-Lr16	1+	R	1+	R	1+	R	1+	R	23	R
Tc-Lr22a	2+3-	R	3+	S	23	R	3	S	3+	S
Tc-Lr37	3+	S	3+	S	3+	S	3+	S	3+	S
Tc-Lr32	2-	R	2-	R	2+	R	2+	R	2-	R
Tc-Lr60	12	R	X	R	;12	R	2+	R	2+/3-	R
Tc-LrCen	X	R	3+	S	3+	S	3+	S	3+	S

Appendix 7.12. Summary of SNP derived KASP markers that demonstrated linkage to *Lr2a* in the Superb/BW278 DH population.

Lab KASP ID	90 K Infinium ID	Allele Specific Forward Primer A1 Allele Specific Forward Primer A2 Common Reverse Primer C1
kwh735	wsnp_CAP12_c1503_764765	GAAGGTGACCAAGTTCATGCTATTGTACAACCAAAAATCCAGTGTGT
		GAAGGTCGGAGTCAACGGATTATTGTACAACCAAAAATCCAGTGTGC
		GCCAAGTTCCTCAGCATCTAGCAAA
kwh736	Kukri_c25843_669	GAAGGTGACCAAGTTCATGCTAACATCAGATTCAAATGCTTTATCAAATGC
		GAAGGTCGGAGTCAACGGATTCTAACATCAGATTCAAATGCTTTATCAAATGT
		GGACTAGAAGTTTGGCAGTGTCGTT
kwh737	D_GBUVHFX02GV41H_67	GAAGGTGACCAAGTTCATGCTCAGATATTCCGGTTCAACTTGGCA
		GAAGGTCGGAGTCAACGGATTAGATATTCCGGTTCAACTTGGCG
		TTCTCTCGGCGCGGCAGTTGTT
kwh738	Kukri_c20972_618	GAAGGTGACCAAGTTCATGCTGCCCTTTCTTGGACCATAAGCTTA
		GAAGGTCGGAGTCAACGGATTCCCTTTCTTGGACCATAAGCTTC
		CACGAGGCCCTTGACATACATGTAT
kwh739	Excalibur_c20175_370	GAAGGTGACCAAGTTCATGCTAAAACAGATTTCTCTGCACCTAGACAT
		GAAGGTCGGAGTCAACGGATTAACAGATTTCTCTGCACCTAGACAC
		CCGGGTGTGTTGATATCCACTAAGAT
kwh740	Excalibur_c1944_1017	GAAGGTGACCAAGTTCATGCTCCCATGAGATAAAGCTTGAGGCA
		GAAGGTCGGAGTCAACGGATTCCCATGAGATAAAGCTTGAGGCG
		GGCTCAGCCCCGCAGGGAA
kwh741	RAC875_c65419_229	GAAGGTGACCAAGTTCATGCTGCAAGGACTAGCGTGTTGTCAAC
		GAAGGTCGGAGTCAACGGATTAGCAAGGACTAGCGTGTTGTCAAA
		ACCACGGCAAGTTTCTCCACCG

Appendix 7.13. Comparison between 90K Infinium assay, corresponding KASP marker, and converted infection type (IT) ratings to *Puccina triticina* isolate 74-2 MGBJ for Superb/BW278. Lines with red font indicate that one or more KASP markers did not have the same genotype as the corresponding 90 K Infinium SNP.

DH Line	wsnp_CAP12_c1503_764765	kwh735	Kukri_c25843_669	kwh736	D_GBUVHFX02GV41H_67	kwh737	Kukri_c20972_618	kwh738	Excalibur_c20175_370	kwh739	Excalibur_c1944_1017	kwh740	RAC875_c65419_229	kwh741	74-2 MGBJ
98B08*A002	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A005	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A006	A	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A007	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A009	A	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A014	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A018	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A019	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A022	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A026	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A029	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A033	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A037	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A038	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A039	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A042	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A044	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A046	A	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A048	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A049	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A050	B	B	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A051	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A052	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A054	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A055	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A056	A	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A059	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A

98B08*A061	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A064	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A067	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A075	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A076	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A077	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A079	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A081	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A082	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A083	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A084	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A086	B	B	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A090	B	B	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A091	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A092	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A093	B	B	B	-	B	-	B	B	B	B	B	B	B	B	B
98B08*A094	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A095	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A102	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A103	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A104	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A108	B	B	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A109	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A110	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A111	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A113	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A117	A	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A119	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A121	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A122	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A125	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A126	B	B	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A127	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A128	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A132	A	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A135	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A136	B	B	B	-	B	B	B	B	B	B	B	-	B	B	B
98B08*A141	A	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A143	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A144	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*A145	-	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A146	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A149	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*A150	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B

98B08*B003	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B005	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B008	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B011	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B013	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B014	B	A	B	-	B	A	B	A	B	A	B	A	B	A	B
98B08*B016	B	A	B	-	B	A	B	A	B	A	B	A	B	A	B
98B08*B021	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B022	B	A	B	-	B	A	B	A	B	A	B	A	-	A	B
98B08*B024	A	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B025	B	A	B	-	B	A	B	A	B	A	B	A	B	A	B
98B08*B026	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B027	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B028	A	B	A	-	A	B	A	B	A	B	A	B	A	B	A
98B08*B029	A	B	A	-	A	B	A	B	A	B	A	B	A	B	A
98B08*B030	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B031	A	B	A	-	A	B	A	B	A	B	A	B	A	B	A
98B08*B034	B	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B035	A	B	A	-	A	B	A	B	A	B	A	B	A	B	A
98B08*B036	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B037	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B044	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B048	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B050	A	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B052	B	B	A	-	A	A	A	A	A	A	A	A	A	-	A
98B08*B053	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B055	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B058	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B062	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B063	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B065	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B066	A	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B069	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B070	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B071	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B073	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B077	-	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B078	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B080	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B081	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B082	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B084	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B085	B	B	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B088	-	A	A	-	A	A	A	A	A	A	A	A	A	A	A

98B08*B093	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B096	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B098	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B099	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B100	B	B	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B101	B	B	B	-	B	B	B	B	B	-	B	B	B	B	B
98B08*B102	A	A	A	-	A	A	A	A	A	-	A	A	A	A	A
98B08*B104	A	A	A	-	A	A	A	A	A	-	A	A	A	A	A
98B08*B105	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B109	A	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B110	A	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B111	A	-	A	-	A	-	A	-	A	-	A	-	A	-	A
98B08*B119	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B122	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B123	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B124	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B126	A	A	-	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B129	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B134	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B135	A	A	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B137	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B138	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B139	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B140	A	A	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B141	B	B	B	-	B	B	B	B	B	B	B	B	B	B	B
98B08*B142	B	B	A	-	A	A	A	A	A	A	A	A	A	A	A
98B08*B143	B	B	B	-	B	B	B	B	B	-	B	B	B	B	B
Superb	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Superb	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Superb	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
BW278	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
BW278	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
BW278	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

Appendix 7.14. Comparison between 90 K Infinium assay, corresponding KASP marker, and converted infection type (IT) ratings to *Puccina triticina* isolate 12-3 MBDS for Superb/86ISMN 2137. Lines with red font indicate that one or more KASP markers did not have the same genotype as the corresponding 90 K Infinium SNP.

DH Line	wsnp_CAP12_c1503_764765	kwh735	Kukri_c25843_669	kwh736	D_GBUVHFX02GV41H_67	kwh737	Kukri_c20972_618	kwh738	Excalibur_c20175_370	kwh739	Excalibur_c1944_1017	kwh740	RAC875_c65419_229	kwh741	12-3 MBDS
09WP01*A0023	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0025	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0026	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0036	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0037	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0040	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0041	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0042	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0043	A	A	-	-	-	-	-	-	-	-	A	A	-	-	-
09WP01*A0057	A	A	-	-	-	-	-	-	-	-	A	A	-	-	-
09WP01*A0058	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0059	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0061	B	B	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0062	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0064	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0065	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0066	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0067	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0183	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0185	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0187	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0188	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0190	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0192	A	A	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0195	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0198	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0199	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0200	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B

09WP01*A0243	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0244	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0245	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0247	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0249	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0272	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0273	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0275	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0291	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0292	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0293	B	B	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0294	B	B	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0297	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*A0298	B	B	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0300	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*A0458	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0070	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0074	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0077	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0079	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0080	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0083	A	A	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0084	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0085	A	B	-	-	-	-	-	-	-	-	A	B	-	-	A
09WP01*B0088	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0090	B	A	-	-	-	-	-	-	-	-	B	A	-	-	B
09WP01*B0125	A	B	-	-	-	-	-	-	-	-	A	B	-	-	A
09WP01*B0130	B	A	-	-	-	-	-	-	-	-	B	A	-	-	B
09WP01*B0133	A	B	-	-	-	-	-	-	-	-	A	B	-	-	A
09WP01*B0134	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0136	B	A	-	-	-	-	-	-	-	-	B	A	-	-	B
09WP01*B0201	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0204	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0205	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0207	B	B	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0208	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0210	B	B	-	-	-	-	-	-	-	-	B	-	-	-	B
09WP01*B0211	B	B	-	-	-	-	-	-	-	-	B	B	-	-	-
09WP01*B0212	A	A	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0213	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0214	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0215	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0220	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0225	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A

09WP01*B0227	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0303	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0307	A	A	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0311	B	B	-	-	-	-	-	-	-	-	B	B	-	-	-
09WP01*B0312	B	B	-	-	-	-	-	-	-	-	B	B	-	-	-
09WP01*B0315	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0316	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0318	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0320	B	B	-	-	-	-	-	-	-	-	B	B	-	-	-
09WP01*B0323	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0324	A	A	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0325	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0326	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
09WP01*B0328	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
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09WP01*B0333	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0334	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
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09WP01*B0341	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
09WP01*B0343	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
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09WP01*B0355	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
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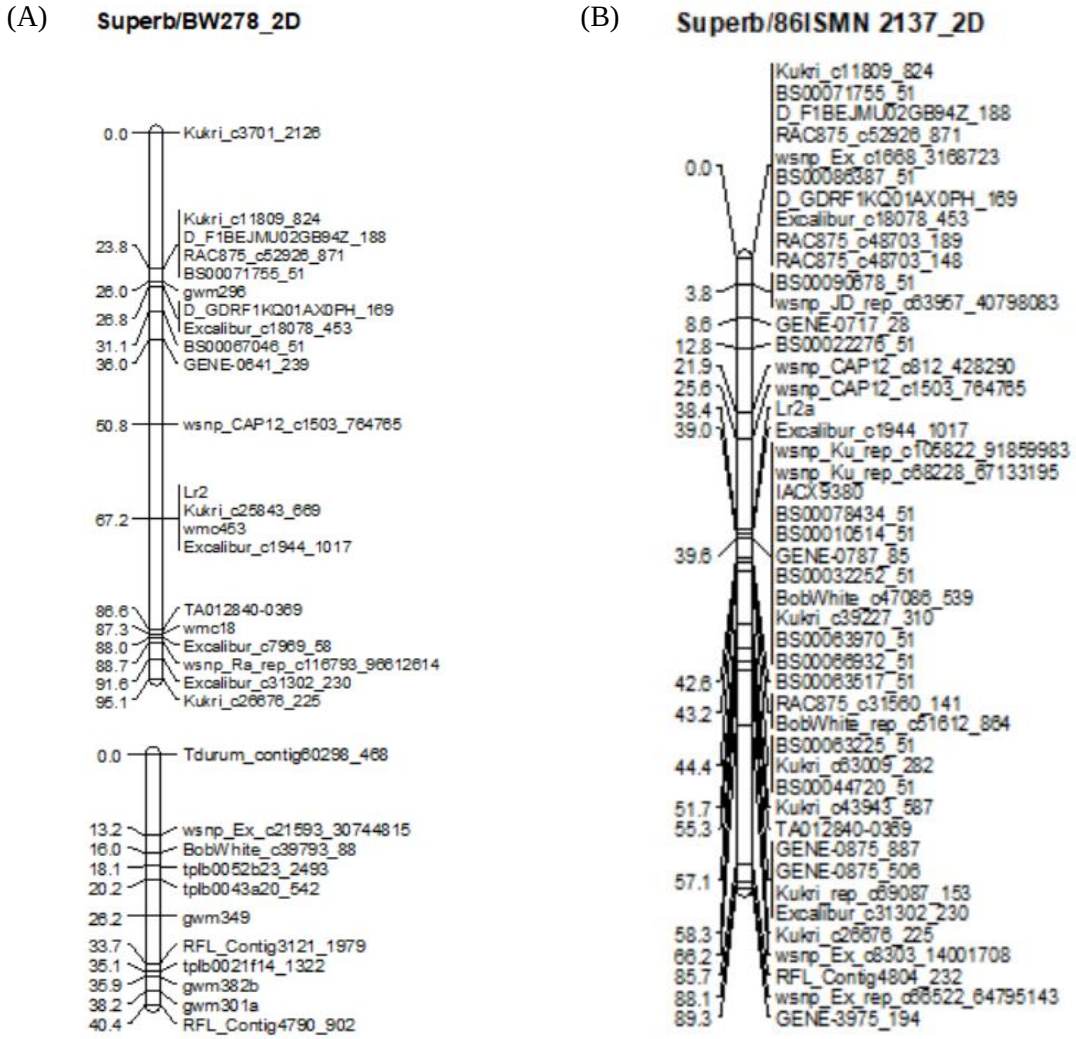
09WP01*E0279	B	A	-	-	-	-	-	-	-	-	B	A	-	-	B
09WP01*E0282	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B
Superb	A	A	-	-	-	-	-	-	-	-	A	A	-	-	A
86ISMN 2137	B	B	-	-	-	-	-	-	-	-	B	B	-	-	B

Appendix 7.15. Simplified genetic maps of chromosome 2D in (A) Superb/BW278 and (B) Superb/86ISMN 2137.



Appendix 7.16. Table of abbreviations.

Abbreviation	Term
AAFC	Agriculture and Agri-Food Canada
AAFC-CRC	Agriculture and Agri-Food Canada- Cereal Research Centre
AFLP	Amplified Fragment Length Polymorphism
AI	Active Ingredient
APR	Adult Plant Resistance
CAPS	Cleaved Amplified Polymorphic Sequence
CI	Coefficient of Infection
cM	Centimorgan
CWRS	Canada Western Red Spring
DH	Double Haploid
DNA	Deoxyribonucleic Acid
FHB	Fusarium Head Blight
FRET	Fluorescence Resonance Energy Transfer
I	Intermediate
ICIM	Inclusive Composite Interval Mapping
IM	Interval Mapping
IT	Infection Type
KASP	Kompetitive Allele Specific PCR
LG	Linkage Group
LOD	Logarithm of Odds
LSmeans	Least Squares Means
MAS	Marker Assisted Selection
MS	Moderately Susceptible
MSTMap	Minimum Spanning Tree of a Graph
Mt	Million tonnes
MR	Moderately Resistant
MRDC	Morden Research and Development Centre
PCR	Polymerase Chain Reaction
PVE	Phenotypic Variation Explained
QTL	Quantitative Trait Loci
R	Resistant
RAPD	Random Amplified Polymorphic DNA
RCBD	Randomized Complete Block Design
RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant Inbred Line
S	Susceptible
SEV	Severity
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats