Mapping Quantitative Trait Loci Associated with Agronomic Traits and Disease Resistance in a Canadian Spring Wheat Mapping Population

by

Darcy Hayes Bemister

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Plant Science

Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

Due to reduced genotyping costs and high-throughput technologies, marker assisted selection has become a valuable tool for plant breeders, allowing for identifying traits of interest in screened germplasm. Marker assisted selection requires the identification of stable, and consistent quantitative trait loci (QTL) that will become reliable markers. The Canada Western Red Spring (CWRS) class of common wheat (*T. aestivum*) is the most produced class of wheat in western Canada and requires a complex arrangement of agronomic traits and adequate disease resistance. The objective of this thesis was to identify QTL associated with economically important diseases in western Canada such as stripe rust (*Puccinia striiformis* f. sp. *tritici*), leaf rust (*Puccinia triticina*), and the leaf spot complex, and agronomic traits that are important to producers and end users including earliness, grain yield, protein content and gluten strength. A total of 208 recombinant inbred lines derived from crossing Canadian spring wheat (*T. aestivum*) cultivars 'Peace' and 'Carberry' were evaluated from 2014 to 2017 in disease nurseries located in Alberta and British Columbia, and conventional and organic yield trials in 2016 and 2017 in Edmonton, Alberta and genotyped with DArTseq markers. Using the least squares means of the combined environments, three QTL associated with stripe rust, four QTL associated with leaf rust, and three QTL associated with leaf spotting were identified. We confirm the presence of a stripe rust QTL on chromosome 4B, with the allele conferring resistance contributed by 'Carberry', that has been previously reported by others. We also identified two QTL associated with stripe rust and leaf rust on chromosome 2A, in which the alleles conferring resistance were contributed by 'Peace', corresponding with previous studies that identified QTL on chromosome 2A that were contributed by a close relative of 'Peace'. Phenotyping of agronomic traits was conducted in conventional and organic environments to identify consistent QTL across

management systems. We identified thirty-eight QTL for nine agronomic traits and QTL clusters on chromosomes 4B and 7D were identified consistently across conventional and organic environments. The largest QTL was identified as an allele contributed by 'Carberry', and is most likely the *Rht-B1b* height reducing gene, due to explaining 53% of the total phenotypic variance and being located on 4B. The second largest QTL was located on chromosome 1A and associated with sedimentation volume and explained 41% of the total phenotypic variance. Results from this study suggest that 'Carberry' could be an attractive germplasm for breeders to enhance resistance against stripe rust and leaf spot with minor resistance alleles, and 'Peace' consistently contributed an allele on 7D that reduced plant height by six centimeters, and maturity by two days, but reduced grain yield by 300 kg ha^{-1}. Minor effect QTL with LOD scores as low as 3.4 were consistently identified across management-specific environments and suggests that stable QTL may not need to be large in effect.

Preface

A version of the second chapter has been submitted to *Crop Science* titled as "Mapping QTL associated with stripe rust, leaf rust and leaf spotting in a Canadian spring wheat population", and authored by Bemister, Darcy H; Semagn, Kassa; Iqbal, Muhammad; Randhawa, Harpinder; Strelkov, Stephen; Spaner, Dean. I was responsible for DNA extraction of the mapping population, phenotyping a portion of the field experiment, curating the genotypic and phenotypic data, analysis of phenotypic data, QTL analysis and writing the manuscript. Kassa Semagn assisted with QTL analysis, interpretation of the results, and manuscript editing. Muhammad Iqbal, and Dean Spaner assisted with manuscript editing. Harpinder Randhawa and Stephen Strelkov assisted with phenotyping.

A version of the third chapter has been submitted to *Crop Science* titled as "Mapping QTL associated with agronomic traits under conventionally and organically managed systems in a Canadian spring wheat population", and authored by Bemister, Darcy H; Semagn, Kassa; Iqbal, Muhammad, Randhawa, Harpinder; Strelkov, Stephen; Dean, Spaner. I was responsible for DNA extraction of the mapping population, phenotyping of the field experiment, curating the genotypic and phenotypic data, analysis of phenotypic data, QTL analysis and writing the manuscript. Kassa Semagn assisted with QTL analysis, interpretation of the results, and manuscript editing. Muhammad Iqbal, Harpinder Randhawa, Stephen Strelkov, and Dean Spaner assisted with manuscript editing.

"... a scientific man ought to have no wishes, no affections, -- a mere heart of stone. --"

Charles Robert Darwin

1857

Acknowledgements

I am sincerely thankful to my supervisor, Dr. Dean Spaner, for giving me the opportunity to complete an MSc. under his supervision, supporting me financially, and seeing potential in me. I am grateful for his professional and personal advice I have received over the two and a half years and will always view this chapter of my life as a significant achievement. I extend my gratitude towards my thesis committee members: Dr. Habibur Rahman, and Dr. Nat Kav for their advice, expertise, and time.

This research would not have been possible without the help of the University of Alberta wheat breeding program, and I thank Dr. Muhammad Iqbal, Dr. Hua Chen, Klaus Strenzke, Izabela Ciechanowska, Fabiana Dias, Joeseph Moss, Russell Puk, Thomas Keady, Katherine Chabot, Lindsay Jessup, Brianna White and Parisa Beheshti for their hard work and expertise. I also thank Dr. Kassa Semagn for his technical expertise, and non-sugar-coated advice, which I always appreciated. I extend my thanks to my fellow graduate students Graham Collier, Hiroshi Kubota, Khang Nguyen, Enid Perez Lara, Rongrong Xiang for their help and support.

I personally thank each agency for their financial contribution. This research was supported by grants to the University of Alberta wheat breeding program from the Alberta Crop Industry Development Fund, Alberta Wheat Commission, Agriculture and Agri-Food Canada, Western Grains Research Foundation Endowment Fund, and Core Program check-off funds to Dr. Dean Spaner. This work was conducted in part within the project “Canadian Triticum Advancement Through Genomics” (CTAG).

I thank my family for their constant encouragement throughout this degree. Lastly, I thank my partner, Sandra Ngo, for her support and perspective as I experienced the highs and lows of being a graduate student.

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1 Literature Review

1.1 Canadian Wheat Production

The production of wheat in Canada is as early as the country itself, and as the country expanded so did the acres of wheat, along with an interest in breeding wheat cultivars adapted to the Canadian environment. During the early 1800s when the country was first settled, Manitoba was the primary "bread basket" of this young country, and Canada was eventually given the title "Granary of the British Empire" (Buller 1919 p. 34). The settlers had to discover through trial and error an appropriate seeding date that did not result in frost damage to a young seedling, and were witness to the devastating damage that was possible from locusts (Buller 1919 p. 11), and spring flooding (Buller 1919 p. 18). The settlers of the Red River Colony were not millers, and the flour was said to be "heated, sour, and altogether of so very bad quality as to be only fit to poison pigs" (Buller 1919 p. 21). This low-quality flour produced by the settlers during the 1820s prompted the Hudson's Bay Company to enter the business of flour milling and provide consultation on the adequate drying and milling of wheat. The storage of grain was also difficult to manage and a heated "… compound of wheat, smut, icicles, dried meat, mice, and mice nests" (Buller 1919 p. 23) was created on at least one occasion.

Around 1842, David Fife discovered the hard red spring cultivar 'Red Fife', which he received from a friend in Scotland. Scholars believe this cultivar to have originated from the Ukraine where it was known as 'Halychanka' (Symko 1999). The success of 'Red Fife' went beyond Canada though, as producers from the northern great plains of the United States began to sow the seed, and therefore it became the first hard spring to be adopted by North American

farmers (Olmstead and Rhode 2002). The widespread success of this cultivar was attributed to the high yields it produced, and the exceptional milling and baking quality of the flour – which ultimately provided a premium price on the British grain markets.

A new cultivar was released in 1909 with the same superior attributes as 'Red Fife', but additionally this cultivar reached maturity 3 to 4 days earlier. This new cultivar, 'Marquis', was a cross between 'Hard Red Calcutta' and 'Red Fife', and eventually replaced 90% of spring wheat within Canada, and 60 to 70% within the United States (Newman (1928) as quoted by McCallum and DePauw (2008)). There were several stem rust epidemics in the first half of the 20th century, and a lack of rust resistance from 'Marquis' resulted in the resistant cultivar, 'Thatcher', to be released in 1935. 'Thatcher' became the dominant hard red spring cultivar in western Canada well into the 1960s and was eventually succeeded by cultivars possessing superior rust resistance, but many of the pedigrees from modern registered cultivars can be traced back to 'Thatcher', and ultimately 'Red Fife'.

Today, the prairie region of Canada: Manitoba, Saskatchewan, and Alberta, produces most of the wheat in Canada, with over 90% of 2017 total wheat production (Statistics Canada 2018). Wheat is the largest crop commodity exported from Canada, with total export quantity ranked 2nd in the world, behind only the United States (Food and Agriculture Organization of the United Nations 2018). The success of wheat in Canada can be partially attributed to the diversity and breadth of cultivars that have been grown in Canada since the time of 'Red Fife', which have produced high yields for farmers, and high quality for the end users.

Remaining a world leader in wheat exports requires the purchaser (importer of wheat) to feel confident in the quality of wheat they are receiving, which Canada enforces through federally regulated standards, including wheat classes and grades (Canadian Grain Commission

2017). There are currently nine milling classes of western wheat that are used to differentiate characteristics such as red or white grain, spring or winter cultivars, and gluten strength. The nine classes are: Canada Northern Hard Red (CNHR), Canada Prairie Spring Red (CPSR), Canada Prairie Spring White (CPSW), Canada Western Amber Durum (CWAD), Canada Western Extra Strong (CWES), Canada Western Hard White Spring (CWHWS), Canada Western Red Spring (CWRS), Canada Western Red Winter (CWRW), and Canada Western Soft White Spring (CWSWS). The three largest western classes are CWRS, CWAD, and CPSR, and account for 69%, 20%, and 4% of total 2017 acreage, respectively (Canadian Grain Commission 2018).

1.2 Canada Western Red Spring Breeding Goals and Objectives

CWRS is the predominant class of wheat found in Canada due to several factors including cultivar adaptability, attractive milling characteristics, and flexibility when producing baked goods (McCallum and DePauw 2008). Over 9.6 million acres of CWRS wheat were seeded in 2017, which accounted for over 82% of common wheat (*T. aestivum)* production in the Prairie Provinces (Canadian Grain Commission 2018). CWRS has proven to be a versatile class of wheat because of the bread making characteristics, but also the ability for the flour to be blended when producing noodles, and other products such as pan bread flour, cakes, or cookies. The superior milling and bake quality of CWRS is recognized worldwide, and therefore the range of acceptable physical dough properties is kept narrow, ensuring CWRS remains a consistent class of wheat that buyers can repeatedly rely upon (Dexter et al. 2006).

The importance of satisfying the end user's demand, and maintaining high cultivar registration standards is illustrated by the 'Garnet' cultivar of the 1920s (Blanchard 1990). The

milling quality of ‘Garnet’ was inferior to the check ‘Marquis’ but this was not acknowledged during the registration process. ‘Garnet’ became a popular choice for producers because it matured ten days before ‘Marquis’, but the disdain from the international milling community grew, and eventually the cultivar was removed from the Number One class.

Host plant resistance remains an important trait that wheat breeders focus on when developing new disease resistant cultivars, and disease resistance has historically been found to be a qualitative trait (a single resistance gene, or *R* gene). Recently, the polygenic control of resistance has received much attention because of the lack of durability associated with *R* genes, and the field of genetics have proven far more complex than Flor’s original gene for gene concept (Flor 1971). Changing disease populations of leaf rust (*Puccinia triticina)* (Wang et al. 2010), and stripe rust (*Puccinia striiformis* f. sp. *tritici*) (Chen 2005) pathogens have resulted in the establishment of several virulent races, and reduced the effectiveness of leaf rust resistance genes, such as *Lr10*, *Lr13, Lr16,* and *Lr14a* (McCallum et al. 2007). Regional differences of stripe rust virulence have been reported in western Canada, suggesting subpopulations of races are present (Brar and Kutcher 2016). Disease resistance is not only a concern because of virulent races, but also the maintenance or improvement of agronomic and quality traits is difficult when breeders concentrate on genetic resistance (Knott 1993; Brevis et al. 2008; Klindworth et al. 2013). Fitness penalties due to linkage drag, and pleiotropic effects are frequent challenges for wheat breeders looking to acquire resistance (Summers and Brown 2013).

1.2.1 Baking and Milling Quality

The protein content and resulting gluten strength of CWRS produces dough strength and mixing tolerances admired by bakers, and the premium world market looks for a protein content

greater than 13.5% (Prairie Grain Development Committee 2016). Kernel hardness provides excellent water absorption of the flour and the dough strength is directly related to the gluten content, such as glutenin proteins (Rooke et al. 1999). The strength of the dough determines the end-use quality of the flour. The strongest dough will produce noodles, while the weakest produces cakes and pastries. Kernel hardness, and the milling process affect starch damage, but some degree of starch damage will provide optimal water absorption (Dexter et al. 1994a). Canada and Australia have been successful in producing high yielding cultivars supplemented by a high grain protein content (Balyan et al. 2013), and therefore command much of the world market, as other wheat growing nations are unable to produce a similar quality of wheat.

The discovery and mapping of a major protein content gene, *Gpc-6B1* (Avivi 1978; Cantrell and Joppa 1991; Olmos et al. 2003) resulted in its introduction to cultivars around the world including the Canadian CWRS cultivars 'Lillian' (DePauw et al. 2005) and 'Somerset' (Fox et al. 2006). Further improvement of grain protein content (GPC) is not a simple Mendelian solution, because of the strong influence from the environment (Fowler and De La Roche 1975; Johansson et al. 2003) and the quantitative nature of GPC (see Balyan et al. (2013) for table of quantitative trait loci (QTL) and chromosome locations).

Kernel hardness cannot be attributed to a single gene, but researchers agree that the gene, *Ha* (hard), located on the short arm of chromosome 5D enacts a major influence on hardness in wheat (Symes 1965). Additional genes *Gsp-1*, *Pina*, and *Pinb*, were reported on chromosome 5D to be tightly linked with the *Ha* locus, and responsible for grain softness (Morrison et al. 1989; Jolly et al. 1996; Sourdille et al. 1996; Turnbull et al. 2003; Wang et al. 2014). Kernel hardness does not pose a significant problem for wheat breeders because of the trait's high heritability ranging from 0.7 to 0.9 (Williams and Sobering (1984), as cited by Sourdille et al. (1996)). The

puroindoline genes, *Pina* and *Pinb*, are absent from the tetraploid species of wheat: durum, and emmer (both wild and domesticated), but the genes were reintroduced through the D genome (Gautier et al. 2000; Chantret et al. 2005). The genetic diversity in CWRS cultivars has decreased due to the demand from the end user (McCallum and DePauw 2008), reducing the opportunity for genetic recombination to improve quality traits.

1.2.2 Early Maturity

Due to the short growing season in western Canada (~ 95 to 125 days), CWRS cultivars must be early maturing in order to minimize potential frost, drought, or heat damage, and successfully set seed (King and Heide 2009). Environmental stress has an impact on grain yield and quality, and frost damaged kernels are a concern for late maturing wheat. Frost damage has been shown to reduce yields from 13% to 33% in winter wheat (Cromey et al. 1998), and reduce kernel size and test weight in durum wheat (Dexter et al. 1994b). The damage is associated with several factors that affect bakers and millers such as protein content, loaf volume, and yield of flour and semolina (Preston et al. 1991; Dexter et al. 1994b). Tolerance to freezing is acquired through cold acclimation, and enables winter wheat cultivars to survive through the winter (Li et al. 2018), but in spring wheat, an appropriate seeding data is the primary method to avoid frost damage. In the greenhouse, neonicotinoid seed treatment was reported to positively affect freezing tolerance in spring wheat seedlings (Larsen and Falk 2013), providing evidence of further management options available to producers. The authors also identified variation in the regrowth of cultivars, suggesting a genetic influence in the freezing tolerance of spring wheat cultivars.

The environmental adaptability of wheat is controlled by three gene groups (Snape et al. 2001): vernalization (*Vrn)*, photoperiod (*Ppd)* and earliness *per se* (*Eps*) genes, and this complex genetic network receives signals from the environment such as temperature and light for the downregulation and upregulation of these genes (Distelfeld et al. 2009). These genes have additive effects on days to flowering and early maturity (Iqbal et al. 2007a), and the mechanisms of flowering time in wheat remain an active research area (as reviewed by Kamran et al. (2014a)).

The *Vrn* group of genes is responsible for winter or spring wheat phenotype (Yan et al. 2004; Santra et al. 2009; Chen and Dubcovsky 2012). Spring wheat cultivars will possess a dominant allele at one or more loci, but winter wheat must possess the recessive allele, *vrn*, at all loci (Iqbal et al. 2007b). Epistasis between the dominant *Vrn* alleles will result in different combinations of alleles producing genotypes with different phenotypes. The *Vrn-A1, Vrn-B1*, and *Vrn-D5* combination, and *Vrn-A1*, *Vrn-B1* combination produce the earliest maturing genotypes (Iqbal et al. 2007b), and the *Vrn-A1a* and *Vrn-B1* alleles are the most common and significant genotypes in CWRS (Kamran et al. 2013a). The inclusion of a *vrn* allele in the combination has shown to not have a negative effect on maturity (Kamran et al. 2014b).

Early maturing cultivars must begin flowering regardless of day length, and the mutation of photoperiod sensitivity genes has allowed wheat to adapt to environments with short day lengths (Shaw et al. 2012), and *Ppd* genes have been shown to influence the heading (ear emergence) of wheat (Scarth and Law 1983). Three photoperiod insensitive alleles exist at the *Photoperiod-1* (*Ppd-1*) loci: *Ppd-A1a*, *Ppd-B1a*, and *Ppd-D1a*. The *Ppd-D1a* allele has been proposed to provide the earliest flowering time, but 78% of elite CIMMYT durum germplasm were reported to carry the *Ppd-A1a* allele (Bentley et al. 2011). The regulation of photoperiod

insensitivity genes has an effect on the expression of other genes involved in the photoperiod pathway, which suggests flowering time may be further explored beyond *Ppd* genes.

Earliness *per se* genes influence flowering time via initiation of floral organs (Worland 1996), and are responsible for the general rate of development (Chen et al. 2015) without receiving signals from the environment (Kamran et al. 2013b). The *Eps* group of genes also interact with *Vrn* and *Ppd* genes, and affect time to heading, with GxE interactions also present (Bullrich et al. 2002). Earliness *per se* genes have received less attention due to the quantitative nature, the overshadowing effects from *Vrn* and *Ppd* alleles, and temperature interactions (Kato and Wada 1999; Appendino and Slafer 2003).

1.2.3 Disease and Insect Resistance

All three rust diseases are polycyclic fungi with sexual and asexual reproduction and possess the ability to develop into a destructive epidemic. The asexual urediniospores are the cause of the distinct visual infection on the leaf tissue, and are the primary source of infection carried by wind currents (Roelfs et al. 1992). Leaf rust of wheat, caused by *Puccinia triticina*, is a prevalent and annual problem across the Canadian prairies with yield reductions from 1 to 20% (McCallum et al. 2007). Annual field surveys of leaf rust have been conducted in Canada since 1931 (Wang et al. 2010), with 310 unique phenotypes collected throughout Canada in 2009 (McCallum et al. 2013).

Stem rust of wheat, caused by *Puccinia graminis* f. sp. *tritici*, resulted in several epidemics during the early 1900s (Craigie (1945), as cited by McCallum et al. (2016)). Control of stem rust began in 1954 with the release of 'Selkirk', containing resistance genes *Sr2*, *Sr6*, *Sr7b*, *Sr9d*, *Sr17*, and *Sr23* (Martens and Dyck 1989; Kolmer et al. 1991). Stem rust of wheat has

not been a concern for Canadian producers since the 1950s (McCallum et al. 2007), and the population of stem rust in Canada remains very small, with 97.3% of isolates belonging to the QFCSC race when surveyed in 2007/08 (Fetch et al. 2015). Maintaining stem rust resistance remains a priority of the wheat industry due to the historic devastation caused by stem rusts in North America, and the transportation of rust spores from the south, via the *Puccinia* pathway.

Stripe (yellow) rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici*, has been gaining virulence and prevalence since 2000 (Chen et al. 2010), and the current genetic resistance against stripe rust may not be enough to prevent significant damage in the future (McCallum et al. 2007). The current resistance genes were reported to successfully provide sufficient yield protection, when low to moderate disease pressure caused significant yield loss in susceptible cultivars (Xi et al. 2015). Rust spores have been reported to survive on winter wheat over the winter months if snow cover is adequate (Kumar et al. 2013), when historically the only source of rust spores were from Mexico and the southern wheat belt of the United States. Thirty-three races were isolated in western Canada, with at least three sub-populations detected due to inoculum sources from differing regions of the United States (Brar and Kutcher 2016). The asexual reproduction, and clonal population structure of stripe rust in Alberta (Holtz et al. 2014) suggests control can be attained with adequate yellow rust (*Yr)* gene stacking.

Common bunt of wheat, caused by *Tilletia tritici* and *Tilletia laevis,* are soil borne fungi that overwinter as teliospores within the soil, or on the seed coat. Sufficient control of the disease has been obtained by use of fungal seed treatments, which began with the use of hexachlorobenzene in the 1950s (Hoffmann 1971). Several genetic sources of resistance exist for common bunt control (He and Hughes 2003), but most winter wheat varieties remain susceptible (Gaudet et al. 2013).

Loose smut of wheat, caused by *Ustilago tritici,* is a fungus that infects during anthesis, but grain quality is not reduced. The fungus is a "Priority 2" disease, and therefore sufficient resistance is not required for cultivar registration, but control in Canada has been successfully maintained through seed treatment, clean source seed, and genetic resistance (Bailey et al. 2003). Further enhancement of genetic resistance is desirable due to environmental, health and cost benefits (Menzies et al. 2009).

Leaf spot of wheat has also been classified as a "Priority 2" disease and may be caused by several pathogens. The three most important in Canada include: Tan spot of wheat, caused by *Pyrenophora tritici-repentis*, the Septoria leaf blotch complex which includes *Mycosphaerella graminicola*, and Spot blotch caused by *Cochliobolus sativus* (Fernandez et al. 2014). Crop management such as rotations involving non-cereal crops have reduced the severity of leaf spot (Fernandez et al. 2016), but similar to loose smut, further genetic resistance will provide additional management options for producers.

Fusarium head blight (FHB), caused by *Fusarium* species including *F. graminearum*, are fungi that overwinter in crop residue until conditions allow sexual and asexual spores to infect the spike between anthesis and the soft dough stage (Sutton 1982). Successful infection of FHB requires a humid environment for spore production, and susceptible cultivars are prone to yield loss and mycotoxin concentrations resulting in economic loss to the producer (Munger et al. 2014). No single management strategy has proven to effectively control FHB (Jeannie Gilbert and Haber 2013), including tillage (Miller et al. 1998). The prevalence of FHB continues to shift across western Canada, and was first reported in Alberta in 2001 (Alberta Agriculture and Forestry 2018b), having previously been characterized as ‘‘infrequent" (Turkington et al. 2011).

Orange wheat blossom midge, *Sitodiplosis mosellana*, has developed into an important pest in western Canadian spring wheat cultivars (Lamb et al. 1999). The insect survives in the soil as a cocoon and emerges to pupate and lay eggs in wheat spikes once they reach the adult stage. The hatched larvae are responsible for yield and quality reduction (Kassa et al. 2016a). One resistance gene, *Sm1*, has been discovered and mapped in spring wheat on the short arm of 2B (McKenzie et al. 2002; Thomas et al. 2005), and is currently available as a "varietal blend" only in several commercial cultivars. A single major R gene such as *Sm1* enacts a strong selection pressure on wheat midge to adapt and overcome their susceptibility, and therefore by seeding a blend of 90% midge resistant and 10% midge susceptible, this creates a refuge system that reduces the selection pressure on the wheat midge. The inclusion of *Sm1* has resulted in larger yields than susceptible cultivars, but resistant wheat still resulted in market downgrading (Smith et al. 2014).

Cereal leaf beetle, *Oulema melanopus*, is the most recent pest in western Canada. The insect originated from Europe and was first observed within Alberta in 2005 (Alberta Agriculture and Forestry 2014). It has since expanded to all three Prairie Provinces (Dosdall et al. 2011). Currently, no Canadian cultivars provide resistance against the beetle, but natural biological control from the parasitoid wasp *Tetrastichus julis* has provided adequate control (Evans et al. 2006). Previous research provided by Gallun et al. (1966) suggests leaf pubescence has an important role in resistance against the cereal leaf beetle. The pest is currently in the "early establishment phase", providing researchers opportunities to develop appropriate management strategies (Kher et al. 2016).

1.3 Molecular Markers

Molecular marker technologies have proven to be a vital resource for modern wheat breeders by assisting with the deciphering of the wheat genome, increasing the speed and efficiency of screening potential cultivars, and providing selection on traits that are difficult to select by phenotype (Randhawa et al. 2013). These markers are an important tool for genetic studies and the development of linkage maps capable of showing the position of genes of interest on a particular chromosome. Botstein et al. (1980) originally used Restriction Fragment Length Polymorphisms (RFLP) markers for a mapping study within the human genome, but the markers were expensive to implement, and laborious to manage (Mammadov et al. 2012). The published method of Polymerase Chain Reaction (PCR) in the 1980s (Mullis and Faloona 1987) allowed researchers to begin working with molecular markers based on PCR technology, and RFLP markers were soon supplemented by Simple Sequence Repeats (SSR) (Litt and Luty 1989; Weber and May 1989), Randomly Amplified Polymorphic DNA (RAPD) (Williams et al. 1990), Amplified Fragment Length Polymorphisms (AFLP) (Vos et al. 1995), Single Nucleotide Polymorphisms (SNP) (Chee et al. 1996), and Diversity Array Technology (DArT™) markers (Jaccoud et al. 2001). As PCR protocols became well known, SSRs (microsatellites) replaced RFLPs and became the marker of choice for genetic mapping, because of the speed of processing afforded by PCR followed by acrylamide gel electrophoresis, and the high allelic variation that occurs within a microsatellite locus (Vignal et al. 2002). Over the past several years, SNPs have become widely adopted because of their high frequency within the genome, and the availability of high throughput detection technologies (Mammadov et al. 2012).

Several important factors that will influence the choice of marker include: the level of polymorphism, dominant versus co-dominant inheritance, the financial costs of the marker

system, and the size of the population (Miah et al. 2013). Wheat breeders are faced with additional concerns due to the ploidy level of the genome, which has resulted in lower resolution maps produced by some markers (Akbari et al. 2006), and also duplication within the genome may result in the discovery of poor genetic markers due to paralogous loci (Mammadov et al. 2012; Hayward et al. 2015).

1.4 Linkage-Based Quantitative Trait Loci

Quantitative trait loci (QTL) are regions of a chromosome known to impact the phenotypic variation of complex traits, and the mapping of these chromosomal regions with genetic markers will improve breeding programs utilizing Marker Assisted Selection (MAS) (Singh et al. 2014). The first study contributing to the mapping of QTL can be attributed to Sax (1923), who concluded that the size of a bean was controlled by multiple "factors" (genes) that may have an additive effect on the trait. He further postulated that the genes located on different chromosomes might not provide an equal effect. Thoday (1961) provided the next significant publication on quantitative traits, and outlined a method for locating quantitative genes on the chromosome via markers. Lander and Botstein (1989) published a seminal paper that brought together the first molecular markers (RFLP), and logarithm of odds (LOD) to explain the phenotypic variation in QTL, and their location on a chromosome; known as interval mapping. The reporting of significant QTL by an LOD score has become the standard, and an LOD score of 3.0 is proportional to a genome-wide false positive rate of 0.05 (Lander and Kruglyak 1995). Haley and Knott (1992) introduced a marker regression-based method due to the computational burden (at the time) associated with maximum likelihood estimation. This newfound methodology to quantify the variation amongst QTL prompted Churchill and Doerge (1994) to

establish an empirical threshold for QTL significance using a permutation test. Composite interval mapping was introduced by Jiang and Zeng (1995) to account for correlation of traits by inclusion of background markers (covariates), and remove the "noise" of these markers. This method increased the statistical power of QTL mapping and increased the precision of estimation. Multiple interval mapping was proposed to model epistasis, and multiple QTL simultaneously (Kao et al. 1999), whereas interval and composite methods only map a single QTL.

The methodologies of QTL mapping are numerous and well-reviewed in the literature (Broman 2001; Knapp 2001; Semagn et al. 2006; Semagn et al. 2010), and consist of three main steps: 1) Analysis of the phenotype data gathered in the field, 2) Creation of a linkage map using the marker genotype data, and 3) Combination of the trait and linkage data to estimate QTL location and effect. Each step has several different methods available, and the researcher will need to choose an appropriate course of action.

Mixed models have become the predominant method to estimate phenotypes across different environments, and replicates. Restricted Estimate Maximum Likelihood (REML) and Best Linear Unbiased Prediction (BLUP) (as reviewed by (Thompson 2008)) are the prevalent estimation approaches used by plant and animal breeders, respectively. Mixed models allow for partitioning of variance components and the prediction of breeding values through estimates of additive genetic and phenotypic variances (Kennedy 1981). The modelling of unbalanced data, and limitations due to inference space are also addressed by mixed models (McLean et al. 1991; Yang 2010). There are numerous ways to analyze field trials using mixed models (as reviewed by Smith et al. (2005)), and the method of choice is at the discretion of the researcher.

Marker linkage maps are constructed by genetic map functions that estimate the distance between markers by the recombination frequency in offspring. Several map functions exist, and their differences can be attributed to how each function accounts for crossover interference (Zhao and Speed 1996). The Haldane (1919) and Kosambi (1944) mapping functions have been the most widely adopted, as the former assumes no crossover interference, and the later allows for crossover interference. The optimized marker order is calculated using a heuristic algorithm, as the true marker order (*n!/2* for *n* loci) remains unfeasible to calculate (Mester et al. 2003). The algorithm used, and the criteria for optimal order is dependent on the software used to construct the linkage map (as reviewed by Tan and Fu (2006)).

The final step of QTL mapping involves the combination of data (phenotype, genotype, and genetic/physical map), and the choice of an appropriate QTL mapping method. This is accomplished by mapping software, and several packages are available (Manly and Olson 1999). Each QTL mapping method has limitations that must be considered by the researcher (Semagn et al. 2010), and estimations of QTL effect and position may be compromised, as Mayer (2005) reported in a regression versus multiple interval mapping comparison. Increased marker density due to the adoption of SNP markers has increased the precision of QTL mapping, but the overestimation of QTL effects due to a reduced mapping population, termed the "Beavis Effect" (Beavis 1994; Xu 2003), remains an un-addressed issue. The publication of a QTL mapping study is the end point for much research (Mammadov et al. 2012), but marker validation must also be conducted to assess the value of proposed markers (Sharp et al. 2001).

The methodologies associated with QTL mapping and marker selection have provided the foundation for emerging technologies such as genomic selection (introduced by Meuwissen et al. (2001) and reviewed by Heffner et al. (2009)), and the potential for characterizing the

variation of gene expression, known as Expression Quantitative Trait Locus (eQTL), first proposed by Jansen and Nap (2001). The current expenses associated with transcript profiling has limited the number of studies undertaken, and completed studies have small sample sizes and reduced statistical power (Mackay et al. 2009). Intuitively, however, this is a promising area for breeders as the delicacies of gene expression are just as revealing as confirming the physical presence of a gene.

1.5 Quantitative Trait Loci in Wheat

Plant and animal breeders focus on the improvement of complex traits including grain and milk yield, dough and carcass quality, and disease resistance, due to the associated economic incentives. These traits may be quantitatively inherited and variation is expressed via numerous QTL with minor effects (Mackay 2001). Non-genetic factors (such as the environment) may also be important (Hill 2010), which compounds the complexity facing breeders. Major variation due to a single locus is a rare occurrence, because many of these mutations have negative effects on the individual and natural selection would remove them from the population (Tanksley 1993).

QTL mapping requires the collection of phenotypic data on the traits of interest, and the genotyping of a population with markers chosen by the researcher (Semagn et al. 2010). The first consensus map of wheat was created in 2004 from four independent studies, and once aligned, the map consisted of 1,235 SSR loci (Somers et al. 2004). By 2008, over 2,500 SSRs were mapped in wheat (Gupta et al. 2008), showcasing the widespread adoption of the technology by breeding groups over this short period of time. The most recent consensus map of *T. aestivum* reported the mapping of over 46,000 markers based on the combination of eight reference populations (Wang et al. 2014). Amongst the major cereals, wheat has been reported to have the

highest SNP frequency (Barker and Edwards 2009), but the polyploid wheat genome presents difficulties with SNP markers, as SNP technologies that do not require a PCR step, such as chip-based systems, are problematic with the hexaploid genome due to primer binding to more than one sub-genome (Ganal and Röder 2007). The discovery of sufficient polymorphic SNPs across different breeding populations is also difficult, and developing chip assays that are population-specific is not practical (Semagn et al. 2014). PCR-based SNP genotyping such as KASP (Kompetitive Allele Specific PCR) also have inherent difficulties, as they require pure DNA samples, due to contaminants (including polyphenols and polysaccharides) which may interfere with PCR binding. High-density SNP arrays of various size have been developed, including 9K (Cavanagh et al. 2013), 35K (Allen et al. 2017), 90K (Wang et al. 2014), and 820K (Winfield et al. 2016).

Genotype-by-environment interactions require additional diligence from researchers to ensure identified QTL are reliable amongst different environments, but the interactions have also suggested that a larger number of environments will produce a greater diversity of QTL associations, and a lack of environments may underestimate the number of QTL (Young 1996). The effect of a QTL may vary in different environments due to QTL-by-environment (QxE) interactions (El-Soda et al. 2014), and requires multi-location trials to confirm the reliability of a QTL as a molecular marker. The identification of inconsistent QTL for quantitative traits remains a significant issue when developing selection markers in plant breeding (Bernardo 2008).

1.6 Quantitative Trait Loci Mapping of Canada Western Red Spring

QTL discovery in wheat can be directed towards essentially any trait with continuous variation that a researcher can collect phenotypic data on, including: grain yield (Quarrie et al. 2005; Bennett et al. 2012a), morphological traits such as flag leaf glaucousness (Bennett et al. 2012b), length of coleoptiles (Spielmeyer et al. 2007), flag leaf senescence (Verma et al. 2004), and physiological traits such as delayed senescence, known as the 'stay green' trait (Kumar et al. 2010), and low temperature tolerance (Fowler et al. 2016). Researchers in western Canada have focused on traits that are important for increasing wheat production across the Prairie Provinces, and the following traits are valuable in CWRS cultivars due to their relationship with the producer and end-user.

Pre-harvest sprouting (PHS) has a negative effect on the grain yield, and the end-use quality, which results in a significant economic loss to the producer (DePauw et al. 2012). 'RL4137' was trialed in the 1960s, and CWRS breeding has included 'RL4137' derived parents for population development since the 1990s (DePauw et al. 2012). Rasul et al. (2009) used 356 microsatellite markers to map the PHS resistance QTL using a doubled haploid mapping population derived from the 'RL4452' × 'AC Domain' cross; this resulted in the discovery of six major QTL. They were mapped to chromosomes 3A, 3B, 4A, 4B, and 7A, and furthermore the QTL on 4B provided the most significant phenotypic variance - this region explained 22% of the total phenotypic variance of falling number, 26% of the variance of sprouting index, and 67% of the variance of germination index. PHS resistance within the 'RL4452' × 'AC Domain' population was revisited by Cabral et al. (2014), who genotyped the population with over 12,200 markers, with 11,200 being SNPs. Interestingly, this study only revealed four significant QTL located on chromosomes 3B, 4A, 7B, and 7D. The largest QTL identified in each study also

differed, as the QTL on 4A provided the greatest amount of phenotypic variance in this study - 26% for the falling number, 32% for the sprouting index, and 58% for the germination index. The sizes of the phenotypic variances are similar between both studies, but the chromosome responsible for the variance differed between studies. The unique results between studies may have been attributable to marker choice, or the statistical analysis used, as both studies used the same data collected from 2003 to 2005, but the model created by Rasul et al. (2009) consisted of four environments, and Cabral et al. (2014) used six environments. The contrasts between these two QTL mapping studies on the same population provide an excellent example of the need to discover a stable QTL in order to prove valuable in MAS. Pre-harvest sprouting resistance remains difficult to quantify genetically due to different QTL associated with different measurement types and the duration of data collection, as reported in a durum RIL population (Knox et al. 2012). Kumar et al. (2015) reported twenty-six QTL associated with pre-harvest sprouting across all twenty-one chromosomes, but 2B, 4A, 5D and 6D had QTL associated with all measurement traits (germination percentage at different germination durations, germination resistance, and germination index).

A review of the literature suggests QTL mapping studies for quality traits is currently not a large area of study when compared to agronomic traits. Huang et al. (2006) reported a grain protein content QTL, *QGpc.crc-4D,* and a flour protein content (FPC) QTL, *QFpc.crc-4D,* which explained a large percentage of phenotypic variance (32.7% and 28.6% respectively), and shared the same closest marker, *Xwmc52*. The most significant phenotypic variance regarding traits involved with bread quality was associated with the *Glu-1* loci – which are responsible for the encoding of high molecular weight glutenin subunits (Anjum et al. 2007). McCartney et al. (2006) reported the mapping of fourteen quality QTL near the *Glu-B1* locus on chromosome 1B,

and the cluster of QTL had a range of 50 cM. Another cluster of ten quality QTL were mapped to chromosome 7D, and this cluster was much closer together with a range of only 14 cM. Similar to Huang et al. (2006), the McCartney et al. (2006) study found *QGpc.crc-4D* and *QFpc.crc-4D* were the most significant GPC and FPC QTL and accounted for 30% and 29% of the phenotypic variance, respectively. QTL associated with GPC and FPC were also reported on chromosome 2B, with *QGpc.crc-2B* explaining 10% of the phenotypic variance, and *QFpc.crc-2B* explaining 17% (McCartney et al. 2006). Both studies reported the plant height QTL, *QHt.crc-4D,* as the most significant plant height QTL, which is presumably the dwarfing gene *Rht-D1b*, although only McCartney et al. (2006) reported a negative association of this height QTL with various end-use traits including grain and protein flour content, and reduced dough and baking quality. A negative association with *Rht-1* and grain protein content was also reported by Chen et al. (2016). Asif et al. (2015) reported a major QTL on chromosome 1B associated with grain test weight across both organic and conventional environments. The sodium dodecyl sulfate (SDS) sedimentation test provides a reliable indication of wheat gluten strength, and a QTL located on chromosome 1A was reported to explain 24% of the phenotypic variance (Chen et al. 2015). A study conducted on seventy-eight Canada western red spring cultivars released from 1845 to 2004 reported that only four loci were significant for the improvement of quality related traits, while the average across traits was thirty-five, with yield exhibiting the highest number at sixty-eight significant loci (Fu and Somers 2011).

The “Green Revolution” successfully introduced Mendelian height reducing genes, but many agronomic traits of interest are quantitatively inherited and require numerous mapping studies to ensure stability in light of epistatic relationships and GxE interactions. Significant plant height QTL will generally map to existing locations of *Rht* genes as reported by McCartney

et al. (2005), and Cabral et al. (2018) identified *Rht-D1* in the 'RL4452' × 'AC Domain' DH population, to be associated with a reduction in plant height, test weight and grain weight. Height reducing genes are not always to source of significant QTL though, and two QTL associated with height were reported on chromosomes 6A and 7A, with no parent possessing semi-dwarf height genes (Fowler et al. 2016). Singh et al. (2016) reported two plant height QTL, one on chromosome 4B and another on 6D, that co-located with common bunt resistance QTL. Further fine mapping of these regions will provide insight into whether these may be individual genes that are linked, or genes with pleiotropic effects. The scenario of an agronomic trait mapping to the same region as a disease resistance QTL was also reported in a study conducted by McCartney et al. (2005), except this involved a maturity QTL on chromosome 3B and FHB resistance.

Chen et al. (2015) reported two earliness *per se* QTL, located on chromosomes 1A and 4A, provided an additive effect with *Vrn-B1*, one of the dominant vernalization genes differentiating spring versus winter wheat. This interaction resulted in a fitness cost in the form of a yield reduction with maturity four days earlier. Similarly, Kamran et al. (2013b) also reported an additive effect involving earliness *per se* QTL and *Ppd-D1a*, a photoperiod insensitive gene. This additive interaction resulted in earlier flowering and maturity, with no fitness penalty reported. A QTL located on chromosome 5A was reported to explain 17% of the phenotypic variance for both days to flowering and maturity in organic environments, resulting in a two to three day reduction (Zou et al. 2017a). The QTL was also reported in conventional environments, and explained 17% and 14% of the phenotypic variance associated with flowering and maturity, respectively (Zou et al. 2017b).

Cuthbert et al. (2008) identified fifty-three QTL associated with yield and yield components, with five major QTL on four chromosomes (1A, 2D, 3B, and 5A) that each explained between 4% and 20% of the phenotypic variance and the consistency of all five QTL across environment suggests they may be potential sources of stable markers. The DH population used for this study also segregated for the *B1* gene, which provides the awned / awnless phenotype, and the authors concluded the awned lines produced a 6% yield increase across all environments. Perez-Lara et al. (2016) reported a major QTL on chromosome 2D to have a major effect on grain yield, maturity, and flowering, but marker density was not sufficient enough to conclude if more than one gene was involved. In organic environments, a major grain yield QTL was reported on chromosome 6A to explain 18% of the phenotypic variance, and 22% of the variance in conventional environments (Asif et al. 2015).

Common bunt, caused by *Tilletia tritici* and *T. laevis*, are fungi of concern for both western and eastern Canada, because of the yield loss, quality downgrading, and potential delivery refusal (Bailey et al. 2003). Because the fungus is soil-borne, control strategies using cultural practices require longer crop rotations, and sanitizing machinery that may carry soil to un-colonized fields. Chemical control in the form of fungicidal seed treatments has been the primary method of minimizing infection, but the financial and environmental costs suggest obtaining control through genetic resistance may be the best solution. Genetic resistance to common bunt may be race specific, or non-specific, and several bunt resistance genes (*Bt)* have been surveyed for resistance (Gaudet and Puchalski 1989). Breeders prefer to introduce non-specific resistance, because of the selection pressures that a particular pathogen race will put on a resistant plant conferring race specific resistance. The *Bt10* gene, located on the short arm of chromosome 6D (Menzies et al. 2006), is one such example of non-specific resistance, and has

become a significant source of resistance within cultivars of western Canada. PCR markers have been developed for the screening of genotypes that confer resistance (Laroche et al. 2000), but the literature suggests that QTL mapping studies of common bunt resistance remain uncommon, presumably because of the numerous resistance sources that are currently available in the form of race specific, and non-specific genes. A study conducted by Singh et al. (2016) used the 'Carberry' × 'AC Cadillac' mapping population to conclude that 'Carberry' contributed five QTL associated with common bunt resistance, while 'AC Cadillac' only one, corresponding to the *Bt10* gene. The cumulative effect of the five QTL from 'Carberry' was unable to confer the same successful resistance level as the single *Bt10* gene. Fofana et al. (2008) used the 'RL4452' × 'AC Domain' DH mapping population discussed earlier to discover three QTL associated with common bunt resistance. One of the QTL had a minor effect that only accounted for 3% of the phenotypic variance, while the other two explained 29% when combined. Wang et al. (2009) located a QTL on chromosome 1BS that derived from the winter wheat cultivar 'Blizzard', and Knox et al. (2013) reported a QTL on chromosome 7B, designated as *QCbt.spa-7B.1,* from the hard red spring cultivar 'McKenzie'.

Loose smut (*Ustilago tritici*) is a yield reducing fungus, which infects the head of wheat, and replaces the kernels with smut spores. Loose smut resistance genes are race specific and therefore resistance genes need to be stacked to confer broad resistance against all races prevalent within Canada. The Canada Western Extra Strong cultivar 'Glenlea' currently possesses the most superior loose smut resistance of any Canadian wheat cultivar, and has been suggested to carry at minimum three major genes, and two minor genes (Knox et al. 2008). 'Glenlea' was reported to have three QTL (on chromosome 3A, 5B, and 7B) with race specific resistance, which provided complete resistance against all races known in Canada (Knox et al.

2014). These three QTL corresponded with existing known resistance genes *Ut8*, *Ut6*, and *Ut7*. The Knox et al. (2014) study crossed eight unique lines (including 'Glenlea') to create four mapping populations, and although some of the lines also carried the same QTL as 'Glenlea', they did not have the same level of resistance. For example, 'Glenlea' conferred resistance against five races of loose smut with the *Ut6* gene, but 'AC Karma' was only resistant against one race (T39), even though it also has *Ut6*. These results suggest that 'Glenlea' carries additional QTL that are still undiscovered, or potential gene interactions (epistasis) may exist. Procunier et al. (1997) previously discovered RAPD and RFLP markers that flanked a gene associated with race T10 resistance, and provided an error rate of only 4%. The resistance gene *Ut6*, located on the long arm of chromosome 5B (Kassa et al. 2014), has only recently been mapped and provides breeders with opportunities for the widespread introgression of this gene into future cultivars. Thirty-eight races of loose smut have been identified within Canada and race T10 is currently the most prevalent in hexaploid wheat (Menzies et al. 2003).

The damage inflicted by rust pathogens continues to remain a burden on Canadian wheat, but the discovery of the virulent Ug99 race in Africa (race TTKSK), has sparked a worldwide interest in developing greater rust resistance, because it developed virulence against *Sr31,* which provided worldwide and durable resistance for over 30 years (Wanyera et al. 2006). The pathogen has currently spread to thirteen countries, with several variants of the original race now present (CIMMYT 2016). Across Canada, it has been suggested that almost 80% of spring wheat cultivars are susceptible to the Ug99 race (Fetch et al. (2012), as cited by Kassa et al. (2016b)). One particular gene, *SrCad*, has proven to provide resistance against Ug99 stem rust (Hiebert et al. 2011), and stable SNP markers have been developed for the accurate detection of genotypes with the resistance gene (Kassa et al. 2016b). These markers are reliable for marker-assisted

selection because of the proven testing amongst different wheat cultivars, but additionally they also provide precision towards *SrCad* only, as *SrTmp* and *Sr42* are also within the same chromosome region.

Gene stacking, or the inclusion of several resistance genes into a single cultivar, has become the primary method of creating CWRS cultivars with long lasting, and durable resistance against the rust pathogens (Singh et al. 2014). Durability of resistance is also obtained by combining three to five adult plant resistance (APR) genes in a single cultivar, as APR genes have an additive effect and are generally non-race specific (Singh et al. 2000). On the other hand, seedling resistance genes have generally been viewed as race specific and non-durable (Singh et al. 2013), as the selection pressure against susceptible races will inevitably result in a virulent new race.

Canadian breeders have been developing rust resistant cultivars since the first stem rust resistant cultivar 'Thatcher' was released in 1935, and 'Renown' was released in 1937 with leaf rust resistance (McCallum et al. 2016). Over two hundred rust resistance genes and alleles have been characterized throughout the world (Park 2015), with at least sixty-nine conveying leaf rust resistance (McCallum et al. 2012), sixty-five numerically designated resistance genes or alleles against stem rust (Yu et al. 2014), and seventy stripe rust resistance genes (Xi et al. 2015). These resistance genes have also been known to confer resistance against more than one type of rust, or even other diseases – for example, *Lr34/Yr18/Pm38,* is the same gene that confers resistance against leaf rust, stripe (yellow) rust, and powdery mildew (Lagudah et al. 2009); also known as *Sr57* for the resistance conferred against stem rust (Kerber and Aung 1999). Several QTL with broad rust resistance across different countries have been reported by Singh et al. (2014), suggesting genes similar to *Lr34*'s versatility. *Lr34* has been deployed in many modern cultivars

including ‘CDC Imagine’, ‘CDC Stanley’, ‘CDC Teal’, ‘Carberry’, ‘Lillian’, and ‘Peace’ (Randhawa et al. 2013), but continues to provide strong resistance since introduction in the 1970s (McCallum et al. 2016). Resistance conferred by *Lr34* is enhanced due to synergistic effects when stacked with additional rust resistance genes (German and Kolmer 1992; Hiebert et al. 2016). Stripe rust resistance genes *Yr24, Yr32*, and *YrSP* have shown to provide resistance across several countries, but *Yr5* and *Yr15* currently provide resistance against all races that were collected in thirteen countries (Sharma-Poudyal et al. 2013). QTL associated with stripe rust were identified in five mapping populations derived from registered Canadian cultivars, and several parents contributed QTL located on the same chromosomes (Bokore et al. 2017). Results such as these are important contributions to the literature, as they provide assurance to the stability and consistency of identified QTL.

1.7 Conclusion

Modern wheat breeders use molecular techniques and technologies supported by phenotype selection in order to develop *T. aestivum* cultivars which benefits producers and end users, including consumers and bakers. The mapping of QTL associated with CWRS characteristics of importance has provided breeders with the chromosomal region of many loci that could potentially become diagnostic markers for MAS if stability is ensured. Ultimately, the current lack of stability suggests that wheat breeders have much more to learn about the wheat genome, and for quantitative traits, QTL mapping results are largely statistical, and not biological (Bernardo 2016). As these technologies involving gene expression and transcriptome analysis become more widespread and the associated costs decrease, this area will provide a valuable next step in understanding the biological basis of quantitative traits (Mackay et al.

2009). The discovery of a sequence of nucleotides does not give the necessary details regarding the intimacies of gene regulation including expression and gain or loss of function, and therefore the ability to locate a locus of interest is only the beginning of molecular wheat breeding, and the pursuit of CWRS cultivars with superior disease resistance, yield, and end-use quality.

1.8 Thesis Objectives

Due to the segregating traits of the ‘Peace’ × ‘Carberry’ population, this thesis is based on the desire to discover further knowledge related to disease resistance, and agronomic traits in different environments.

I propose the following thesis objectives:

1) Determine if there are minor disease resistance QTL associated with stripe rust, leaf rust, and leaf spotting, and estimate their location and effect.
2) Determine if there are QTL associated with agronomic and quality related traits in organic and conventional environments and estimate their location and effect.

These objectives will be tested with the following hypotheses:

1) Null: Minor disease resistance QTL do not exist.
2) Null: No QTL are associated with agronomic and quality related traits in organic and conventional environments.

2 Mapping QTL associated with stripe rust, leaf rust, and leaf spotting in a Canadian spring wheat population[1]

2.1 Introduction

Western Canada is a significant contributor to the worldwide supply of wheat, ranking 5th in the world for total tonnage produced (Food and Agriculture Organization of the United Nations 2018), and the prairie provinces, comprising of Manitoba, Saskatchewan and Alberta, were responsible for over 97% of total spring wheat production across Canada in 2017 (Statistics Canada 2018). The management and control of diseases that affect wheat yield and quality will help ensure Canadian producers remain global leaders in wheat production, and genetic host resistance remains the most efficient and successful method to provide protection against diseases, due to the input costs associated with fungicide applications, and the environmental concerns (McCallum et al. 2007; Bokore et al. 2017). The management of leaf diseases through fungicide control is further complicated due to the relationship between disease development and climatic conditions, and the success of many fungicides being dependent on an application in the early stages of disease development (Kutcher et al. 2011).

Canadian wheat breeders have been concerned with obtaining disease resistance since the early 1900s, as stem rust epidemics repeatedly swept across the prairies and destroyed the crop of early settlers (Buller 1919; McCallum et al. 2007). Today, leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*), along with the leaf spot complex consisting of tan

[1] A version of this chapter with the same title has been submitted to *Crop Science* in May 2018; authored as Bemister et al.

spot (*Pyrenophora tritici-repentis*), septoria leaf blotch (*Phaeosphaeria nodorum*, *Mycosphaerella graminicola*, and *Phaesosphaeria avenaria)*, and spot blotch (*Cochliobolus sativus)* are the prominent leaf diseases in western Canada, and an active area of research for genetic resistance. Leaf and stripe rust are "Priority 1" diseases (along with stem rust, common bunt, and Fusarium head blight) and require at least an Intermediate level of resistance in order to be registered under bread wheat classes, including the Canada Western Red Spring (CWRS) class, and the leaf spot complex is "Priority 2", meaning resistance is desirable (Prairie Recommending Committee for Wheat Rye and Triticale 2015). Over 86% of the spring wheat acres were seeded to CWRS in 2017 (Canadian Grain Commission 2018), and there is a constant need to search for novel resistance genes, because of the continually evolving disease populations (Singh et al. 2014), and reduced genetic diversity in registered cultivars (Zwart et al. 2010).

Stripe rust of wheat (yellow rust) caused by *Puccinia striiformis* f. sp. *tritici* has become an increasing concern across the western provinces, and these epidemics result in a reduction of yield and grain quality, as the pathogen reduces the green leaf tissue and photosynthetic ability of the plant (Wellings 2011; Singh et al. 2014). Due to changing weather patterns, and an increased number of virulent races, the risk posed by stripe rust has elevated since the year 2000 (Lyon and Broders 2017; Liu et al. 2017). These new races produced a greater number of urediniospores and a shorter latency period in warmer temperatures, with an average sporulation being two days early, and twice the germination percentage (Milus et al. 2006). Traditionally, rust spores have been carried through the wind from the Pacific Northwest, or along the *Puccinia* Pathway, but given the appropriate conditions, stripe rust has the ability to overwinter with winter wheat, and use a "green bridge" to inoculate spring wheat the following season (Xi et al. 2015). The ability

of stripe rust to overwinter adds complexity to the successful management of the disease, which already requires an understanding of host resistance, inoculum source, distribution, frequency, and race structure (Xi et al. 2015). Further improvements in genetic host resistance will provide the most reliable method to manage stripe rust. Randhawa et al. (2012) screened CWRS varieties for stripe rust resistance and found that 60% possessed resistance against the disease, but most resistance was conferred by only one gene, *Yr18.* The authors also concluded that uncharacterized sources of additional stripe rust resistance were present in spring wheat cultivars. To date, over 80 numerically designated stripe rust (*Yr*) genes have been catalogued (McIntosh et al. 2017), but an ineffectiveness in the currently deployed genes has recently been reported, such as *Yr10* in southern Alberta (Puchalski and Gaudet 2011).

Leaf rust of wheat (brown rust) caused by *Puccinia triticina* is a much older leaf disease on the Canadian prairies and the first leaf rust resistance gene (*Lr14A)* was introduced in the 1930s (McCallum et al. 2007). Over 100 leaf rust (*Lr*) genes have been numerically designated (McIntosh et al. 2017), but many older *Lr* genes have reduced effectiveness (such as *Lr13,* and *Lr16)* due to evolving pathogen populations (McCallum et al. 2007). These race-specific genes must be supplemented with durable, long-lasting resistance genes such as *Lr34*, and *Lr46,* as they confer slow-rusting resistance (Singh et al. 1998; Singh and Huerta-Espino 2003). The ineffectiveness of older *Lr* genes suggests the importance of continual discovery of novel resistance genes, and further characterization of genetic factors beyond main effects including genetic interactions and synergistic relationships (Singh et al. 2014). When comparing historic and modern cultivars, Martens et al. (2014) reported modern cultivars possessing partial or complete resistance against leaf rust, with historic cultivars highly susceptible. The modern cultivars involving *Lr21, Lr22a*, and large gene stacks with *Lr34* were highly resistant to

infection, but the effectiveness of *Lr34* was not consistent across cultivars, and some were reported to have intermediate susceptibility. It was previously reported by Kolmer (1996) that " … *Lr13* and *Lr34* singly and together have provided the most durable resistance to leaf rust in wheat throughout the world", but today this statement no longer holds, as races of *P. triticina* have continued to adapt over the past twenty years.

Leaf spot of wheat is caused by a number of different pathogens, and the most significant leaf spot diseases in western Canada include tan spot (*Pyrenophora tritici-repentis*), septoria leaf blotch (*Phaeosphaeria nodorum*, *Mycosphaerella graminicola*, and *Phaesosphaeria avenaria)*, and spot blotch (*Cochliobolus sativus).* It may be difficult to visually distinguish between these diseases, and laboratory analysis may be required as they all produce leaf lesions as the symptom of infection. These pathogens have similar characteristics as crop residue is the most significant source of primary inoculum, and weather conditions are a significant contributor to successful disease development (Bailey et al. 2003). Currently, the registration of a CWRS cultivar does not require resistance against leaf spots, and therefore most CWRS cultivars have moderate susceptibility, or intermediate resistance against leaf spotting (Alberta Seed Guide 2018; Government of Saskatchewan 2018). In Australia, yield loses between 18% to 31% have been reported (Bhathal et al. 2003), but generally within western Canada, leaf spots remains a minor leaf disease complex compared to leaf and stripe rust. The frequency and prevalence of leaf spot diseases are associated with warmer and wetter regions (Gilbert and Woods 2001), and tan spot has been reported as the most prevalent in western Canada due to the reduced humidity requirement for disease development (Fernandez et al. 2010). This disease forms tan-coloured lesions that are frequently surrounded by chlorotic haloes and depending on the eight characterized races and their host-selective toxins, leaf tissue may be necrotic and / or chlorotic

(Lamari et al. 2003; Faris et al. 2013). It is yet to be determined if the shift towards minimal and no-till systems, along with a changing climate will have a significant impact on the future of leaf spot severity and prevalence across the prairies, but it would be wise if breeders were proactive and considered leaf spot resistance in their breeding programs.

Single gene resistance is not durable, nor long-lasting, and therefore breeders must look for horizontal resistance – resistance that is conferred by several genes. Both parents used in this study, 'Peace' and 'Carberry', are moderately resistant to stripe rust (Alberta Seed Guide 2018), confer rust resistance from *Lr34/Yr18*, but additionally each carry unique resistance genes. Conducting linkage-based QTL analysis on this population allows for the same / similar genetic region to be mapped for multiple diseases, and gaining knowledge on the clustering of traits can increase selection in breeding programs (Singh et al. 2016). QTL analysis is an effective first step into the discovery of markers that are tightly linked to gene(s) associated with trait improvement (Singh et al. 2014). Marker assisted selection (MAS) has become a tool in breeding programs due to the low-cost and high-throughput genotyping that has become available. The DArTseq platform used in our study is a genotyping by sequencing (GBS) method, that uses restriction enzymes to separate the informative sequences from the repetitive portions of the wheat genome (Li et al. 2015), and genotyping costs are kept low, because this method relies on complexity reduction and multiple sequencing.

The objectives of this present study were to: (1) identify and genetically map quantitative trait loci (QTL) associated with leaf rust, stripe rust, and leaf spot, (2) locate genomic regions associated with multiple traits, in the 'Peace' × 'Carberry' recombinant inbred line population.

2.2 Materials and Methods

2.2.1 Population Development and Phenotyping

The population in this study consisted of 208 recombinant inbred lines (RILs) that were advanced to F_6 by single seed descent method. Seed from the RILs and two parents were sown in 2015 as double head rows for seed multiplication purposes, and F_6-derived F_7 seed was used in this study. The population was derived from a cross involving two registered Canadian spring wheat cultivars, 'Peace' and 'Carberry'. Both parents meet the end-use quality specifications of the Canada Western Red Spring market class. 'Peace' (Humphreys et al. 2014) is a tall, awnless cultivar with the *SrCad* gene, which confers resistance against the highly virulent Ug99 race (Hiebert et al. 2011), and also the rust resistance gene, *Lr34/Yr18* (Humphreys et al. 2014) and *Lr1*, *Lr13*, and *Lr27* (Randhawa et al. 2013). 'Carberry' (DePauw et al. 2011) is an awned, high yielding semi-dwarf wheat with *Lr34/Yr18*, and *Lr16* (Randhawa et al. 2013).

Stripe rust was assessed from 2014 – 2017 in disease nurseries that were located near Creston, British Columbia, Lethbridge, Alberta and in Edmonton, Alberta. All environments were exposed to natural infection, but stripe rust infection levels were only sufficient in 2016 for analysis. These three environments were replicated twice and consisted of the RIL population, parents, and five checks planted in hill plots. Spreader rows of the susceptible cultivar ('Park') was planted around, and within the nursery to increase the level of epidemic. The proportion of rust pustules on leaf tissue was assessed visually on a 1 – 9 scale (ranging from no infection to highly susceptible), based on the Cobb scale (Peterson et al. 1948), when spreader rows reached a highly susceptible level of infection.

Leaf rust was assessed in Edmonton, Alberta from 2014 to 2017, and near Creston, British Columbia during the 2016 field season. The trials were single replicate in 2014 and 2015,

and two replicates in 2016 and 2017. The Edmonton leaf rust nursery was artificially inoculated by spraying the spreader rows with urediniospores of the prevalent races in the region, that were collected from infected plants the previous field season. The visual assessment was conducted in the same manner as stripe rust, on a 1 – 9 scale, and the Edmonton nursery included 'Park' as the susceptible check, and 'Carberry' as the resistant check. Insufficient disease pressure was present in Edmonton 2016, and the data was excluded from analysis.

Leaf spot was assessed in Edmonton, Alberta from 2014 to 2017, and consisted of single replicates in 2014 and 2015 and two replicates in 2016 and 2017. The spores from two isolates (AB50-2 and AB7-2) of *Pyrenophora triciti-repentis* were sprayed as inoculant over the nursery to enhance the level of infection. These isolates contain the *ToxA* gene and belong to Race 1, which is the most common race in Alberta (Aboukhaddour et al. 2013). The level of severity was assessed visually in the same manner as stripe and leaf rust, on a 1 – 9 scale.

2.2.2 Genotyping and QTL Analysis

Four seeds from each RIL and the two parents were sown in trays containing professional growing mix (Sun Gro Horticulture Canada Ltd., Seba Beach AB) and grown in a greenhouse. The greenhouse growth conditions consisted of a 16-hour photoperiod, and a temperature of 21°C. Approximately 100-200 mg fresh leaf tissue was collected at the two-leaf grown stage, and frozen with liquid nitrogen in 2-mL nuclease-free tubes. Samples were stored in a -80°C freezer until DNA extraction. Genomic DNA was extracted following the Cetyl Trimethylammonium Bromide (CTAB) protocol supplied by Diversity Arrays Technology (DArT, Bruce Australia). One hundred microliters of DNA from each RIL and the two parents was sent to Diversity Arrays Technology for genotyping using sequencing-based DArT (DArTseq), which generated both dominant

(silicoDArT), and co-dominant (SNP) markers (Akbari et al. 2006). Allele scoring, and quality control was conducted by the service provider.

Least squares means were calculated for each individual using a mixed effects model:

$$\Upsilon_{iblt} = \mu_t + G_{it} + R_{blt} + E_t + GE_{ilt} + \varepsilon_{iblt}$$

where Υ_{iblt} is the observation of genotype *i* in replicate *b* in environment *l* of trait *t*, μ_t is the mean effect of trait *t,* G_{it} is the effect of genotype *i* on trait *t*, R_{blt} is the effect of replicate *b* in environment *l* on trait *t*, E_t is the effect of environment on trait *t*, GE_{ilt} is the interaction of genotype *i* and environment *l* of trait *t*, and ε_{iblt} is the residual error. The effect of genotype was treated as a fixed effect, and the effects of replication, environment, and G×E interaction were treated as random. Analysis was conducted in SAS version 9.4 (SAS Institute, Cary NC) using PROC MIXED and the Restricted Maximum Likelihood (REML) method. Plots of residuals were displayed through the SAS Output Delivery System (ODS), and frequency distributions were displayed using R statistical software (version 3.2.3) and the ggplot2 package (Wickham 2009). Boxplots and histograms of frequency distribution were used to identify outliers, and extreme phenotypes were removed from QTL analysis. SAS code published by Holland (2003; 2006) was used to calculate genetic and phenotypic correlation and broad-sense heritability on a line mean basis.

Linkage maps were constructed using silicoDArT and SNP markers that have positions confirmed in 'Wheat consensus map version 4.0' provided by Diversity Arrays Technology (2018). The genotyping data was curated as follows: SNP markers with heterozygous calls were replaced with missing values, all markers with > 20% missing data were removed, markers with extreme segregation distortion (1:1, p-value < 0.01) were removed, and genotypes with > 20% missing calls were removed. Linkage groups were created by a two-step process, in which 'draft'

groups were first identified with MSTmap (Wu et al. 2008), and marker order and distances within these groups were calculated in MapDisto 2.0 (Heffelfinger et al. 2017) using the Kosambi mapping function (Kosambi 1944), a minimum logarithm of odds (LOD) threshold of 3.0, and a minimum recombination frequency threshold of 0.35. The order of markers was calculated with the 'seriation' algorithm, and the SARF (Sum of Adjacent Recombination Frequencies) criteria. Linkage groups and their orientation were assigned to the 21 chromosomes using the prior information provided by the consensus map.

QTL analysis was performed using the least squares means of disease severity combined across environments, and in each individual environment using QTL IciMapping v4.1 (Meng et al. 2015) and the Inclusive Composite Interval Mapping Additive (ICIM-ADD) model (Li et al. 2007). The minimum LOD threshold was 2.5 with a 1.0 cM walking step; missing phenotypes were replaced with the mean value of the trait, and the genetic maps were drawn using MapChart v2.32 (Voorrips 2002). Putative QTL were labelled as per the *Recommended Rules for Gene Symbolization in Wheat* and begin with 'Q' (symbol for QTL), followed by the trait designator, laboratory designator (dms = Dean Michael Spaner), and the chromosome, respectively.

2.3 Results

2.3.1 Disease Severity Assessment

The frequency distribution of least squares means for combined environments are shown in Figure 2.6.1, with severity scores of the parents shown as a dotted line for 'Carberry', and solid line for 'Peace', and transgressive segregation was present in the RIL population and seen in all three disease severity assessments. As expected due to the rust resistance conferred by the

parents, the RIL population was generally resistant, with a skewed tail towards increased disease severity scores of leaf and stripe rust. The leaf spot distribution is approximately normal, as both parents are moderately susceptible to the disease complex. Figure 2.6.2 shows the frequency distribution of disease severity in the single environments (four leaf rust, four leaf spot, and three stripe rust environments). In the single environments, the leaf rust severity scores ranged from 1 – 2.25 for 'Carberry', and 2 – 5.75 for 'Peace', the stripe rust severity scores ranged from 1.75 – 3 for 'Carberry', and 3.75 – 4 for 'Peace', and the leaf spot severity scores ranged from 3.5 – 6 for 'Carberry', and 3.8 – 4.25 for 'Peace'. 'Park' was the susceptible check for leaf and stripe rust, with leaf rust scores ranging from 6.6 – 9, and stripe rust scores ranging from 5 – 7. 'AC Barrie' was one of the moderately susceptible checks for leaf spot, and scores ranged from 3 – 6, and 'Lillian' was the moderately resistant check for leaf spot, and scores ranged from 2 – 6. The descriptive statistics of the least squares means for combined environments were calculated and shown in Table 2.6.1 for both parents, and the RIL population. The least squares means for combined environments of leaf spot was 4.4 for 'Peace', 5.1 for 'Carberry', and 5.3 for the RIL population across the four environments, leaf rust was 4.3 for 'Peace', 1.7 for 'Carberry', and 2.5 for the RIL population across the four environments, and stripe rust was 3.9 for 'Peace', 2.3 for 'Carberry', and 2.7 for the RIL population across the three environments. Broad-sense heritability was calculated on a line mean basis for each disease and ranged from 0.50 – 0.73 (Table 2.6.2).

2.3.2 QTL Analysis

After curating ~ 36,500 markers received from the service provider, 8,413 polymorphic markers were used for linkage map construction (approximately 23% of the original dataset), and

the final population size used for analysis consisted of 204 individuals after four individuals were removed due to > 20% missing genotype data. Markers showing sufficient linkage were separated into the 21 chromosomes of *T. aestivum*, with a final genetic map length of 2,851 cM, and 4,439 markers (consisting of 1,015 SNPs and 3,424 DArTs). Only a single marker was kept if markers mapped to the same position. The shortest linkage group (4D) has a distance of 27 cM, and density of 24 markers, while the longest linkage group (7A) has a distance of 219 cM, and a density of 416 markers. The average marker density across the entire linkage map is 1.6 markers per cM, with individual linkage groups ranging from 0.7 to 2.3 markers per cM. A summary of the genetic map is shown in Table 2.6.3.

By using the inclusive composite interval mapping (ICIM) additive model and the least squares means of the combined environments, a total of ten QTL were identified: three being associated with leaf spot, four with leaf rust, and three with stripe rust (Table 2.6.4; Figure 2.6.3). ICIM was also conducted on each individual environment to provide insight into the stability of identified QTL, and a total of thirty QTL were identified (Table 2.6.5).

Three QTL were identified to be associated with leaf spot, and together explained 28% of total phenotypic variance (Table 2.6.2). 'Carberry' was the primary source of resistance, as *QLs.dms-2A* (with an LOD of 5.7 and located at 75 cM on 2A) and *QLs.dms-7D* (with an LOD of 5.9 and located at 13 cM on 7D) each explained 10% of the phenotypic variance. These alleles from 'Carberry' reduced the disease score by 0.6 and 0.7, respectively, in homozygous RILs carrying them. *QLs.dms-4B* at 43 cM on 4B originated from 'Peace' and explained 8% of the phenotypic variance and was responsible for a 0.7 reduction in severity scores. A total of twelve QTL associated with leaf spot were identified in the individual environment analysis (Table 2.6.5). QTL on 4B were identified in two individual environments (Edmonton 2014, and

Edmonton 2016) that map to the same position as *QLs.dms-4B*, and QTL suggestive of *QLs.dms-2A* were also identified in three individual environments (Edmonton 2015, Edmonton 2016, and Edmonton 2017). *QLs.dms-7D* was identified in two individual environments (Edmonton 2016 and Edmonton 2017).

Four QTL were identified to be associated with leaf rust and together explained 25% of the total phenotypic variance (Table 2.6.2). The most significant QTL was *QLr.dms-5B*, with an LOD of 4.3, located at 123 cM on 5D, and explaining 8% of the phenotypic variance. This resistance allele was contributed by 'Peace', and RILs that were homozygous for this allele reduced their severity scores by 0.3. Additionally, 'Peace' was the parental source for *QLr.dms-2A* located at 71 cM on 2A, which explained 5% of the phenotypic variance. 'Carberry' was the parental source of two QTL associated with leaf rust, *QLr.dms-4A* and *QLr.dms-3D*, located at 64 cM on 4A, and 45 cM on 3D, respectively. The QTL on 4A explained 6% of the phenotypic variance, and the QTL on 3D explained 5%. A total of eleven QTL associated with leaf rust were identified in the individual analysis (Table 2.6.5). A QTL on 3D was identified in two environments (Creston 2016, and Edmonton 2017) that mapped to the same position, or near the leaf rust QTL, *QLr.dms-3D,* located at 45 cM. *QLr.dms-4A* was identified in Creston, and no QTL were identified in any environments that correspond to *QLr.dms-2A* and *QLr.dms-5B*.

Three QTL were identified to be associated with stripe rust and together explained 27% of the total phenotypic variance (Table 2.6.2). The most significant QTL was *QYr.dms-4B*, with an LOD of 7.9, located at 21 cM on 4B, and explaining 16% of the phenotypic variance. This allele was contributed by 'Carberry', and RILs that were homozygous at the flanking markers had a reduction of 0.7 in their disease severity score. In addition to this QTL, 'Carberry' was the parental source of *QYr.dms-3A*, located at 70 cM on 3A, which explained 6% of the phenotypic

variance, and reduced the severity score of homozygous RILs by 0.5. 'Peace' was the source of a single relatively minor stripe rust QTL, *QYr.dms-2A*, that was located at 75 cM on 2A, and explained 5% of the phenotypic variance. A total of seven QTL associated with stripe rust were identified in the individual environment analysis (Table 2.6.5). *QYr.dms-4B* was identified in all three individual environments, *QYr.dms-2A* was identified in two environments (Creston 2016, and Edmonton 2016), and *QYr.dms-3A* was identified in a single environment (Creston 2016), and

2.4 Discussion

In our study, *QYr.dms-4B*, was the largest QTL identified and explained 16% of the phenotypic variance associated with stripe rust and was located at 21 cM on chromosome 4B. This QTL was also the most stable of the stripe rust QTL and was identified in all three individual environments, in addition to the combined analysis. An extensive review of stripe rust QTL conducted by Rosewarne et al. (2013) reported eight published identifications of QTL located between 12 cM – 27 cM on 4B. More recently, Naruoka et al. (2015) identified two minor stripe rust QTL on 4B (*Qyr.wpg-4B.1* and *Qyr.wpg-4B.2*) by association mapping of 402 winter wheat accessions, and Bokore et al. (2017) conducted linkage-based QTL mapping on the 'Carberry' × 'AC Cadillac' doubled haploid (DH) population and identified a single QTL on 4B (*QYr.spa-4B*). QTL associated with stripe rust have also been identified on 3A (Lillemo et al. 2008; Singh et al. 2014; Zou et al. 2017c) and 2A (Jighly et al. 2015; Maccaferri et al. 2015; Naruoka et al. 2015; Vazquez et al. 2015) and in this study we identified a QTL on 3A, *QYr.dms-3A*, that explained 6% of the phenotypic variance, and a QTL on 2A , *QYr.dms-2A*, that

explained 5% of the variance. In addition to the combined analysis, *QYr.dms-2A* was identified in two individual environments, and *QYr.dms-3A* was identified in one.

QLs.dms-7D and *QLs.dms-2A* were the second largest QTL identified in our study, and each explained 10% of the phenotypic variance associated with leaf spotting. Studies have reported QTL on 7D for leaf spot (Adhikari et al. 2012), tan spot (Gurung et al. 2011), and septoria tritici blotch (*Mycosphaerella graminicola*) (Kosellek et al. 2013), and on 2A for septoria tritici blotch (Adhikari et al. 2015; Ando et al. 2018), but in our study we were unable to look at disease specific QTL, because the leaf spot complex is difficult to phenotype due to the large number of diseases that produced similar symptoms. QTL associated with leaf spotting have also been identified on 4B (Zwart et al. 2010; Gurung et al. 2014) and we identified a QTL on 4B, *QLs.dms-4B*, that explained 8% of the phenotypic variance. In addition to the combined analysis, this QTL was identified in two of the four individual environments.

All four leaf rust QTL identified in our combined analysis were relatively minor, and each explained < 10% of the phenotypic variance. QTL associated with leaf rust have been identified on 3D (Singh et al. 2009; Rosewarne et al. 2015) and in our study we identified a QTL on 3D, *QLr.dms-3D*, that explained 5% of the phenotypic variance, and in addition to the combined analysis, was the most stable leaf rust QTL and was identified in two of the four individual environments. *QLr.dms-4A* explained 6% of the phenotypic variance, and was identified in a single environment, but to our knowledge, only one other study has identified a QTL on 4A (Zhang et al. 2017). The QTL they reported, *QLr.hebau-4AL*, was identified in three environments and explained between 3% to 8% of the phenotypic variance. QTL associated with leaf rust have been identified on 5B (Messmer et al. 2000; Zhou et al. 2014), and in our study we identified *QLr.dms-5B* to explain 8% in the combined analysis, and a QTL similar to *QLr.dms-*

5B was identified in one environment. No QTL were identified on 2A in the individual environments, and therefore the stability of *QLs.dms-2A* remains unknown, but QTL on 2A have been identified by others. Rosewarne et al. (2012) reported a QTL on 2A in two environments that explained 5% and 7% of the phenotypic variance, and Schnurbusch et al. (2004) identified a QTL on 2A that explained 12% of the infection response. The 'Carberry' × 'AC Cadillac' DH population was analyzed by Singh et al. (2014) and 'Carberry' was reported to be the source of leaf rust resistance on 2B and 4B. The leaf rust QTL they identified on 4B, *QLr.spa-4B*, was located between 0 – 18 cM, and our results identified a QTL at 21 cM on 4B in one of the four leaf rust environments (Creston 2016) originating from 'Carberry', and may be the same QTL identified by Singh et al. (2014). In our study, this QTL explained 8% phenotypic variance, and their QTL was identified in one environment and explained 5% of the phenotypic variance.

QTL that map to the same or similar position for different traits can be indicative of tight linkage, or pleiotropy, and the pleiotropic effects of rust resistance genes have been well recognized for several genes including *Lr34/Yr18* (Lagudah et al. 2009), *Lr46/Yr29* (Kolmer et al. 2015) and *Lr27/Sr2* (Mago et al. 2011). In our study, we found the same QTL that mapped to 21 cM on 4B associated with leaf rust in Creston 2016, and stripe rust in Edmonton 2016 and Lethbridge 2016. 'Carberry' was the parental source of this resistance allele, and it explained 8% of the phenotypic variance associated with leaf rust, and 8 – 10% of the variance associated with stripe rust. 'Carberry' was the source of resistance for a QTL that explained 9% of the phenotypic variance associated with stripe rust was also identified at 20 cM on 4B in Creston 2016. In the combined analysis, *QYr.dms-4B* was identified at 21 cM, and associated with stripe rust, but this QTL was not detected in the combined analysis associated with leaf rust. In the combined analysis, *QLs.dms-2A* was associated with leaf spotting, and co-located with the stripe

rust QTL *QYr.dms-2A*, but these QTL originated from different parents, and therefore this region on 2A may consist of tightly linked QTL.

The mapping population we used in our study, 'Peace' × 'Carberry', has very similar genetics to the 'Carberry' × 'AC Cadillac' population used by Singh et al. (2014) and Bokore et al. (2017), because in addition to 'Carberry' being a parent in both populations, 'AC Cadillac' is a close relative to 'Peace' due to both cultivars originating from BW90 and BW553. 'Peace' and 'AC Cadillac' both inherited the stem rust resistance gene, *SrCad*, from BW553 (Hiebert et al. 2011), providing further evidence of related genetics. Bokore et al. (2017) reported that 'AC Cadillac' contributed stripe rust QTL from chromosomes 2A, 2B, 3B, and 5B, while Singh et al. (2014) reported stripe rust QTL from 'AC Cadillac' on chromosomes 2A, 2B, 3A, 3B, 5B, and 7B, and a single leaf rust QTL on 2A. Singh et al. (2014) found the leaf rust QTL on 2A, *QLr.spa-2A*, to explain 6% of the phenotypic variance, and located at 127.8 cM, which may be the same leaf rust QTL as identified in our study's combined analysis (*QLr.dms-2A* was identified at 71 cM and explained 5% variance). They also identified a stripe rust QTL, *QYr.spa-2A*, nearby at 111.8 cM that explained 6% of the stripe rust infection response, and the authors concluded that further research would be needed to confirm if this region had multiple genes, or a single gene with pleiotropic effects. Bokore et al. (2017) only conducted QTL analysis associated with stripe rust, but confirmed the significance of *QYr.spa-2A* (located at 50.5 cM in their study) in conferring resistance. We identified *QYr.dms-2A* only 4 cM away from *QLr.dms-2A,* which also originated from 'Peace', and explained 5% of the phenotypic variance. The two QTL we identified from 'Peace' in our combined analysis, *QYr.spa-2A* and *QLr.dms-2A*, correspond with the two QTL identified by Singh et al. (2014) and confirm the presence of minor QTL on 2A that confer leaf and stripe rust resistance. The largest QTL in our combined analysis,

QYr.dms-4B, originated from 'Carberry', but the position of this QTL was ~ 100 cM from the stripe rust QTL on 4B from 'Carberry' that was reported in other studies (Singh et al. 2014; Bokore et al. 2017). Further research is required to clarify if 'Carberry' contributes two alleles associated with stripe rust resistance on 4B, but in our study we did identify two stripe rust QTL on 4B in the single environment analysis (Edmonton 2016). One of the QTL corresponds to *QYr.dms-4B* at 21 cM, but the second QTL was located at 44 cM and providing evidence that 'Carberry' may indeed confer stripe rust resistance from two QTL on 4B.

The comparison of QTL studies becomes difficult due to a number of issues, including different markers used for linkage map construction, reduced recombination due to population development (RIL versus DH population), potential genotype by environment interaction, and the choice of software used for QTL analysis. The markers deployed by both Singh et al. (2014) and Bokore et al. (2017) were different from our study, the 'Carberry' × 'AC Cadillac' population was doubled haploid, and QTL mapping software utilized was different. Although there was not a direct agreement between the QTL identified in our study and the previously published studies using 'Carberry' as a parental source, there is significance evidence to suggest that 4B is a source of leaf and stripe rust resistance, and this study has provided further evidence that there are two rust resistance QTL on 2A, and these alleles were contributed by 'Peace'.

'Peace' has been reported to carry *Lr1, Lr13 Lr27, Lr34/Yr18* and 'Carberry' to carry *Lr16, Lr34/Yr18* (Randhawa et al. 2013), but virulence towards *Lr13* and *Lr16* has been reported, making these genes no longer durable sources of resistance (McCallum et al. 2016). *Lr34* remains an effective source of resistance since being introduced in 'Glenlea' during the 1970s, and is a very important resistance gene because of the resistance it confers against stripe rust, stem rust, powdery mildew, and barley yellow dwarf virus in addition to leaf rust (McCallum et

al. 2012). *Lr1* is located on 5D (Cloutier et al. 2007), *Lr13* is located on 2BS (Zhang et al. 2016), *Lr16* on the distal end of 2BS, and *Lr27* on 3BS (Singh and McIntosh, 1992). In both the combined and individual analysis, no QTL associated with leaf rust were identified on chromosomes 5D, 2B, or 3B that could correspond to the leaf rust genes that are segregating in the RIL population. Possible reasons these genes were not identified in the QTL analysis may be because the genetic map is not comprehensive and recombination regions are not adequately mapped, and / or these genes may not confer resistance against the modern races that are in the wild. Gene-specific primers were not developed to screen the RIL population for the segregating rust resistance genes, but future research using this population could address the reported synergistic effect of *Lr34* on other resistance genes (German and Kolmer 1992). The interaction of resistance genes has been recognized to enhance resistance (Kolmer 1996), and in this study we did not consider synergistic relationships, or complementary genes, which may both contribute to explaining additional genetic factors.

The linkage map constructed in our study, with a density of 4,439 markers, a total length of 2,851 cM, and an average of 1.6 markers per cM is very similar to recently published studies. Zou et al. (2017c) produced a map consisting of 1,203 markers, over 27 linkage groups with total length of 3,442 cM (0.4 markers per cM), and Steffan et al. (2017) constructed a map considering of 34 linkage groups using 1,734 markers with a total of 2,882 cM (0.60 markers per cM). The genetic map produced by McCartney et al. (2016) was a higher density map with 3,081 markers over 2,467 cM across 25 linkage groups (1.2 markers per cM), but Dong et al. (2017) published a much lower density map using only 689 unique markers over 2,425 cM (0.3 markers per cM). Lastly, Vazquez et al. (2015) constructed linkage maps for two populations, and both these maps had an average density of 0.2 markers per cM, across 49 and 32 linkage groups using

229 and 198 markers, respectively. The literature suggests that our constructed genetic map is very appropriate compared to recently published studies.

2.5 Conclusion

As the resistance conferred by currently deployed major genes continues to decline, there is an increasing need to discover minor resistance QTL and begin characterizing and implementing them into current breeding programs. The objective of this study was to identify QTL associated with disease resistance in the 'Peace' × 'Carberry' RIL population that did not segregate for *Lr34/Yr18*, and both parents carried moderate resistance to stripe rust. Through ICIM analysis we reported four QTL associated with leaf rust, three QTL associated with stripe rust, and three QTL associated with leaf spot in the combined analysis. 'Carberry' provided the majority of resistance alleles to the RIL population, and these alleles were also responsible for explaining the majority of total phenotypic variance. The leaf spot QTL, *QLs.dms-2A* and *QLs.dms-7D*, the leaf rust QTL, *QLr.dms-5B*, and the stripe rust QTL, *QYr.dms-4B*, explained the highest proportion of phenotypic variance for each respective trait, contributing 10%, 10%, 8% and 16% of the variance, respectively. The leaf spot and stripe rust QTL were also the most stable, as they were repeatedly identified in the individual environment analysis. *QYr.dms-4B* was identified in all three of the stripe rust environments; *QLs.dms-2A* and *QLs.dms-7D* were identified in two of the four leaf spot environments. This study also confirmed the results of related studies by identifying QTL on 4B that were contributed by 'Carberry' (*QYr.dms-4B*), and two QTL on 2A contributed by 'Peace' (*QYr.dms-2A* and *QLr.dms-2A*). The 'Peace' × 'Carberry' RIL population has shown to be segregating for several alleles associated with leaf rust, stripe rust, and leaf spot, but the leaf rust QTL we identified were all relatively minor and

explained only between 5 – 8% of the phenotypic variance. The largest QTL identified in this study were contributed by ‘Carberry’ and associated with leaf spotting and stripe rust resistance, suggesting that ‘Carberry’ may be an attractive parental source for breeders looking to enhance resistance against stripe rust and leaf spot with minor resistance alleles.

2.6 Tables and Figures

Table 2.6.1. Summary of descriptive statistics of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population using the least squares (LS) means of the combined environments.

Trait	No. of Environments	Mean disease score of parents		RIL population descriptive stats				LS means F-Value	
		'Peace'	*'Carberry'*	*Min*	*Max*	*Mean*	*SD*	*F-Value*	*Significance*
Leaf Spot	3	4.4	5.1	3.0	7.5	5.3	1.1	2.89	<0.0001
Leaf Rust	4	4.3	1.7	1.2	5.5	2.5	0.8	2.03	<0.0001
Stripe Rust	3	3.9	2.3	1.2	6.7	2.7	1.1	3.05	<0.0001

Table 2.6.2. Summary of quantitative trait loci (QTL) identified in the combined environment analysis of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population. Broad-sense heritability was calculated on a line-mean basis, with standard error shown in brackets. The total phenotypic variance was calculated using the sum of individual QTL phenotypic variances.

Trait	Heritability	No. of QTL Identified	Total phenotypic variance explained (%)
Leaf Spot	0.70 *(0.04)*	3	27.7
Leaf Rust	0.50 *(0.06)*	4	24.5
Stripe Rust	0.73 *(0.04)*	3	27.1

Table 2.6.3. Summary of DArT and single nucleotide polymorphism (SNP) markers used in the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population. The average marker density across the genetic map is 1.6 per cM (centimorgan).

Chromosome	Linkage Map (cM)	Total Markers	Markers per cM
1A	132	268	*2.0*
2A	129	183	*1.4*
3A	158	306	*1.9*
4A	129	182	*1.4*
5A	140	156	*1.1*
6A	101	214	*2.1*
7A	219	416	*1.9*
Sub-Genome A Total	1008	1725	
1B	145	339	*2.3*
2B	189	412	*2.2*
3B	202	239	*1.2*
4B	104	143	*1.4*
5B	214	450	*2.1*
6B	177	345	*1.9*
7B	149	200	*1.3*
Sub-Genome B Total	1180	2128	
1D	97	73	*0.8*
2D	89	99	*1.1*
3D	139	118	*0.8*
4D	27	24	*0.9*
5D	71	66	*0.9*
6D	105	107	*1.0*
7D	135	99	*0.7*
Sub-Genome D Total	663	586	
Total cM	2851		
Total Markers	4439		

Table 2.6.4. Detailed summary of quantitative trait loci (QTL) identified in the combined environment analysis of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population. Flanking markers are identified by the "Clone ID" provided by Diversity Arrays Technology. (D) and (S) designates a DArT or SNP marker, respectively.

Trait	QTL	Chr.†	Position (cM)	Confidence Interval (cM)	Left Marker	Right Marker	LOD ‡	R^2 (%)	Additive Effect	Difference §	Parental Resistance Allele
Leaf Spot	*QLs.dms-2A*	2A	75	74.5 - 75.5	2A_d1117218	2A_d1089684	5.7	10	0.3	0.6	Carberry
Leaf Spot	*QLs.dms-4B*	4B	43	41.5 - 44.5	4B_d3384830	4B_s4991673	4.3	8	-0.3	-0.7	Peace
Leaf Spot	*QLs.dms-7D*	7D	13	11.5 - 14.5	7D_s1047233	7D_s1022494	5.9	10	0.3	0.7	Carberry
Leaf Rust	*QLr.dms-2A*	2A	71	70.5 - 71.5	2A_d3958592	2A_d3953769	2.9	5	-0.1	-0.3	Peace
Leaf Rust	*QLr.dms-4A*	4A	64	63.5 - 64.5	4A_d1164232	4A_s1118950	3.4	6	0.1	0.3	Carberry
Leaf Rust	*QLr.dms-5B*	5B	123	122.5 - 123.5	5B_s4991398	5B_d2278329	4.3	8	-0.2	-0.3	Peace
Leaf Rust	*QLr.dms-3D*	3D	45	44.5 - 45.5	3D_d977229	3D_d1269275	2.9	5	0.1	0.2	Carberry
Stripe Rust	*QYr.dms-2A*	2A	75	74.5 - 75.5	2A_d1117218	2A_d1089684	2.9	5	-0.2	-0.5	Peace
Stripe Rust	*QYr.dms-3A*	3A	70	66.5 - 72.5	3A_s3948706	3A_d1103494	3.4	6	0.2	0.5	Carberry
Stripe Rust	*QYr.dms-4B*	4B	21	20.5 - 21.5	4B_d1111841	4B_d1087899	7.9	16	0.3	0.7	Carberry

† Chromosome

‡Logarithm of odds

§Difference is calculated using the mean disease score difference between flanking markers that have the same parental allele at both markers (AA – BB).

Table 2.6.5. Detailed summary of quantitative trait loci (QTL) identified in the individual environment analysis of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population. Flanking markers are identified by the "Clone ID" provided by Diversity Arrays Technology. (D) and (S) designates a DArT or SNP marker, respectively.

Trait	Environment	Chr.†	Position (cM)	Confidence Interval (cM)	Left Marker	Right Marker	LOD‡	R^2 (%)	Additive Effect
Leaf Spot	Edmonton 2014	4B	43	41.5 - 44.5	4B_d3384830	4B_s4991673	2.8	6	-0.5
Leaf Spot	Edmonton 2015	2A	75	74.5 - 75.5	2A_d1117218	2A_d1089684	5.0	10	0.6
Leaf Spot	Edmonton 2015	5A	15	13.5 - 16.5	5A_d2275889	5A_d3034030	2.7	5	-0.4
Leaf Spot	Edmonton 2015	5A	119	118.5 - 120.5	5A_d2303648	5A_s1228740	2.9	6	-0.4
Leaf Spot	Edmonton 2016	1A	51	50.5 - 51.5	1A_s1217762	1A_d3948456	3.9	6	0.2
Leaf Spot	Edmonton 2016	2A	64	62.5 - 66.5	2A_d1118293	2A_d1164587	4.2	6	0.2
Leaf Spot	Edmonton 2016	5A	85	84.5 - 85.5	5A_d3022239	5A_d1126765	4.0	6	-0.2
Leaf Spot	Edmonton 2016	4B	43	42.5 - 44.5	4B_d3384830	4B_s4991673	8.6	14	-0.3
Leaf Spot	Edmonton 2016	3D	40	39.5 - 40.5	3D_d1163317	3D_d3064748	3.5	5	-0.2
Leaf Spot	Edmonton 2016	7D	12	11.5 - 12.5	7D_d1105401	7D_s1047233	3.0	4	0.2
Leaf Spot	Edmonton 2017	2A	76	75.5 - 76.5	2A_d1128367	2A_d4004105	6.2	10	0.4
Leaf Spot	Edmonton 2017	4B	20	18.5 - 21.5	4B_d1111841	4B_d1087899	3.8	5	-0.3
Leaf Spot	Edmonton 2017	2D	22	20.5 - 22.5	2D_d1160995	2D_d3939639	3.7	5	0.3
Leaf Spot	Edmonton 2017	3D	34	32.5 - 37.5	3D_d2251907	3D_d1089277	2.8	4	-0.3
Leaf Spot	Edmonton 2017	7D	12	11.5 - 12.5	7D_d1105401	7D_s1047233	7.7	11	0.5
-	-	-	-	-	-	-	-	-	-
Leaf Rust	Edmonton 2014	2D	22	20.5 - 23.5	2D_d1160995	2D_d3939639	2.8	6	0.2
Leaf Rust	Edmonton 2015	1A	56	55.5 - 56.5	1A_d1124870	1A_d1764604	3.6	5	0.3
Leaf Rust	Edmonton 2015	4B	24	21.5 - 26.5	4B_d3025629	4B_d2276030	4.5	8	-0.3
Leaf Rust	Creston 2016	1A	108	106.5 - 108.5	1A_d3024760	1A_s1864148	4.0	5	0.3
Leaf Rust	Creston 2016	4A	63	62.5 - 63.5	4A_s1397352	4A_d1106380	3.0	3	0.3

Trait	Environment	Chr.†	Position (cM)	Confidence Interval (cM)	Left Marker	Right Marker	LOD‡	R^2 (%)	Additive Effect
Leaf Rust	Creston 2016	4B	21	20.5 - 22.5	4B_d1111841	4B_d1087899	6.4	8	0.4
Leaf Rust	Creston 2016	5B	172	170.5 - 173.5	5B_d2276721	5B_d3959142	3.6	5	-0.3
Leaf Rust	Creston 2016	3D	45	44.5 - 45.5	3D_d977229	3D_d1269275	2.8	3	0.3
Leaf Rust	Edmonton 2017	3A	74	73.5 - 74.5	3A_s5323857	3A_s1012499	3.1	6	0.2
Leaf Rust	Edmonton 2017	7B	141	140.5 - 141.5	7B_s100008598	7B_d3034274	3.0	6	0.2
Leaf Rust	Edmonton 2017	3D	63	62.5 - 63.5	3D_d1109776	3D_d3027742	5.5	11	0.3
-	-	-	-	-	-	-	-	-	-
Stripe Rust	Creston 2016	2A	76	75.5 - 76.5	2A_d1128367	2A_d4004105	6.8	12	-0.3
Stripe Rust	Creston 2016	3A	70	67.5 - 72.5	3A_s3948706	3A_d1103494	4.3	7	0.3
Stripe Rust	Creston 2016	4B	20	19.5 - 21.5	4B_d1111841	4B_d1087899	5.4	9	0.3
Stripe Rust	Creston 2016	7B	7	6.5 - 7.5	7B_s100006160	7B_d1059164	2.6	4	0.2
Stripe Rust	Creston 2016	3D	46	45.5 - 46.5	3D_d1265681	3D_s1058686	5.1	8	0.3
Stripe Rust	Edmonton 2016	2A	75	74.5 - 75.5	2A_d1117218	2A_d1089684	3.4	6	-0.3
Stripe Rust	Edmonton 2016	4B	21	18.5 - 21.5	4B_d1111841	4B_d1087899	5.7	10	0.4
Stripe Rust	Edmonton 2016	4B	44	42.5 - 44.5	4B_s4991673	4B_d1258252	3.2	6	0.3
Stripe Rust	Lethbridge 2016	4B	21	20.5 - 22.5	4B_d1111841	4B_d1087899	4.1	8	0.4
Stripe Rust	Lethbridge 2016	5B	91	90.5 - 91.5	5B_d1106609	5B_d1193595	4.0	7	-0.4

†Chromosome

‡Logarithm of odds

Figure 2.6.1. Frequency distribution of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population for stripe rust, leaf rust, and leaf spot severity using the least squares means of the combined environments, on a 1 to 9 scale. The horizontal lines represent the mean disease scores of the parents, with the dotted line representing 'Carberry', and the solid line representing 'Peace'.

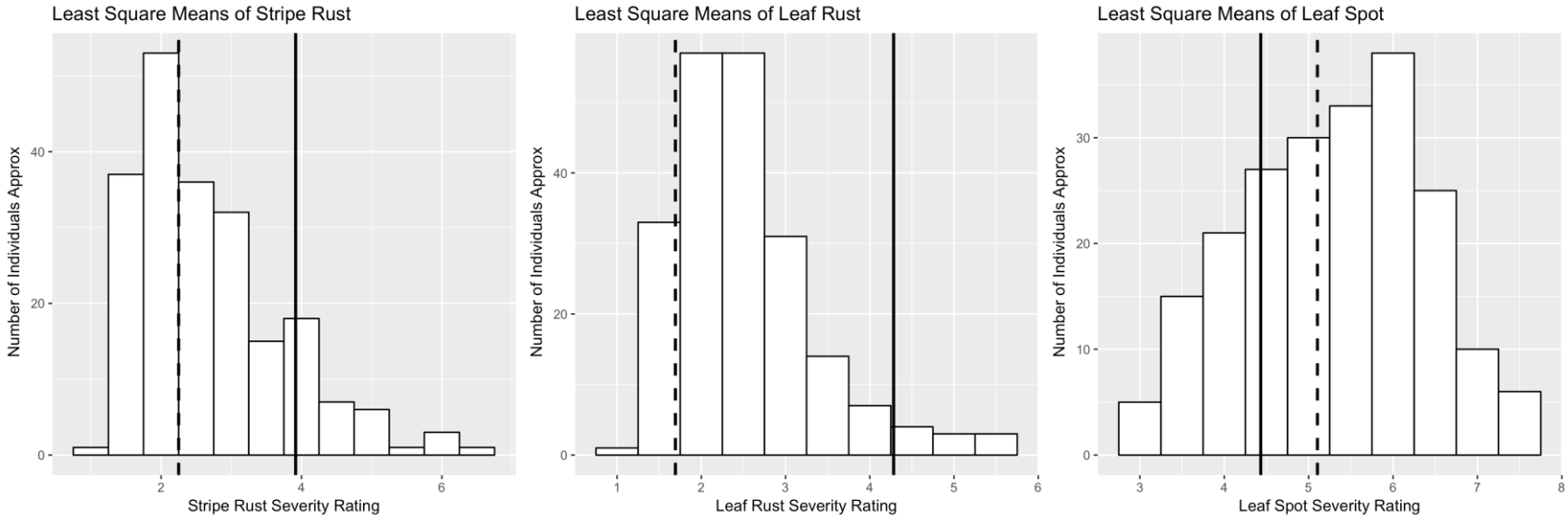


Figure 2.6.2. Frequency distribution of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population for stripe rust, leaf rust, and leaf spot severity in each individual environment, on a 1 to 9 scale. Disease severity scores were taken in field nurseries located in Edmonton Alberta, and near Creston British Columbia, and Lethbridge Alberta from 2014 to 2017.

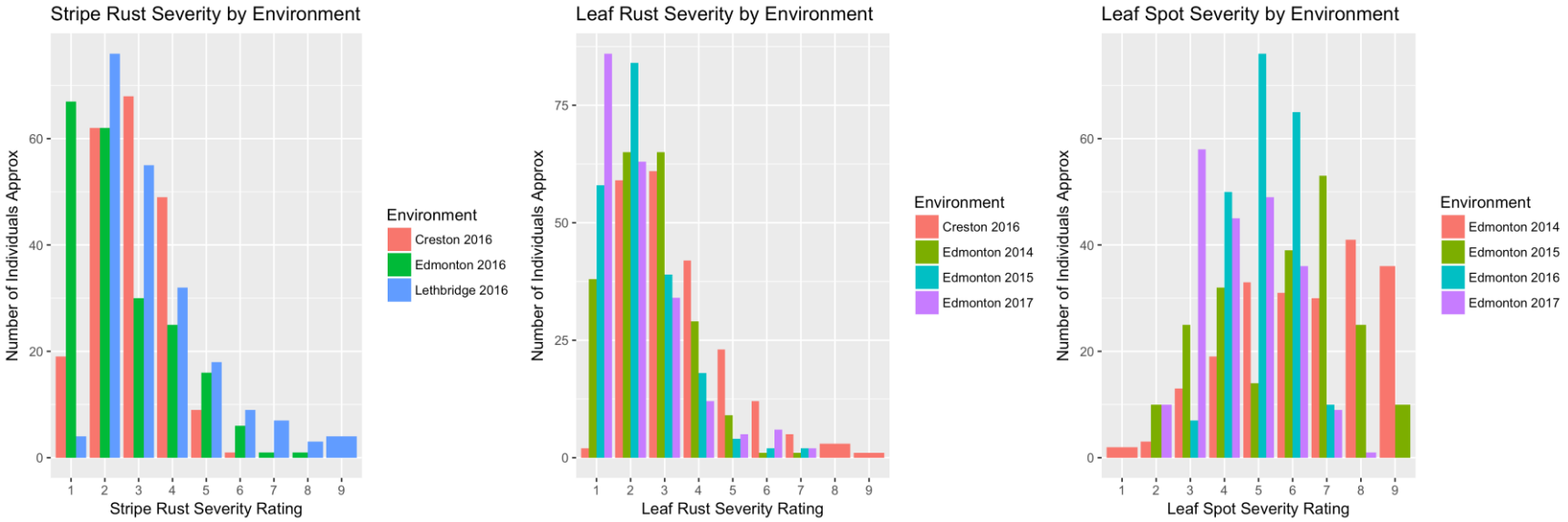
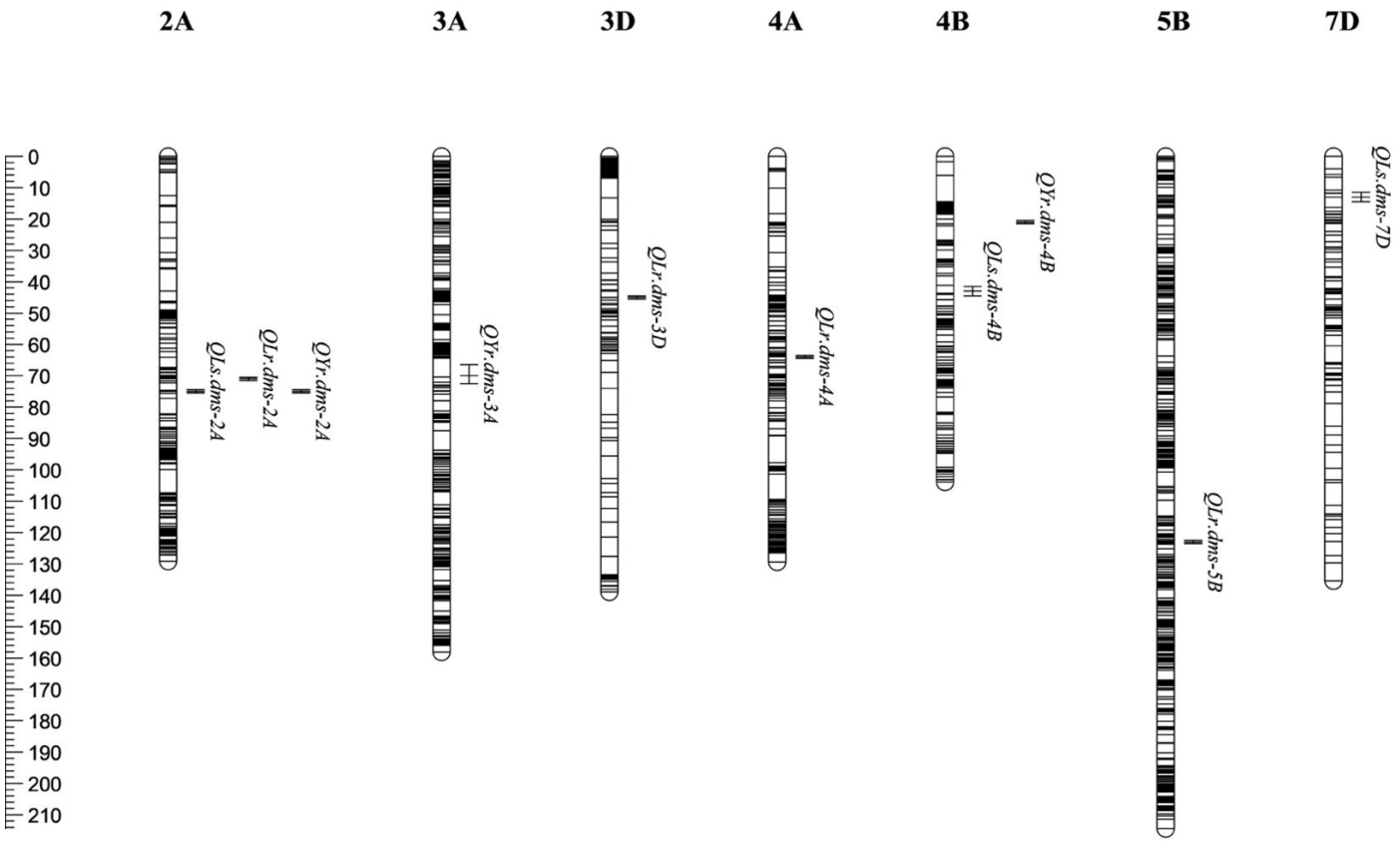


Figure 2.6.3. The location of the ten quantitative trait loci (QTL) identified in the combined environment analysis of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population. The map position is located on the left side in centimorgans (cM), and the horizontal lines on each chromosome represent mapped markers. QTL are shown on the right side of the chromosome with the position and 95% confidence interval.



3 Mapping QTL associated with agronomic traits under conventionally and organically managed systems in a Canadian spring wheat population[2]

3.1 Introduction

The Canada Western Red Spring (CWRS) class of wheat is the most produced wheat type in western Canada, and was responsible for 69% of total wheat acres across British Columbia, Alberta, Saskatchewan and Manitoba in 2017 (Canadian Grain Commission 2018). Wheat breeders must focus on the improvement of both agronomic and quality traits, as this will ensure that CWRS remains an attractive class for producers, and a sought-after product for millers in the international export market. The relationship between agronomic and quality traits is complex though, and the most well-known being the negative relationship between grain yield and grain protein (Metzger 1935) and the positive relationship between grain yield and maturity. Therefore, cultivar development requires a thorough understanding of genetic factors affecting the traits that are important to the producer and end-user. The development of a genetic mapping population from two elite parents minimizes the chance for linkage drag, and creates individuals that are well-adapted to growing in western Canada (Singh et al. 2014).

The identification of novel quantitative trait loci (QTL) associated with agronomic and quality traits in CWRS germplasm is a first step to enable breeders to deploy marker assisted

[2] A version of this chapter with the same title has been submitted to *Crop Science* in August 2018; authored as Bemister et al.

selection (MAS) in their breeding programs. Several studies on identifying QTL for agronomic traits have been conducted using Canadian germplasm (McCartney et al. 2005; Cuthbert et al. 2008; Chen et al. 2015; Perez-Lara et al. 2016; Zou et al. 2017a, 2017b), but QTL associated with quality has been less studied (Fowler et al. 2016; Cabral et al. 2018). Agronomic traits of particular interest for western Canada include early maturity, because of potential frost damage in the Spring and Fall occurs regularly across the prairies. McCartney et al. (2005) identified four QTL associated with maturity, as did Chen et al. (2015) and Perez-Lara et al. (2016). Arguably, grain yield is the most important consideration for producers as it's directly related to economic profit, and Cuthbert et al. (2008) identified five QTL associated with grain yield, and Perez-Lara et al. (2016) identified two.

Crop competitiveness has been recognized as an important characteristic for wheat development under organic environments (as reviewed by Mason and Spaner (2006)), and traits such as height, seedling ground cover, early maturity, leaf size and tillers were associated with grain yield in competitive environments (Huel and Hucl 1996; Lemerle et al. 1996; Mason et al. 2007). The differences in ideal genotypes for wheat cultivars growing in conventional versus organic environments has resulted in the registration of organic-specific cultivars (Rolland et al. 2017), and the mapping of QTL in organic environments has been reported (Asif et al. 2015; Zou et al. 2017a). Although many breeding objectives are comparable regardless of management system (Asif et al. 2015), it has been suggested that direct selection of germplasm in organic environments produced genotypes superior in yield in organic environments versus genotypes selected under conventional environments (Kirk et al. 2012). Management-specific QTL were identified by Asif et al. (2015), but consistent QTL were also identified across management systems, and associated with grain yield, test weight, thousand kernel weight, and flowering. The

stability and consistency of identified QTL remains a problematic concern for plant breeding (Bernardo 2008), but due to the significant differences in biotic and abiotic stresses between conventional and organic systems (such as soil nutrients, soil moisture, and weed competition) QTL that are identified across management systems may be highly stable, and reliable markers for breeders.

The objectives of the present study were to: 1) map QTL associated with agronomic and quality traits, and 2) compare QTL identified in conventional and organic environments in a recombinant inbred line (RIL) mapping population derived from two registered CWRS cultivars, 'Peace' and 'Carberry'.

3.2 Materials and Methods

3.2.1 Population Development and Phenotyping

The population in this study remains the same as Chapter 2 of this thesis, and consisted of 208 recombinant inbred lines (RILs) that were advanced to F_6 by single seed descent. The population was derived from two registered Canadian spring wheat cultivars, 'Peace' and 'Carberry', and both parents meet the end-use quality specifications of the Canada Western Red Spring market class. 'Peace' (Humphreys et al. 2014) is a tall, awnless cultivar that carries the *Vrn-A1a* allele (Iqbal et al. 2007c) in addition to the *SrCad* gene, which confers resistance against the highly virulent Ug99 race (Hiebert et al. 2011). 'Carberry' (DePauw et al. 2011) is a awned, high yielding semi-dwarf that carries the *Vrn-A1a*, *Ppd-D1b* and *Rht-B1b* alleles (Chen et al. 2016). Both parents carry the resistance gene *Lr34/Yr18/Pm38* (Randhawa et al. 2013).

Field experiments were conducted at the University of Alberta South Campus Crop Research station in Edmonton, AB, Canada (Latitude: 53° 29' N, Longitude: 113° 32' W). The 208 RILs, two parents, and five check CWRS cultivars including 'Glenn', 'Go Early', 'CDC Kernen', 'Parata', and 'Splendor' were seeded on May 11th in 2016, and May 20th in 2017 on conventionally and organically managed land as paired trials each year. The trials were designed as randomized incomplete block design with two replicates both years. Experimental plots were seeded at a rate of 300 seeds m^{-2} with the 2016 plot size of 3m × 1.14m and consisting of 6 rows with 19 cm spacing. Plot size in 2017 was increased to 4m long due to increased seed available. Soil samples were taken each year in the early spring and analyzed for nutrient content and soil quality. Nutrient levels of Nitrogen (N), Phosphorus (P), Potassium (K), Sulfate (S) of conventional land in 2016 were 34, 53, >600, 17 ppm, respectively, and pH was 6.0 with 13.1% organic matter. The 2016 organic land levels were 24, >60, 426, 11 ppm, respectively, and pH was 6.7 with 11.8% organic matter. In 2017, conventional land levels were 12, 14, 198, 6 ppm, respectively, and pH was 6.1 with 11.0% organic matter. The 2017 organic land levels were taken in the fall and were 59, >60, 310, 14 ppm, respectively, and pH was 6.6 with 12.9% organic matter. In both years, conventional trials were broadcast fertilized with 70 kg ha^{-1} of 46-0-0 (N-P_2O_5-K_2O) in early spring, and band fertilized during seeding with 36 kg ha^{-1} of 11-52-0 (N-P_2O_5-K_2O). Weed control in the conventional trials was maintained through local recommendations (Alberta Agriculture and Forestry 2018a) by appropriate use of registered herbicides according to directions on the label. Organic land did not receive any inputs, and the four-year rotation was wheat, rye plough-down, field peas, canola. The conventional land followed a three-year rotation of wheat, field peas, canola.

Phenotypic data was collected for days to heading, physiological maturity, plant height, lodging, grain yield, grain protein content, sodium dodecyl sulfate (SDS) sedimentation volume, thousand kernel weight (TKW), and test weight (TWt). Days to heading was recorded when 50% of the plants in a plot had heads fully emerged from the flag leaf sheath, and days to flowering was recorded when 50% of the plants in a plot had begun displaying anthers outside the floret. Physiological maturity of the plant was recorded when 50% of peduncles lost their green colour. Plant height was recorded from the base of the plant to the tip of the head excluding awns. Lodging was recorded at time of harvest, on a 1 – 9 scale, with 1 representing zero lodging, and 9 as completely lodged. Plots were harvested using Wintersteiger Nursery Master Elite plot combine (Wintersteiger Inc., Salt Lake City, UT), and grain was dried and subsequently weighed to obtain grain yield. Grain protein content was determined with near-infrared reflectance spectroscopy using a Monochromator NIR system model 6500 (NIRSystems, Inc., Silver Springs, MD)**.** SDS sedimentation volume was measured on whole grain flour samples gathered from a Udy cyclone mill (UDY Corporation, Fort Collins, CO) as per AACC International method 56-70.01 (http://methods.aaccnet.org/). Thousand kernel weight was measured by randomly selecting 200 seeds using a digital seed counter (Agriculex Inc., Guelph, ON) and multiplying the weight by five. Test weight was measured by filling a standard one-pint cup with a sample of clean grain using a hopper and stand (Seedburo Equipment Co., Des Plaines, IL) and recording the weight.

3.2.2 Genotyping and QTL Analysis

Section 2.2.2 of this thesis provides detailed information regarding the methods related to DNA extraction, genotyping, and linkage map construction. Briefly, genomic DNA was delivered

to Diversity Arrays Technology (DArT, Bruce Australia) for DArTseq genotyping by sequencing. The least squares means were estimated for each individual in the population using a mixed effects model. The effect of genotype was fixed, and effects of replicate, environment, and genotype by environment interact were random. Analysis was conducted in SAS version 9.4 (SAS Institute, Cary NC) using PROC MIXED and the Restricted Maximum Likelihood (REML) method. Linkage maps were constructed using silicoDArT and SNP markers that have positions confirmed in 'Wheat consensus map version 4.0' provided by Diversity Arrays Technology (2018). Linkage groups were created by a two-step process, in which linkage groups were identified in MSTmap (Wu et al. 2008), and marker order and distances within these groups were calculated in MapDisto 2.0 (Heffelfinger et al. 2017) using the Kosambi mapping function (Kosambi 1944). Refer to Section 2.2.2 for details relating to curation of genotypic data, and linkage map thresholds.

QTL analysis was performed using the least squares means of the trait measurement combined across environments, the least squares means of management system (conventional and organic), and each individual environment using QTL IciMapping v4.1 (Meng et al. 2015) and the Inclusive Composite Interval Mapping Additive (ICIM-ADD) model (Li et al. 2007). Logarithm of Odds threshold and walking step remain the same as Chapter Two (LOD = 2.5 and 1.0 cM step) and the genetic maps were drawn using MapChart v2.32 (Voorrips 2002). Putative QTL were labelled as per the *Recommended Rules for Gene Symbolization in Wheat* and begin with 'Q' (symbol for QTL), followed by the trait designator, laboratory designator (dms = Dean Michael Spaner), and the chromosome, respectively.

3.3 Results

3.3.1 Phenotype Assessment

The descriptive statistics of the least squares means for combined environments and management system are presented in Table 3.6.1 for both parents, and the RIL population. In the combined environments (two conventional and two organic) 'Carberry' began heading 3 days earlier than 'Peace', matured 1 day later, was 14 cm shorter, yielded 150 kg ha^{-1} less, had a test weight that was 0.9 kg hL^{-1} heavier, 1.3% less grain protein content, 1 gram heavier thousand kernel weight, and the same sedimentation measurement of 20 ml. The RIL population on average had a lodging score of two, began heading at 52 days, matured at 91 days, was 89 cm tall, yielded 4.28 ton ha^{-1}, had a test weight of 80.6 kg hL^{-1}, a protein content of 16.1%, a thousand kernel weight of 38.2 grams, and a sedimentation of 20 ml. Genetic correlations ranged from -0.68 (grain protein – grain yield) to 0.84 (heading – maturity), and phenotypic correlations ranged from -0.27 (test weight – lodging) to 0.68 (maturity – heading) (Table 3.6.2). Broad sense heritability was calculated on a line mean basis for each trait and ranged from 0.35 (lodging) to 0.78 (heading) in combined environments, 0.32 (lodging) to 0.82 (sedimentation) in conventional environments, and 0.40 (grain yield) to 0.80 (heading) in organic environments (Table 3.6.3).

3.3.2 QTL Analysis

The linkage map used in this study remains the same as Chapter Two (Section 2.3.2), which consisted of 8,413 polymorphic markers (approximately 23% of the original dataset), and the final population size used for analysis consisted of 204 individuals after four individuals were removed due to > 20% missing genotype data. Markers that exhibited sufficient linkage were

separated into the 21 chromosomes of *T. aestivum*, with a final genetic map length of 2,851 cM, and 4,439 markers (consisting of 1,015 SNPs and 3,424 DArTs). Only a single marker was kept if markers mapped to the same position. The average marker density across the entire linkage map was 1.6 markers per cM, with individual linkage groups ranging from 0.7 - 2.3 markers per cM (Table 3.6.4).

By using the inclusive composite interval mapping (ICIM) additive model and the least squares means of the combined environments, a total of thirty-eight QTL were identified: four being associated with lodging, three with heading, four with maturity, four with plant height, five with grain yield, five with test weight, four with grain protein, seven with thousand kernel weight, and two with sedimentation (Table 3.6.5; Figure 3.6.1). A detailed summary of the QTL identified in the combined environment is provided in Table 3.6.6, and the total phenotypic variance explained by the QTL identified in the combined analysis ranged from 28 to 72% depending on the trait (Table 3.6.7). ICIM was also conducted for each management system (conventional and organic) and each individual environment to provide insight into the stability of identified QTL, and a total of forty-seven QTL were identified in the conventional environment (Table 3.6.8), and forty-three in the organic environment (Table 3.6.9).

A total of four QTL were identified in combined environments that were associated with lodging (*QLdg.dms-4A*, *QLdg.dms-7A*, *QLdg.dms-3B*, and *QLdg.dms-4B*), and explained 28% of the total phenotypic variance. These QTL were located on chromosomes 4A, 7A, 3B, and 4B and each explained 10%, 6%, 5% and 8% of the phenotypic variance, respectively. Out of the four lodging QTL identified, none of these QTL were also found in the organic environment, but two were found in the conventional environment (*QLdg.dms-7A* and *QLdg.dms-3B*).

Three QTL associated with heading were identified in combined environments (*QHd.dms-1A*, *QHd.dms-5A*, and *QHd.dms-7D*), and explained 29% of the total phenotypic variance. These QTL were located on chromosomes 1A, 5A, and 7D and each explained 4%, 5% and 20% of the phenotypic variance, respectively. Of the three QTL associated with heading, one QTL (*QHd.dms-1A*) was also identified in the organic environment, and one QTL (*QHd.dms-7D*) was identified in both the conventional and organic environments.

Four QTL associated with maturity were identified in the combined analysis (*QMat.dms-3A*, *QMat.dms-4B*, *QMat.dms-5B*, and *QMat.dms-7D*) and explained a total of 40% of the total phenotypic variance. These QTL were located on chromosomes 3A, 4B, 5B, and 7D and each explained 6%, 15%, 10%, and 10% of the phenotypic variance, respectively. Three of these QTL (*QMat.dms-4B*, *QMat.dms-7D*, *QMat.dms-3A*) were also identified in both the conventional and organic environments.

Four QTL associated with plant height were identified in the combined analysis (*QPht.dms-5A*, *QPht.dms-4B*, *QPht.dms-7D.1*, and *QPht.dms-7D.2*) and explained a total of 72% of the phenotypic variance. These QTL were located on chromosomes 5A, 4B, and 7D, and each explained 4%, 53%, 11%, and 5% of the phenotypic variance, respectively. All four of the QTL associated with plant height were identified in both the conventional and organic environments.

Five QTL associated with grain yield were identified in the combined analysis (*QYld.dms-6A*, *QYld.dms-4B.1*, *QYld.dms-4B.2*, *QYld.dms-5B*, and *QYld.dms-7D*) and explained 28% of the total phenotypic variance. These QTL were located on chromosomes 6A, 4B, 5B, and 7D and each explained 3%, 5%, 5%, 8%, and 6% of the phenotypic variance, respectively. The two QTL on 4B, *QYld.dms-4B.1* and *QYld.dms-4B.2*, were identified in both the conventional

and organic environments, and one QTL was also identified in the organic environment (*QYld.dms-7D)*.

Five QTL associated with test weight were identified (*QTwt.dms-1A*, *QTwt.dms-3A*, *QTwt.dms-4B*, *QTwt.dms-6D*, and *QTwt.dms-7D*) and explained a total of 34% of the total phenotypic variance. These QTL were located on chromosomes 1A, 3A, 4B, 6D, and 7D and each explained 7%, 7%, 5%, 10% and 5% of the phenotypic variance, respectively. Two of the five QTL were identified in the conventional environment (*QTwt.dms-1A* and *QTwt.dms-3A*), two were identified in the organic environment (*QTwt.dms-4B* and *QTwt.dms-7D*), and one was identified in both the conventional and organic environments (*QTwt.dms-6D*).

Four QTL associated with grain protein content (GPC) were identified in the combined environments (*QGpc.dms-2A*, *QGpc.dms-3A*, *QGpc.dms-7A*, and *QGpc.dms-1B*) and explained 30% of the total phenotypic variance. These QTL were located on chromosomes 2A, 3A, 7A and 1B and each explained 5%, 6%, 9%, and 9% of the phenotypic variance, respectively. Two of the four GPC QTL were identified in both conventional and organic environments (*QGpc.dms-7A* and *QGpc.dms-1B*).

Seven QTL associated with thousand kernel weight (TKW) were identified in the combined environments (*QTkw.dms-5A*, *QTkw.dms-7A*, *QTkw.dms-4B*, *QTkw.dms-6B.1*, *QTkw.dms-6B.2*, *QTkw.dms-2D*, and *QTkw.dms-5D*) and explained a total of 41% of the phenotypic variance. These QTL were located on chromosomes 5A, 7A, 4B, 6B, 2D, and 5D and each explained 10%, 5%, 6%, 4%, 4%, 7%, and 4% of the phenotypic variance, respectively. Two of the seven QTL were identified in both the conventional and organic environments (*QTkw.dms-5A* and *QTkw.dms-4B*), four were identified in the organic environment (*QTkw.dms-*

6B.1, *QTkw.dms-6B.2*, *QTkw.dms-2D*, and *QTkw.dms-5D*), and one was identified in the conventional environment (*QTkw.dms-7A*).

Two QTL associated with sedimentation volume were identified in the combined environments (*QSds.dms-1A* and *QSds.dms-1B*) and explained 58% of the total phenotypic variance. These QTL were located on chromosomes 1A and 1B and each explained 41% and 17% of the phenotypic variance, respectively. *QSds.dms-1A* was identified in both the conventional and organic environments, and *QSds.dms-1B* was identified in the conventional environment.

3.4 Discussion

This study was conducted to identify QTL associated with agronomic traits that were stable across management-specific environments. We identified a total of thirty-eight QTL associated with nine agronomic traits, and sixteen of these QTL were consistently identified in both conventional and organic environments.

QTL associated with lodging are not frequently reported in the literature, which may be due to the low heritability of the trait and the significant role of the environment to induce lodging (such as wind and rain). However, McCartney et al. (2005) reported a heritability of 0.19 for lodging, and also identified a QTL on 4B associated with lodging, *QLd.crc-4B*, that explained 10% of the phenotypic variance. Lodging had a very low heritability estimate in our study as well, and across all environments (0.35 combined, 0.32 in the conventional, and 0.43 in the organic environment). The lodging QTL on 4B identified by McCartney et al. (2005) also mapped to the same genomic region as a QTL associated with plant height and test weight. In our study, we did identify a plant height QTL (*QPht.dms-4B*) that was 10 cM away from the

lodging QTL, *QLdg.dms-4B*, and a test weight QTL (*QTwt.dms-4B*) that was 7 cM away, and 'Carberry' was the contributor of all three alleles.

Due to the short growing season in western Canada (~95 to 125 days), wheat cultivars must be early maturing in order to minimize potential frost, drought, or heat damage, and successfully set seed (King and Heide 2009). The environmental adaptability of wheat is controlled by three gene groups (Snape et al. 2001): vernalization (*Vrn)*, photoperiod (*Ppd)* and earliness *per se* (*Eps*) genes, and this complex genetic network receives signals from the environment such as temperature and light for the downregulation and upregulation of these genes (Distelfeld et al. 2009). In our combined analysis, several QTL clustered near the heading QTL, *QHd.dms-7D*, and were associated with maturity, plant height, grain yield, and test weight; this clustering also occurred in the organic environment, and to a lesser extent in the conventional environment (no QTL associated with grain yield and test weight were identified on 7D). QTL clustering associated with heading, maturity, grain yield, and thousand kernel weight was also identified by Tahmasebi et al. (2017) on chromosome 7D. Additionally, QTL associated with heading on 7D, 1A, and 5A have been reported by others (Bennett et al. 2012b; Lopes et al. 2013; Fowler et al. 2016).

VRN-B3 has been mapped to the short arm of chromosome 7B (Chao et al. 1989; Yan et al. 2006), but the heading (*QHd.dms-7D*) and maturity QTL (*QMat.dms-7D*) we identified were located on 7D. This region on 7D appears to be associated with earliness and reduction in plant height (*QPht.dms-7D.1*) and lower grain yield (*QYld.dms-7D*). The maturity QTL on 4B, *QMat.dms-4B*, co-localized with the plant height QTL, *QPht.dms-4B*, and grain yield QTL *QYld.dms-4B.1* (in the conventional and organic environments), and also increased days to maturity by two days. 'Peace' was the contributor of the allele on 7D associated with reduced

maturity, and the height reduction allele on 4B was contributed by 'Carberry', but even though the QTL co-located for the same traits (maturity, height and grain yield), the 'Peace' allele reduced height and days to maturity at the expense of yield, while the 'Carberry' allele reduced height and increased the days to maturity with an increase in grain yield. The positive relationship between days to maturity and grain yield has been previously reported in spring wheat (Iqbal et al. 2007d). Several studies have identified QTL associated with maturity on the same chromosomes as our study, including 7D (McCartney et al. 2005; Carter et al. 2011; Tahmasebi et al. 2017), 4B (Asif et al. 2015; Zou et al. 2017a, 2017b), 5B (Asif et al. 2015; Zou et al. 2017b), and 3A (Tahmasebi et al. 2017).

One of the parents of the RIL population used in this study, 'Carberry', is a semi-dwarf cultivar, and therefore we expected this population to segregate for height. Previously, Chen et al. (2016) screened spring wheat cultivars, including 'Carberry', and confirmed the presence of the height reducing mutant allele *Rht-B1b*. In our study we have identified the plant height QTL, *QPht.dms-4B*, to be the *Rht-B1b* gene due to its large effect, and location on 4BS (Sourdille et al. 1998). This QTL is flanked by markers 4B_s4991673 and 4B_d1258252, and to the author's knowledge, are the first reported DArTseq markers to be identified in the Canadian literature associated with *Rht-B1b*. Several other studies have reported plant height QTL on chromosome 4B (McCartney et al. 2005; Asif et al. 2015; Singh et al. 2016; Tahmasebi et al. 2017; Yu et al. 2017; Zou et al. 2017a, 2017b; Cabral et al. 2018), and to a lesser extent on 5A (Zou et al. 2017b) and 7D (Tahmasebi et al. 2017).

Grain yield and GPC are important traits for successful CWRS cultivars because producers desire high-yielding cultivars, and export markets demand high protein content, which provides producers with a premium price. We reported a genetic correlation of -0.68 between

grain yield and GPC in the present study, which is very similar to the correlation of -0.63 reported by others (Asif et al. 2015). The grain yield QTL we identified in our combined analysis were all relatively minor, and each explained less than 10% of phenotypic variance. Minor effect grain yield QTL in wheat are frequently reported in the literature (McCartney et al. 2005; Cuthbert et al. 2008; Zou et al. 2017a). We did not identify any grain yield QTL with moderate effects (greater than 10%), unlike some studies (Carter et al. 2011; Fowler et al. 2016). The identification of stable and consistent grain yield QTL is critical, because these QTL are heavily affected by the environment, and many QTL associated with grain yield are due to the genotype being adapted to the environment (Carter et al. 2011). We identified only one grain yield QTL (*QYld.dms-4B.1*) that was consistent across the combined, conventional and organic environments, but it was present in only one of the two respective individual environments. Many studies also report environment-specific grain yield QTL (Cuthbert et al. 2008; Carter et al. 2011; Zou et al. 2017b). QTL associated with grain yield have been reported on chromosomes 5B (Perez-Lara et al. 2016; Assanga et al. 2017; Zou et al. 2017a; Liu et al. 2018), 6A (Asif et al. 2015; Fowler et al. 2016; Assanga et al. 2017), and 7D (Tahmasebi et al. 2017; Zou et al. 2017b), but further testing in different environments is necessary to confirm the stability of any of the grain yield QTL we identified.

Three of the four GPC QTL we identified were contributed by 'Peace' and reduced the grain protein content by 0.4 to 0.5% but did not co-locate with any other QTL. Co-location of QTL including GPC has been reported in some studies (Zou et al. 2017b), but not in others (Zou et al. 2017a). QTL associated with GPC have been identified on chromosomes 3A (Fowler et al. 2016; Zou et al. 2017b) and 7A (Zou et al. 2017a), but a review of the literature suggests that QTL analysis on GPC has not been conducted in many studies. The results of this study suggest

that ‘Peace’ may possess several alleles that have a detrimental effect on protein content with no recognizable benefit.

In this study, we identified management-specific QTL associated with test weight, but to the author’s knowledge, only one other similar study exists to draw comparisons. Asif et al. (2015) identified a test weight QTL on 1B in both conventional and organic environments, but a QTL on 1A was only identified in the conventional environment. In our study, *QTwt.dms-7D* was identified in the organic environment, and *QTwt.dms-3A* was identified in the conventional environment. Test weight QTL have been located on all the same chromosomes as we found, except for 6D (McCartney et al. 2005; Asif et al. 2015; Zou et al. 2017a).

QTL associated with TKW have been identified on all the chromosomes we identified in the combined analysis, including 5A (Cuthbert et al. 2008; Fowler et al. 2016; Assanga et al. 2017; Zou et al. 2017b), 7A (Cuthbert et al. 2008; Raman et al. 2009; Asif et al. 2015; Cabral et al. 2018), 6B (Raman et al. 2009; Assanga et al. 2017; Zou et al. 2017a), 2D (Cuthbert et al. 2008; Liu et al. 2018; Cabral et al. 2018), 4B (McCartney et al. 2005; Yu et al. 2017), and 5D (Liu et al. 2018). In our study, TKW had moderate genetic correlations with plant height, grain yield, and test weight (0.33, 0.39, and 0.37, respectively), and the TKW QTL we identified (*QTkw.dms-4B*) mapped near the QTL cluster on 4B that was associated with several traits including plant height, grain yield and test weight. McCartney et al. (2005) identified a cluster of QTL on 4D that were associated with several agronomic traits including TKW, and Cuthbert et al. (2008) identified a similar cluster on 5A. These clusters of QTL are attractive regions for future research, as their reliability is confirmed partially by the genetic correlation of traits, and they have the prospect of a single allele affecting several agronomic traits important for Canadian wheat breeders.

The sodium dodecyl sulfate (SDS) sedimentation test provides a reliable indication of wheat gluten strength, but very few studies have conducted QTL analysis associated with this measure of gluten. The two sedimentation QTL we identified, *QSds.dms-1A* and *QSds.dms-1B*, both had a large effect on gluten strength, and are, therefore, important alleles for future CWRS cultivars. This is one of the very few studies to provide analysis on this important trait, but QTL associated with sedimentation have been reported on chromosome 1A previously (Chen et al. 2015; Yu et al. 2017). Chen et al. (2015) reported their QTL, *QSed.dms-1A*, at 18.83 cM and explaining 24% of the phenotypic variance in their overall analysis, Yu et al. (2017) reported their QTL, *Qsed.swust-1A.1*, at 46 cM explaining 38% of the phenotypic variance in their overall analysis. Despite few in number, the mentioned studies reporting QTL for sedimentation have strong similarities to our results on 1A, as we identified our QTL at 3 cM, and it explained 41% of the phenotypic variance. The *Glu-1* genes (high-molecular weight glutenin subunits) are responsible for glutenin protein in wheat and are located on the long arms of chromosome 1A, 1B, and 1D. Therefore, the positioning of *QSds.dms-1A* does not correspond with *Glu-A1*, but in our study, *QSds.dms-1B* was identified at 96 cM on 1B, which is positioned at the expected location of *Glu-B1*. Additional storage protein genes *Gli-A1*, *Gli-B1*, and *Gli-D1* are located on the short arms of 1A, 1B, and 1D (Payne et al. 1984), and correspond to the location of our QTL on 1A. Diagnostic DNA markers exist for quality traits including protein storage genes (Gale 2005), but a review of the literature suggests that these genes are largely neglected in QTL analysis, and therefore further research using this population would provide much needed insight and knowledge towards this important quality trait.

Organic farming is a low-input system where producers have limited management options compared to conventional systems that rely heavily on synthetic and chemical inputs. It

has been suggested that crops must possess different archetypes for success in organic systems, and characteristics such as weed competition, disease resistance, tolerance to mechanical weed control, and efficient nutrient use are important considerations (Wolfe et al. 2008; Van Bueren et al. 2011). Furthermore, the lack of cultivars adapted to organic environments is considered one of the reasons of lower yields in organic agriculture (Yousef et al. 2015). The literature suggests there are indeed characteristics that improve the yield of crops grown in organic systems, and organic environments have increased diversity and richness in soil microbial communities (Nelson et al. 2011), but the effects of management system on agronomic traits of wheat remains less clear, and conflicting. Earliness has been identified as an important trait in spring wheat to increase organic yields (Mason et al. 2007), but Kamran et al. (2014c) did not find early maturity to improve yield. Mason et al. (2006) reported no difference in the grain protein content of spring wheat between management systems, but Nelson et al. (2011) found that organically managed spring wheat had an average 1.3% increase. Not all traits have management-specific effects, as Mikó et al. (2014) identified only seven of fifteen traits in winter wheat with significant genotype by management interactions. Kamran et al. (2014c) reported three of nine traits in spring wheat had significant genotype by management interactions, including grain yield, and grain protein content, but Mikó et al. (2014) did not find grain protein content to have a significant interaction.

To the author's knowledge, only one study has been published that has identified management-specific QTL in organic and conventional systems (Asif et al. 2015). They identified consistent QTL under organic and conventional systems but did not combine data from the two systems for QTL analysis. We conducted QTL analysis using the least squares means of all environments, in addition to management-specific QTL analysis and have identified several agronomic QTL that were stable and consistent across both systems. QTL mapping using diverse

environments has been previously conducted under drought and heat stress (Lopes et al. 2013; Tahmasebi et al. 2017). Management-specific QTL were to be expected and were identified in this study for all traits, but plant height was notably consistent across environments, and all four of the plant height QTL identified in the combined analysis were also found in the management-specific analysis. Additionally, QTL were found on 7D that were consistent across organic and conventional environments and were associated with earlier heading (*QHd.dms-7D*) and maturity (*QMat.dms-7D*), and reduced plant height (*QPht.dms-7D.1*). 'Peace' was the parental source of these stable QTL, and they were found in all four of the individual environments. 'Carberry' was the parental source of a QTL on 4B that was consistently identified across environments and is most likely the *Rht-B1b* height reducing gene. This allele reduced height (*QPht.dms-4B*), and increased days to maturity (*QMat.dms-4B*), grain yield (*QYld.dms-4B.1*), and test weight (*QTwt.dms-4B*). The plant height and maturity QTL were consistently expressed in all individual environments, but the grain yield and test weight QTL were not.

The heritability of traits differed by management system, as reported by others (Asif et al. 2015). The estimates were higher in the conventional environments for all traits except lodging (0.32 versus 0.43), heading (0.75 versus 0.80), and plant height (0.73 versus 0.76). In the organic environments, the RIL population had an average lodging score of two (versus three in the conventional environments), matured seven days earlier, was 6 centimetres shorter and TKW was 0.7 grams heavier. On average, the conventional environments yielded 1.3 t/ha greater, test weight was 0.8 kg/hL heavier, protein content was 1.5% and the measure of gluten strength was one millilitre greater.

Generally, large effect QTL have been the focus of plant breeders due to inconsistency across environments (Bernardo 2008), but in our study, the effects of the QTL on 7D were not

large, but they were still consistently identified. A total of thirty-eight QTL were identified in the combined analysis, but sixteen of these QTL were also identified in both management-specific environments. All traits except lodging were represented in these sixteen QTL, but the number of QTL per trait differed and ranged from one to four. One QTL each for heading (*QHd.dms-7D*), test weight (*QTwt.dms-6D*), and gluten strength (*QSds.dms-1A*); two QTL each for grain yield (*QYld.dms-4B.1*, *QYld.dms-4B.2*), grain protein content (*QGpc.dms-7A*, *QGpc.dms-1B*), and thousand kernel weight (*QTkw.dms-5A*, *QTkw.dms-4B*); three QTL for maturity (*QMat.dms-3A*, *QMat.dms-4B*, *QMat.dms-7D*), and four QTL for plant height (*QPht.dms-5A*, *QPht.dms-4B*, *QPht.dms-7D.1*, *QPht.dms-7D.2*) were identified across management systems. The LOD scores of these consistent QTL ranged from 3.4 to 45.7 and the phenotypic variance explained ranged from 4% to 53%.

These results suggest that QTL stability across diverse environments do not require large effects, and minor QTL may show stability as markers. Additional research is necessary to understand why some, but not all QTL, are consistently identified, and if some traits (such as heading, maturity and plant height) have a predisposition to becoming stable and reliable QTL.

3.5 Conclusion

CWRS is the most important class of wheat in western Canada, and breeders must consider several important agronomic and quality traits as they work to improve future cultivars. We identified thirty-nine QTL across nine traits in the combined analysis, and we also identified sixteen QTL that were consistently identified across conventional and organic environments. The largest QTL we identified was associated with plant height (*QPht.dms-4B*) and is most likely the plant height reducing gene, *Rht-B1b,* as it explained 53% of the phenotypic variance, and the

allele was contributed by ‘Carberry’. The second largest QTL was associated with sedimentation volume (*QSds.dms-1A*) and explained 41% of the phenotypic variance. QTL clusters were found on 4B at ~ 44 cM, and 7D at ~ 11 cM and associated with several agronomic traits including heading, maturity, height, grain yield, and test weight. Consistent QTL were identified across both organic and conventional environments, even though they were minor effect QTL with LOD scores as low as 3.4. Consistent QTL were found for all traits except lodging, and these results provide evidence that minor effect QTL may be as consistent as large effect QTL. Additionally, the large effect QTL associated with sedimentation volume on 1A was identified in all environments, and previously published studies collaborate our results, but further research is necessary to confirm that it is the *Gli-1* protein storage gene. Additional field experiments should be conducted using this population, as it segregates for traits of importance, such as earliness, and the QTL on 7D was consistently identified across diverse environments and may be a useful marker for selection.

3.6 Tables and Figures

Table 3.6.1a. Summary of descriptive statistics of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population using the least squares means of the combined environments.

Trait	Parental Descriptive Statistics		RIL Population Descriptive Statistics					F Statistics	
	'Peace'	*'Carberry'*	*Min*	*Max*	*Mean*	*SD*	*CV (%)*	*F-Value*	*Significance*
Lodging	3	2	1	6	2	0.7	28	4.2	<.0001
Heading (days)	53	50	46	66	52	3.1	6	25.6	<.0001
Maturity (days)	91	92	85	104	91	3.4	4	12.7	<.0001
Height (cm)	96	82	69	111	89	8.6	10	19.8	<.0001
Yield (t ha^{-1})	4.8	4.6	1.81	5.54	4.28	0.7	16	8.5	<.0001
TWt (kg hL^{-1})	81.0	81.9	74.6	83.0	80.6	1.4	2	14.3	<.0001
Protein (%)	16.2	15.5	13.1	19.8	16.1	1.0	6	7.5	<.0001
TKW (g)	38.3	39.3	31.6	46.5	38.2	2.6	7	18.4	<.0001
SDS (ml)	20	20	15	27	20	2.3	12	18.0	<.0001

Min: Minimum; Max: Maximum; SD: Standard Deviation; CV: Coefficient of Variation; TWt: Test Weight; TKW: Thousand Kernel Weight; SDS: Sedimentation.

Table 3.6.1b. Summary of descriptive statistics of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population using the least squares means of the conventional environments.

Trait	Parental Descriptive Statistics		RIL Population Descriptive Statistics					F Statistics	
	'Peace'	*'Carberry'*	*Min*	*Max*	*Mean*	*SD*	*CV (%)*	*F-Value*	*Significance*
Lodging	3	2	2	7	3	0.9	30	2.4	<.0001
Heading (days)	53	50	46	67	52	3.2	6	10.7	<.0001
Maturity (days)	94	97	88	111	95	3.7	4	9.0	<.0001
Height (cm)	101	84	69	117	92	9.0	10	8.2	<.0001
Yield (t ha^{-1})	5.3	5.3	1.8	6.5	4.9	0.8	16	7.9	<.0001
TWt (kg hL^{-1})	81.6	82.7	73.3	83.7	81.2	1.5	2	9.3	<.0001
Protein (%)	17.1	15.9	14.2	20.4	16.8	1.0	6	10.9	<.0001
TKW (g)	38.0	38.4	30.3	46.9	37.9	2.8	7	10.5	<.0001
SDS (ml)	20	21	15	29	20	2.5	12	14.6	<.0001

Min: Minimum; Max: Maximum; SD: Standard Deviation; CV: Coefficient of Variation; TWt: Test Weight; TKW: Thousand Kernel Weight; SDS: Sedimentation.

Table 3.6.1c. Summary of descriptive statistics of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population using the least squares means of the organic environments.

Trait	Parental Descriptive Statistics		RIL Population Descriptive Statistics					F Statistics	
	'Peace'	*'Carberry'*	*Min*	*Max*	*Mean*	*SD*	*CV (%)*	*F-Value*	*Significance*
Lodging	2	1	1	5	2	0.6	31	3.6	<.0001
Heading (days)	53	50	45	65	52	3.0	6	15.7	<.0001
Maturity (days)	87	88	81	99	88	3.3	4	4.8	<.0001
Height (cm)	91	79	65	106	86	8.6	10	12.0	<.0001
Yield (t ha^{-1})	4.2	3.9	1.5	5.2	3.6	0.7	18	3.2	<.0001
TWt (kg hL^{-1})	80.4	81.2	75.3	82.8	80.0	1.4	2	6.9	<.0001
Protein (%)	15.3	15.0	12.1	19.3	15.3	1.1	7	3.2	<.0001
TKW (g)	38.6	40.3	32.6	46.2	38.6	2.6	7	12.3	<.0001
SDS (ml)	19	20	14	25	19	2.3	12	7.3	<.0001

Min: Minimum; Max: Maximum; SD: Standard Deviation; CV: Coefficient of Variation; TWt: Test Weight; TKW: Thousand Kernel Weight; SDS: Sedimentation.

Table 3.6.2. Genetic (above diagonal) and phenotypic (below diagonal) correlation of agronomic and quality traits in the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population.

	Ldg	**Head**	**Mat**	**PHt**	**GY**	**TWt**	**GPC**	**TKW**	**SDS**
Ldg	-	0.25*	0.12	0.23*	-0.44*	-0.32*	0.07	-0.25*	-0.03
Head	0.14*	-	0.84*	0.21*	-0.13	-0.25*	0.07	-0.03	-0.02
Mat	0.09	0.68*	-	-0.12	-0.08*	-0.18*	-0.06	-0.04	-0.13
PHt	0.16*	0.16*	-0.10	-	0.14*	0.38*	0.15*	0.33*	-0.02
GY	-0.23*	-0.14	-0.04	0.18*	-	0.70*	-0.68*	0.39*	-0.14*
TWt	-0.27*	-0.20*	-0.18*	0.29*	0.55*	-	-0.26*	0.37*	-0.12
GPC	0.11	0.04	0.10	0.12	-0.21*	-0.21*	-	0.03	0.16*
TKW	-0.21*	-0.06	-0.04	0.26*	0.39*	0.36*	0.10	-	0.09
SDS	0.01	-0.05	-0.07	-0.01	-0.03	-0.11	0.30*	0.09	-

* Statistically Significant ($\propto = 0.05$)

Ldg: Lodging; Head: Heading; Mat: Maturity; PHt: Plant Height, GY: Grain Yield; TWt: Test Weight; GPC: Grain Protein Content; TKW: Thousand Kernel Weight; SDS: Sedimentation.

Table 3.6.3. Broad-sense heritability estimates on a line-mean basis of agronomic and quality traits of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population.

Combined Environments

Trait	Heritability	Standard Error
Lodging	0.35	0.03
Heading	0.78	0.02
Maturity	0.64	0.03
Plant Height	0.75	0.02
Grain Yield	0.54	0.03
Test Weight	0.71	0.02
Grain Protein	0.48	0.03
TKW	0.74	0.02
Sedimentation	0.72	0.02

Conventional Environments

Trait	Heritability	Standard Error
Lodging	0.32	0.05
Heading	0.75	0.03
Maturity	0.68	0.03
Plant Height	0.73	0.03
Grain Yield	0.69	0.03
Test Weight	0.75	0.03
Grain Protein	0.77	0.02
TKW	0.77	0.02
Sedimentation	0.82	0.02

Organic Environments

Trait	Heritability	Standard Error
Lodging	0.43	0.04
Heading	0.80	0.02
Maturity	0.57	0.04
Plant Height	0.76	0.02
Grain Yield	0.40	0.04
Test Weight	0.67	0.03
Grain Protein	0.37	0.04
TKW	0.76	0.02
Sedimentation	0.63	0.03

TKW: Thousand Kernel Weight.

Table 3.6.4. Summary of DArT and single nucleotide polymorphism (SNP) markers used in the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population. The average marker density across the genetic map is 1.6 per cM (centimorgan).

Chromosome	Linkage Map (cM)	Total Markers	Markers / cM
1A	132	268	*2.0*
2A	129	183	*1.4*
3A	158	306	*1.9*
4A	129	182	*1.4*
5A	140	156	*1.1*
6A	101	214	*2.1*
7A	219	416	*1.9*
Sub-Genome A	1008	1725	
1B	145	339	*2.3*
2B	189	412	*2.2*
3B	202	239	*1.2*
4B	104	143	*1.4*
5B	214	450	*2.1*
6B	177	345	*1.9*
7B	149	200	*1.3*
Sub-Genome B	1180	2128	
1D	97	73	*0.8*
2D	89	99	*1.1*
3D	139	118	*0.8*
4D	27	24	*0.9*
5D	71	66	*0.9*
6D	105	107	*1.0*
7D	135	99	*0.7*
Sub-Genome D	663	586	
Total cM	2851		
Total Markers	4439		

Table 3.6.5. Summary of quantitative trait loci (QTL) identified in each environment of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population.

Environment	Total QTL	Number of QTL Identified								
		Lodging	*Heading*	*Maturity*	*Plant Height*	*Grain Yield*	*Test Weight*	*Grain Protein*	*TKW*	*Sedimentation*
Combined	38	4	3	4	4	5	5	4	7	2
Conventional	47	4	2	6	5	4	6	12	6	2
Conventional 2016	37	5	2	6	5	3	3	5	5	3
Conventional 2017	41	4	4	5	4	5	5	4	7	3
Organic	43	4	3	5	6	5	6	6	7	1
Organic 2016	33	2	2	7	3	4	3	7	4	1
Organic 2017	34	3	3	3	5	2	5	5	5	3

TKW: Thousand Kernel Weight.

Table 3.6.6. Detailed summary of quantitative trait loci (QTL) identified in the combined environment analysis of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population. Flanking markers are identified by the "Clone ID" provided by Diversity Arrays Technology. (d) and (s) in marker names designate a DArT or SNP marker, respectively. Difference is calculated using the mean disease score difference between flanking markers that have the same parental allele at both markers (AA – BB).

Trait	QTL	Chr	Pos (cM)	C.I. (cM)	Left Marker	Right Marker	LOD	R^2 (%)	Add Effect	Diff	Parental Allele Reducing Score
Lodging	*QLdg.dms-4A*	4A	76	75.5 - 76.5	4A_d3533210	4A_d1094320	5.8	10	0.2	0.3	Carberry
Lodging	*QLdg.dms-7A*	7A	46	44.5 - 47.5	7A_d4733607	7A_d2372979	3.3	6	-0.1	-0.1	Peace
Lodging	*QLdg.dms-3B*	3B	112	111.5 - 112.5	3B_s1008939	3B_d1023777	2.9	5	-0.1	-0.3	Peace
Lodging	*QLdg.dms-4B*	4B	54	53.5 - 54.5	4B_d1254295	4B_d4405282	4.6	8	0.1	0.4	Carberry
Heading	*QHd.dms-1A*	1A	122	121.5 - 123.5	1A_d1125646	1A_d1009935	2.8	4	0.4	0.7	Carberry
Heading	*QHd.dms-5A*	5A	106	105.5 - 106.5	5A_d1101279	5A_d3384817	3.3	5	0.5	1.5	Carberry
Heading	*QHd.dms-7D*	7D	13	11.5 - 14.5	7D_s1047233	7D_s1022494	12.8	20	-1.0	-2.2	Peace
Maturity	*QMat.dms-3A*	3A	56	55.5 - 57.5	3A_d1096296	3A_d1036906	3.8	6	0.7	1.2	Carberry
Maturity	*QMat.dms-4B*	4B	44	43.5 - 45.5	4B_s4991673	4B_d1258252	9.9	15	-1.1	-2.3	Peace
Maturity	*QMat.dms-5B*	5B	122	121.5 - 122.5	5B_s3029177	5B_d1112858	6.8	10	0.9	1.9	Carberry
Maturity	*QMat.dms-7D*	7D	12	11.5 - 12.5	7D_d1105401	7D_s1047233	6.9	10	-0.9	-2.2	Peace
Plant Height	*QPht.dms-5A*	5A	84	83.5 - 84.5	5A_d1100228	5A_d1108380	5.3	4	1.5	4.5	Carberry
Plant Height	*QPht.dms-4B*	4B	44	42.5 - 44.5	4B_s4991673	4B_d1258252	45.7	53	5.8	12.9	Carberry
Plant Height	*QPht.dms-7D.1*	7D	12	11.5 - 12.5	7D_d1105401	7D_s1047233	14.4	11	-2.6	-6.2	Peace
Plant Height	*QPht.dms-7D.2*	7D	56	55.5 - 56.5	7D_d1049127	7D_d2303374	6.7	5	-1.8	-2.6	Peace
Grain Yield	*QYld.dms-6A*	6A	79	78.5 - 79.5	6A_d4992823	6A_d4395023	2.5	3	0.1	0.2	Carberry
Grain Yield	*QYld.dms-4B.1*	4B	49	48.5 - 50.5	4B_d1083787	4B_d3534171	4.0	5	-0.1	-0.2	Peace

Trait	QTL	Chr	Pos (cM)	C.I. (cM)	Left Marker	Right Marker	LOD	R^2 (%)	Add Effect	Diff	Parental Allele Reducing Score
Grain Yield	*QYld.dms-4B.2*	4B	102	101.5 - 103	4B_d4404439	4B_d1113608	3.4	5	0.1	0.3	Carberry
Grain Yield	*QYld.dms-5B*	5B	159	158.5 - 159.5	5B_s1205825	5B_d1256946	6.0	8	-0.2	-0.3	Peace
Grain Yield	*QYld.dms-7D*	7D	11	8.5 - 11.5	7D_s1215756	7D_d4262395	4.4	6	-0.1	-0.3	Peace
Test Weight	*QTwt.dms-1A*	1A	130	129.5 - 131	1A_d1119273	1A_d1105551	4.1	7	-0.3	-0.8	Peace
Test Weight	*QTwt.dms-3A*	3A	48	46.5 - 50.5	3A_d1104125	3A_d3026062	4.4	7	0.3	0.6	Carberry
Test Weight	*QTwt.dms-4B*	4B	47	45.5 - 47.5	4B_d3935712	4B_d1048997	3.2	5	0.3	0.5	Carberry
Test Weight	*QTwt.dms-6D*	6D	27	26.5 - 27.5	6D_s1091343	6D_s1085677	6.4	10	-0.4	-0.7	Peace
Test Weight	*QTwt.dms-7D*	7D	6	4.5 - 6.5	7D_d1119043	7D_d3935878	3.0	5	-0.2	-0.4	Peace
Grain Protein	*QGpc.dms-2A*	2A	30	26.5 - 32.5	2A_s1053961	2A_d1400152	2.9	5	0.2	0.4	Carberry
Grain Protein	*QGpc.dms-3A*	3A	115	114.5 - 115.5	3A_d1130719	3A_d1117269	3.6	6	-0.2	-0.5	Peace
Grain Protein	*QGpc.dms-7A*	7A	197	196.5 - 197.5	7A_d1101008	7A_d1092869	5.4	9	-0.3	-0.4	Peace
Grain Protein	*QGpc.dms-1B*	1B	115	114.5 - 115.5	1B_d1672015	1B_d1095529	5.3	9	-0.3	-0.4	Peace
TKW	*QTkw.dms-5A*	5A	138	137.5 - 140	5A_d4542591	5A_d993093	6.4	10	-0.8	-1.6	Peace
TKW	*QTkw.dms-7A*	7A	218	217.5 - 218	7A_d1114756	7A_d1109546	3.1	5	-0.5	-1.6	Peace
TKW	*QTkw.dms-4B*	4B	69	68.5 - 69.5	4B_s1094836	4B_d3534297	4.5	6	0.6	1.4	Carberry
TKW	*QTkw.dms-6B.1*	6B	16	15.5 - 16.5	6B_d1126615	6B_d1103792	2.8	4	-0.5	-1.4	Peace
TKW	*QTkw.dms-6B.2*	6B	147	145.5 - 149.5	6B_d989571	6B_d1094956	2.7	4	0.5	1	Carberry
TKW	*QTkw.dms-2D*	2D	62	60.5 - 67.5	2D_d1134631	2D_d1109826	4.4	7	-0.7	-1.1	Peace
TKW	*QTkw.dms-5D*	5D	45	43.5 - 45.5	5D_d4539071	5D_d2244762	3.0	4	0.5	0.5	Carberry
SDS	*QSds.dms-1A*	1A	3	2.5 - 3.5	1A_d1077302	1A_s1106928	34.1	41	-1.5	-3	Peace
SDS	*QSds.dms-1B*	1B	96	95.5 - 96.5	1B_d5970172	1B_s1110494	17.2	17	0.9	-1.9	Carberry

Chr: Chromosome; Pos: Position; C.I.: Confidence Interval; LOD: Logarithm of Odds; Add Effect: Additive Effect; Diff: Difference; TKW: Thousand Kernel Weight; SDS: Sedimentation.

Table 3.6.7. Summary of quantitative trait loci (QTL) identified in the combined environment analysis of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population. Broad-sense heritability was calculated on a line-mean basis. The total phenotypic variance was calculated using the sum of individual QTL phenotypic variances.

Trait	Heritability	No. of QTL Identified	Total phenotypic variance explained (%)
Lodging	0.35	4	28
Heading	0.78	3	29
Maturity	0.64	4	40
Plant Height	0.75	4	72
Grain Yield	0.54	5	28
Test Weight	0.71	5	34
Grain Protein	0.48	4	30
TKW	0.74	7	41
Sedimentation	0.72	2	58

TKW: Thousand Kernel Weight.

Table 3.6.8. Detailed summary of quantitative trait loci (QTL) identified in the conventional environment analysis of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population. Flanking markers are identified by the "Clone ID" provided by Diversity Arrays Technology. (d) and (s) in marker names designate a DArT or SNP marker, respectively. Difference is calculated using the mean disease score difference between flanking markers that have the same parental allele at both markers (AA – BB).

Trait	Chr	Pos (cM)	C.I. (cM)	Left Marker	Right Marker	LOD	R^2 (%)	Add Effect	Individual Env
Lodging	3A	39	38.5 - 39.5	3A_d1235934	3A_s1111138	2.7	4	0.2	1/2
Lodging	4A	76	75.5 - 76.5	4A_d3533210	4A_d1094320	6.0	10	0.2	0/2
Lodging	7A	46	44.5 - 47.5	7A_d4733607	7A_d2372979	4.3	7	-0.2	0/2
Lodging	3B	112	111.5 - 112.5	3B_s1008939	3B_d1023777	4.8	8	-0.2	0/2
Heading	5B	122	121.5 - 122.5	5B_s3029177	5B_d1112858	14.0	21	1.1	1/2
Heading	7D	13	11.5 - 13.5	7D_s1047233	7D_s1022494	13.0	20	-1.1	2/2
Maturity	3A	56	55.5 - 57.5	3A_d1096296	3A_d1036906	3.4	2	0.7	0/2
Maturity	4A	21	20.5 - 21.5	4A_d1230298	4A_d4543987	3.9	3	-0.7	0/2
Maturity	5A	132	129.5 - 137.5	5A_d2258770	5A_d4542591	2.7	2	-0.6	0/2
Maturity	4B	44	43.5 - 45.5	4B_s4991673	4B_d1258252	9.9	7	-1.2	2/2
Maturity	5B	118	117.5 - 118.5	5B_s1125898	5B_d3959851	25.0	21	-2.1	1/2
Maturity	7D	13	11.5 - 14.5	7D_s1047233	7D_s1022494	12.1	9	-1.4	2/2
Plant Height	5A	84	83.5 - 84.5	5A_d1100228	5A_d1108380	4.0	3	1.4	0/2
Plant Height	4B	43	42.5 - 44.5	4B_d3384830	4B_s4991673	45.4	54	6.3	2/2
Plant Height	1D	66	65.5 - 66.5	1D_d1111633	1D_s3021667	3.0	2	-1.2	0/2
Plant Height	7D	12	11.5 - 12.5	7D_d1105401	7D_s1047233	13.9	10	-2.8	2/2
Plant Height	7D	54	52.5 - 54.5	7D_d1108030	7D_d994906	4.9	3	-1.6	2/2

Trait	Chr	Pos (cM)	C.I. (cM)	Left Marker	Right Marker	LOD	R^2 (%)	Add Effect	Individual Env
Grain Yield	7A	197	196.5 - 197.5	7A_d1101008	7A_d1092869	4.7	7	0.2	1/2
Grain Yield	4B	43	41.5 - 44.5	4B_d3384830	4B_s4991673	4.7	8	-0.2	0/2
Grain Yield	4B	102	101.5 - 103	4B_d4404439	4B_d1113608	5.3	8	0.2	1/2
Grain Yield	6D	34	33.5 - 34.5	6D_d1241743	6D_d4439991	2.8	4	-0.1	0/2
Test Weight	1A	50	49.5 - 50.5	1A_d1125251	1A_d1113511	3.3	4	0.2	1/2
Test Weight	1A	127	126.5 - 127.5	1A_d3024089	1A_d1117369	6.5	10	-0.4	1/2
Test Weight	3A	48	46.5 - 49.5	3A_d1104125	3A_d3026062	5.3	8	0.3	1/2
Test Weight	5A	8	7.5 - 10.5	5A_d1108615	5A_d4008619	3.1	4	-0.2	1/2
Test Weight	4B	102	101.5 - 103	4B_d4404439	4B_d1113608	4.2	6	0.3	1/2
Test Weight	6D	27	26.5 - 27.5	6D_s1091343	6D_s1085677	5.7	8	-0.3	1/2
Grain Protein	1A	36	35.5 - 36.5	1A_d1138691	1A_d3938417	7.8	4	-0.2	0/2
Grain Protein	2A	54	53.5 - 54.5	2A_s993063	2A_d3935452	8.4	4	0.2	0/2
Grain Protein	3A	14	13.5 - 14.5	3A_d2289860	3A_d3949168	7.4	4	-0.2	0/2
Grain Protein	6A	79	78.5 - 79.5	6A_d4992823	6A_d4395023	31.8	20	-0.5	0/2
Grain Protein	7A	194	193.5 - 194.5	7A_s1213119	7A_s1090541	5.2	3	-0.2	0/2
Grain Protein	1B	114	113.5 - 114.5	1B_s4910661	1B_d1672015	6.4	3	-0.2	0/2
Grain Protein	2B	142	141.5 - 143.5	2B_d1094913	2B_d3533636	12.9	7	-0.3	0/2
Grain Protein	4B	44	42.5 - 44.5	4B_s4991673	4B_d1258252	11.8	6	0.3	2/2
Grain Protein	3D	77	72.5 - 80.5	3D_d1050189	3D_d2270346	5.3	3	-0.2	0/2
Grain Protein	3D	116	113.5 - 116.5	3D_d1205852	3D_d1212202	4.9	2	0.2	0/2
Grain Protein	6D	30	29.5 - 31.5	6D_d1160681	6D_s994751	6.6	3	0.2	1/2
Grain Protein	6D	104	103.5 - 104	6D_d1248579	6D_d1102905	4.9	3	0.2	0/2
TKW	5A	138	137.5 - 140	5A_d4542591	5A_d993093	5.3	9	-0.8	2/2
TKW	7A	165	164.5 - 166.5	7A_d976804	7A_d4539336	2.9	4	0.5	0/2
TKW	7A	218	217.5 - 218	7A_d1114756	7A_d1109546	3.6	6	-0.6	0/2
TKW	4B	69	68.5 - 69.5	4B_s1094836	4B_d3534297	3.8	6	0.6	1/2
TKW	6B	43	42.5 - 43.5	6B_d1113473	6B_s1121615	3.8	6	-0.6	0/2

Trait	Chr	Pos (cM)	C.I. (cM)	Left Marker	Right Marker	LOD	R^2 (%)	Add Effect	Individual Env
TKW	2D	35	34.5 - 36.5	2D_d1137522	2D_d1039425	3.6	6	-0.6	1/2
Sedimentation	1A	3	2.5 - 3.5	1A_d1077302	1A_s1106928	31.6	38	-1.5	2/2
Sedimentation	1B	97	96.5 - 97.5	1B_s987187	1B_d1276568	18.7	19	1.1	2/2

Env: Environment; Chr: Chromosome; Pos: Position; C.I.: Confidence Interval; LOD: Logarithm of Odds; Add Effect: Additive Effect; TKW: Thousand Kernel Weight.

Table 3.6.9. Detailed summary of quantitative trait loci (QTL) identified in the organic environment of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population. Flanking markers are identified by the "Clone ID" provided by Diversity Arrays Technology. (d) and (s) in marker names designate a DArT or SNP marker, respectively. Difference is calculated using the mean disease score difference between flanking markers that have the same parental allele at both markers (AA – BB).

Trait	Chr	Pos (cM)	C.I. (cM)	Left Marker	Right Marker	LOD	R^2 (%)	Add Effect	Individual Env
Lodging	2A	82	80.5 - 82.5	2A_d4004105	2A_d3936135	2.6	4	-0.1	0/2
Lodging	4B	42	40.5 - 43.5	4B_d3384830	4B_s4991673	5.9	12	0.1	1/2
Lodging	4B	54	53.5 - 54.5	4B_d1254295	4B_d4405282	7.0	13	0.2	0/2
Lodging	3D	47	46.5 - 48.5	3D_s1058686	3D_d4393992	2.7	5	-0.1	0/2
Heading	1A	122	121.5 - 124.5	1A_d1125646	1A_d1009935	3.8	4	0.5	0/2
Heading	1B	11	9.5 - 13.5	1B_d1120108	1B_d1265591	3.5	4	0.5	1/2
Heading	7D	12	11.5 - 12.5	7D_d1105401	7D_s1047233	14.4	17	-1.0	2/2
Maturity	3A	56	55.5 - 56.5	3A_d1096296	3A_d1036906	12.7	10	1.1	1/2
Maturity	4B	44	43.5 - 45.5	4B_s4991673	4B_d1258252	10.6	8	-1.0	2/2
Maturity	5B	119	118.5 - 119.5	5B_s1125898	5B_d3959851	20.8	18	-1.5	1/2
Maturity	4D	26	24.5 - 26	4D_d1111555	4D_d2271901	3.6	3	0.6	0/2
Maturity	7D	13	11.5 - 14.5	7D_s1047233	7D_s1022494	9.5	7	-0.9	2/2
Plant Height	3A	62	61.5 - 62.5	3A_s1111624	3A_d1279592	4.7	3	1.5	0/2
Plant Height	5A	84	83.5 - 84.5	5A_d1100228	5A_d1108380	6.9	5	1.8	2/2
Plant Height	4B	44	43.5 - 44.5	4B_s4991673	4B_d1258252	42.9	49	5.6	2/2
Plant Height	6B	124	122.5 - 124.5	6B_s3024454	6B_s2277702	3.0	2	-1.2	0/2
Plant Height	7D	11	10.5 - 11.5	7D_s1215756	7D_d4262395	14.2	11	-2.7	2/2
Plant Height	7D	56	55.5 - 56.5	7D_d1049127	7D_d2303374	5.0	4	-1.5	0/2
Grain Yield	2A	83	82.5 - 83.5	2A_d2299870	2A_d3023429	2.6	4	-0.1	0/2

Trait	Chr	Pos (cM)	C.I. (cM)	Left Marker	Right Marker	LOD	R^2 (%)	Add Effect	Individual Env
Grain Yield	4B	44	43.5 - 45.5	4B_s4991673	4B_d1258252	3.5	5	-0.1	1/2
Grain Yield	4B	102	101.5 - 103	4B_d4404439	4B_d1113608	3.6	5	0.1	0/2
Grain Yield	5B	159	158.5 - 159.5	5B_s1205825	5B_d1256946	4.5	6	-0.1	0/2
Grain Yield	7D	11	10.5 - 11.5	7D_s1215756	7D_d4262395	4.3	6	-0.1	1/2
Test Weight	5A	121	120.5 - 125.5	5A_d1090968	5A_d4536234	2.7	4	-0.2	0/2
Test Weight	4B	44	43.5 - 45.5	4B_s4991673	4B_d1258252	4.3	6	0.3	1/2
Test Weight	7B	26	25.5 - 26.5	7B_d2256891	7B_s16530556	4.6	7	-0.3	1/2
Test Weight	2D	6	5.5 - 6.5	2D_s1095078	2D_d1088274	5.6	9	0.3	1/2
Test Weight	6D	27	26.5 - 27.5	6D_s1091343	6D_s1085677	5.5	8	-0.3	2/2
Test Weight	7D	12	11.5 - 12.5	7D_d1105401	7D_s1047233	4.4	7	-0.3	1/2
Grain Protein	1A	35	34.5 - 35.5	1A_d1100401	1A_d7345050	3.4	6	-0.2	0/2
Grain Protein	3A	154	153.5 - 154.5	3A_s1296360	3A_d1212480	2.6	4	-0.2	0/2
Grain Protein	4A	70	69.5 - 70.5	4A_s990343	4A_d2322968	2.7	4	-0.2	1/2
Grain Protein	7A	194	193.5 - 194.5	7A_s1213119	7A_s1090541	3.2	6	-0.2	0/2
Grain Protein	1B	113	112.5 - 113.5	1B_d1123036	1B_d1160821	6.7	12	-0.3	2/2
Grain Protein	5B	130	129.5 - 130.5	5B_d1088234	5B_d2279136	3.4	6	0.2	1/2
TKW	4A	40	38.5 - 40.5	4A_d3952176	4A_d3950696	2.5	4	-0.5	0/2
TKW	5A	138	137.5 - 140	5A_d4542591	5A_d993093	6.5	10	-0.8	2/2
TKW	4B	69	68.5 - 70.5	4B_s1094836	4B_d3534297	4.9	7	0.7	1/2
TKW	6B	16	15.5 - 16.5	6B_d1126615	6B_d1103792	3.3	5	-0.6	1/2
TKW	6B	142	141.5 - 143.5	6B_d3954836	6B_d2280668	4.5	7	0.7	2/2
TKW	2D	62	60.5 - 63.5	2D_d1134631	2D_d1109826	4.5	8	-0.7	2/2
TKW	5D	45	43.5 - 45.5	5D_d4539071	5D_d2244762	3.2	5	0.6	0/2
Sedimentation	1A	3	2.5 - 3.5	1A_d1077302	1A_s1106928	30.6	41	-1.5	2/2

Env: Environment; Chr: Chromosome; Pos: Position; C.I.: Confidence Interval; LOD: Logarithm of Odds; Add Effect: Additive Effect; TKW: Thousand Kernel Weight.

Figure 3.6.1a. The location of the fifteen quantitative trait loci (QTL) identified in the combined environment analysis on sub-genome A of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population. The map position is located on the left side in centimorgans (cM), and the horizontal lines on each chromosome represent mapped markers. QTL are shown on the right side of the chromosome with the position and 95% confidence interval.

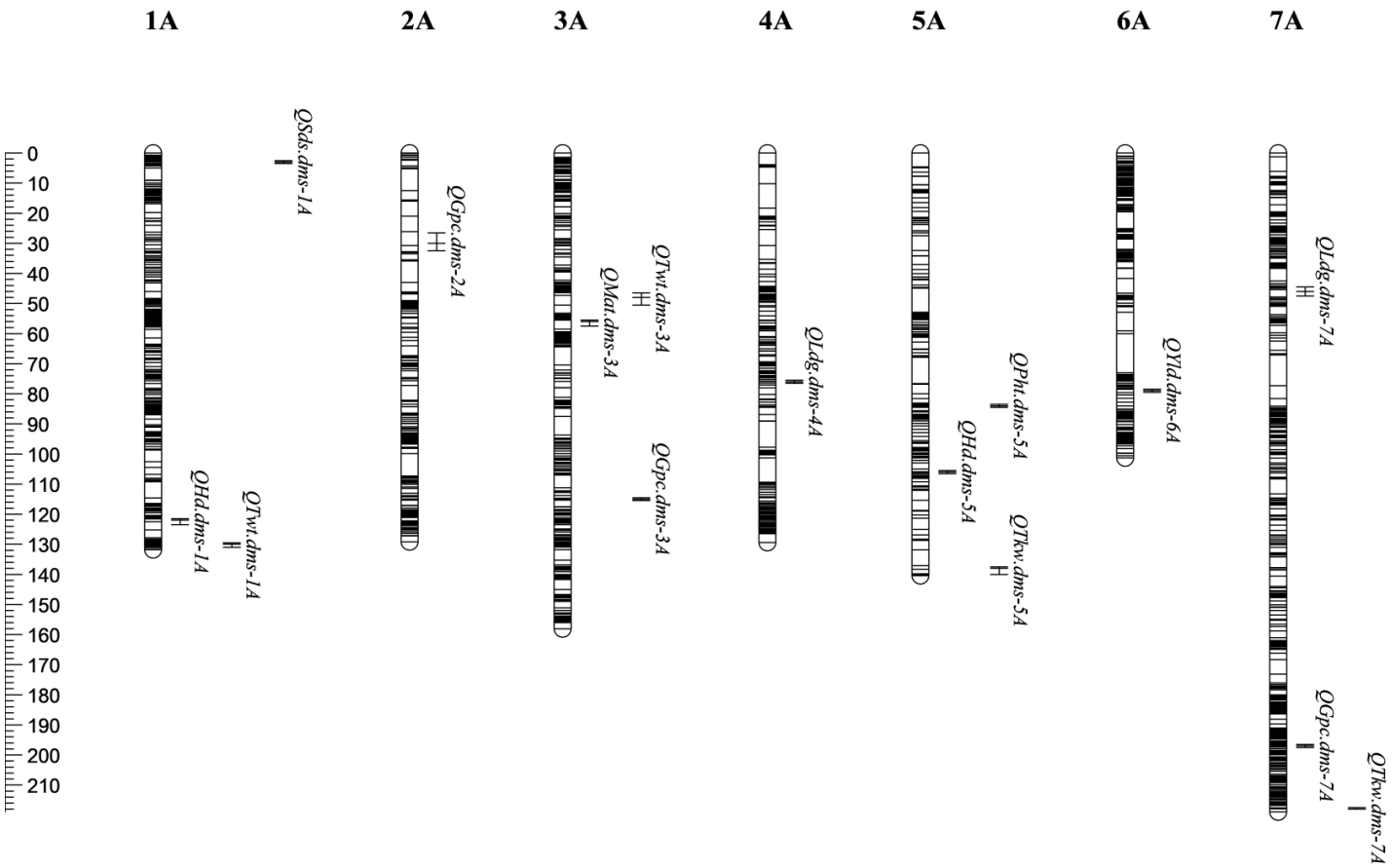


Figure 3.6.1b. The location of the fourteen quantitative trait loci (QTL) identified in the combined environment analysis on sub-genome B of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population. The map position is located on the left side in centimorgans (cM), and the horizontal lines on each chromosome represent mapped markers. QTL are shown on the right side of the chromosome with the position and 95% confidence interval.

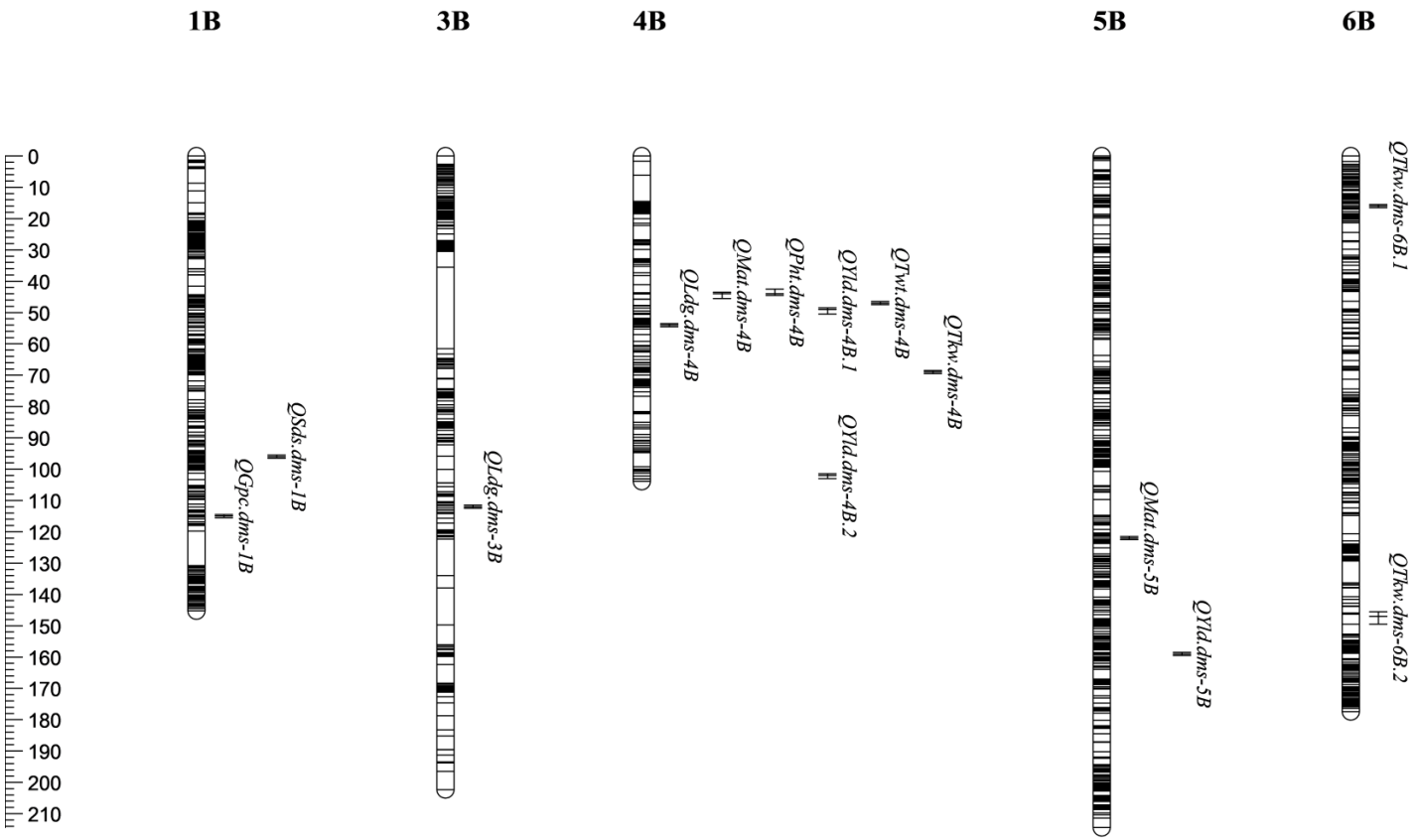
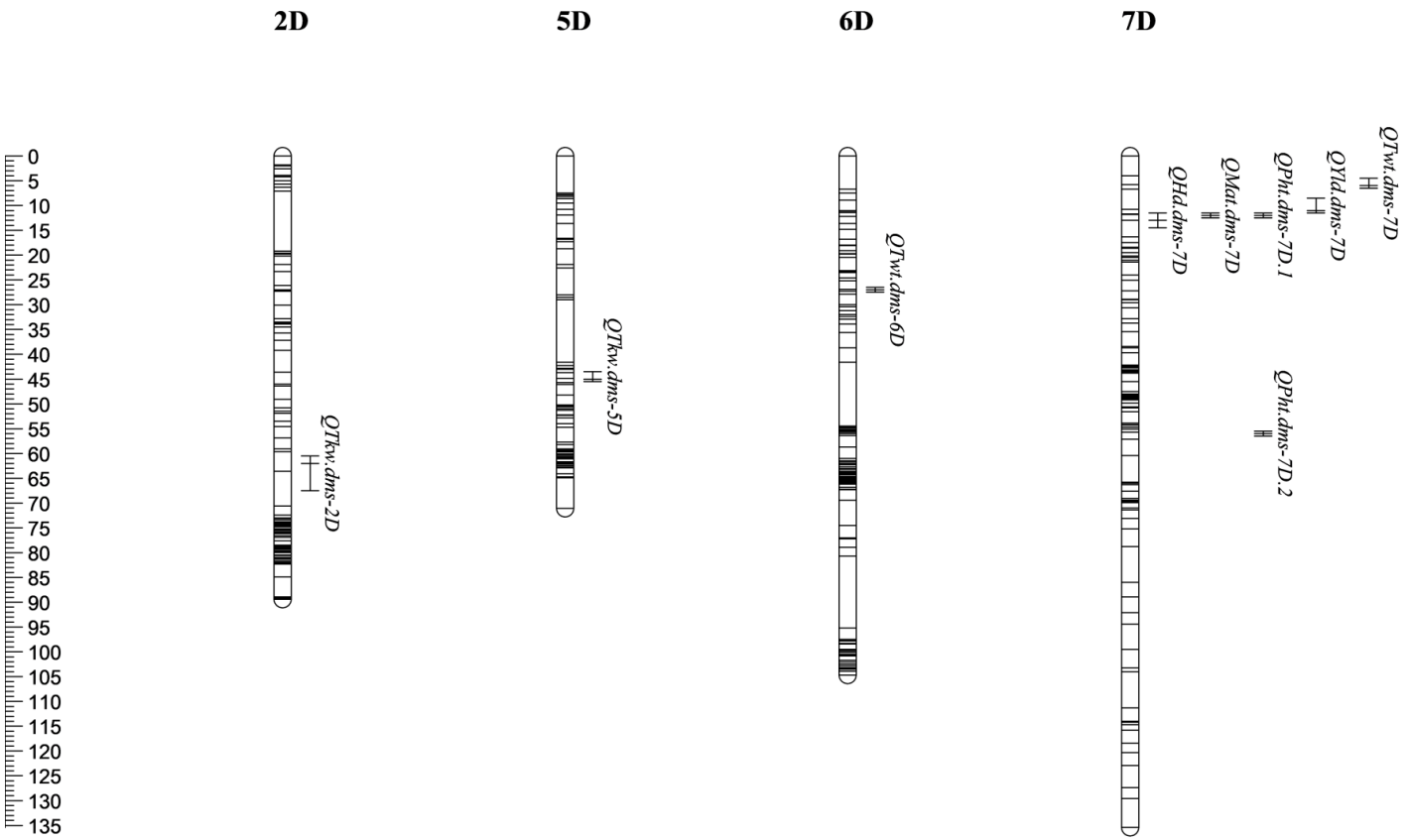


Figure 3.6.1c. The location of the nine quantitative trait loci (QTL) identified in the combined environment analysis on sub-genome D of the 'Peace' × 'Carberry' RIL (recombinant inbred line) spring wheat (*T. aestivum*) mapping population. The map position is located on the left side in centimorgans (cM), and the horizontal lines on each chromosome represent mapped markers. QTL are shown on the right side of the chromosome with the position and 95% confidence interval.



4 General Discussion and Conclusions

4.1 Introduction

Western Canada, encompassing Manitoba, Saskatchewan and Alberta, was responsible for over 90% of total 2017 wheat production in Canada (Statistics Canada 2018), and over 82% of common wheat was attributed to the Canada Western Red Spring (CWRS) class (Canadian Grain Commission 2018). CWRS is the predominant class of wheat in Canada due to several factors including cultivar adaptability, attractive milling characteristics, and flexibility when producing baked goods (McCallum and DePauw 2008). CWRS requires a complex assortment of traits to remain desirable to producers, and end-users, including agronomic traits such as early maturity, grain yield, and protein content, and disease resistance against important pathogens in western Canada including leaf rust, and stripe rust. Quantitative trait loci (QTL) analysis is an effective first step into the discovery of markers that are tightly linked to gene(s) associated with trait improvement (Singh et al. 2014), and marker assisted selection has become a tool in breeding programs due to the low-cost and high-throughput genotyping that has become available.

Canadian wheat breeders have been concerned with obtaining disease resistance since the early 1900s, as stem rust epidemics repeatedly swept across the prairies and destroyed crops of early settlers (Buller 1919; McCallum et al. 2007). Today, leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*), along with the leaf spot complex consisting of tan spot (*Pyrenophora tritici-repentis*), septoria leaf blotch (*Phaeosphaeria nodorum*, *Mycosphaerella graminicola*, and *Phaesosphaeria avenaria)*, and spot blotch (*Cochliobolus sativus)* are the

prominent leaf diseases in western Canada, and an active area of research for genetic resistance. Single gene resistance is not durable, nor long-lasting, and therefore breeders must look for horizontal resistance – resistance that is conferred by several genes.

Due to the short growing season in western Canada (~95 to 125 days), CWRS cultivars must be early maturing in order to minimize potential frost, drought, or heat damage, and successfully set seed (King and Heide 2009). Additionally, bakers admire the dough strength and mixing tolerances produced by CWRS due to its high protein content and resulting gluten strength; the premium world market looks for a protein content greater than 13.5% (Prairie Grain Development Committee 2016), and grain producers are rewarded with a price premium if protein content is acceptable. Complex traits such as grain yield are quantitatively inherited and variation is expressed via numerous QTL with minor effects (Mackay 2001), and non-genetic factors (such as the environment) may also be important (Hill 2010). The stability and consistency of identified QTL remains a problematic concern for plant breeding (Bernardo 2008), but due to the significant differences in biotic and abiotic stresses between conventional and organic systems (such as soil nutrients, soil moisture, and weed competition), QTL that are identified across management systems may be highly stable, and reliable markers for breeders.

The objectives of this thesis were to: (1) identify and genetically map QTL associated with leaf rust, stripe rust, and leaf spot, (2) locate genomic regions associated with multiple traits, (3) identify and genetically map QTL associated with agronomic and quality traits, and (4) compare QTL identified in conventional and organic environments, in the ‘Peace’ × ‘Carberry’ recombinant inbred line population.

4.2 Contribution to Knowledge

In the first study (Chapter Two), we identified three QTL associated with leaf spotting, four QTL associated with leaf rust, and three QTL associated with stripe rust. The total phenotypic variance explained was 28% for the leaf spot QTL, 25% for the leaf rust QTL, and 27% for the stripe rust QTL. The leaf spot QTL were located on chromosomes 2A, 4B and 7D and individually explained 8 to 10% of the phenotypic variance. The leaf rust QTL were located on 2A, 4A, 5B, and 3D and individually explained 5 to 8% of the phenotypic variance. The stripe rust QTL were located on chromosomes 2A, 3A and 4B and individually explained 5 to 16% of the phenotypic variance. The stripe rust QTL on 4B (*QYr.dms-4B)* was the largest QTL identified in the study and was identified in all three individual environments. Previous studies have also identified a stripe rust QTL on 4B that was contributed by 'Carberry' (Singh et al. 2014; Bokore et al. 2017). *QYr.dms-2A* and *QLr.dms-2A* were both contributed by 'Peace', and associated with stripe rust and leaf rust resistance, respectively. Both these QTL correspond with two QTL identified by Singh et al. (2014), who used a mapping population derived from a close relative of 'Peace'. Results of this study suggest that 'Carberry' may be an attractive parental source for breeders to enhance resistance against stripe rust and leaf spotting, and 'Peace' contributed alleles associated with stripe and leaf rust resistance that had been previously identified in a close relative.

In the second study (Chapter Three), we identified a total of thirty-eight QTL associated with nine agronomic traits. Clusters of QTL were found on chromosomes 4B and 7D, and associated with maturity, plant height, grain yield, and test weight. Of the thirty-eight QTL identified, sixteen were consistently identified across management systems, and associated with all traits except lodging. The LOD scores of these QTL ranged from 3.4 to 45.7 and the

phenotypic variance explained ranged from 4 to 53%. The largest QTL (*QPht.dms-4B*) explained 53% of the phenotypic variance and was associated with plant height. This allele was contributed by 'Carberry' and is most likely the *Rht-B1b* height reduction gene. In addition to reducing height by thirteen centimetres, *QPht.dms-4B / Rht-B1b* increased the days to maturity by two and grain yield by 200 kg ha^{-1}. The second largest QTL (*QSds.dms-1A*) was associated with sedimentation volume, explained 41% of the phenotypic variance and was contributed by 'Peace'. The QTL identified on 7D were contributed by 'Peace', and this allele reduced height by six centimetres, days to maturity by two days, and grain yield by 300 kg ha^{-1}. Results of this study suggest that QTL with minor effects may be stable and consistent QTL, even though large effect QTL have predominantly been the focus of plant breeders (Bernardo 2008).

4.3 General Discussion

The largest stripe rust resistance QTL, *QYr.dms-4B*, originated from 'Carberry', but the position of this QTL was ~ 100 cM from the stripe rust QTL on 4B from 'Carberry' that was reported in other studies (Singh et al. 2014; Bokore et al. 2017). Further research is required to clarify if 'Carberry' contributes two alleles associated with stripe rust on 4B, but in our study we did identify two stripe rust QTL on 4B in the single environment analysis (Edmonton 2016). One of the QTL corresponds to *QYr.dms-4B* at 21 cM, but the second QTL was located at 44 cM and provides evidence that 'Carberry' may indeed confer stripe rust resistance from two alleles on 4B. It is a rare opportunity to compare QTL analysis results with another study that derived a mapping population from the same parent and this provides a unique perspective on QTL stability in light of the numerous differences between studies such as genetic markers and software utilized.

Previous research has identified QTL associated with sedimentation volume at a similar position to *QSds.dms-1A* (Chen et al. 2015), and the *Gli-A1* gene located on the short arm of 1A corresponds to the same location (Payne et al. 1984). Few studies have analyzed QTL associated with gluten strength, and the identification of this possible storage protein gene remains unknown. A previous study has identified management-specific QTL (Asif et al. 2015), and therefore it was expected that we would also produce similar results, but the relatively large number of consistent QTL that we identified across management systems was surprising. The literature suggests that QTL analysis in conventionally and organically managed systems is an unexplored area of research, and further research is warranted.

4.4 Future Research

I. The consistency of QTL across management systems, and why QTL within traits are not consistent. Potential QTL-by-environment interactions should be explored in order to gain knowledge of QTL stability.

II. Several leaf rust genes segregate in the 'Peace' × 'Carberry' population (*Lr1*, *Lr13*, *Lr16*, and *Lr27*), but no leaf rust QTL were identified on chromosomes 5D, 2B, or 3B that could correspond to these leaf rust genes. Screening this population for leaf rust genes and exploring synergistic relationships with *Lr34* (non-segregating), and the reported virulence towards *Lr13* and *Lr16* should be explored.

III. Molecular analysis of *QSds.dms-1A*, and identification of this unknown storage protein gene. The fact that this major QTL associated with gluten strength is segregating within elite CWRS cultivars may suggest a negative correlation with another trait of importance. Development of a DArTseq molecular marker may aid in germplasm development.

IV. Additional phenotyping in leaf rust nurseries should be conducted to further investigate whether 'Carberry' is the parental source of one or two leaf rust resistance alleles on chromosome 4B.

V. Validation of DArTseq molecular markers for *QPht.dms*-4B, the height reducing gene *Rht-B1b*, for deployment in marker assisted selection.

References

Aboukhaddour R, Turkington TK, and Strelkov SE. (2013). Race structure of *Pyrenophora triciti-repentis* (tan spot of wheat) in Alberta, Canada. *Canadian Journal of Plant Pathology*, *35*(2), 256–268. https://doi.org/10.1080/07060661.2013.782470

Adhikari TB, Gurung S, Hansen JM, Jackson EW, and Bonman JM. (2012). Association mapping of quantitative trait loci in spring wheat landraces conferring resistance to bacterial leaf streak and spot blotch. *The Plant Genome Journal*, *5*(1), 1–16. https://doi.org/10.3835/plantgenome2011.12.0032

Adhikari TB, Mamidi S, Gurung S, and Bonman JM. (2015). Mapping of new quantitative trait loci (QTL) for resistance to Septoria tritici blotch in spring wheat (*Triticum aestivum* L.). *Euphytica*, *205*(3), 699–706. https://doi.org/10.1007/s10681-015-1393-4

Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang S, … Kilian A. (2006). Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. *Theoretical and Applied Genetics*, *113*(8), 1409–1420.

Alberta Agriculture and Forestry. (2014). *Cereal leaf beetle*. Retrieved from http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex14735

Alberta Agriculture and Forestry. (2018a). *Crop Protection 2018*. (H. Brook & M. Cutts, Eds.). Edmonton AB: Alberta Agriculture and Forestry, Information Management and Innovation. Retrieved from https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex32/$FILE/606-1.pdf

Alberta Agriculture and Forestry. (2018b). *Fusarium head blight - frequently asked questions*. Retrieved from http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/faq12513

Alberta Seed Guide. (2018). Alberta Seed Guide - Spring 2018, Mitigation Strategies. Retrieved

from http://www.seed.ab.ca/

Allen AM, Winfield MO, Burridge AJ, Downie RC, Benbow HR, Barker GLA, … Edwards KJ. (2017). Characterization of a Wheat Breeders' Array suitable for high-throughput SNP genotyping of global accessions of hexaploid bread wheat (*Triticum aestivum*). *Plant Biotechnology Journal*, *15*(3), 390–401. https://doi.org/10.1111/pbi.12635

Ando K, Rynearson S, Muleta KT, Gedamu J, Girma B, Bosque-Pérez NA, … Pumphrey MO. (2018). Genome-wide associations for multiple pest resistances in a Northwestern United States elite spring wheat panel. *PLOS ONE*, *13*(2), 1–25. https://doi.org/10.1371/journal.pone.0191305

Anjum FM, Khan MR, Din A, Saeed M, Pasha I, and Arshad MU. (2007). Wheat gluten: High molecular weight glutenin subunits-structure, genetics, and relation to dough elasticity. *Journal of Food Science*, *72*(3), R56–R63.

Appendino ML, and Slafer GA. (2003). Earliness per se and its dependence upon temperature in diploid wheat lines differing in the major gene *Eps-Am1* alleles. *The Journal of Agricultural Science*, *141*(2), 149–154.

Asif M, Yang R-C, Navabi A, Iqbal M, Kamran A, Lara EP, … Spaner D. (2015). Mapping QTL, selection differentials, and the effect of under organic and conventionally managed systems in the "Attila" × "CDC Go" spring wheat mapping population. *Crop Science*, *55*(3), 1129–1142.

Assanga SO, Fuentealba M, Zhang G, Tan CT, Dhakal S, Rudd JC, … Liu S. (2017). Mapping of quantitative trait loci for grain yield and its components in a US popular winter wheat "TAM 111" using 90K SNPs. *PLOS ONE*, *12*(12). https://doi.org/10.1371/journal.pone.0189669

Avivi L. (1978). High grain protein content in wild tetraploid wheat *Triticum dicoccoides* Korn. In *Proc. 5th Int. Wheat Genet. Symp., New Delhi* (Vol. 1, pp. 372–380).

Bailey KL, Gossen BD, Gugel RK, and Morrall RAA. (2003). *Diseases of field crops in Canada. Third Edition, Second Printer*. Saskatoon, Sask: Canadian Phytopathological Society.

Balyan HS, Gupta PK, Kumar S, Dhariwal R, Jaiswal V, Tyagi S, … Kumari S. (2013). Genetic improvement of grain protein content and other health-related constituents of wheat grain. *Plant Breeding*, *132*(5), 446–457.

Barker GLA, and Edwards KJ. (2009). A genome-wide analysis of single nucleotide polymorphism diversity in the world's major cereal crops. *Plant Biotechnology Journal*, *7*(4), 318–325.

Beavis WD. (1994). The power and deceit of QTL experiments: Lessons from comparative QTL studies. In *Proceedings of the Forty-Ninth Annual Corn and Sorghum Industry Research Conference* (pp. 250–266). Washington, DC: American Seed Trade Association.

Bennett D, Reynolds M, Mullan D, Izanloo A, Kuchel H, Langridge P, and Schnurbusch T. (2012a). Detection of two major grain yield QTL in bread wheat (*Triticum aestivum* L.) under heat, drought and high yield potential environments. *Theoretical and Applied Genetics*, *125*(7), 1473–1485.

Bennett D, Izanloo A, Edwards J, Kuchel H, Chalmers K, Tester M, … Langridge P. (2012b). Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (*Triticum aestivum* L.) population adapted to southern Australian conditions. *Theoretical and Applied Genetics*, *124*(4), 697–711. https://doi.org/10.1007/s00122-011-1740-3

Bentley AR, Turner AS, Gosman N, Leigh FJ, Maccaferri M, Dreisigacker S, … Laurie DA.

(2011). Frequency of photoperiod-insensitive *Ppd-A1a* alleles in tetraploid, hexaploid and synthetic hexaploid wheat germplasm. *Plant Breeding*, *130*(1), 10–15.

Bernardo R. (2008). Molecular markers and selection for complex traits in plants: Learning from the last 20 years. *Crop Science*, *48*(5), 1649–1664.

Bernardo R. (2016). Bandwagons I, too, have known. *Theoretical and Applied Genetics*, *129*(12), 2323–2332. https://doi.org/10.1007/s00122-016-2772-5

Bhathal JS, Loughman R, and Speijers J. (2003). Yield reduction in wheat in relation to leaf disease from yellow (tan) spot and septoria nodorum blotch. *European Journal of Plant Pathology*, *109*(5), 435–443. https://doi.org/10.1023/A:1024277420773

Blanchard J. (1990). The Garnet wheat controversy, 1923-1938. *Manitoba History*, *Spring*(19), 12–22.

Bokore FE, Cuthbert RD, Knox RE, Randhawa HS, Hiebert CW, DePauw RM, … Bhavani S. (2017). Quantitative trait loci for resistance to stripe rust of wheat revealed using global field nurseries and opportunities for stacking resistance genes. *Theoretical and Applied Genetics*, *130*(12), 2617–2635. https://doi.org/10.1007/s00122-017-2980-7

Botstein D, White RL, Skolnick M, and Davis RW. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, *32*(3), 314–331.

Brar GS, and Kutcher HR. (2016). Race characterization of *Puccinia striiformis* f. sp. *tritici*, the cause of wheat stripe rust, in Saskatchewan and southern Alberta, Canada and virulence comparison with races from the United States. *Plant Disease*, *100*(8), 1744–1753.

Brevis JC, Chicaiza O, Khan IA, Jackson L, Morris CF, and Dubcovsky J. (2008). Agronomic and quality evaluation of common wheat near-isogenic lines carrying the leaf rust resistance

gene. *Crop Science*, *48*(4), 1441–1451.

Broman KW. (2001). Review of statistical methods for QTL mapping in experimental crosses. *Lab Animal*, *30*(7), 44–52. https://doi.org/11469113

Buller AH. (1919). *Essays on wheat*. New York: Macmillan Company.

Bullrich L, Appendino M, Tranquilli G, Lewis S, and Dubcovsky J. (2002). Mapping of a thermo-sensitive earliness *per se* gene on *Triticum monococcum* chromosome 1Am. *Theoretical and Applied Genetics*, *105*(4), 585–593.

Cabral AL, Jordan MC, McCartney CA, You FM, Humphreys DG, MacLachlan R, and Pozniak CJ. (2014). Identification of candidate genes, regions and markers for pre-harvest sprouting resistance in wheat (*Triticum aestivum* L.). *BMC Plant Biology*, *14*(1), 1–12.

Cabral AL, Jordan MC, Larson G, Somers DJ, Humphreys DG, and McCartney CA. (2018). Relationship between QTL for grain shape, grain weight, test weight, milling yield, and plant height in the spring wheat cross "RL4452" / "AC Domain." *PLOS ONE*, *13*(1). https://doi.org/10.1371/journal.pone.0190681

Canadian Grain Commission. (2017). *Official Grain Grading Guide*. Retrieved from https://www.grainscanada.gc.ca/oggg-gocg/ggg-gcg-eng.htm

Canadian Grain Commission. (2018). *Grain varieties by acreage insured*. Retrieved from http://www.grainscanada.gc.ca/statistics-statistiques/variety-variete/varieties-en.htm

Cantrell RG, and Joppa LR. (1991). Genetic analysis of quantitative traits in wild emmer (*Triticum turgidum* L. var. *dicoccoides*). *Crop Science*, *31*(3), 645–649.

Carter AH, Garland-Campbell K, and Kidwell KK. (2011). Genetic mapping of quantitative trait loci associated with important agronomic traits in the spring wheat (*Triticum aestivum* L.) cross "Louise" × "Penawawa." *Crop Science*, *51*(1), 84–95.

https://doi.org/10.2135/cropsci2010.03.0185

Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S, Kiani S, … Akhunov E. (2013). Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proceedings of the National Academy of Sciences*, *110*(20), 8057–8062. https://doi.org/10.1073/pnas.1217133110

Chantret N, Salse J, Sabot F, Rahman S, Bellec A, Laubin B, … Chalhoub B. (2005). Molecular basis of evolutionary events that shaped the *hardness* locus in diploid and polyploid wheat species (*Triticum* and *Aegilops*). *The Plant Cell*, *17*(4), 1033–1045.

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, and Gale MD. (1989). RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. *Theoretical and Applied Genetics*, *78*(4), 495–504. https://doi.org/10.1007/BF00290833

Chee M, Yang R, Hubbell E, Berno A, Huanh XC, Stern D, … Fodor SPA. (1996). Accessing genetic information with high-density DNA arrays. *Science*, *274*(5287), 610–614.

Chen XM. (2005). Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. *Canadian Journal of Plant Pathology*, *27*(3), 314–337.

Chen X, Penman L, Wan A, and Cheng P. (2010). Virulence races of *Puccinia striiformis* f. sp. *tritici* in 2006 and 2007 and development of wheat stripe rust and distributions, dynamics, and evolutionary relationships of races from 2000 to 2007 in the United States. *Canadian Journal of Plant Pathology*, *32*(3), 315–333.

Chen A, and Dubcovsky J. (2012). Wheat TILLING mutants show that the vernalization gene *VRN1* down-regulates the flowering repressor *VRN2* in leaves but is not essential for flowering. *PLOS Genetics*, *8*(12), 1–13.

Chen H, Iqbal M, Perez-Lara E, Yang R-C, Pozniak C, and Spaner D. (2015). Earliness *per se*

quantitative trait loci and their interaction with *Vrn-B1* locus in a spring wheat population. *Molecular Breeding*, *35*(9), 1–14.

Chen H, Moakhar NP, Iqbal M, Pozniak C, Hucl P, and Spaner D. (2016). Genetic variation for flowering time and height reducing genes and important traits in western Canadian spring wheat. *Euphytica*, *208*(2), 377–390.

Churchill GA, and Doerge RW. (1994). Empirical threshold values for quantitative trait mapping. *Genetics*, *138*(3), 963–971.

CIMMYT. (2016). *Pathotype tracker - where is Ug99?* Retrieved from http://rusttracker.cimmyt.org/?page_id=22

Cloutier S, McCallum BD, Loutre C, Banks TW, Wicker T, Feuillet C, … Jordan MC. (2007). Leaf rust resistance gene *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large psr567 gene family. *Plant Molecular Biology*, *65*(1–2), 93–106. https://doi.org/10.1007/s11103-007-9201-8

Craigie JH. (1945). Epidemiology of stem rust in western Canada. *Scientific Agriculture*, *25*(6), 285–402.

Cromey MG, Wright DSC, and Boddington HJ. (1998). Effects of frost during grain filling on wheat yield and grain structure. *New Zealand Journal of Crop and Horticultural Science*, *26*(4), 279–290.

Cuthbert JL, Somers DJ, Brûlé-Babel AL, Brown PD, and Crow GH. (2008). Molecular mapping of quantitative trait loci for yield and yield components in spring wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *117*(4), 595–608.

DePauw RM, Townley-Smith TF, Humphreys G, Knox RE, Clarke FR, and Clarke JM. (2005). Lillian hard red spring wheat. *Canadian Journal of Plant Science*, *85*(2), 397–401.

https://doi.org/10.4141/P04-137

DePauw RM, Knox RE, McCaig TN, Clarke FR, and Clarke JM. (2011). Carberry hard red spring wheat. *Canadian Journal of Plant Science*, *91*(3), 529–534. https://doi.org/10.4141/cjps10187

DePauw RM, Knox RE, Singh AK, Fox SL, Humphreys DG, and Hucl P. (2012). Developing standardized methods for breeding preharvest sprouting resistant wheat, challenges and successes in Canadian wheat. *Euphytica*, *188*(1), 7–14.

Dexter JE, Preston KR, Martin DG, and Gander EJ. (1994a). The effects of protein content and starch damage on the physical dough properties and bread-making quality of Canadian durum wheat. *Journal of Cereal Science*, *20*(2), 139–151.

Dexter JE, Marchylo BA, and Mellish VJ. (1994b). Effects of frost damage and immaturity on the quality of durum wheat. *Cereal Chemistry*, *71*(5), 494–501.

Dexter JE, Preston KR, and Woodbeck N. (2006). Future of flour: A compendium of flour improvement. In L. Popper, S. Werner, & W. Fruend (Eds.), *Canadian Wheat* (pp. 43–62). Agrimedia.

Distelfeld A, Li C, and Dubcovsky J. (2009). Regulation of flowering in temperate cereals. *Current Opinion in Plant Biology*, *12*(2), 178–184.

Diversity Arrays Technology. (2018, August 7). *Genetic Maps*. Retrieved from https://www.diversityarrays.com/index.php/technology-and-resources/genetic-maps/

Dong Z, Hegarty JM, Zhang J, Zhang W, Chao S, Chen X, … Dubcovsky J. (2017). Validation and characterization of a QTL for adult plant resistance to stripe rust on wheat chromosome arm 6BS (*Yr78*). *Theoretical and Applied Genetics*, *130*(10), 2127–2137. https://doi.org/10.1007/s00122-017-2946-9

Dosdall LM, Cárcamo H, Olfert O, Meers S, Hartley S, and Gavloski J. (2011). Insect invasions of agroecosystems in the western Canadian prairies: Case histories, patterns, and implications for ecosystem function. *Biological Invasions*, *13*(5), 1135–1149.

El-Soda M, Malosetti M, Zwaan BJ, Koornneef M, and Aarts MGM. (2014). Genotype × environment interaction QTL mapping in plants: Lessons from *Arabidopsis*. *Trends in Plant Science*, *19*(6), 390–398.

Evans EW, Karren JB, and Israelsen CE. (2006). Interactions over time between cereal leaf beetle (Coleoptera: Chrysomelidae) and larval parasitoid *Tetrastichus julis* (Hymenoptera: Eulophidae) in Utah. *Journal of Economic Entomology*, *99*(6), 1967–1973.

Faris JD, Liu Z, and Xu SS. (2013). Genetics of tan spot resistance in wheat. *Theoretical and Applied Genetics*, *126*(9), 2197–2217. https://doi.org/10.1007/s00122-013-2157-y

Fernandez MR, Clarke FR, Knox RE, Clarke JM, and Singh AK. (2010). Quantification of effects of leaf spotting diseases on grain yield and market quality of durum wheat using near-isogenic lines. *Canadian Journal of Plant Pathology*, *32*(2), 177–187. https://doi.org/10.1080/07060661003740025

Fernandez MR, Fox SL, Hucl P, and Singh AK. (2014). Leaf spotting reaction of spring common, durum and spelt wheat, and Kamut under organic management in western Canada. *Canadian Journal of Plant Science*, *94*(5), 929–935.

Fernandez MR, Wang H, Cutforth H, and Lemke RL. (2016). Climatic and agronomic effects on leaf spots of spring wheat in the western Canadian Prairies. *Canadian Journal of Plant Science*, *96*(5), 895–907.

Fetch T, Zegeye T, Singh D, Wanyera R, Penner M, and Rashid K. (2012). Virulence of Ug99 (race TTKSK) and race TRTTF on Canadian wheat cultivars. *Canadian Journal of Plant*

Science, *92*(3), 602.

Fetch T, Fetch JM, and Xue A. (2015). Races of *Puccinia graminis* on barley, oat, and wheat in Canada in 2007 and 2008. *Canadian Journal of Plant Pathology*, *37*(3), 331–341.

Flor HH. (1971). Current status of the gene-for-gene concept. *Annual Review of Phytopathology*, *9*(1), 275–296.

Fofana B, Humphreys DG, Cloutier S, McCartney CA, and Somers DJ. (2008). Mapping quantitative trait loci controlling common bunt resistance in a doubled haploid population derived from the spring wheat cross "RL4452" × "AC Domain." *Molecular Breeding*, *21*(3), 317–325.

Food and Agriculture Organization of the United Nations. (2018). *FAOSTAT*. Retrieved from http://www.fao.org/faostat/en/

Fowler DB, and De La Roche IA. (1975). Wheat quality evaluation. 3. Influence of genotype and environment. *Canadian Journal of Plant Science*, *55*(1), 263–269.

Fowler DB, N'Diaye A, Laudencia-Chingcuanco D, Pozniak CJ, Laudenci-Chingcuanco D, and Pozniak CJ. (2016). Quantitative trait loci associated with phenological development, low-temperature tolerance, grain quality, and agronomic characters in wheat (*Triticum aestivum* L.). *PLOS ONE*, *11*(3), 1–21. https://doi.org/10.1371/journal.pone.0152185

Fox SL, Townley-Smith TF, Humphreys DG, McCallum BD, Fetch TG, Gaudet DA, … Howes NK. (2006). Somerset hard red spring wheat. *Canadian Journal of Plant Science*, *86*(1), 163–167.

Fu Y-B, and Somers DJ. (2011). Allelic changes in bread wheat cultivars were associated with long-term wheat trait improvements. *Euphytica*, *179*(2), 209–225.

Gale KR. (2005). Diagnostic DNA markers for quality traits in wheat. *Journal of Cereal Science*,

41(2), 181–192. https://doi.org/10.1016/j.jcs.2004.09.002

Gallun RL, Ruppel R, and Everson EH. (1966). Resistance of small grains to the cereal leaf beetle. *Journal of Economic Entomology*, *59*(4), 827–829.

Ganal MW, and Röder MS. (2007). Microsatellite and SNP markers in wheat breeding. In R. K. Varshney & R. Tuberosa (Eds.), *Genomics-Assisted Crop Improvement: Volume 2 Genomics Applications in Crops* (pp. 1–24). Springer.

Gaudet DA, and Puchalski BL. (1989). Races of common bunt (*Tilletia caries* and *T. foetida*) of wheat in western Canada. *Canadian Journal of Plant Pathology*, *11*(4), 415–418.

Gaudet DA, Puchalski BJ, Despins T, McCartney C, Menzies JG, and Graf RJ. (2013). Seeding date and location affect winter wheat infection by common bunt (*Tilletia tritici* and *T. laevis*) in western Canada. *Canadian Journal of Plant Science*, *93*(3), 483–489.

Gautier M-F, Cosson P, Guirao A, Alary R, and Joudrier P. (2000). Puroindoline genes are highly conserved in diploid ancestor wheats and related species but absent in tetraploid *Triticum* species. *Plant Science*, *153*(1), 81–91.

German SE, and Kolmer JA. (1992). Effect of gene *Lr34* in the enhancement of resistance to leaf rust of wheat. *Theoretical and Applied Genetics*, *84*(1), 97–105.

Gilbert J, and Woods SM. (2001). Leaf spot diseases of spring wheat in southern Manitoba farm fields under conventional and conservation tillage. *Canadian Journal of Plant Science*, *81*(1784), 551–559. https://doi.org/10.4141/cjps2001-

Gilbert J, and Haber S. (2013). Overview of some recent research developments in Fusarium head blight of wheat. *Canadian Journal of Plant Pathology*, *35*(2), 149–174.

Government of Saskatchewan. (2018). *Varieties of Grain Crops - 2018*. Retrieved from http://publications.gov.sk.ca/documents/20/96889-Varieties of Grain Crops_2018.pdf

Gupta PK, Mir RR, Mohan A, and Kumar J. (2008). Wheat genomics: Present status and future prospects. *International Journal of Plant Genomics*, *2008*, 1–36.

Gurung S, Mamidi S, Bonman JM, Jackson EW, del Río LE, Acevedo M, … Adhikari TB. (2011). Identification of novel genomic regions associated with resistance to *Pyrenophora tritici-repentis* races 1 and 5 in spring wheat landraces using association analysis. *Theoretical and Applied Genetics*, *123*(6), 1029–1041. https://doi.org/10.1007/s00122-011-1645-1

Gurung S, Mamidi S, Bonman JM, Xiong M, Brown-Guedira G, and Adhikari TB. (2014). Genome-wide association study reveals novel quantitative trait loci associated with resistance to multiple leaf spot diseases of spring wheat. *PLOS ONE*, *9*(9). https://doi.org/10.1371/journal.pone.0108179

Haldane JBS. (1919). The combination of linkage values, and the calculation of distances between the loci of linked factors. *Journal of Genetics*, *8*(29), 299–309. https://doi.org/10.1016/j.biortech.2011.07.096

Haley CS, and Knott SA. (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*, *69*(4), 315–324. https://doi.org/10.1038/hdy.1992.131

Hayward AC, Tollenaere R, Dalton-Morgan J, and Batley J. (2015). Molecular marker applications in plants. In J. Batley (Ed.), *Plant Genotyping: Methods and Protocols* (pp. 13–27). New York: SpringerSpringer.

He C, and Hughes GR. (2003). Inheritance of resistance to common bunt in spelt and common wheat. *Canadian Journal of Plant Science*, *83*(1), 47–56.

Heffelfinger C, Fragoso CA, and Lorieux M. (2017). Constructing linkage maps in the genomics

era with MapDisto 2.0. *Bioinformatics*, *33*(14), 2224–2225. https://doi.org/10.1093/bioinformatics/btx177

Heffner EL, Sorrells ME, and Jannink J-L. (2009). Genomic selection for crop improvement. *Crop Science*, *49*(1), 1–12.

Hiebert CW, Fetch TG, Zegeye T, Thomas JB, Somers DJ, Humphreys DG, … Knott DR. (2011). Genetics and mapping of seedling resistance to Ug99 stem rust in Canadian wheat cultivars "Peace" and "AC Cadillac." *Theoretical and Applied Genetics*, *122*(1), 143–149.

Hiebert CW, Kolmer JA, McCartney CA, Briggs J, Fetch T, Bariana H, … Spielmeyer WS. (2016). Major gene for field stem rust resistance co-locates with resistance gene *Sr12* in "Thatcher" wheat. *PLOS ONE*, *11*(6), 1–16. https://doi.org/10.1371/journal.pone.0157029

Hill WG. (2010). Understanding and using quantitative genetic variation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1537), 73–85. https://doi.org/10.1098/rstb.2009.0203

Hoffmann JA. (1971). Control of common and dwarf bunt of wheat by seed treatment with thiabendazole. *Phytopathology*, *61*, 1071–1074.

Holland J, Nyquist W, and Cervantes-Martínez C. (2003). Estimating and Interpreting Heritability for Plant Breeding: An Update. *Plant Breeding Reviews*, *22*, 9 - 112.

Holland JB. (2006). Estimating genotypic correlations and their standard errors using multivariate restricted maximum likelihood estimation with SAS Proc MIXED. *Crop Science*, *46*(2), 642–654. https://doi.org/10.2135/cropsci2005.0191

Holtz MD, Kumar K, Zantinge JL, and Xi K. (2014). Genetic diversity of *Puccinia striiformis* from cereals in Alberta, Canada. *Plant Pathology*, *63*(2), 415–424.

Huang XQ, Cloutier S, Lycar L, Radovanovic N, Humphreys DG, Noll JS, … Brown PD.

(2006). Molecular detection of QTLs for agronomic and quality traits in a doubled haploid population derived from two Canadian wheats (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *113*(4), 753–766.

Huel DG, and Hucl P. (1996). Genotypic variation for competitive ability in spring wheat. *Plant Breeding*, *115*(5), 325–329.

Humphreys DG, Townley-Smith TF, Lukow OM, McCallum BD, Fetch TG, Gilbert JA, … Fox SL. (2014). Peace hard red spring wheat. *Canadian Journal of Plant Science*, *94*(7), 1297–1302. https://doi.org/10.4141/cjps2013-286

Iqbal M, Navabi A, Salmon DF, Yang R-C, Murdoch BM, Moore SS, and Spaner D. (2007a). Genetic analysis of flowering and maturity time in high latitude spring wheat. *Euphytica*, *154*(1), 207–218.

Iqbal M, Navabi A, Yang R-C, Salmon DF, and Spaner D. (2007b). The effect of vernalization genes on earliness and related agronomic traits of spring wheat in northern growing regions. *Crop Science*, *47*(3), 1031–1039.

Iqbal M, Navabi A, Yang R-C, Salmon DF, and Spaner D. (2007c). Molecular characterization of vernalization response genes in Canadian spring wheat. *Genome*, *50*, 511–516. https://doi.org/10.1139/G07-028

Iqbal M, Navabi A, Salmon DF, Yang RC, and Spaner D. (2007d). Simultaneous selection for early maturity, increased grain yield and elevated grain protein content in spring wheat. *Plant Breeding*, *126*(3), 244–250. https://doi.org/10.1111/j.1439-0523.2007.01346.x

Jaccoud D, Peng K, Feinstein D, and Kilian A. (2001). Diversity arrays: A solid state technology for sequence information independent genotyping. *Nucleic Acids Research*, *29*(4), 1–7. https://doi.org/10.1093/nar/29.4.e25

Jansen RC, and Nap J-P. (2001). Genetical genomics: The added value from segregation. *Trends in Genetics*, *17*(7), 388–391.

Jiang C, and Zeng ZB. (1995). Multiple trait analysis of genetic mapping for quantitative trait loci. *Genetics*, *140*(3), 1111–1127. Retrieved from https://www.genome.gov/10000715

Jighly A, Oyiga BC, Makdis F, Nazari K, Youssef O, Tadesse W, … Ogbonnaya FC. (2015). Genome-wide DArT and SNP scan for QTL associated with resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in elite ICARDA wheat (*Triticum aestivum* L.) germplasm. *Theoretical and Applied Genetics*, *128*(7), 1277–1295. https://doi.org/10.1007/s00122-015-2504-2

Johansson E, Prieto-Linde ML, Svensson G, and Jönsson JÖ. (2003). Influences of cultivar, cultivation year and fertilizer rate on amount of protein groups and amount and size distribution of mono- and polymeric proteins in wheat. *The Journal of Agricultural Science*, *140*(3), 275–284.

Jolly CJ, Glenn GM, and Rahman S. (1996). *GSP-1* genes are linked to the grain hardness locus (*Ha*) on wheat chromosome 5D. *Proceedings of the National Academy of Sciences*, *93*(6), 2408–2413.

Kamran A, Randhawa HS, Pozniak C, and Spaner D. (2013a). Phenotypic effects of the flowering gene complex in Canadian spring wheat germplasm. *Crop Science*, *53*(1), 84–94.

Kamran A, Iqbal M, Navabi A, Randhawa H, Pozniak C, and Spaner D. (2013b). Earliness per se QTLs and their interaction with the photoperiod insensitive allele *Ppd-D1a* in the "Cutler" × "AC Barrie" spring wheat population. *Theoretical and Applied Genetics*, *126*(8), 1965–1976.

Kamran A, Iqbal M, and Spaner D. (2014a). Flowering time in wheat (*Triticum aestivum* L.): A

key factor for global adaptability. *Euphytica*, *197*(1), 1–26.

Kamran A, Randhawa HS, Yang R-C, and Spaner D. (2014b). The effect of *VRN1* genes on important agronomic traits in high-yielding Canadian soft white spring wheat. *Plant Breeding*, *133*(3), 321–326.

Kamran A, Kubota H, Yang R-C, Randhawa HS, and Spaner D. (2014c). Relative performance of Canadian spring wheat cultivars under organic and conventional field conditions. *Euphytica*, *196*(1), 13–24. https://doi.org/10.1007/s10681-013-1010-3

Kao CH, Zeng ZB, and Teasdale RD. (1999). Multiple interval mapping for quantitative trait loci. *Genetics*, *152*(3), 1203–1216. https://doi.org/10.1534/genetics.108.099028

Kassa MT, Menzies JG, and McCartney CA. (2014). Mapping of the loose smut resistance gene *Ut6* in wheat (*Triticum aestivum* L.). *Molecular Breeding*, *33*(3), 569–576.

Kassa MT, Haas S, Schliephake E, Lewis C, You FM, Pozniak CJ, … others. (2016a). A saturated SNP linkage map for the orange wheat blossom midge resistance gene *Sm1*. *Theoretical and Applied Genetics*, *129*(8), 1507–1517.

Kassa MT, You FM, Fetch TG, Fobert P, Sharpe A, Pozniak CJ, … Hiebert CW. (2016b). Genetic mapping of *SrCad* and SNP marker development for marker-assisted selection of Ug99 stem rust resistance in wheat. *Theoretical and Applied Genetics*, *129*(7), 1–10.

Kato K, and Wada T. (1999). Genetic analysis and selection experiment for narrow-sense earliness in wheat by using segregating hybrid progenies. *Breeding Science*, *49*(4), 233–238.

Kennedy BW. (1981). Variance component estimation and prediction of breeding values. *Canadian Journal of Genetics And Cytology*, *23*(4), 565–578.

Kerber ER, and Aung T. (1999). Leaf rust resistance gene *Lr34* associated with nonsuppression

of stem rust resistance in the wheat cultivar “Canthatch.” *Phytopathology*, *89*(6), 518–521.

Kher S V, Dosdall LM, and Cárcamo HA. (2016). Biology, host preferences and fitness of *Oulema melanopus* (Coleoptera: Chrysomelidae), a recent invasive pest in western Canada. *Arthropod-Plant Interactions*, *10*(5), 365–376.

King RW, and Heide OM. (2009). Seasonal flowering and evolution: The heritage from Charles Darwin. *Functional Plant Biology*, *36*(12), 1027–1036.

Kirk AP, Fox SL, and Entz MH. (2012). Comparison of organic and conventional selection environments for spring wheat. *Plant Breeding*, *131*(6), 687–694. https://doi.org/10.1111/j.1439-0523.2012.02006.x

Klindworth DL, Hareland GA, Elias EM, and Xu SS. (2013). Attempted compensation for linkage drag affecting agronomic characteristics of durum wheat 1AS/1DL translocation lines. *Crop Science*, *53*(2), 422–429.

Knapp SJ. (2001). Mapping quantitative trait loci. In R. L. Phillips & I. K. Vasil (Eds.), *DNA-Based Markers in Plants* (2nd ed., pp. 59–99). Kluwer Academic Publishers. Retrieved from https://link.springer.com/content/pdf/10.1007%2F978-94-015-9815-6.pdf

Knott DR. (1993). Agronomic and quality characters of near-isogenic lines of wheat carrying genes for stem rust resistance. *Euphytica*, *68*(1), 33–41.

Knox RE, Campbell H, Clarke JM, DePauw RM, Procunier JD, and Howes NK. (2008). Genetics of resistance to *Ustilago tritici* in “Glenlea” wheat (*Triticum aestivum*). *Canadian Journal of Plant Pathology*, *30*(2), 267–276.

Knox RE, Clarke FR, Clarke JM, Fox SL, DePauw RM, and Singh AK. (2012). Enhancing the identification of genetic loci and transgressive segregants for preharvest sprouting resistance in a durum wheat population. *Euphytica*, *186*(1), 193–206.

Knox RE, Campbell HL, DePauw RM, Gaudet D, Puchalski B, and Clarke FC. (2013). DNA markers for resistance to common bunt in "McKenzie" wheat. *Canadian Journal of Plant Pathology*, *35*(3), 328–337.

Knox RE, Campbell HL, Clarke FR, Menzies JG, Popovic Z, Procunier JD, … Somers DJ. (2014). Quantitative trait loci for resistance in wheat (*Triticum aestivum*) to *Ustilago tritici*. *Canadian Journal of Plant Pathology*, *36*(2), 187–201.

Kolmer JA, Dyck PL, and Roelfs AP. (1991). An appraisal of stem and leaf rust resistance in North American hard red springs wheats and the probability of multiple mutations to virulence in populations of cereal rust fungi. *Phytopathology*, *81*(3), 237–239.

Kolmer JA. (1996). Genetics of resistance to wheat leaf rust. *Annual Reviews Phytopathology*, *34*(96), 435–55.

Kolmer JA, Lagudah ES, Lillemo M, Lin M, and Bai G. (2015). The *Lr46* gene conditions partial adult-plant resistance to stripe rust, stem rust, and powdery mildew in "Thatcher" wheat. *Crop Science*, *55*(6), 2557–2565. https://doi.org/10.2135/cropsci2015.02.0082

Kosambi DD. (1944). The estimation of map distances from recombination values. *Annals of Eugenics*, *12*, 172–175. https://doi.org/10.1111/j.1469-1809.1943.tb02321.x

Kosellek C, Pillen K, Nelson JC, Weber WE, and Saal B. (2013). Inheritance of field resistance to Septoria tritici blotch in the wheat doubled-haploid population "Solitär" × "Mazurka." *Euphytica*, *194*(2), 161–176. https://doi.org/10.1007/s10681-013-0898-y

Kumar U, Joshi AK, Kumari M, Paliwal R, Kumar S, and Röder MS. (2010). Identification of QTLs for stay green trait in wheat (*Triticum aestivum* L.) in the "Chirya 3" × "Sonalika" population. *Euphytica*, *174*(3), 437–445.

Kumar K, Holtz MD, Xi K, and Turkington TK. (2013). Overwintering potential of the stripe

rust pathogen (*Puccinia striiformis*) in central Alberta. *Canadian Journal of Plant Pathology*, *35*(3), 304–314.

Kumar S, Knox RE, Clarke FR, Pozniak CJ, DePauw RM, Cuthbert RD, and Fox S. (2015). Maximizing the identification of QTL for pre-harvest sprouting resistance using seed dormancy measures in a white-grained hexaploid wheat population. *Euphytica*, *205*(1), 287–309. https://doi.org/10.1007/s10681-015-1460-x

Kutcher HR, Johnston AM, Bailey KL, and Malhi SS. (2011). Managing crop losses from plant diseases with foliar fungicides, rotation and tillage on a Black Chernozem in Saskatchewan, Canada. *Field Crops Research*, *124*(2), 205–212. https://doi.org/10.1016/j.fcr.2011.05.018

Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, Spielmeyer W, … Keller B. (2009). Gene-specific markers for the wheat gene *Lr34/Yr18/Pm38* which confers resistance to multiple fungal pathogens. *Theoretical and Applied Genetics*, *119*(5), 889–898.

Lamari L, Strelkov SE, Yahyaoui A, Orabi J, and Smith RB. (2003). The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. *Phytopathology*, *93*(4), 391–396. https://doi.org/10.1094/PHYTO.2003.93.4.391

Lamb RJ, Wise IL, Olfert OO, Gavloski J, and Barker PS. (1999). Distribution and seasonal abundance of *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) in spring wheat. *The Canadian Entomologist*, *131*(3), 387–397.

Lammerts Van Bueren ET, Jones SS, Tamm L, Murphy KM, Myers JR, Leifert C, and Messmer MM. (2011). The need to breed crop varieties suitable for organic farming, using wheat, tomato and broccoli as examples: A review. *NJAS - Wageningen Journal of Life Sciences*,

58(3–4), 193–205. https://doi.org/10.1016/j.njas.2010.04.001

Lander ES, and Botstein D. (1989). Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, *121*(1), 185–199.

Lander E, and Kruglyak L. (1995). Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. *Nature Genetics*, *11*(3), 241–247. https://doi.org/10.1038/nm0495-365

Laroche A, Demeke T, Gaudet DA, Puchalski B, Frick M, and McKenzie R. (2000). Development of a PCR marker for rapid identification of the *Bt-10* gene for common bunt resistance in wheat. *Genome*, *43*(2), 217–223.

Larsen RJ, and Falk DE. (2013). Effects of a seed treatment with a neonicotinoid insecticide on germination and freezing tolerance of spring wheat seedlings. *Canadian Journal of Plant Science*, *93*(3), 535–540. https://doi.org/10.4141/cjps2012-127

Lemerle D, Verbeek B, Cousens RD, and Coombes NE. (1996). The potential for selecting wheat varieties strongly competitive against weeds. *Weed Research*, *36*(6), 505–513.

Li H, Ye G, and Wang J. (2007). A modified algorithm for the improvement of composite interval mapping. *Genetics*, *175*(1), 361–374. https://doi.org/10.1534/genetics.106.066811

Li H, Vikram P, Singh RP, Kilian A, Carling J, Song J, … Singh S. (2015). A high density GBS map of bread wheat and its application for dissecting complex disease resistance traits. *BMC Genomics*, *16*(1). https://doi.org/10.1186/s12864-015-1424-5

Li Q, Byrns B, Badawi MY, Diallo AB, Danyluk J, Sarhan F, … Fowler DB. (2018). Transcriptomic insights into phenological development and cold tolerance of wheat grown in the field. *Plant Physiology*, *176*(March), 2376–2394. https://doi.org/10.1104/pp.17.01311

Lillemo M, Asalf B, Singh RP, Huerta-Espino J, Chen XM, He ZH, and Bjørnstad Å. (2008).

The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line "Saar." *Theoretical and Applied Genetics*, *116*(8), 1155–1166. https://doi.org/10.1007/s00122-008-0743-1

Litt M, and Luty JA. (1989). A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics*, *44*(3), 397–401.

Liu T, Wan A, Liu D, and Chen X. (2017). Changes of races and virulence genes in *Puccinia striiformis* f. sp. *tritici*, the wheat stripe rust pathogen, in the United States from 1968 to 2009. *Plant Disease*, *101*(8), 1522–1532. https://doi.org/10.1094/PDIS-12-16-1786-RE

Liu Y, Wang R, Hu Y, and Chen J. (2018). Genome-wide linkage mapping of quantitative trait loci for late-season physiological and agronomic traits in spring wheat under irrigated conditions. *Agronomy*, *8*(5). https://doi.org/10.3390/agronomy8050060

Lopes MS, Reynolds MP, McIntyre CL, Mathews KL, Jalal Kamali MR, Mossad M, … Baum M. (2013). QTL for yield and associated traits in the "Seri" / "Babax" population grown across several environments in Mexico, in the West Asia, North Africa, and South Asia regions. *Theoretical and Applied Genetics*, *126*(4), 971–984. https://doi.org/10.1007/s00122-012-2030-4

Lyon B, and Broders K. (2017). Impact of climate change and race evolution on the epidemiology and ecology of stripe rust in central and eastern USA and Canada. *Canadian Journal of Plant Pathology*, *39*(4), 385–392. https://doi.org/10.1080/07060661.2017.1368713

Maccaferri M, Zhang J, Bulli P, Abate Z, Chao S, Cantu D, … Dubcovsky J. (2015). A genome-wide association study of resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in a

worldwide collection of hexaploid spring wheat (*Triticum aestivum* L.). *G3: Genes|Genomes|Genetics*, *5*(3), 449–465. https://doi.org/10.1534/g3.114.014563

Mackay TFC. (2001). The genetic architecture of quantitative traits. *Annual Review of Genetics*, *35*(1), 303–339. https://doi.org/10.1146/annurev.genet.35.102401.090633

Mackay TFC, Stone EA, and Ayroles JF. (2009). The genetics of quantitative traits: Challenges and prospects. *Nature Reviews Genetics*, *10*(8), 565–577.

Mago R, Tabe L, McIntosh RA, Pretorius Z, Kota R, Paux E, … Spielmeyer W. (2011). A multiple resistance locus on chromosome arm 3BS in wheat confers resistance to stem rust (*Sr2*), leaf rust (*Lr27*) and powdery mildew. *Theoretical and Applied Genetics*, *123*(4), 615–623. https://doi.org/10.1007/s00122-011-1611-y

Mammadov J, Aggarwal R, Buyyarapu R, and Kumpatla S. (2012). SNP markers and their impact on plant breeding. *International Journal of Plant Genomics*, *2012*, 1–11.

Manly KF, and Olson JM. (1999). Overview of QTL mapping software and introduction to map manager QT. *Mammalian Genome*, *10*(4), 327–334. https://doi.org/10.1007/s003359900997

Martens JW, and Dyck PL. (1989). Genetics of resistance to rust in cereals from a Canadian perspective. *Canadian Journal of Plant Pathology*, *11*(1), 78–85.

Martens G, Lamari L, Grieger A, Gulden RH, and McCallum B. (2014). Comparative yield, disease resistance and response to fungicide for forty-five historic Canadian wheat cultivars. *Canadian Journal of Plant Science*, *94*(2), 371–381. https://doi.org/10.4141/cjps2013-193

Mason HE, and Spaner D. (2006). Competitive ability of wheat in conventional and organic management systems: A review of the literature. *Canadian Journal of Plant Science*, *86*(2), 333–343. https://doi.org/10.4141/P05-051

Mason HE, Navabi A, Frick BL, O'Donovan JT, and Spaner DM. (2007). The weed-competitive

ability of Canada western red spring wheat cultivars grown under organic management. *Crop Science*, *47*(3), 1167–1176. https://doi.org/10.2135/cropsci2006.09.0566

Mayer M. (2005). A comparison of regression interval mapping and multiple interval mapping for linked QTL. *Heredity*, *94*(6), 599–605. https://doi.org/10.1038/sj.hdy.6800667

McCallum BD, Fetch T, and Chong J. (2007). Cereal rust control in Canada. *Australian Journal of Agricultural Research*, *58*(6), 639–647. https://doi.org/10.1071/AR06145

McCallum BD, and DePauw RM. (2008). A review of wheat cultivars grown in the Canadian prairies. *Canadian Journal of Plant Science*, *88*(4), 649–677.

McCallum BD, Hiebert C, Huerta-Espino J, and Cloutier S. (2012). Wheat leaf rust. In I. Sharma (Ed.), *Disease Resistance in Wheat* (pp. 33–62). CABI.

McCallum BD, Humphreys DG, Somers DJ, Dakouri A, and Cloutier S. (2012). Allelic variation for the rust resistance gene *Lr34/Yr18* in Canadian wheat cultivars. *Euphytica*, *183*(2), 261–274. https://doi.org/10.1007/s10681-011-0519-6

McCallum BD, Seto-Goh P, and Xue A. (2013). Physiologic specialization of *Puccinia triticina*, the causal agent of wheat leaf rust, in Canada in 2009. *Canadian Journal of Plant Pathology*, *35*(3), 338–345.

McCallum BD, Hiebert CW, Cloutier S, Bakkeren G, Rosa SB, Humphreys DG, … Wang X. (2016). A review of wheat leaf rust research and the development of resistant cultivars in Canada. *Canadian Journal of Plant Pathology*, *38*(1), 1–18.

McCartney CA, Somers DJ, Humphreys DG, Lukow O, Ames N, Noll J, … McCallum BD. (2005). Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross "RL4452" × "AC Domain." *Genome*, *48*(5), 870–883.

McCartney CA, Somers DJ, Lukow O, Ames N, Noll J, Cloutier S, … McCallum BD. (2006).

QTL analysis of quality traits in the spring wheat cross "RL4452" × "AC Domain". *Plant Breeding*, *125*(6), 565–575.

McCartney CA, Brûlé-Babel AL, Fedak G, Martin RA, McCallum BD, Gilbert J, … Pozniak CJ. (2016). Fusarium head blight resistance QTL in the spring wheat cross "Kenyon" / "86ISMN 2137". *Frontiers in Microbiology*, *7*(OCT). https://doi.org/10.3389/fmicb.2016.01542

McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, and Xia XC. (2017). *Catalogue of Gene Symbols for Wheat: 2017 Supplement*. Retrieved from https://shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp

McKenzie RIH, Lamb RJ, Aung T, Wise IL, Barker P, Olfert OO, and McIntosh RI. (2002). Inheritance of resistance to wheat midge, *Sitodiplosis mosellana*, in spring wheat. *Plant Breeding*, *121*(5), 383–388.

McLean RA, Sanders WL, and Stroup WW. (1991). A unified approach to mixed linear models. *The American Statistician*, *45*(1), 54–64.

Meng L, Li H, Zhang L, and Wang J. (2015). QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *Crop Journal*, *3*(3), 269–283. https://doi.org/10.1016/j.cj.2015.01.001

Menzies JG, Nielsen J, Thomas PL, and Knox RE. (2003). Virulence of Canadian isolates of *Ustilago tritici*: 1964-1998, and the use of the geometric rule in understanding host differential complexity. *Canadian Journal of Plant Pathology*, *25*(1), 62–72.

Menzies JG, Knox RE, Popovic Z, and Procunier JD. (2006). Common bunt resistance gene *Bt10* located on wheat chromosome 6D. *Canadian Journal of Plant Science*, *86*(Special Issue), 1409–1412.

Menzies JG, Turkington TK, and Knox RE. (2009). Testing for resistance to smut diseases of barley, oats and wheat in western Canada. *Canadian Journal of Plant Pathology*, *31*(3), 265–279.

Messmer M, Seyfarth R, Keller M, Schachermayr G, Winzeler M, Zanetti S, … Keller B. (2000). Genetic analysis of durable leaf rust resistance in winter wheat. *Theoretical and Applied Genetics*, *100*, 419–431. https://doi.org/10.1007/s001220050055

Mester D, Ronin Y, Minkov D, Nevo E, and Korol A. (2003). Constructing large-scale genetic maps using an evolutionary strategy algorithm. *Genetics*, *165*(4), 2269–2282.

Metzger WH. (1935). The residual effect of alfalfa cropping periods of various lengths upon the yield and protein content of succeeding wheat crops. *Journal of the American Society of Agronomy*, *27*(8), 653–659. https://doi.org/10.2134/agronj1935.00021962002700080007x

Meuwissen THE, Hayes BJ, and Goddard ME. (2001). Prediction of total genetic value using genome-wide dense marker maps. *Genetics*, *157*(4), 1819–1829.

Miah G, Rafii MY, Ismail MR, Puteh AB, Rahim HA, Islam KN, and Latif MA. (2013). A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *International Journal of Molecular Sciences*, *14*(11), 22499–22528.

Mikó P, Löschenberger F, Hiltbrunner J, Aebi R, Megyeri M, Kovács G, … Rakszegi M. (2014). Comparison of bread wheat varieties with different breeding origin under organic and low input management. *Euphytica*, *199*(1–2), 69–80. https://doi.org/10.1007/s10681-014-1171-8

Miller JD, Culley J, Fraser K, Hubbard S, Meloche F, Ouellet T, … Voldeng H. (1998). Effect of tillage practice on Fusarium head blight of wheat. *Canadian Journal of Plant Pathology*, *20*(1), 95–103.

Milus EA, Seyran E, and Mcnew R. (2006). Aggressiveness of *Puccinia striiformis* f. sp. *tritici*

isolates in the south-central United States. *Plant Disease*, (July), 847–852.

Morrison WR, Law CN, Wylie LJ, Coventry AM, and Seekings J. (1989). The effect of group 5 chromosomes on the free polar lipids and breadmaking quality of wheat. *Journal of Cereal Science*, *9*(1), 41–51.

Mullis KB, and Faloona FA. (1987). Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in Enzymology*, *155*, 335–350.

Munger H, Vanasse A, Rioux S, and Légère A. (2014). Bread wheat performance, Fusarium head blight incidence and weed infestation response to low-input conservation tillage systems in eastern Canada. *Canadian Journal of Plant Science*, *94*(2), 193–201.

Naruoka Y, Garland-Campbell KA, and Carter AH. (2015). Genome-wide association mapping for stripe rust (*Puccinia striiformis* f. sp. *tritici*) in US Pacific Northwest winter wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *128*(6), 1083–1101. https://doi.org/10.1007/s00122-015-2492-2

Nelson AG, Quideau S, Frick B, Niziol D, Clapperton J, and Spaner D. (2011). Spring wheat genotypes differentially alter soil microbial communities and wheat breadmaking quality in organic and conventional systems. *Canadian Journal of Plant Science*, *91*(3), 485–495. https://doi.org/10.4141/cjps10056

Newman LH. (1928). The history and present status of wheat production in Canada. Ottawa, ON.

Olmos S, Distelfeld A, Chicaiza O, Schlatter AR, Fahima T, Echenique V, and Dubcovsky J. (2003). Precise mapping of a locus affecting grain protein content in durum wheat. *Theoretical and Applied Genetics*, *107*(7), 1243–1251.

Olmstead AL, and Rhode PW. (2002). The red queen and the hard reds: Productivity growth in American wheat, 1800-1940. *The Journal of Economic History*, *62*(4), 929–966.

Park RF. (2015). Long term surveys of pathogen populations underpin sustained control of the rust diseases of wheat in Australia. *Journal and Proceedings of the Royal Society of New South Wales*, *148*(455/456), 15–27.

Payne P, Holt L, Jackson E, and Law C. (1984). Wheat storage proteins: Their genetics and their potential for manipulation by plant breeding. *Philosophical Transactions of the Royal Society B*, *304*(1120), 359–371.

Perez-Lara E, Semagn K, Chen H, Iqbal M, N'Diaye A, Kamran A, … Spaner D. (2016). QTLs associated with agronomic traits in the "Cutler" × "AC Barrie" spring wheat mapping population using single nucleotide polymorphic markers. *PLOS ONE*, *11*(8), 1–19.

Peterson RF, Campbell AB, and Hannah AE. (1948). A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian Journal of Research*, *26c*(5), 496–500. https://doi.org/10.1139/cjr48c-033

Prairie Grain Development Committee. (2016). *Wheat quality objectives*. Retrieved from http://www.pgdc.ca/committees_wrt.html

Prairie Recommending Committee for Wheat Rye and Triticale. (2015). *Operating Procedures, Final Draft*. Retrieved from http://www.pgdc.ca/committees_wrt_pd.html

Preston KR, Kilborn RH, Morgan BC, and Babb JC. (1991). Effects of frost and immaturity on the quality of a Canadian hard red spring wheat. *Cereal Chemistry*, *68*(2), 133–138.

Procunier JD, Gray MA, Howes NK, Knox RE, and Bernier AM. (1997). DNA markers linked to a T10 loose smut resistance gene in wheat (*Triticum aestivum* L.). *Genome*, *40*(2), 176–179.

Puchalski BJ, and Gaudet DA. (2011). *Canadian Plant Disease Survey* (No. 91). Retrieved from https://phytopath.ca/wp-content/uploads/2014/10/cpds-archive/vol91/cpds_vol_91_2011.pdf

Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, … Dodig D. (2005). A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross "Chinese Spring" × "SQ1" and its use to compare QTLs for grain yield across a range of environments. *Theoretical and Applied Genetics*, *110*(5), 865–880.

Raman R, Allen H, Diffey S, Raman H, Martin P, and McKelvie K. (2009). Localisation of quantitative trait loci for quality attributes in a doubled haploid population of wheat (*Triticum aestivum* L.). *Genome*, *52*, 701–715. https://doi.org/10.1139/g09-045

Randhawa H, Puchalski BJ, Frick M, Goyal A, Despins T, Graf RJ, … Gaudet DA. (2012). Stripe rust resistance among western Canadian spring wheat and triticale varieties. *Canadian Journal of Plant Science*, *92*(4), 713–722. https://doi.org/10.4141/cjps2011-252

Randhawa HS, Asif M, Pozniak C, Clarke JM, Graf RJ, Fox SL, … Spaner D. (2013). Application of molecular markers to wheat breeding in Canada. *Plant Breeding*, *132*(5), 458–471. https://doi.org/10.1111/pbr.12057

Rasul G, Humphreys DG, Brule-Babel A, McCartney CA, Knox RE, DePauw RM, and Somers DJ. (2009). Mapping QTLs for pre-harvest sprouting traits in the spring wheat cross "RL4452" / "AC Domain". *Euphytica*, *168*(3), 363–378.

Roelfs AP, Singh RP, and Saari EE. (1992). *Rust diseases of wheat: Concepts and methods of disease management*. Mexico, D.F.: CIMMYT.

Rolland B, Fontaine L, Mailliard A, Gardet O, Heumez E, Walczak P, … Oury FX. (2017). From selection to cultivation with the support of all stakeholders: The first registration in France of two winter bread wheat varieties after value for cultivation and use evaluation in organic farming systems. *Organic Agriculture*, *7*(1), 73–81. https://doi.org/10.1007/s13165-015-0140-4

Rooke L, Bekes F, Fido R, Barro F, Gras P, Tatham AS, … Shewry PR. (1999). Overexpression of a gluten protein in transgenic wheat results in greatly increased dough strength. *Journal of Cereal Science*, *30*(2), 115–120.

Rosewarne GM, Singh RP, Huerta-Espino J, Herrera-Foessel SA, Forrest KL, Hayden MJ, and Rebetzke GJ. (2012). Analysis of leaf and stripe rust severities reveals pathotype changes and multiple minor QTLs associated with resistance in an "Avocet" × "Pastor" wheat population. *Theoretical and Applied Genetics*, *124*(7), 1283–1294. https://doi.org/10.1007/s00122-012-1786-x

Rosewarne GM, Herrera-Foessel SA, Singh RP, Huerta-Espino J, Lan CX, and He ZH. (2013). Quantitative trait loci of stripe rust resistance in wheat. *Theoretical and Applied Genetics*, *126*(10), 2427–2449. https://doi.org/10.1007/s00122-013-2159-9

Rosewarne GM, Li ZF, Singh RP, Yang EN, Herrera-Foessel SA, and Huerta-Espino J. (2015). Different QTLs are associated with leaf rust resistance in wheat between China and Mexico. *Molecular Breeding*, *35*(6). https://doi.org/10.1007/s11032-015-0317-2

Santra DK, Santra M, Allan RE, Campbell KG, and Kidwell KK. (2009). Genetic and molecular characterization of vernalization genes *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* in spring wheat germplasm from the Pacific Northwest region of the USA. *Plant Breeding*, *128*(6), 576–584.

Sax K. (1923). The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics*, *8*(6), 552–560.

Scarth R, and Law CN. (1983). The location of the photoperiod gene, *Ppd2* and an additional genetic factor for ear-emergence time on chromosome 2B of wheat. *Heredity*, *51*(3), 607–619.

Schnurbusch T, Paillard S, Schori A, Messmer M, Schachermayr G, Winzeler M, and Keller B. (2004). Dissection of quantitative and durable leaf rust resistance in Swiss winter wheat reveals a major resistance QTL in the *Lr34* chromosomal region. *Theoretical and Applied Genetics*, *108*(3), 477–484. https://doi.org/10.1007/s00122-003-1444-4

Semagn K, Bjørnstad Å, and Ndjiondjop MN. (2006). Principles, requirements and prospects of genetic mapping in plants. *African Journal of Biotechnology*, *5*(25), 2569–2587. https://doi.org/10.5897/AJB2006.000-5112

Semagn K, Bjørnstad Å, and Xu Y. (2010). The genetic dissection of quantitative traits in crops. *Electronic Journal of Biotechnology*, *13*(5), 1–45.

Semagn K, Babu R, Hearne S, and Olsen M. (2014). Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): Overview of the technology and its application in crop improvement. *Molecular Breeding*, *33*(1), 1–14.

Sharma-Poudyal D, Chen XM, Wan AM, Zhan GM, Kang ZS, Cao SQ, … Patzek LJ. (2013). Virulence characterization of international collections of the wheat stripe rust pathogen, *Puccinia striiformis* f. sp. *tritici*. *Plant Disease*, *97*(3), 379–386.

Sharp PJ, Johnston S, Brown G, McIntosh RA, Pallotta M, Carter M, … Jones MGK. (2001). Validation of molecular markers for wheat breeding. *Crop and Pasture Science*, *52*(12), 1357–1366.

Shaw LM, Turner AS, and Laurie DA. (2012). The impact of photoperiod insensitive *Ppd-1a* mutations on the photoperiod pathway across the three genomes of hexaploid wheat (*Triticum aestivum*). *The Plant Journal*, *71*(1), 71–84.

Singh R, and McIntosh R. (1992). Genetic association of wheat stem rust resistance gene *Sr12* and leaf rust resistance gene *Lr27*. *Cereal Research Communications*, 217–220.

Singh RP, Mujeeb-Kazi A, and Huerta-Espino J. (1998). *Lr46*: A gene conferring slow-rusting resistance to leaf rust in wheat. *Phytopathology*, *88*(9), 890–894. https://doi.org/10.1094/PHYTO.1998.88.9.890

Singh RP, Huerta-Espino J, and Rajaram S. (2000). Achieving near-immunity to leaf and stripe rusts in wheat by combining slow rusting resistance genes. *Acta Phytopathologica et Entomologica Hungarica*, *35*(1/4), 133–139.

Singh RP, and Huerta-Espino J. (2003). Effect of leaf rust resistance gene *Lr34* on components of slow rusting at seven growth stages in wheat. *Euphytica*, *129*(3), 371–376. https://doi.org/10.1023/A:1022216327934

Singh D, Simmonds J, Park RF, Bariana HS, and Snape JW. (2009). Inheritance and QTL mapping of leaf rust resistance in the European winter wheat cultivar "Beaver." *Euphytica*, *169*(2), 253–261. https://doi.org/10.1007/s10681-009-9959-7

Singh A, Knox RE, DePauw RM, Singh AK, Cuthbert RD, Campbell HL, … Clarke F. (2013). Identification and mapping in spring wheat of genetic factors controlling stem rust resistance and the study of their epistatic interactions across multiple environments. *Theoretical and Applied Genetics*, *126*(8), 1951–1964.

Singh RP, Herrera-Foessel S, Huerta-Espino J, Singh S, Bhavani S, Lan C, and Basnet BR. (2014). Progress towards genetics and breeding for minor genes based resistance to Ug99 and other rusts in CIMMYT high-yielding spring wheat. *Journal of Integrative Agriculture*, *13*(2), 255–261.

Singh AK, Knox RE, DePauw RM, Singh AK, Cuthbert RD, Campbell HL, … Bhavani S. (2014). Stripe rust and leaf rust resistance QTL mapping, epistatic interactions, and co-localization with stem rust resistance loci in spring wheat evaluated over three continents.

Theoretical and Applied Genetics, *127*(11), 2465–2477. https://doi.org/10.1007/s00122-014-2390-z

Singh AK, Knox RE, DePauw RM, Singh AK, Cuthbert RD, Kumar S, and Campbell HL. (2016). Genetic mapping of common bunt resistance and plant height QTL in wheat. *Theoretical and Applied Genetics*, *129*(2), 243–256. https://doi.org/10.1007/s00122-015-2624-8

Smith AB, Cullis BR, and Thompson R. (2005). The analysis of crop cultivar breeding and evaluation trials: An overview of current mixed model approaches. *Journal of Agricultural Science*, *143*(6), 449–462. https://doi.org/10.1017/S0021859605005587

Smith MAH, Wise IL, Fox SL, Vera CL, DePauw RM, and Lukow OM. (2014). Seed damage and sources of yield loss by *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) in resistant wheat varietal blends relative to susceptible wheat cultivars in western Canada. *Canadian Entomologist*, *146*(3), 335–345.

Snape JW, Butterworth K, Whitechurch E, and Worland AJ. (2001). Waiting for fine times: Genetics of flowering time in wheat. *Euphytica*, *119*(1), 185–190.

Somers DJ, Isaac P, and Edwards K. (2004). A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *109*(6), 1105–1114.

Sourdille P, Perretant MR, Charmet G, Leroy P, Gautier MF, Joudrier P, … Bernard M. (1996). Linkage between RFLP markers and genes affecting kernel hardness in wheat. *Theoretical and Applied Genetics*, *93*(4), 580–586.

Sourdille P, Charmet G, Trottet M, Tixier MH, Boeuf C, Nègre S, … Bernard M. (1998). Linkage between RFLP molecular markers and the dwarfing genes *Rht-B1* and *Rht-D1* in wheat. *Hereditas*, *128*(1), 41–46. https://doi.org/10.1111/j.1601-5223.1998.00041.x

Spielmeyer W, Hyles J, Joaquim P, Azanza F, Bonnett D, Ellis ME, … Richards RA. (2007). A QTL on chromosome 6A in bread wheat (*Triticum aestivum*) is associated with longer coleoptiles, greater seedling vigour and final plant height. *Theoretical and Applied Genetics*, *115*(1), 59–66.

Statistics Canada. (2018). *CANSIM Table 001-0017. Estimated areas, yield, production, average farm price and total farm value of principal field crops, in metric and imperial units, annual* . Retrieved from http://www5.statcan.gc.ca/cansim/a26?lang=eng&id=10017

Steffan PM, Torp AM, Borgen A, Backes G, and Rasmussen SK. (2017). Mapping of common bunt resistance gene *Bt9* in wheat. *Theoretical and Applied Genetics*, *130*(5), 1031–1040. https://doi.org/10.1007/s00122-017-2868-6

Summers RW, and Brown JKM. (2013). Constraints on breeding for disease resistance in commercially competitive wheat cultivars. *Plant Pathology*, *62*(S1), 115–121.

Sutton JC. (1982). Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. *Canadian Journal of Plant Pathology*, *4*(2), 195–209.

Symes KJ. (1965). The inheritance of grain hardness in wheat as measured by the particle size index. *Australian Journal of Agricultural Research*, *16*(2), 113–123.

Symko S. (1999). From a single seed: Tracing the Marquis wheat success story in Canada to its roots in the Ukraine. Retrieved from http://www5.agr.gc.ca/resources/prod/doc/publications/marquis/wheat-ble_e.pdf

Tahmasebi S, Heidari B, Pakniyat H, and McIntyre CL. (2017). Mapping QTLs associated with agronomic and physiological traits under terminal drought and heat stress conditions in wheat (*Triticum aestivum* L.). *Genome*, *60*(1), 26–45. https://doi.org/10.1139/gen-2016-0017

Tan Y-D, and Fu Y-X. (2006). A novel method for estimating linkage maps. *Genetics*, *173*(4), 2383–2390. https://doi.org/10.1534/genetics.106.057638

Tanksley SD. (1993). Mapping polygenes. *Annual Review of Genetics*, *27*(1), 205–233.

Thoday JM. (1961). Location of polygenes. *Nature*, *191*, 368–370.

Thomas J, Fineberg N, Penner G, McCartney C, Aung T, Wise I, and McCallum BD. (2005). Chromosome location and markers of *Sm1*: A gene of wheat that conditions antibiotic resistance to orange wheat blossom midge. *Molecular Breeding*, *15*(2), 183–192.

Thompson R. (2008). Estimation of quantitative genetic parameters. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1635), 679–686. https://doi.org/10.1098/rspb.2007.1417

Turkington TK, Clear RM, Demeke T, Lange R, Xi K, and Kumar K. (2011). Isolation of *Fusarium graminearum* from cereal, grass and corn residues from Alberta, 2001-2003. *Canadian Journal of Plant Pathology*, *33*(2), 179–186.

Turnbull K-M, Turner M, Mukai Y, Yamamoto M, Morell MK, Appels R, and Rahman S. (2003). The organization of genes tightly linked to the *Ha* locus in *Aegilops tauschii*, the D-genome donor to wheat. *Genome*, *46*(2), 330–338.

Vazquez MD, Zemetra R, Peterson CJ, Chen XM, Heesacker A, and Mundt CC. (2015). Multi-location wheat stripe rust QTL analysis: Genetic background and epistatic interactions. *Theoretical and Applied Genetics*, *128*(7), 1307–1318. https://doi.org/10.1007/s00122-015-2507-z

Verma V, Foulkes MJ, Worland AJ, Sylvester-Bradley R, Caligari PDS, and Snape JW. (2004). Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. *Euphytica*, *135*(3), 255–263.

Vignal A, Milan D, SanCristobal M, and Eggen A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics. *Genetics Selection Evolution*, *34*(3), 275–305.

Voorrips RE. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, *93*(1), 77–78. https://doi.org/10.1093/jhered/93.1.77

Vos P, Hogers R, Bleeker M, Reijans M, de Lee T, Hornes M, … Zabeau M. (1995). AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research*, *23*(21), 4407–4414.

Wang S, Knox RE, DePauw RM, Clarke FR, Clarke JM, and Thomas JB. (2009). Markers to a common bunt resistance gene derived from "Blizzard" wheat (*Triticum aestivum* L.) and mapped to chromosome arm 1BS. *Theoretical and Applied Genetics*, *119*(3), 541–553.

Wang X, Bakkeren G, and McCallum BD. (2010). Virulence and molecular polymorphisms of the wheat leaf rust fungus *Puccinia triticina* in Canada from 1997 to 2007. *Botany*, *88*(6), 575–589.

Wang G, Leonard JM, von Zitzewitz J, Peterson CJ, Ross AS, and Riera-Lizarazu O. (2014). Marker-trait association analysis of kernel hardness and related agronomic traits in a core collection of wheat lines. *Molecular Breeding*, *34*(1), 177–184.

Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, … Akhunov E. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant Biotechnology Journal*, *12*(6), 787–796.

Wanyera R, Kinyua MG, Jin Y, and Singh RP. (2006). The spread of stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on *Sr31* in wheat in eastern Africa. *Plant Disease*, *90*(1), 113.

Weber JL, and May PE. (1989). Abundant class of human DNA polymorphisms which can be

typed using the polymerase chain reaction. *American Journal of Human Genetics*, *44*(3), 388–396.

Wellings CR. (2011). Global status of stripe rust: A review of historical and current threats. *Euphytica*, *179*(1), 129–141. https://doi.org/10.1007/s10681-011-0360-y

Wickham H. (2009). *ggplot2: Elegant graphics for data analysis*. New York: Springer-Verlag.

Williams PC, and Sobering DC. (1984). Influence of variables on wheat hardness. *Cereal Foods World*, *29*, 498.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, and Tingey S V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, *18*(22), 6531–6535.

Winfield MO, Allen AM, Burridge AJ, Barker GLA, Benbow HR, Wilkinson PA, … Edwards KJ. (2016). High-density SNP genotyping array for hexaploid wheat and its secondary and tertiary gene pool. *Plant Biotechnology Journal*, *14*(5), 1195–1206. https://doi.org/10.1111/pbi.12485

Wolfe MS, Baresel JP, Desclaux D, Goldringer I, Hoad S, Kovacs G, … Lammerts Van Bueren ET. (2008). Developments in breeding cereals for organic agriculture. *Euphytica*, *163*(3), 323–346. https://doi.org/10.1007/s10681-008-9690-9

Worland AJ. (1996). The influence of flowering time genes on environmental adaptability in European wheats. *Euphytica*, *89*(1), 49–57.

Wu Y, Bhat PR, Close TJ, and Lonardi S. (2008). Efficient and accurate construction of genetic linkage maps from the minimum spanning tree of a graph. *PLOS Genetics*, *4*(10), 1–11. https://doi.org/10.1371/journal.pgen.1000212

Xi K, Kumar K, Holtz MD, Turkington TK, and Chapman B. (2015). Understanding the

development and management of stripe rust in central Alberta. *Canadian Journal of Plant Pathology*, *37*(1), 21–39. https://doi.org/10.1080/07060661.2014.981215

Xu S. (2003). Theoretical basis of the Beavis effect. *Genetics*, *165*(4), 2259–2268.

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, … Dubcovsky J. (2004). The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. *Science*, *303*(5664), 1640–1644.

Yan L, Fu D, Li C, Blechl A, Tranquilli G, Bonafede M, … Dubcovsky J. (2006). The wheat and barley vernalization gene *VRN3* is an orthologue of FT. *Proceedings of the National Academy of Sciences*, *103*(51). https://doi.org/10.1073/pnas.0607142103

Yang R-C. (2010). Towards understanding and use of mixed-model analysis of agricultural experiments. *Canadian Journal of Plant Science*, *90*(5), 605–627. https://doi.org/10.4141/CJPS10049

Young ND. (1996). QTL mapping and quantitative disease resistance in plants. *Annual Review of Phytopathology*, *34*(1), 479–501.

Yousef EAA, Lampei C, and Schmid KJ. (2015). Evaluation of cauliflower genebank accessions under organic and conventional cultivation in southern Germany. *Euphytica*, *201*(3), 389–400. https://doi.org/10.1007/s10681-014-1225-y

Yu L-X, Barbier H, Rouse MN, Singh S, Singh RP, Bhavani S, … Sorrells ME. (2014). A consensus map for Ug99 stem rust resistance loci in wheat. *Theoretical and Applied Genetics*, *127*(7), 1561–1581.

Yu M, Zhang H, Zhou X-L, Hou D-B, and Chen G-Y. (2017). Quantitative trait loci associated with agronomic traits and stripe rust in winter wheat mapping population using single nucleotide polymorphic markers. *Molecular Breeding*, *37*(8).

https://doi.org/10.1007/s11032-017-0704-y

Zhang P, Hiebert CW, McIntosh RA, McCallum BD, Thomas JB, Hoxha S, … Bansal U. (2016). The relationship of leaf rust resistance gene *Lr13* and hybrid necrosis gene *Ne2m* on wheat chromosome 2BS. *Theoretical and Applied Genetics*, *129*(3), 485–493. https://doi.org/10.1007/s00122-015-2642-6

Zhang P, Yin G, Zhou Y, Qi A, Gao F, Xia X, … Liu D. (2017). QTL mapping of adult-plant resistance to leaf rust in the wheat cross "Zhou 8425B" / "Chinese Spring" using high-density SNP markers. *Frontiers in Plant Science*, *8*(May). https://doi.org/10.3389/fpls.2017.00793

Zhao H, and Speed TP. (1996). On genetic map functions. *Genetics*, *142*(4), 1369–1377.

Zhou Y, Ren Y, Lillemo M, Yao Z, Zhang P, Xia X, … Liu D. (2014). QTL mapping of adult-plant resistance to leaf rust in a RIL population derived from a cross of wheat cultivars "Shanghai 3" / "Catbird" and "Naxos." *Theoretical and Applied Genetics*, *127*(9), 1873–1883. https://doi.org/10.1007/s00122-014-2346-3

Zou J, Semagn K, Iqbal M, N'Diaye A, Chen H, Asif M, … Spaner D. (2017a). Mapping QTLs controlling agronomic traits in the "Attila" × "CDC Go" spring wheat population under organic management using 90K SNP array. *Crop Science*, *57*(1), 365–377. https://doi.org/10.2135/cropsci2016.06.0459

Zou J, Semagn K, Iqbal M, Chen H, Asif M, N'Diaye A, … Spaner D. (2017b). QTLs associated with agronomic traits in the "Attila" × "CDC Go" spring wheat population evaluated under conventional management. *PLOS ONE*, *12*(2), 1–20. https://doi.org/10.1371/journal.pone.0171528

Zou J, Semagn K, Chen H, Iqbal M, Asif M, N'Diaye A, … Spaner D. (2017c). Mapping of

QTLs associated with resistance to common bunt, tan spot, leaf rust, and stripe rust in a spring wheat population. *Molecular Breeding*, *37*(12). https://doi.org/10.1007/s11032-017-0746-1

Zwart RS, Thompson JP, Milgate AW, Bansal UK, Williamson PM, Raman H, and Bariana HS. (2010). QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. *Molecular Breeding*, *26*(1), 107–124. https://doi.org/10.1007/s11032-009-9381-9