Quantitative trait loci mapping of rust resistance and agronomic traits in the doubled haploid spring wheat population 'HYAYT12-10' × 'GP146'

by

Izabela L. Ciechanowska

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

Marker-assisted selection requires the identification of molecular markers associated with major genes and quantitative trait loci (QTL) using linkage analysis. In this study, we used 167 doubled haploid (DH) lines derived from two unregistered spring wheat (*Triticum aestivum* L.) parental lines that belong to the Canada Western Special Purpose (CWSP) class to map QTLs associated with five traits using inclusive composite interval mapping (ICIM). Using ICIM, least square means phenotype data across 3-4 environments, and a genetic map of 2,676 SNPs out of the wheat 90K SNP array, we identified ten QTLs associated with maturity (4A and 5B), plant lodging (4B, 5A, 5D, and 7D), grain yield (2D), leaf rust (4A) and stem rust (1A and 2B). Each QTL individually accounted for 6.0-22.3% of the phenotypic variance and together accounted for 8.6-38.2% of each trait. QTLs identified for rusts using ICIM had a minor effect (6.0-9.0%) or a major effect (22.3%). Our major effect QTL at 22.3% was discovered on chromosome 2B and contributed to stem rust response. Its physical location has been associated with disease response in previous studies. Results from this study provide additional valuable information to wheat researchers, in particular that the area on chromosome 2B should be considered for future analyses.

Preface

A modified version of chapter two has been submitted to *The Canadian Journal of Plant Science* titled as "Quantitative trait loci mapping of rust resistance and agronomic traits in spring wheat" authored by Ciechanowska, I., Semagn, K., McCallum, B., Randhawa, H., Strenzke, K., Virginillo, M., Iqbal, M., and Spaner, D.

The mapping population for this study was created in 2013 by crossing 'HYAYT12-10' with 'GP146' in the greenhouse at the University of Alberta. The line was advanced by the doubled haploid (DH) method in the Lethbridge Research and Development Centre of Agriculture and Agri-Food Canada by Dr. Harpinder Randhawa.

I was responsible for collecting maturity, lodging and yield data in Edmonton 2016 and 2017. Klaus Strenzke and Hua Chen scored the leaf rust nursery in Edmonton 2016. I scored the Edmonton 2017 leaf rust nursery. Brent McCallum scored all Manitoba leaf rust and stem rust nurseries. Mark Virginillo and Harpinder Randhawa grew, maintained and recorded Lethbridge agronomic data. The University of Alberta wheat breeding group grew and maintained the research plots at the Edmonton Research Station at South Campus. I extracted the genomic DNA and Christina Sidebottom of the National Research Council in Saskatoon did the genotyping of the Illumina Wheat 90K iSelect SNP Array. Statistical and QTL analysis was performed by me with significant help from Dr. Kassa Semagn. Considerable editing of this manuscript was done by Dr. Kassa Semagn, Dr. Dean Spaner and Dr. Muhammad Iqbal.

Acknowledgments

I am very grateful to my supervisor, Dr. Dean Spaner. Thank you for the opportunity, financial support, and time to complete my graduate degree, Master of Science in Plant Science. A heartfelt thank you to the University of Alberta wheat breeding group: Dr. Muhammad Iqbal, Klaus Strenzke, Joseph Moss, Tom Keady, Katherine Chabot, Fabiana Perotta Dias, Dr. Hua Chen, Dr. Steliana Paula Barbu Dobre and Momna Farzand. I am very appreciative for their technical support with phenotypic data collection in both field and nurseries and advice on analysis and molecular work. I would not have been able to get through this thesis if it wasn't for Dr. Kassa Semagn. Thank you, Dr. Semagn, for your patience. Lastly, many hugs and thanks to my family and friends. You all encouraged me and endured my many temperaments. A very big thank you especially to my supportive husband, Ian Reynolds, who has held our household together even when I was barely present in body and mind.

This study was supported by grants to the University of Alberta wheat breeding program from the Alberta Crop Industry Development Fund (ACIDF), Alberta Wheat Commission (AWC), Saskatchewan Wheat Development Commission (Sask Wheat), Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery and Collaborative Grant, Agriculture and Agri-Food Canada (AAFC), Western Grains Research Foundation Endowment Fund (WGRF), and Core Program Check-off funds to Dean Spaner.

Table of Contents

List of Tables:

List of Figures:

1. Literature Review

1.1 Canadian Wheat

1.1.1 The Wheat Plant

Wheat (*Triticum aestivum* L.) is a self-pollinating grass plant of the Family *Poaceae* (Acquaah 2012). This grass is cultivated for its seed and is a staple food in most places in the world (Acquaah 2012, DePauw et al. 2007). In Canada, 35.2 million tonnes (Mt) of wheat was produced in 2020 (Statistics Canada 2020a), of which 19.6 Mt was exported.

Wheat is a crop which has adapted and been successful in many different climates, preferring temperate regions without excessive rainfall (Acquaah 2012, DePauw et al. 2007). There are winter and spring wheat cultivars. Spring wheat is sown in spring and harvested in fall. Winter wheat is sown in fall, germinates but then remains dormant when the temperature gets too cold (Acquaah 2012, Distelfeld et al. 2009). This cold period is necessary to shift from a vegetative to reproductive (flowering) state (Rawson et al 1998). This is called vernalization. The seedling continues to grow in spring and is harvested in mid to late summer (Acquaah 2012, McCallum and DePauw 2008). Wheat kernels are either soft or hard and can be red, white or purple in colour (Knievel et al. 2009, McCallum and DePauw 2008).

1.1.2 The History of Canadian Wheat Production

The story of Canadian spring wheat breeding and production began in 1842 with Scottish immigrant David Fife. Near Peterborough, Ontario, the farmer planted what was to be called Red Fife – an homage to its dark kernel colour and its first Canadian producer (Cuthbert 2006). The success of Red Fife was due to its desirable baking and milling characteristics; it spread past

Canadian borders and into the United States' Mid-West (Cuthbert 2006, Olmstead and Rhode 2002). Red Fife was the first hard spring wheat to be adopted by North American farmers (Olmstead and Rhode 2002) and would become the parent to most bread wheat developed in North America (Cuthbert 2006).

In 1892, the cultivars Hard Red Calcutta and Red Fife were crossed to produce Marquis, an early maturing, higher yielding cultivar which still maintained Red Fife's baking quality (McCallum and DePauw 2008). Marquis was officially released in 1911 and remained a popular hard spring wheat until the late 1930s (McCallum and DePauw 2008). However, both Red Fife and Marquis were susceptible to stem and leaf rusts (McCallum and DePauw 2008, McCallum et al. 2016). It was not until 1935 that stem rust resistant Thatcher was developed in response to several rust epidemics in Canada which began as early as 1902 and continued into the 1930s (McCallum et al. 2016). In 1937, Renown was released, and it was the first leaf rust resistant cultivar available (carrying *Lr14a*) (McCallum et al. 2016). Thatcher was the predominant cultivar grown until 1968, when it was replaced by Manitou (McCallum et al. 2016). Semi-dwarf wheat cultivars from the International Maize and Wheat Improvement Centre (CIMMYT) were introduced in the late 1960s and they introduced different traits into the Canadian wheat gene pool, specifically high yield, low protein and low to medium gluten strength (McCallum and DePauw 2008). As wheat diversification in Canada continued, additional wheat classes were added to reflect the profiles of these cultivars and improvements within each class have been the focus of research (McCallum and DePauw 2008). The success of Canadian wheat can be credited to the diversity of cultivars that have been grown since Red Fife, reflecting breeders' efforts in incorporating desirable traits such as high yields, lodging and disease resistance for growers and high quality for end users (McCallum and DePauw 2008).

1.1.3 Standardizing Wheat in Canada

Wheat did not always guarantee good performance or high quality grain, especially in the early 1900s. It was not until 1925 that the Canadian Grain Act introduced grading in wheat (to reflect the health of seed) and created classes to categorize grain according to its qualities (McCallum and DePauw 2008). A decade later the Canadian Wheat Board was officially established to regulate the import and export of wheat (McCallum and DePauw 2008). Federal standards have continually adapted to the changing market and production challenges and have developed strict guidelines for the registration of cultivars and their categorization into different classes and grades (McCallum and DePauw 2008, Randhawa et al. 2013). Today, the Canadian Grain Commission recognizes nine wheat classes. Each class is based on end-use characteristics, kernel colour and hardness, protein percentage, gluten strength and spring or winter growth (Canadian Grain Commission 2021, Randhawa et al. 2013): Canada Northern Hard Red (CNHR), Canada Prairie Spring Red (CPSR), Canada Prairie Spring White (CPSW), Canada Western Amber Durum (CWAD), Canada Western Hard White Spring (CWHWS), Canada Western Red Spring (CWRS), Canada Western Red Winter (CWRW), Canada Western Soft White Spring (CWSWS) and Canada Western Special Purpose (CWSP) (Canadian Grain Commission 2021, McCallum and DePauw 2008). Wheat registered in the special purpose class is usually high yielding and not a milling grain due to its high starch and low protein content, which makes it most apt for use as animal feed and ethanol production (Beres et al. 2013, Canadian Grain Commission 2021).

Wheat in Western Canada is graded (from highest to lowest quality) as Grade No.1, No.2, No.3 and Feed Grade. Down-grading factors include abnormal/unhealthy looking seed due to various types of damage (environmental, pest or disease), pathogen spore or mycotoxin presence,

low purity such as foreign seeds, weeds or stones in the grain samples (Canadian Grain Commission 2021, McCallum and DePauw 2008).

The predominant types of wheat in western Canada include durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.), winter hexaploid wheat (*Triticum aestivum* L.), and spring hexaploid wheat (*Triticum aestivum* L.) which accounts for most Canadian wheat production (McCallum and DePauw 2008). According to Agriculture and Agri-Food Canada in their December 2020 report for Outlook for Principal Field Crops, Statistics Canada estimated Canadian western hard red spring (CWRS) wheat production in 2020 at 21.98 Mt; Canada Prairie Spring (CPS) was at 1.82 Mt; Canada Northern Hard Red Spring (CNHR) amounted to 0.82 Mt; soft white spring (CWSWS) reached 0.52 Mt; all other spring wheat totaled 0.62 Mt and eastern hard red spring contributed 0.43 Mt. Durum wheat production in the 2020 season was at 6.57 Mt. Total Canadian wheat production (durum, spring and winter types) for 2020 was 35.2 Mt and over half of this was exported (Agriculture and Agri-Food Canada 2020, Statistics Canada 2020b). In Canada, the Prairie Provinces of Manitoba, Saskatchewan and Alberta grow over 90% of Canada's wheat and all of Canada's Canadian Western Red Spring wheat cultivars (Aboukhaddour et al. 2013, Canadian Grain Commission 2021).

1.2 Wheat Breeding

1.2.1 The Beginning of Domestication

Agriculture was an innovation in the Middle East, about 12,000 years ago (Liu et al. 2014). This was the domestication of plants and animals, which shifted people from a nomadic hunter-gatherer lifestyle into settled agrarian communities (Acquaah 2012). The Fertile Crescent

was one area where civilization and culture emerged along with the Neolithic (Acquaah 2012, Liu et al. 2014).

Selection of certain plants based on their visible traits (phenotype) was the first method of breeding. Early breeders visually selected plants with desirable characteristics, called phenotypic markers. The art of visual selection relies on a person's intuition and an ability to spot unique traits which can arise in nature (Acquaah 2012). To this day, these phenotypic markers are very important as they are the result of a genetic and environmental effects. In general, breeders want wheat that adapts to biotic and abiotic changes, shows stability in yield performance and consistently meets nutritional (end use) expectations (Acquaah 2012, Randhawa et al. 2013).

1.2.2 Canadian Expectations of Wheat Breeding

The Canadian Minister of Agriculture and Agri-Food approves committees which are responsible for overseeing crop registration. These committees form the protocols and standards by which each breeder's proposed cultivar must meet to be considered for registration (Agriculture and Agri-Food Canada 2013, PGDC 2018). Wheat is overseen by the Prairie Grain Development Committee (PGDC) for wheat, rye and triticale. There are three recommending committees, assessing disease, quality and agronomic components (Agriculture and Agri-Food Canada 2013, PGDC 2018). Candidates are measured against existing standard check cultivars over several years to assess their merit (how well they compare to the industry standards) (Agriculture and Agri-Food Canada 2013, PGDC 2018). An intermediate level of resistance is necessary to the five priority diseases, namely leaf rust (*Puccinia Puccinia triticina* Eriks. f.sp. *tritici*), stem rust (*Puccinia graminis* f.sp. *tritici* Eriks. & E. Henn.T), stripe rust (*Puccinia striiformis* Westend. f.sp. *tritici* Eriks.), fusarium head blight (*Fusarium graminerium* Schwabe)

and bunt (*Tilletia tritici* and *T. laevis*) (PGDC 2018). Agronomic traits of interest are lodging resistance, maturity, yield, test weight and kernel weight and good kernel quality (McCallum and DePauw 2008, PGDC 2018). The quality committee assesses protein content, gluten strength, falling number and various milling and baking characteristics. Of course, depending on the wheat class (end-use category), expected quality characteristics will vary (McCallum and DePauw 2008, PGDC 2018). For example, grain intended for animal feed and ethanol production is usually higher yielding with a low protein profile. Even down the production line, a bread baker would select a wheat flour which would fall into a category with a red, hard kernel and strong gluten profile, while a pastry chef would prefer a white and softer kernel with lower gluten content (McCallum and DePauw 2008).

In the end, the breeder must satisfy the demands of the market – its growers, millers and their final customers (McCallum and DePauw 2008). Depending on the required end use of the product, a specific wheat cultivar must be selected to provide the best economic return for the input required to grow it (McCallum and DePauw 2008). In western Canada, farmers need cultivars which resist lodging, disease, pests, harsh environmental conditions and mature as early as possible due to the short growing season (McCallum and DePauw 2008).

1.2.3 Molecular Markers

Plant breeding also partners with scientific advances to help in phenotypic selection. For over 20 years, molecular marker assisted selection or breeding (MMAS or MAB) has allowed plant breeders to quickly identify individual plants carrying desirable traits (Randhawa et al. 2013). Researchers regularly check for molecular markers linked to desired wheat traits such as resistance to leaf rust, stem rust, stripe rust, fusarium head blight, bunt and insects including

wheat stem sawfly (*Cephus cinctus* Nort.) and wheat midge (*Sitodiplosis mosellana* Géhin) (Acquaah 2012, Randhawa et al 2013). Markers are also used to select for characteristics such as earliness and vernalization and several quality factors such as grain texture and protein content (Agrios 2005, Acquaah 2012, Randhawa et al. 2013, Toth et al. 2018). Physical characteristics such as plant height and, in turn, lodging can be selected for by confirming the presence of certain reduced height (*Rht)* genes and their alleles by using MMAS (Sukhikh et al. 2021).

A molecular marker is a known area on the DNA which is closely linked to a DNA fragment (locus) which contributes to a plant's characteristics and/or responses to biotic or abiotic stressors (Pinto da Silva et al. 2018, Korzun 2002). Molecular markers can confirm the plant's genetics when certain traits may be difficult to select for phenotypically (Acquaah 2012, Randhawa et al. 2013). In addition, molecular markers are used to corroborate gene stacking when striving to give a plant superior performance against various pathogens and their races (Randhawa et al. 2013). Three requirements should be met for effective marker assisted selection. They are the accessibility to cost-effective and time-efficient screening methods that are reproduceable, recognized co-segregation between molecular markers and their respective genes (Randhawa et al. 2013) and certainty that the molecular markers are correlated to the traits expressed by the genotypes in response to specific growing conditions (Sharp et al. 2001).

Molecular marker assisted selection can be used to increase selection pressure. As an example, plants can be screened for known markers linked to resistance alleles against pathogens which are not present in Canada, a method to safeguard against future pathogen outbreaks (Toth et al. 2018). This is also a useful technology when seed number is limited, as screening for desired traits will save time and resources. Selection accuracy is also a benefit of MMAS. Phenotypes can vary according to environment; if associated markers of traits are well known,

then molecular selection is one method to choose plants independent of field conditions (Toth et al. 2018). This is a beneficial tool in early generations, while genes are still segregating, in choosing a diverse set of desired genes in the population by indirectly selecting for traits (Randhawa et al. 2013, Toth et al. 2018). Molecular markers can also confirm sources of genetic variance which cause phenotypic deviation from the norm (Toth et al. 2018). There are many documented markers linked with minor alleles and areas contributing to agronomically important traits which are continually being discovered and studied (Toth et al. 2018). Evolving MMAS techniques can assay up to thousands of samples for numerous traits in several hours, and procedures are easily scaled up or down according to experiment size. A DNA sample can be prepared from any tissue at any point in the plant's life cycle, and the DNA amount necessary is in the nanograms (Toth et al. 2018). This high throughput, experiment adaptability and ease in sampling are what make MMAS an accessible tool when selecting based on established marker-trait associations (Toth et al. 2018).

Toth et al. (2018) caution that it is important to remember that molecular markers should not be used as the sole sources of data in ideal situations. Some genes conferring known traits have been cloned, such as *Lr34* (Huang et al. 2003, Prasad et al. 2020), and their markers are very closely linked or on the coding region of the gene (McCallum et al. 2011). However, this is not always possible in large-genome-species, as cloning a specific locus requires fine genetic and physical mapping of the area (Huang et al. 2003). The goal is to find molecular markers that are as tightly linked as possible to the area of interest. This is the only way to try to limit recombination events between markers and loci, which can cause the presence of a marker but lack the desired locus (false positive), or the observation of a trait but no supporting evidence of a marker in screening (false negative) (Toth et al. 2018). Some genes may be present but they

may have suppressors which change the phenotype, such as the Anza-derived *Yr17* suppressor (Helguera et al. 2003) and Thatcher-derived *Lr23* suppressor (McIntosh and Dyck 1975). Given these types of ambiguous circumstances, MMAS should always be used in conjunction with phenotypic selection and never replace it (Ellis et al. 2002, Liu et al. 2008, Toth et al. 2018).

Finely mapping areas of interest may result in better linked molecular markers for predicting the presence of loci and can even lead to the cloning of certain loci. This renders MMAS more accurate for the breeder (Pinto da Silva et al. 2018, Prasad et al. 2020, Toth et al. 2018). Molecular technology is constantly improving and has facilitated the creation of genetic maps for species with large genomes, such as hexaploid wheat at 17 gigabase pairs (Benchley et al. 2012, Pinto da Silva et al. 2018). For screening a desired locus, single nucleotide polymorphisms (SNPs) are considered the most widespread form of sequence variability in wheat. A SNP is one nucleotide difference between two genotypes at a locus (Semagn et al. 2014, Toth et al. 2018, Würschum et al. 2013). For SNP detection, an allele specific polymerase chain reaction (AS-PCR) method is used which involves two primers, each designed for a specific allele at the locus. Only if the allele is present, will there be a successful PCR product (amplicon) (Toth et al. 2018). Kompetitive allele specific PCR (KASP) is an improved SNP detection method where the primers are tagged at their 5' ends and, depending what allele is present, the associated fluorescent signal will be recorded (Semagn et al. 2014, Toth et al. 2018). The benefit of KASP is its discrimination to verify homozygosity or heterozygosity in a locus by observing both types of fluorescence (Biosearch Technologies 2020, Semagn et al. 2014).

Even though SNPs are the most common sequence changes, simple sequence repeats (SSRs) are easy to use for their detection and reproducibility, codominant inheritance and wide genome coverage (Pinto da Silva et al. 2018, Toth et al. 2018). A SSR is a sequence of

nucleotides in a locus which differs in copy number between two genotypes, resulting in different amplicon length. In AS-PCR SNP and SSR detection, DNA products are traditionally verified using fluorescence such as ethidium bromide or SYBrsafe and run on agarose or polyacrylamide gels to assess for amplicon size (Toth et al. 2018). However, in recent years, new technologies have been developed for fast and accurate screening of many samples, and for thousands of different loci discrepancies. These are known as 'next generation sequencing' (NGS) marker technologies and they have become readily available to researchers. The first generation methods rely on the Sanger sequencing method, such as capillary electrophoresis (CE) (Pinto da Silva et al. 2018, Toth et al. 2018). In later and more current NGS methods, DNA fragments are sequenced and their bases are read by emitting different fluorescent colours, such as the 90K SNP array method (Walker and Rapley 2008). These fast evolving marker tools along with resources such as the International Wheat Genome Sequencing Consortium's reference genome of bread wheat, lastly updated on July 25, 2019 (IWGSC RefSeq v2.0), offer a wide option of methods for the researcher and have made breeding theoretically more efficient and practical (Pinto da Silva et al. 2018).

1.2.4 Genes and Quantitative Trait Loci

Loci are given gene designations when they are recognized as being genetically different than already established loci (Pinto da Silva et al. 2018). Rust resistance genes are named depending on the specific pathogen and given a number, for example *Lr12* (leaf rust gene 12) (Pinto da Silva et al. 2018). A gene can be qualitative (or major) and its expression is noticeably absent or present. This is known also as vertical resistance, or qualitatively inherited resistance, and is specific in its function (e.g. a wheat plant that carries *Lr12* is race-specific, meaning it

expresses full resistanceto only a specific subset of *Puccinia triticina* races) (Agrios 2005). A quantitative (or minor) gene is responsible for a range of smaller contributions to one or more traits and its function can sometimes be affected by certain environments (Lagudah et al. 2006, Pinto da Silva et al. 2018). This partial protection is also known as horizontal resistance, or quantitatively inherited resistance. For example, the partially resistant stripe rust gene *Yr17* is also linked to leaf and stem rust resistance genes *Lr37* and *Sr38*, respectively (Brar et al. 2019, Helguera et al. 2002, Plotnikova and Stubei 2013). In addition, this complex is strongly expressed at higher temperatures, which makes it ideal in warmer environments (Brar et al. 2018, Brar et al. 2019). Loci which cause quantitative phenotypic variation are known as quantitative trait loci (QTL). Once QTL have been pinpointed to an area on the chromosome through analysis, molecular approaches narrow down the QTL to potential genes and their functions (Miles and Wayne 2008, Pinto da Silva et al. 2018). QTL mapping/linkage studies are important to uncovering loci which could contribute as sources of resistance and the markers linked to them (Sing et al. 2014). In addition, different loci can interact in different ways and these relationships are important to understand (Sing et al. 2014).

Minor QTLs are significantly altered by environment and they usually contribute less than 15% variation to a trait. Major QTLs are typically expressed in more than one environment and are usually considered responsible for 15 – 20% of variation observed in experimental results (Du et al. 2015, Pinto da Silva et al. 2018). It is important to consider the possibility that one QTL can cause different phenotypic responses in different genetic backgrounds (epistatic interactions) and in different environmental conditions (Pinto da Silva et al. 2018, Miles and Wayne 2008). A QTL is suspected when the average phenotype of a gene's alleles in the experimental population significantly deviates from what is expected (Pinto da Silva et al. 2018).

This observation signals that the expected phenotype is skewed due to other factors which are affecting known genes and their responses. For accurate QTL identification, the parental lines used to create the population should ideally be genetically varied to increase different QTL combination possibilities within the population (Pinto da Silva et al. 2018, Xu et al. 2017). This can be done by choosing unlike parents in biparental crossing methods or using a few parents to create a multiparent population. This is a strategic way to increase the assortment of QTL alleles and markers and to allow more recombination events - and therefore better data for linkage analysis - among them (Pinto da Silva et al. 2017, Xu et al. 2017).

QTLs can easily be missed or misinterpreted, so it is always better to have a large population size for less bias and more precision in QTL effect calculation and location estimation (Charcosset and Gallais 1996, Miles and Wayne 2008). An inadequate population size can result in a bias towards overestimation of contribution from certain loci and overlook smaller contributors to phenotype. This is known as the "Beavis effect" (Beavis 1994 and 1997, Miles and Wayne 2008). QTL with smaller effects can also be lost due to large experimental error, and a QTL mapping process with low power (Zhang et al. 2020). Furthermore, QTLs can also be missed if they are closely linked. If near connected QTLs confer a similar effect then only one will be incorrectly identified, and if they confer two opposing functions then they may cancel each other out and no QTL will be detected (Zhang et al. 2020).

Common population types which are used for QTL mapping (known as interval mapping) are F_2 backcrosses, doubled haploids (DH), backcrosses (BC), recombinant inbred lines (RIL), near-isogenic lines (NIL), association mapping (AM) and multiparent advanced generation crosses (MAGIC) (Pinto da Silva et al. 2018, Xu et al. 2017). Quantitative trait loci linkage analysis is a statistical genetic mapping method, using the recombination frequency of the

population (Meng et al. 2015, Zhang et al. 2020). One of the most common approaches is Composite Interval Mapping (CIM), but the model does not account for background influence. Inclusive Composite Interval Mapping (ICIM) identifies possible markers and performs interval mapping using phenotypes recorded in each environment (Meng et al. 2015). Well developed and widely used software for performing various methods of linkage analysis include QGene, Map Manager, IciMapping, QTLnetwork, MapQTL, and win QTL Cartographer, to name a few (Zhang et al. 2020).

QTLs are reported by their proximity to known markers by either their genetic positions in centimorgans (cM) or their physical positions in base pairs (bp or b), by their total associated variance (r^2) in percentage to explain phenotypic variation (% PVE) and by their logarithm of the odds score (LOD) which is the statistical strength of data used to estimate a QTL's position (Broman 2001, Pinto da Silva et al. 2018, Semagn et al. 2021a).

1.3 Wheat and the Rust Pathogen

1.3.1 History of Common Rust Diseases of Wheat

Cereal rust diseases are a problem for modern cereal production and there is evidence they have historically plagued human crops. Wheat remnants with stem rust have been found in Israel and have been dated from the Bronze Age (Kislev 1982, Leonard and Szabo 2005). Rust outbreaks are recorded in the Old Testament as penance for people's sins (Leonard and Szabo 2005). Even Ancient Romans (Liu et al. 2014, Pinto da Silva et al. 2018) celebrated the festival Robigalia on April 25th, to appease the god Robigus so their fields would be free of rust and blight diseases (Leonard and Szabo 2005, Merriam-Webster 2021). Rust severity can be

controlled by management practices, foliar fungicides, and the development of resistant cultivars (Wegulo and Byamukama 2012). Earlier seeding of crops in western Canada is one strategy to try to evade the heaviest weeks of infection in early summer when spores are blown in from the United States (McCallum et al. 2007). However, this is not a feasible approach to farmers in areas of longer winters and shorter growing seasons, such as parts of western Canada, where late spring frosts and early fall frosts are a greater risk when compared to the eastern provinces and southwest British Columbia (Qian et al. 2012, Semagn et al. 2021b). Spraying of fungicides can be expensive to the grower and controversial to the public (Dakouri et al. 2013, McCallum et al. 2007). Chemicals must also be applied at specific growth stages and there is a risk that pathogens could develop resistance (Dakouri et al. 2013, McCallum et al. 2007). The most effective tool to prevent infection is through genetic resistance and it is a mandatory requirement when registering wheat cultivars in Canada (PGDC 2018). Since before the 1900s, it has been understood that a plant's response to various diseases is an inherited trait; this information led to the incorporation of these inherited traits to future crops (Agrios 2005). Selecting plants with the desired genotype (and therefore the desired phenotype) is an environmentally mindful and cost-effective long-term approach for disease control (Dakouri et al. 2013).

1.3.2 Rust Disease Cycle

Rust is a devastating fungal disease which, like wheat, has adapted to many different environments worldwide. This fungal pathogen is of importance because it has a quick infection to sporulation period (about 10 days, which then repeats many times during the growing season), has a high spore release count (about 1 trillion spores per acre for an intermediate stem rust infection), and travels very far by wind (Dakouri et al.2013, Fetch et al. 2011). Rust fungi use the

plant as a living host and produce spore-covered pustules on leaves or stems which stifle the plant's photosynthetic potential (Agrios 2005). The pathogen causes sickly plants leading to significant yield losses (less grain per head) and a reduction in grain quality (shriveled grain) (Agrios 2005).

Rusts are found on many different food crops and they are very specialized (Agrios 2005). Specific rusts, called *formae speciales* (f. sp.) only attack specific plant species. For example, a barley rust will not be able to infect a wheat plant (Agrios 2005, Fetch et al. 2011). Furthermore, rust pathogens are then identified by their physiological race (Agrios 2005, Fetch et al. 2011). Each specific rust strain varies in virulence and how it infects (or fails to infect) different host crop cultivars (Agrios 2005, Fetch et al. 2011).

Wheat rusts are caused by several fungi of the genus *Puccinia* f.sp. *tritici*. In Canada, there are three wheat rusts of interest: leaf rust (*Lr*), *Puccinia triticina* Eriks. f.sp. *tritici*, (*Pt*); stripe rust (*Yr*), *Puccinia striiformis* Westend. f.sp. *tritici* Eriks. (*Pst*); and stem rust (*Sr*), *Puccinia graminis* f.sp. *tritici* Eriks. & E. Henn.T (*Pgt*) (Kassa et al 2017, Lan et al. 2017). The fungus is biotrophic; it needs a living host to grow and to produce spores (Agrios 2005).

Rusts are macrocyclic fungi – they can go through all five different fungal spore stages in their life cycle (Agrios 2005). *Puccinia* sp. are heteroecious; they have two alternate hosts in their life cycle (Agrios 2005, Randhawa et al. 2013). Their main host for their asexual cycle is wheat and they go through their sexual stage in the presence of the common barberry plant (*Berberis vulgaris*) (Agrios 2005, Fetch et al. 2011, Leonard and Szabo 2005, Randhawa et al. 2013). The barberry plant was originally an ornamental bush (Fetch et al. 2011) brought by Europeans to North America in the mid-1800s (Pederson 2013). Its popularity grew in North America into the 1900s (Pederson 2013). However, the plant's contribution to devastating stem

rust epidemics led to its forced eradication from 1916 until the late 1970s in the northern United States and Canada (Fetch et al 2011, Pederson 2013). The public was extensively educated to identify the bush and over 500 million plants were destroyed in this period (Pederson 2013). Hence, today the pathogen rarely goes through its sexual stage and it continually reinfects wheat as urediniospores (Fetch et al. 2011).

The asexual urediniospores are released from a uredium, a pustule visible on the surface of wheat leaves and/or stems (Agrios 2005). Spores are carried by wind currents, they land on their host and germinate to produce a germ tube which finds a plant stomate (Leonard and Szabo 2005, Rawson et al. 1998). An appressorium forms over the opening, a penetration peg develops, grows in between the guard cells and forms a substomatal vesicle. From this, an infection hypha grows and creates a haustorial mother cell, which penetrates a cell wall and grows a haustorium in the cell cytoplasm. The haustorium is a structure which takes in the plant cell's nutrients and stifles host responses (Duplessis et al. 2011, Garanica et al. 2014). The fungus continues to infect other plant cells via secondary infection hyphae and creates more haustoria to absorb nutrients for growth and sporulation (Leonard and Szabo 2005, Wang et al. 2015).

This infecting stage can repeat a few times in the season over a large area, causing more devastation due to this characteristic polycyclic nature (Agrios 2005, Fetch et al. 2011). Near the end of the season, the pustules release urediniospores and teliospores; the teliospores are black and overwinter on stubble and the whole cycle restarts the next season (Agrios 2005).

1.3.3 Rust Resistance in Wheat

Plants exhibit three types of resistance: race specific seedling resistance or all stage resistance (ASR), race specific adult plant resistance (APR) and race non-specific resistance (PR) (Zhang et al. 2019). However, resistance is usually divided into race specific and race non-specific responses (Lan et al. 2017). Most resistance is race specific which confers near full immunity in all conditions, however, it is also prone to being short lived (Zhang et al. 2019). This is known as qualitative or vertical resistance; these major genes are usually only effective for a particular race or races and are usually expressed in the seedling to adult plant stages (Pilet-Nayel et al. 2017; Pinto da Silva et al. 2018; Rollar et al. 2021). Race non-specific genes are known to confer partial resistance and can be more dependent on environment (Lan et al. 2017); they delay the onset of disease and leaves tend to have smaller uredia which produce less spores for a shorter period of time (Zhang et al. 2019). This is quantitative or horizontal resistance, and these minor genes can confer a tiny to a moderate range of responses and are usually expressed in later growth stages and provide adult plant resistance (Lan et al. 2017, Pilet-Nayel et al. 2017, Pinto da Silva et al. 2018, Rollar et al. 2021).

Race specific resistance is known as gene for gene interaction (Agrios 2005, Prasad et al. 2020, Wang et al. 2015). A plant's R gene encodes receptors which recognize the Avr gene's elicitor molecules (Agrios 2005). This recognition causes a hypersensitive response (HR) in the area of infection (Agrios 2005, Wang et al. 2015). A hypersensitive response employs mechanisms such as programmed cell death, and release of various toxic compounds and oxidative reactions (Agrios 2005, Wang et al. 2015). If the Avr gene mutates and changes its product/function, it can avoid this gene to gene recognition and prevent any defensive response. This now makes the race virulent to an R gene which makes a gene no longer effective (Agrios

2005, McCallum et al. 2016). This race specific recognition is common of major genes, such as *Lr10* and *Lr14a,* which have both been overcome and are no longer effective against Canadian leaf rusts (Agrios 2005, McCallum et al. 2007). The constant wheat rust reinfections of crops in a single season allows rust pathogens more opportunities to undergo random mutations and then high selection pressure when afflicting new host plants in surrounding areas (Prasad et al. 2020). The rust spores carrying the newly mutated Avr gene will successfully infect the host plants which can no longer recognize the pathogen's new product (Agrios 2005). Research estimates that leaf rust resistance can be overcome by a pathogen race in a short five to seven years (Kilpatrick 1975), which is why studying host-pathogen interactions and tracking their continued effectiveness to disease resistance is integral to cultivar development (McCallum et al. 2007).

However, plant-pathogen interactions are usually not so simple. Within a plant species, different cultivars will respond with varying susceptibility or resistance to certain pathogen races and even environments (Agrios 2005). Races can also show different levels of avirulence or virulence toward plant cultivars. These differences in responses between host and pathogen can be very large to very minute (Agrios 2005, McCallum et al. 2016). These sources of resistance are considered quantitative (minor) and their non-race specificity confers a more durable source of resistance to pathogens (Agrios 2005, Fetch et al. 2011). Because they do not have a specific Avr gene target, they are not defunct if races change. At the same time, they do not show complete resistance to pathogens and need to be deployed in combination with other major and minor genes for near complete disease resistance (Agrios 2005, Fetch et al. 2011). The exact mechanism of these interactions is not understood, but what is known is that using numerous genes in combination improves a plant's resistance to pathogens and can delay pathogen virulence (Agrios 2005, Kassa et al. 2017).

Krattinger et al. (2016) proposed that rust genes fall into three families according to the genes' longevity and race and pathogen specificity. Each group is also characterized by specific protein domains (Peng and Yang 2017). Group one is composed of race-specific resistance genes which target only one race of one pathogen (Peng and Yang 2017). An example of this is *Lr10*, a race-specific seedling leaf rust resistance gene that is cloned but has lost effectiveness to current Canadian leaf rust races (Kassa et al. 2017). Most genes are in this family and are receptor-like proteins which consist of nucleotide-binding sites and leucine-rich repeats (hence these genes are known as NLR, NBS-LRR or R genes) (Krattinger et al. 2016, Peng and Yang 2017). When a pathogen suppresses a plant's basal immunity (the first immune response which sends warning signals), R gene proteins detect the fungal effectors and initiate the second immune response (Shamrai 2014, Peng and Yang 2017). Group two consists of genes which are race-nonspecific and exhibit an immune response to other fungal pathogens as well. A perfect example is *Lr34*, an ABC transporter with transmembrane (TM) and nucleotide binding (NB) domains (Krattinger et al. 2009, Krattinger et al. 2011). *Lr34* is a complex with *Sr57*, *Yr18* and *Pm38*; conferring partial resistance to leaf rust (*Puccinia triticina*), stem rust (*Puccinia graminis*), stripe rust (*Puccinia striiformis*) and powdery mildew (*Blumeria graminis*), respectively, and enhancing the resistance level of many other resistance genes (Hiebert et al. 2010). Group three confers race-nonspecific immunity against all races of one pathogen. This group's genes are known as START for short: proteins they encode have steroidogenic acute regulatory (StAR) protein-related lipid transfer domains. START proteins have a few functions: the first, is plant immunity to pathogens; second, is plant responses to environmental stressors such as excess salinity or heat; and third, there is evidence for transcription regulation in Arabidopsis research (Peng and Yang 2017). The temperature sensitive *Yr36* belongs to this family; it confers adult resistance to many stripe rust

races in wheat. Interestingly, no *Lr* genes appear to encode START proteins (Krattinger et al. 2016, Peng and Yang 2017).

Peng and Yang's (2017) recent studies indicated that genes belonging to group one (NLR genes) would probably lie within seedling and race-specific resistant QTL areas. While groups two and three (ABC and START genes, respectively), would be most likely found within adult and race-nonspecific resistant QTL regions.

1.4 Summary of Three Rusts

1.4.1 Leaf Rust

Leaf rust of wheat, caused by *Puccinia triticina* Eriks. f.sp. *tritici* (*Pt*), poses a challenge to farmers globally (Kassa et al. 2017). Leaf rust can cause yield reductions from 1 to 20% (McCallum et al. 2007). Out of the three types of rusts, leaf rust is the least devastating, but it is also the most prevalent in Canada and therefore accounts for most economic losses (Fetch et al. 2011, Kassa et al. 2017). Leaf rust attacks the foliage of wheat and forms red brown pustules, about 1-3 mm wide and up to 10 mm long, parallel to the leaf axis (Agrios 2005).

Some of Canada's first cultivars, such as Marquis and Thatcher, were grown widely throughout the country in the early to mid 1900s but were susceptible to leaf rust (McCallum et al. 2007). In the late 1920s, *Lr14a* was the first leaf rust gene introduced into Canada via the cultivar Hope and it was bred into Canadian cultivars Renown, Regent and Redman. In 1953, Selkirk was introduced, and it carried a collection of *Lr10*, *Lr14a* and *Lr16* and several stem rust genes (Hucl and Baker 1987, McCallum et al. 2007). Shortly after, *Puccinia triticina* races developed virulence to *Lr10* and *Lr14a*. *Lr16* lost some of its resistance in the mid-1960s, but upon Selkirk's loss of popularity to newer cultivars, virulence to this gene has fallen (Fetch et al.

2011, McCallum et al. 2007). It went through another rise of virulence in the 1990s and fall of virulence a decade later but has managed to still confer partial resistance to leaf rust and is found in current cultivars such as AC Domain and AC Splendor, where it is used in conjunction with other leaf rust genes to enhance the plant's resistance response (Fetch et al. 2011, Kassa et al. 2017, McCallum et al. 2007). In the 1960s until the 1990s, the cultivars Manitou, Neepawa and Katepwa were grown extensively. Their genetics included *Lr13*, which lost some of its effectiveness in the mid-1980s and again in the 1990s (McCallum et al. 2007). The popular cultivar AC Barrie, released in 1994, relies on its leaf rust resistance from *Lr13* and *Lr16* (McCaig et al. 1996, McCallum et al. 2007). However, in 2002 it was found that 71 out of 72 leaf rust isolates overcame *Lr13* resistance, which has left AC Barrie relying on *Lr16* as a moderately leaf rust susceptible plant (McCallum and Seto-Goh 2005, McCallum et al. 2007). *Lr16* is a seedling leaf rust resistance gene that has been finely mapped on chromosome 2BS and recently linked to five SNP markers (BS00108724_kwm461, 2BS-5203447_kwm742, 2BS-5194460_kwm747, 2BS -175914_kwm847, and 2BS-5175914_kwm849) by Kassa et al. (2017). *Lr16*'s moderate response to *P. triticina* is improved when deployed with adult plant resistance genes such as *Lr34* (Fetch et al. 2011, Kassa et al. 2017). Combinations of resistance genes with well studied *Lr34* are almost fully resistant to leaf rust and the gene has never been defeated by a *Puccinia triticina* mutation worldwide (Fetch et al. 2011, McCallum et al. 2007, McCallum et al. 2011).

The leaf rust gene *Lr34* is believed to have originated in Asia as a gain of function mutation (Dakouri et al. 2014). It is categorized as a slow rusting adult plant resistance (APR) gene conferring partial resistance to all leaf rust pathogens and enhancing resistance when paired with other major or minor genes (Hiebert et al. 2010, McCallum et al. 2007, McCallum et al.

2011, Pinto da Silva et al. 2018). It shows varying responses depending on the environment and genetic background (Pinto da Silva et al. 2018). *Lr34* is a complex with *Yr18* (stripe rust), *Sr5* (stem rust), *Pm38* (powdery mildew) and *Bdv1* (barley yellow dwarf virus) resistance genes and confers moderate resistance to all when used as the only source of resistance (McCallum et al. 2011, Pinto da Silva et al. 2018, Randhawa et al. 2013, Singh 1993). Glenlea, released in 1972, was the first major Canadian cultivar to carry *Lr34* and it remains moderately resistant to leaf rust today (Dyck et al. 1985, McCallum et al. 2011). Many Canadian cultivars rely on gene stacking with *Lr34* such as AC Splendor (*Lr13*, *Lr16*, and *Lr34*) (Kolmer and Liu 2002), AC Domain (*Lr10*, *Lr16*, *Lr34*) (Liu and Kolmer 1997*a*) and Laura (*Lr1*, *Lr10*, *Lr34*) (Kolmer 1994). *Lr34* is located on chromosome 7DS and is the first APR gene to be cloned (McCallum et al. 2011, Pinto da Silva et al. 2018).

Lr46 is a similar APR gene as *Lr34.* In fact, it is also part of a complex with *Yr29* (stripe rust), *Sr58* (stem rust) and *Pm39* (powdery mildew) (Pinto da Silva et al. 2018). This gene is located on chromosome 1BL and linked markers have been found in various genetic backgrounds, e.g. between SSRs Xwmc719 and Xhbe248 in Saar (Pinto da Silva et al. 2018). When compared with *Lr34*, *Lr46* has a less noticeable resistance response in some conditions (Lagudah 2011).

A third complex of *Lr67/Yr46/Sr55/Pm46* (leaf rust/stipe rust/stem rust/powdery mildew, respectively) was first identified in Pakistan (Pinto da Silva et al. 2018). There are two alleles of this gene, *Lr67res* (resistant) and *Lr67sus* (susceptible), and they only differ by two amino acids in a region which causes the resistance allele to reduce glucose uptake and therefore is thought to inhibit fungal growth (Moore et al. 2015, Pinto da Silva et al. 2018). This complex has been cloned and is located on chromosome 4DL (Moore et al. 2015, Pinto da Silva et al. 2018).

Lr34, Lr46, and *Lr67* APR complexes can be found using MMAS and they also cause leaf tip necrosis, a useful marker in the field (Hiebert et al. 2010, McCallum et al. 2011). Combining these APR genes with other resistance genes is a strategy to extend the longevity of resistance; to date, the most reported are the benefits of stacking with *Lr34* (Ellis et al. 2014). Pyramiding leaf rust genes has been successful in combinations such as *Lr13* and *Lr16* (Samborski and Dyck 1982), *Lr13* and *Lr34* (Kolmer et al. 1991), *Lr12* and *Lr34* (Dyck et al. 1966), *Lr13* and *Lr67*, *Lr16* and *Lr34* and *Lr67* (Che et al. 2019, McCallum and Hiebert 2012).

Leaf rust resistance genes which continue to be effective in Canada are *Lr2,* incorporated in AC Cora (1994) and McKenzie (1997), *Lr22a* in AC Minto (1991) and cultivar Pasqua (1991) which carries (and remains resistant) *Lr11*, *Lr13*, *Lr14b*, *Lr30*, *Lr34* (McCallum et al. 2007). Other potential resistance genes of interest are *Lr52* (*LrW)* (Hiebert et al. 2005), *LrW2*, *Lr18*, *Lr35, Lr37* (McCallum et al. 2007), *Lr16* (Kassa et al. 2017), *Lr21* (Fetch et al. 2011), *Lr33* (Che et al. 2019), *Lr48, Lr49* (Pinto da Silva et al. 2018) and the three APR gene complexes, *Lr34, Lr46* and *Lr67* (Che et al. 2019). Once common in Western Canadian wheat, the genes *Lr1*, *Lr10*, *Lr13*, and *Lr14a* have been overcome by *Puccinia triticina* races in Canada and are not useful to further breeding efforts (Fetch et al. 2011, Randhawa et al. 2013).

Since the first leaf rust gene discovered in 1946, ~80 *Lr* genes have been identified (https://wheat.pw.usda.gov/GG3/wgc), with most mapped onto chromosomes using DNA markers, and with the genes *Lr1, Lr10, Lr21, Lr34* and *Lr67* cloned (Prasad et al. 2020). Pinto da Silva et al. (2018) recently reviewed leaf rust resistance conferred by QTL in hexaploid wheat and reported 249 QTL found in studies between January 1971 and January 2018. Bemister et al. (2019) found two minor QTL affecting 10% of leaf rust in a Peace x Carberry population in Canada. A recent study by Bokore et al. (2020) looked at the Canadian cultivars Carberry, AC

Cadillac, Vesper, Lillian, Stettler and Red Fife and mapped 20 QTL, which demonstrated elevated resistance responses and are prospective resistance sources to western Canadian wheat breeders.

1.4.2 Stem Rust

Stem rust of wheat is caused by *Puccinia graminis* f.sp. *tritici* Eriks. & E. Henn.T (*Pgt*), (Kassa et al. 2017, Singh et al. 2008) and it has historically been the cause of some of the most devastating crop epidemics in North America (McCallum et al. 2007). From the early 1900s until the last epidemic in the mid 1950s (Ghazvini et al. 2013), producers of wheat struggled as *P. graminis* reduced crop yields up to 40% (Khan et al. 2005, Hiebert et al. 2017). Stem rust appears as oblong pustules mostly on the wheat sheaths, just below the auricles and along the plant stem, and can also be found on the actual plant stem, sometimes even on leaves and glumes (Agrios 2005, Singh et al. 2008). The heavy toll of infection leads to poor yield, poor grain quality and, in major cases, can cause severe lodging (Agrios 2005, Fetch et al. 2011).

The first stem rust resistant Canadian wheat cultivar was Thatcher, a popular choice in fields from 1939 and into the 1970s, carrying *Sr5*, *Sr9 g*, *Sr12*, and *Sr16* (Kolmer et al. 2011, McCallum et al. 2007). However, this cultivar lost resistance to *Pgt* race 15B in the 1950s epidemic and was also susceptible to leaf rust, another problem at the time (McCallum et al. 2007, Peturson 1958). In 1954 a stem rust resistant cultivar, Selkirk, with stacked genes *Sr2*, *Sr6*, Sr*7b*, Sr*9d*, Sr*17*, and Sr*23* (Kolmer et al. 1991), was released (McCallum et al. 2007). Around this time, the barberry eradication program was also in action across North America which helped maintain stability of the newly deployed genes by slowing down the number of pathogen races and plateauing their population sizes (McCallum et al. 2007, Roelfs 1982).

Currently, there are 61 stem rust genes identified in wheat and its ancestors (https://wheat.pw.usda.gov/GG3/wgc) and most are race specific (Gao et al. 2017, Leonard and Szabo 2005, Wang et al. 2015). Most Canadian spring wheat cultivars carry some form of resistance to *P. graminis* races across North America (Fetch et al. 2011) and the gene combinations responsible are probably *Sr5*, *Sr7a*, *Sr9b*, *Sr12*, *Sr16,* (Kolmer et al.1991, McCallum et al. 2007), *Sr13, Sr14, Sr22, Sr28, Sr33, Sr35, Sr42*, *Sr45* (Hiebert et al. 2011), *Sr54* (Ghazvini et al. 2013), *SrCad* (Hiebert et al. 2010, Randhawa et al. 2013), *Sr2* (Randhawa et al. 2013) and *Sr6*, which confers resistance to all stem rust races in North America (Fetch and Dunsmore 2004, McCallum et al. 2007,), but is temperature sensitive and confers susceptibility at 25°C and resistance at 20°C (Manocha 1975). Of these, *Sr13, Sr14, Sr22, Sr28, Sr33, Sr35, Sr42* (proposed the same as *SrCad*) and *Sr45* are known to carry resistance to TTKSK (Ug99), a new *P. graminis* race from Africa.

Stem rust is also found in three adult plant resistance gene complexes: *Lr34*/*Yr18/Sr5/Pm38, Lr46*/*Yr29/Sr58*/*Pm39* and *Lr67/Yr46/Sr55/Pm46,* which confer resistance to leaf rust/stripe rust/stem rust/powdery mildew (Pinto da Silva et al. 2018). These APR genes are found to enhance all forms of rust resistance and, on their own, deliver moderate levels of resistance to these pathogens (Pinto da Silva et al. 2018). In particular, *Lr34* has had major benefits to a plant's pathogen response when pyramided with other genes (Ellis et al. 2014). Data from stem rust nurseries in Kenya showed that genotypes with *SrCad* and *Lr34* had a near immune resistance to Ug99 races, while *SrCad* on its own gave a moderately resistant phenotype (Hiebert et al. 2011).

In general, the control of stem rust has remained successful for the past 50 years and therefore research has slowed toward diversifying the Canadian stem rust gene pool (Martens

and Dyck 1989). However, pathogens evolve and researchers are now revisiting stem rust genes as a new race, TTKSK, was reported in Uganda in 1999 and it is virulent to most *Sr* genes used in breeding programs (Ghazvini et al. 2013, Hiebert et al. 2011). The race group is also called Ug99 (Pretorius et al. 2000) and has caused a number of epidemics in Africa (Randhawa et al. 2013). Ug99 variants have been reported in South Africa (Randhawa et al. 2013, Visser et al. 2011), and one of the most aggressive variants is TRTTF, found in Yemen in 2006 and later in Ethiopia (Hiebert et al. 2017, Olivera et al. 2012). About 90% of wheat grown worldwide is susceptible to Ug99 races (Gao et al. 2017, Hiebert et al. 2017), however, *Sr8a, Sr22, Sr24, Sr26, Sr27, Sr31, Sr32, Sr33, Sr35, Sr39, Sr40, Sr46, Sr47, Sr50,* and *SrSatu* are identified as conferring resistance to the TRTTF isolate (Guerrero-Chavez et al. 2015, Olivera et al. 2012). Resistance genes to TRTTF in Canadian wheat are unknown, although the majority of Ug99 resistance relies on *SrCad* in the Canadian wheat germplasm, a gene which confers both TTKSK and TRTTF resistance (Hiebert et al. 2017). Other deployed genes are *Sr2* and *Sr57,* which confer TTKSK resistance (Randhawa et al. 2013). Molecular markers have been linked to *Sr2*, csSr2 and Xgwm533, and *SrCad*, FSD_RSA and cfd49, for MMAS (Hiebert et al. 2016a, Randhawa et al. 2013). *SrCad* is mapped onto chromosome 6DS, and it has been linked to the bunt resistance gene *Bt10* (Hiebert et al. 2011) and the gene is found in Canadian cultivars AC Cadillac and Peace. Further investigation found the *Lr34* complex (*Lr34*/*Yr18/Sr5/Pm38*) in both genotypes as well, which most likely explains the durability and high resistance response to stem rust from these two cultivars (Hiebert et al. 2011, Randhawa et al. 2013). Both AC Cadillac and Peace confer resistance at both the seedling and adult stage for Ug99 races (Hiebert et al. 2011) and all existing stem rust isolates in North America (Randhawa et al. 2013), but they are not widely grown across Canada (McCallum and DePauw 2008).

Hiebert et al. (2016a) found *SrCad* genotypes which segregated with *SrTrmp,* and *Sr42* and, upon analysis, found that all map to a similar area on the 6DS chromosome region, making them difficult to differentiate. *SrCad* is the most predictable in its marker linkage precision, and further studies into *SrTrmp* and *Sr42* are proposed, with *SrTrmp* discovered as a source of resistance to TTKSK (Hiebert et al. 2016a). That same year, Hiebert et al. (2016b) also found a QTL in the area of *Sr12,* which was responsible for *Pgt* resistance in Kenyan nurseries. In 2017, Hiebert et al. reported findings of a source of resistance in Harvest identified in the area on chromosome 6AS, which has two proposed alleles, *Sr8a* and *Sr8b* (Hiebert et al. 2017, Sing and McIntosh 1986). Of the two, *Sr8a* has been reported as resistant to TRTTF (Olivera et al. 2012). The results from Hiebert et al. (2017) were corroborated with results from a previous study in South Dakota by Guerrero-Chavez et al. (2015), where a line also had TRTTF resistance on 6AS believed to be *Sr8*. Several QTLs, with varying degrees of effects, have also been identified in nine Kenyan spring wheat cultivars which confer higher than expected levels of resistance to the aggressive Ug99 race (Bajgain et al. 2016).

Canadian breeding programs are returning focus on stem rust research and gene pyramiding for resistance longevity in stem rust, especially in combinations incorporating APR gene complexes such as *Lr34, Lr46* and *Lr67* (Hiebert et al. 2011, Pinto da Silva et al. 2018, Randhawa et al. 2013). With the information of new emerging pathogen races across the world, breeders can be proactive and raise the selection pressure with MMAS for preventing unforeseen epidemics in a possible introduction of Ug99 into North America (Hiebert et al. 2017, Khan et al. 2005, McCallum et al. 2007).

1.4.3 Stripe Rust

Stripe rust is caused by *Puccinia striiformis* Westend. f.sp. *tritici* Eriks. (*Pst*), (Fetch et al. 2011, Kassa et al. 2017) and is a worldwide problem, reducing yields from 10% up to 70% (Coriton et al. 2020). It has been a concern mostly in late maturing soft white spring wheat in irrigated areas in southern Alberta, although now it has spread into British Columbia, Saskatchewan and Manitoba (Fetch et al. 2011, McCallum et al. 2007, Xi et al. 2015). Stripe rust appears as bright yellow orange oval pustules along the axes of the leaves. It is a rust which prefers cooler temperatures, flourishing best in conditions no higher than 20°C (Fetch et al. 2011).

The first report of stripe rust was in central Alberta in 1918 (McCallum et al. 2007) and problems elevated in the 1980s as the predominant cultivar in southern Alberta, Fielder which carries *Yr6* and *Yr20* (Brar et al. 2019), became susceptible to stripe rust (Conner & Kuzyk 1988, Xi et al. 2015). The cultivar Owens was released during this time and conferred stripe rust resistance with *Yr7* (Xi et al. 2015). However, it performed poorly agronomically and was not grown extensively in Canada (Conner & Kuzyk 1988) and was replaced by the stripe resistant cultivar AC Reed, which covered 90% of southern Alberta fields (Su et al. 2003, Xi et al. 2015). Stripe rust research slowed at this time as AC Reed performed well and the pathogen was not a huge problem in most wheat growing areas in Canada (McCallum et al. 2007, Xi et al. 2015). *Puccinia striiformis* got more attention in the late 1990s when it overcame AC Reed's resistance and plagued fields in central Alberta (Xi et al. 2015) and then appeared in Saskatchewan and Manitoba in 2000 (Fetch et al. 2011). It has caused major epidemics in 2005 and 2006 in the southern prairies, where nurseries reported lines with 100% susceptibility (McCallum et al. 2006), and has reoccurred annually since (Fetch et al. 2011, Xi et al. 2015). Research has

identified new, more aggressive races (Chen 2005), which have adapted to higher temperatures (Milus et al. 2006, Milus et al. 2015) and have been attributed to the some of these outbreaks in Canada and the United States (McCallum et al. 2007). Its constant appearance in fields since 2000 has led to a renewed interest in studying stripe rust (Xi et al. 2015) and incorporating resistance genes into western Canadian cultivars (Fetch et al. 2011). Since the focus before this was breeding stripe rust resistance into the Soft White Spring class, other wheat classes are now in need of stripe rust protection (Brar et al. 2019, Fetch et al. 2011).

The *Yr1* gene was overcome in the mid-1930s (Brar et al. 2019). Virulence on *Yr6, Yr7, Yr8, Yr9, Yr27,* and *Yr32* has been rising since 2000 and has mostly rendered these genes ineffective (Brar et al. 2019). In 2009, virulence was reported in southern Alberta to stripe rust resistance gene *Yr10* (Brar et al. 2019, Randhawa et al. 2012) and the loss of *Yr24* followed. Gene *Yr24* was not available in Canadian cultivars, but it is of no use to any breeding program now (Brar et al. 2019). The loss of *Yr10*'s effectiveness with the release of the winter wheat Radiant was due to its popularity, which increased selection pressure (Brar et al. 2019, Puchalski and Gaudet 2011). However, other winter wheat cultivars, such as Emerson and McClintock (Graf et al. 2013), carry *Yr17* located on chromosome 2AS and linked to *Lr37*, a leaf rust resistance gene (Helguera et al. 2002, Plotnikova and Stubei 2013), and *Sr38* for stem rust resistance (Brar et al. 2019). This gene offers moderate protection to all three rusts at the adult stage (Randhawa et al. 2012, Xi et al. 2015) and was reported to contribute up to 80% of variation in adult stripe rust resistance responses (Milus et al. 2015). It is strongly linked to a high temperature gene which causes it to be expressed in warmer environments, making it ideal for southern prairie conditions (Brar et al. 2018, Brar et al. 2019) and a good contender against the aggressive, warmer temperature *P. striiformis* races (Milus et al. 2015). However, in

Canadian cultivars *Yr17* is mostly found in winter wheat and observed in only one western red spring wheat, CDC Stanley (Brar et al. 2019, Randhawa et al. 2012, Statistics Canada 2020). CDC Stanley should be considered as a potential *Yr17* contributor in western red spring wheat creation for better stripe rust resistance expression in warmer areas (Brar et al. 2019).

Similar heat sensitive genes include *Yr28* and *Yr36* (Brar et al. 2019, Li et al. 2016, Segovia et al. 2014). They are both slow rusting adult resistance genes, with *Yr28* less utilized in breeding programs for stripe rust protection, mostly due to a lack of good diagnostic markers until recently developed by Zheng et al. (2020). On the other hand, *Yr36* is present in many Canadian cultivars (Randhawa et al. 2012) and happens to also be linked to *Gpc-B1*, a gene contributing to higher protein content in grain (Uauy et al. 2005). Even though it is recognized as an adult resistance gene expressed at higher temperatures, *Yr36* has been shown to confer some resistance at the seedling stage and at temperatures below 18°C (Segovia et al. 2014).

In total, 83 stripe rust genes have been identified to date (https://wheat.pw.usda.gov/GG3/wgc) and most of the present Canadian cultivars solely rely on *Yr18* for stripe rust resistance (Randhawa et al. 2012). Past cultivars with moderate resistance to stripe rust were also most likely relying on *Yr18* (Fetch et al. 2011). This gene is part of the largely deployed slow rusting *Lr34*/*Yr18/Sr5/Pm38* APR complex on chromosome 7DS (conferring resistance to leaf rust/stripe rust/stem rust/powdery mildew, respectively) (Pinto da Silva et al. 2018, Fetch et al. 2011, Herrera-Foessel et al. 2011). This complex does not provide full protection, but has proved its worth in durability and its prevention in other fungal diseases (Brar et al. 2019). Stripe rust resistance is also found in the other two APR complexes: *Lr46*/*Yr29/Sr58*/*Pm39* and *Lr67/Yr46/Sr55/Pm46* (Pinto da Silva et al. 2018, Herrera-Foessel et al. 2011). These confer resistance to leaf rust/stripe rust /stem rust/powdery mildew and are

recommended for gene pyramiding to enhance the plant's disease response (Pinto da Silva et al. 2018, Herrera-Foessel et al. 2011). Especially due to the more aggressive nature of *Pst* races in the past two decades, it is important for breeders to incorporate a combination of race specific and partial race non-specific genes (such as the APR complexes) for durable forms of stripe rust resistance in future wheat cultivars (Fetch et al. 2011, Xi et al. 2015).

1.5 Conclusion

Wheat, *Triticum aestivum* L., is an important food staple worldwide which is threatened by disease, pests and extreme environmental conditions. In order for producers to maintain healthy grain with superior agronomic performance and end use characteristics, it is necessary for breeders to investigate and incorporate genetic components which can aid in maintaining standards and overcoming the unpredictable. Choosing a cultivar based on a producer's needs and growing region is the most cost effective approach. Not everyone can afford (and blindly accept) the financial and ecological costs which are associated with pest and disease control. Having cultivars with natural resistance to these problems is a benefit to the grower, consumer, and a way to protect the environment. Unfortunately, understanding these genetic interactions and their responses in different environments is a tedious task which can make selection complicated. Continuous field studies paired with evolving molecular technology and sharing of reported results are the methods we rely on to keeping our crops vigorous and our harvest bountiful.

1.6 Present Study

1.6.1 Research Objectives

Given the need for high yielding and disease resistant wheat lines, the two objectives of this research are outlined as follows:

1. Assess the agronomic phenotypes of the doubled haploid population (parentage 'HYAYT12-10' and 'GP146') over two years over four environments for days to maturity, lodging, and yield and determine if there are any quantitative trait loci associated with these traits.
2. Assess the disease phenotypes of the doubled haploid population (parentage 'HYAYT12-10' and 'GP146') over two to three years over five environments for leaf rust and stem rust susceptibility/resistance and determine if there are any quantitative trait loci (QTL) associated with these two diseases.

1.6.2 Null Hypotheses

Based on these two stated objectives, the null hypothesis for each one is as follows:

1. Null: QTL contributing to variation of agronomic responses do not exist.
2. Null: QTL contributing to variation of disease traits do not exist.

2. Quantitative trait loci mapping of rust resistance and agronomic traits in spring wheat. *

Izabela Ciechanowska, Kassa Semagn, Brent McCallum, Harpinder Randhawa, Klaus Strenzke, Mark Virginillo, Muhammad Iqbal, and Dean Spaner

*An edited version of this chapter under the same title has been submitted to *The Canadian Journal of Plant Science* in December 2021; authored as Ciechanowska et al.

2.1 Introduction

Wheat (*Triticum aestivum* L.) is a major crop in Canada with an estimated total production of 35.2 million tonnes (Mt) in 2020 (Statistics Canada 2020c) of which 19.6 Mt was exported. To support the strong demand for modern wheat varieties (cultivars) across 17 market classes (10 classes in western and 7 classes in eastern Canada), breeders both in the public and private sector have registered 591 varieties (cultivars) from 1961 to 2020, which includes 336 spring wheat, 205 winter wheat, and 50 durum wheat cultivars (https://inspection.canada.ca/active/netapp/regvar/regvar_lookupe.aspx). About 39% of these cultivars were registered for production in British Columbia, Alberta, Saskatchewan, and Manitoba, which accounted for over 90% of the total wheat production in the country (https://www150.statcan.gc.ca/). The Canadian Food Inspection Agency (CFIA), with the recommendations from the Prairie Grain Development Committee (PGDC), is responsible for the registration and cancellation of cultivars. CFIA requires that each candidate cultivar for registration should possess a combination of various traits (depending on the market class) of which early maturity, short plant stature, lodging tolerance, high grain yield, standardized grain

protein content (GPC), and enhanced resistance to five priority diseases are mandatory across all market classes (PGDC 2018).

Stem, leaf, and stripe rusts caused by *Puccinia graminis* f. sp. *tritici* (Pgt), *P. triticina* (Ptr), and *P. striiformis* f. sp *tritici* (Pst), respectively, are three of the five priority diseases in Canada. They are responsible for major losses in grain yield and quality. Leaf rust is the most common rust disease of wheat in western Canada annually, and its severity fluctuates every year (McCallum et al. 2021). Stripe rust has been detected in western Canada every year since 2000 with areas reporting serious epidemics in 2005, 2006, and 2011 (McCallum et al. 2007, Randhawa et al. 2012). Multiple stem rust epidemics have been reported in Canada in the early 1900s and from 1953 to 1955, which caused a loss of hundreds of millions of dollars (Peturson 1958). The severity of rusts can be reduced through agronomic management practices, the application of foliar fungicides, and the development of resistant cultivars (Wegulo and Byamukama 2012). The development of resistant cultivars is a more economical and environmentally friendly approach to controlling rust. However, breeding for disease resistance is often challenging due to (i) the need to pyramid different sources of resistance to triple rusts into the same genetic background, and (ii) qualitative and quantitative inheritance of the rusts (Pinto da Silva et al. 2018), which complicates the selection process. Qualitative resistance is controlled by a single gene with a major effect, which is effective against a subset of races. These major genes confer vertical resistance and tend to be expressed from the seedling to adult plant stages, but they lose their effectiveness over time due to changes in pathogen populations. On the other hand, quantitative resistance is a partial level of resistance controlled by two or more minor to moderate effect genes and/or QTLs, which are more durable but require the introgression of multiple genes or QTLs. Quantitative resistances are expressed at later growth

stages and provide adult plant resistance (Pilet-Nayel et al. 2017; Pinto da Silva et al. 2018; Rollar et al. 2021). Currently, a total of 61 stem rust, 80 leaf rust and 83 stripe rust resistance genes have been identified in bread wheat, durum wheat, and their relatives (https://wheat.pw.usda.gov/GG3/wgc). Most of these genes are race-specific (qualitative) resistant in their function, but a few are known to confer partial (quantitative) resistance at the adult stage such as *Lr12*, *Lr13*, *Lr22*a/b, *Lr34*, *Lr46*, *Lr67*, *Lr68*, *Lr71*, *Lr75*, *Lr77*, and *Lr78* (Dakouri et al. 2013; Pinto da Silva et al. 2018). For stem rust, it was reported that on average a combination of 4–5 minor genes reduced stem rust severity to negligible levels at maturity (Sing et al. 2011). Little is known about non-specific stem rust resistance genes, and most cultivars rely on combination of *Sr2* and other unknown slow rusting resistance genes for durable resistance to stem rust in Canada, the U.S.A., and Australia (Singh et al. 2011).

Improved cultivars can be developed using multiple conventional breeding methods and molecular marker-assisted selection (MMAS). MMAS is an indirect selection method that requires mapping genes and major effect QTLs associated with target traits, which involves developing (assembling) appropriate populations followed by coarse mapping, fine mapping, validation, and the development of reliable and breeder-friendly molecular markers (Collard et al. 2005; Schaid et al. 2018; Platten et al. 2019; Jaganathan et al. 2020). There have been continuous efforts to map and characterize genes and QTL associated with target traits of interest using diverse linkage-based analysis (LA). As reviewed by different authors (Collard et al. 2005; Semagn et al. 2010; Gupta et al. 2019), the LA method includes simple interval mapping, composite interval mapping, inclusive composite interval mapping, and multiple interval mapping (Kao et al. 1999; Li et al. 2010; Akond et al. 2019), which all depend on well defined biparental populations, such F_x derived families, backcross, near isogenic lines (NILs), doubled

haploids (DH), and recombinant inbred lines (RILs). Such types of mapping populations are often developed by crossing two parents with a contrasting phenotypic trait(s) of interest. Although LA is the most widely used method since the early 1990s, it has four major drawbacks: (1) the time and cost in developing the mapping populations, (2) low resolution of the method due to a limited number of recombination events, (3) the use of old populations developed five or more years before the mapping studies, and (4) the biparental populations capture only alleles originating from their parents.

Our group at the University of Alberta conducted multiple studies to map genes and QTLs in recombinant inbred lines derived primarily from the Canada Western Red Spring (CWRS) class on agronomic traits (Semagn et al. 2021a, Semagn et al. 2021b) and reaction to diseases (Bemister et al. 2019, Perez-Lara et al. 2017, Zou et al. 2017). However, we have not conducted any mapping study in biparental populations derived from the Canada Western Special Purpose (CWSP) class, which forms the basis in the present study. Advanced breeding lines and cultivars in the CWSP class produce high grain yield with high starch content but low grain protein content, and are considered desirable for ethanol production and animal feed (Beres et al. 2013, Canadian Grain Commission 2021).

The objectives of the present study were to map genes and QTLs associated with rust resistance and major agronomic traits using inclusive composite interval mapping (ICIM).

2.2 Materials and Methods

2.2.1 Phenotyping

This study was conducted on 167 doubled haploid (DH) lines derived from a cross between 'HYAYT12-10' × 'GP146' using the wheat-maize hybridization method (Sadasivaiah et al. 1999). Both parents are unregistered lines that belong to the CWSP class. 'HYAYT12-10' is an advanced breeding line from the University of Alberta derived from a cross between 'Hidhab' × 'AC Andrew' (Sadasivaiah et al. 2004). 'Hidhab' was extracted from HD1220/3*Kal/Nac CM40454 and characterized by a relatively good level of resistance to triple rusts, an average grain yield with a relatively high grain protein content, strong gluten, late maturing, and well adapted to drought (Aissaoui and Fenni 2021). 'GP146' is a high grain yielder with a soft white grain developed from a cross between 'Bhishaj' (Randhawa et al. 2011) of Agriculture and Agri-Food Canada (AAFC) and a synthetic line 'SKAUZ/PASTOR/3/CROC_1/AE.SQUARROSA(224)//OPATA' from the International Maize and Wheat Improvement Center (CIMMYT). 'AC Andrew' is a DH line developed by the Lethbridge Research Center from Dirkwin/SC8021V2// Treasure/Blanca, and characterized by higher grain yield, high lodging tolerance, resistant to the prevalent races of stripe rust, stem rust, and powdery mildew, and moderately resistant to leaf rust (Sadasivaiah et al. 2004). This doubled haploid population 'HYAYT12-10' × 'GP146' was originally chosen to explore QTL for stripe rust resistance, as it exhibited a great deal of rust differential at a contra season nursery in New Zealand in the winter of 2015. This differential was not present in later stripe rust nurseries using the prevailing races in western Canada, but did occur for leaf rust.

The DH lines, the two parents, and four CWSP checks (AAC Awesome, Pasteur, AC Andrew, and Sadash) were planted in hill plots in disease nurseries using randomized complete

block design with two replications. One gram of seed from each entry was planted in a hill. Hills were spaced 20-30 cm apart. Reaction to both leaf and stem rust was evaluated in 2016, 2017, and 2018 in Morden, Manitoba, Canada. Leaf rust was also evaluated for two years in 2016 and 2017 at the University of Alberta South Campus Research Station, Edmonton, Alberta. At each location, urediniospores of both rusts were collected from infected plants in nurseries in mid-August of the previous year and frozen in -80 °C in 1.5 mL vials until needed for inoculation in June of next year. Urediniospores were recovered from -80 °C on the day of inoculation, allowed to acclimate for a few minutes, heat shocked in a 42°C water bath and suspended in 2 L of Soltrol 170. To create homogeneous disease epidemics within each trial, plants were inoculated at the 5-6 leaf stage (Zadok's 15-16) in the early evening using a low-volume sprayer. The nursery was inoculated a second time about 3-5 days later and for a third time after another 3-5 days. Visual disease assessment of both rusts was done on a 1 to 9 scale on each hill plot basis, in which 1 represents no visible signs or symptoms (resistant) and 9 indicates the leaf area is totally covered with spores (highly susceptible). The disease ratings were done when the susceptible and resistant checks in the disease nursery showed contrasting reactions. The resistant check, Carberry, appeared uninfected; the moderately and completely susceptible checks, Barrie and Park, were covered in pustules; the moderately resistant/intermediate check, Peace, was partially covered in rust spores.

The DH population, parents, and checks were also evaluated for agronomic traits in conventionally managed fields both at the University of Alberta South Campus Research Station, Edmonton, AB, and at the Lethbridge Research and Development Centre, Lethbridge, AB, in 2016 and 2017. All agronomic trials were conducted using a randomized incomplete block design, with two replications in Edmonton in 2016 and 2017, and Lethbridge in 2016. Plots were

3.0 x 1.0 m, with six rows each 19 cm apart and seeded on the first and second week of May each year at a rate of 300 seeds m^{-2}. Weeds were controlled using registered herbicides following local recommendations and label directions. The four-year crop rotation in conventionally managed land was a rotation of 2-row barley (*Hordeum vulgare)*, canola (*Brassica*. *napus* L.), field pea (*Pisum sativum*) and wheat (*Triticum aestivum* L.). Each entry was evaluated for plant lodging, days to maturity, and grain yield. Lodging score was recorded on a plot basis at the time of harvest on a 1-to-9 scale with 1 and 9 representing no lodging and completely lodged, respectively. Days to maturity from the time of seeding were scored when more than 50% of the peduncles in a plot turned yellow. Plots were individually harvested with a small plot Wintersteiger Classic combine. Seed was collected into cotton bags and dried for 4 days using an industrial dryer at 35° - 40°C for 4-5 days after harvest. Each bag of grain was cleaned with a Pfeuffer four sieve seed cleaner. Yield per plot was recorded in kg $3m^{-2}$ and converted to t ha^{-1}.

2.2.2 DNA Extraction and Genotyping

The two parents and the 167 DH lines were grown in a growth chamber until the 3-4 leaf stage. Genomic DNA was extracted from tissue samples collected at 3-4 leaf stage using a modified Cetyl Trimethylammonium Bromide (CTAB) method (Doyle and Doyle 1987). DNA quality was verified using 0.8% agarose gel stained with SYBR® Safe. The DNA concentration was normalized to approximately 100 ng μL^{-1} after being assessed with a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, 2010). Fifty microliters of each DNA sample was sent to the National Research Council (NRC) in Saskatoon, Saskatchewan, where each was genotyped by the NRC with the Illumina 90K Infinium Wheat Array (Wang et al. 2014).

2.2.3 Data Analysis: Phenotype and Genotype

For each trait, F statistics and least squares means were computed across all environments using a mixed linear model in R, v3.5.2. Parameters were estimated by the Restricted Maximum Likelihood (REML) method with the nlme package using the lme function. A mixed effects analyses of variance (ANOVA) approach was used to calculate least square means for each entry. The observed variable was explained with the linear model:

$$\Upsilon_{tjqk} = \mu_t + G_{jt} + E_{tj} + (G \times E)_{tjk} + B_{tjqk} + \varepsilon_{jqkt}$$

in which Υ_{jqkt} is the response observed in trait t of entry j in block q in environment k, μ_t is the overall mean effect of trait t, G_{jt} is the effect of genotype of entry j on trait t, E_{tj} is the effect of environment on trait t in entry j, $(G \times E)_{tjk}$ is the interaction effect of genotype and environment on trait t in entry j in environment k, B_{tjqk} is the blocking effect on trait t of entry j in block q in environment k, and ε_{jqkt} is the residual error. Genotypes (G) were considered fixed, while environments (E), replications, and blocks (B) within replications, and $G \times E$ were considered as random effects. Broad-sense heritability was computed using Multi Environment Trial Analysis with R for Windows (MetaR) v6.04 (https://hdl.handle.net/11529/10201). Test for normality on the least squares means, box plots, frequency distribution plots, and regression (R^2) plots from the phenotype data were generated using RStudio Version 1.1.4.

The 90K genotype data were filtered as described in a previous study (Xiang et al. 2021). First, we removed all SNPs that were monomorphic between the two parents, missing or heterozygous in both parents, and those with greater than 20% missing data in the DH lines. This initial stage of filtering resulted to 4,799 SNPs for linkage analysis. We performed linkage analysis using JoinMap v4.0 (van Ooijen 2006) and further excluded all markers that showed segregation distortion at $p < 0.01$, and those that were either unlinked or formed a linkage group

with <5 markers using a minimum LOD score of 3, a recombination frequency of 0.35, and Kosambi mapping function (Kosambi 1943). We finally retained 2,676 SNPs for map construction using MapDisto for Windows v2.1.7.10 (Heffelfinger et al. 2017). For each SNP, we obtained the International Wheat Genome Sequence Consortium (IWGSC) RefSeq v2.0 information in two steps. First, we retrieved 100-bp sequence information of each of the selected SNPs by pasting SNP ID into http://download.txgen.tamu.edu/shichen/flanking_v2.html. We then used a BLASTN search by pasting the sequence against the Chinese spring chromosome survey sequencing (http://download.txgen.tamu.edu/shichen/mapper_v2.html) and obtained both the chromosome name and physical positions. The SNP genotype data and physical information were then sorted using chromosome name and physical position in ascending order (this is a step required to obtain the correct marker order). We then created a temporary new SNP ID that consisted of "Chr" as a prefix for chromosome, followed by 01 to 21 to represent each chromosome, and the physical positions in bp (e.g., Chr01- 29183813 to represent the first SNP on chromosome 1A that mapped at 29,183,813 bp). In cases where two or more SNPs on the same chromosome had the same physical position, we added 1 bp to avoid duplicates and make each position unique to serve as SNP ID. We then loaded the SNP data with the new SNP ID into MapDisto and constructed a linkage map using the "Extract LG's from loci" option. The latter option generates linkage maps based on the pre-defined linage groups (LGs) using the physical positions for locus ordering and to convert the positions into cM.

2.2.4 Data Analysis: Inclusive Composite Interval Mapping

Inclusive composite interval mapping (ICIM) was performed on the least-squares means of each trait, and both the genetic map in cM and the physical map in kilobase pair (kb) using

QTL IciMapping version 4.2.53 (Li et al. 2007; Meng et al. 2015) with the following parameters: mean replacement for missing phenotypic data, a minimum logarithm of odds (LOD) score of 3.0, and an additive model to determine the effect of individual QTL. The walking distance was set to 1 cM for genetic maps and 2 kb for physical maps. In cases where two or more QTLs are detected for the same trait with an overlapping confidence interval or common flanking markers, only one was retained. QTLs that explained <10%, 10-20%, and >20% of the phenotypic variation were arbitrarily classified into minor, moderate, and major effect, respectively. QTL names were assigned by following the International Rules of Genetic Nomenclature (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm), which comprised of trait acronym, lab designation (dms = Dean Michael Spaner), and chromosome number. MapChart v2.1 (Voorrips 2002) was used to construct genetic maps and QTL graphs.

2.3 Results

2.3.1 Phenotypic and Genetic Variation

'HYAYT12-10' matured about 3 days later, yielded 740 kg ha^{-1} more grain, and was more tolerant to lodging, and resistant to both leaf and stem rust than 'GP146' (Table 1). The 167 DH lines required 105-112 days to mature, varied in lodging score from 1 to 5, and yielded 4.6-6.8 t ha^{-1} grain. Leaf and stem rust scores in the DH lines varied from 1 to 9 with an overall average score of 3.5 for leaf rust and 3.8 for stem rust. Of the 167 DH lines, only 10 DH lines produced more grain yield, eleven lines were more resistant to leaf rust, and fifteen lines more resistant to stem rust than the high yielding 'HYAYT12-10' (6.3 Mg ha^{-1}) and rust-resistant (with scores of 1.5 for both leaf and stem rust) parent. The genotype effect was significant ($p < 0.05$) in the model for all traits. Broad-sense heritability was moderate to high, and varied from

0.41 for maturity to 0.78 for leaf rust. The phenotypic distribution of least-square means averaged across all environments was normal ($p < 0.05$) for both maturity and grain yield but skewed for lodging score, leaf rust, and stem rust (Figure 1) with most of the DH lines showing moderate scores in all three traits. The latter three traits are not recorded in the ratio scale while the first two are. Statistically significant ($p < 0.05$) correlations were observed only between maturity and lodging (-0.38), between maturity and grain yield (0.20), and between leaf and stem rust (0.88) (Figure 2).

Of the wheat 90K iSelect array used for genotyping the DH population, we integrated 2,676 SNPs in the final genetic maps (Table 2, Figure 4). The number of mapped markers per chromosome varied from 35 on chromosome 6D to 379 on 3B with an overall average of 127 SNPs per chromosome. The genetic map covers all 21 wheat chromosomes, which spans between 1,797 on chromosome 1D and 5,345 cM on 2A with an overall average of 3,059 cM per chromosome. The corresponding IWGSC RefSeq v2.0 physical map length per chromosome ranged from 494 to 852 Mb. Pearson correlation coefficients between the genetic map in cM and physical map in Mb varied from 0.79 on 1D to 0.99 on 7D (Figure 3), which indicates a very high agreement in marker orders between the two types of maps.

2.3.2 Inclusive Composite Interval Mapping

We identified 10 QTLs using inclusive composite interval mapping and the genetic linkage maps (Table 3 and Figure 4). The QTLs were associated with maturity (2), lodging (4), grain yield (1), leaf rust (1), and stem rust (2). The two QTLs for maturity were mapped at 524 cM on chromosome 4A (*QMat.dms-4A*) and at 2171 cM on 5B (*QMat.dms-5B*), which explained 10.8% and 12.0% of the phenotypic variance, respectively. The four QTLs associated with plant

lodging were mapped at 1517 cM on chromosomes 4B (*QLdg.dms-4B*), at 538 cM on 5A (*QLdg.dms-5A*), at 568 cM on *5D* (*QLdg.dms-5D*), and at 1102 cM on 7D (*QLdg.dms-7D*). Each QTL for lodging explained from 7.7% to 12.2% and together accounted for 38.2% of the phenotypic variance. The single QTL associated with grain yield was mapped at 1221 cM on chromosome 2D (*QYld.dms-2D*) and explained 8.6% of the phenotypic variance. DH lines that were homozygous for the 'HYAYT12-10' alleles at the two flanking markers yielded on average 250 kg ha^{-1} more grain yield than those with the 'GP146' alleles.

The single QTL detected for leaf rust was mapped at 3127 cM on chromosome 4A (*QLr.dms-4A*) and accounted for 9.0% of the phenotypic variance. DH lines that were homozygous for the 'HYAYT12-10' alleles at the two flanking markers of *QLr.dms-4A* had on average 1.6 lower leaf rust scores than those with the GP146 alleles. The two QTLs associated with stem rust were mapped at 1305 cM on chromosomes 1A (*QSr.dms-1A*) and at 3143 cM on 2B (*QSr.dms-2B*), which accounted for 6.0% and 22.3% of the phenotypic variance, respectively. DH lines that were homozygous for the 'HYAYT12-10' alleles at the two flanking markers *QSr.dms-1A* and *QSr.dms-2B* had on average 1.7 and 1.9 less stem rust score than those with the 'GP146' alleles. Overall, all QTLs detected in the present study explained from 8.6% to 38.2% of the total phenotypic variance per trait, so most of the variation in all five traits remained unexplained.

2.4 Discussion

The present study employed 167 DH lines derived from 'HYAYT12-10' × 'GP 146', which are unregistered lines belonging to the Canada Western Special Purpose class. We

uncovered a total of 10 QTLs of which seven were associated with agronomic traits and three for leaf and stem rusts. The development of early maturing wheat cultivars is always a priority in the northern areas of the world (including the Canadian prairies) where frosts can damage crops due to a short growing season (Semagn et al. 2021b). Our study identified two moderate effect QTLs for maturity at 29.2-29.8 Mb on chromosome 4A (*QMat.dms-4A*) from 'HYAYT12-10' and at 581.5-583.5 Mb on 5B (*QMat.dms-5B*) from 'GP146'. QTLs for maturity have been reported across several wheat chromosomes, including 4A (Kamran et al. 2013, McCartney et al. 2005a, Semagn et al. 2021b, Perez-Lara et al. 2016) and 5B (Kamran et al. 2013, Semagn et al. 2021b). One of the minor effect maturity QTLs reported on chromosome 4A (*QMat.dms-4A.1*) by Perez-Lara et al. (2016) was flanked by *CAP12_rep_c4000_432* and *Ra_c7973_1185* SNPs, which are physically located at 24.6 and 37.0 Mb, respectively. The physical confidence interval of that QTL overlaps with the *QMat.dms-4A* identified in the present study (Table 3).

Using the recent IWGSC RefSeq physical map, our group recently reported the physical positions of eight QTLs for heading, flowering, and maturity on chromosomes 5B, which individually accounted for 1.8-19.3% of the phenotypic variance (Semagn et al. 2021b). One of the QTLs was associated with heading (*QHd.dms-5B.3*), flowering (*QFlt.dms-5B.2*) and maturity (*QMat.dms-5B.2*), maps at 574.5-577.0 Mb. *Vrn-B1* (gene ID: *TraesCS5B02G396600*) is one of the major genes that affects the vernalization response and flowering time in wheat (Santra et al. 2009), and is physically located between 573.8 Mb and 577.0 Mb, based on the IWGSC RefSeq v1.0 and IWGSC RefSeq v2.0 maps, respectively. The maturity QTL detected in the present study was, therefore, about 6.5 Mb away from the *Vrn-B1* gene and far from all QTLs reported for the three earliness traits in previous studies.

Plant lodging is another important trait in wheat breeding that directly affects grain yield. The introduction of the *Reduced height* (*Rht*) dwarfing or semi-dwarfing genes (Peng et al. 1999), such as Rht-*B1* and *Rht-D1* have made a significant impact in modern wheat cultivars. Our study uncovered four QTLs for lodging tolerance on chromosomes 4B (*QLdg.dms-4B*), 5A (*QLdg.dms-5A*), 5D (*QLdg.dms-5D*) and 7D (*QLdg.dms-7D*). Recently, our group reported the physical positions of 20 QTLs for lodging and 14 QTLs for plant height in four RILs populations, which individually accounted for 1.5-19.4% and 1.8-49.1% of the phenotypic variance, respectively (Semagn et al. 2021a). However, no QTLs in the previous study were close to the QTLs identified for lodging in the present study. For example, *QLdg.dms-4B* identified in the present study was located at 505.3-512.8 Mb and had a moderate effect (accounting for 12.2% of the phenotypic variance), with the favorable allele originating from 'HYAYT12-10'. Multiple similar QTLs associated either with lodging or plant height on 4B have been reported (e.g., Verma et al. 2005, McCartney et al. 2005a, Hassan et al. 2019) using genetic positions, but none were close to *QLdg.dms-4B. Rht-B1* (*TraesCS4B02G043100*) is one of the genes located on the short arm of chromosome 4B that has been widely used in wheat breeding not only to reduce plant height and increase lodging tolerance but also in increasing yield components and the number of productive tillers (Kertesz et al. 1991, Lanning et al. 2012; Sherman et al. 2014, Jobson et al. 2019). The exact physical position of the *Rht-B1* gene differs depending on the version of the reference sequence and varied from 30.8 Mb (based on IWGS RefSeq v1.0) to 33.6 Mb (based on IWGS RefSeq v2.1), which is far from the QTL detected in the present study. QTLs for lodging tolerance have also been reported on chromosome 5A in different studies (e.g., Keller et al. 1999, Marza et al. 2006), but their positions were reported using genetic maps in cM, which makes direct comparisons among independent studies

unreliable. Song et al. (2021) reported a minor QTL for stem diameter on chromosome 5A between *RAC875_c9617_373* and *RAC875_c9617_395* that maps at 663.9 Mb, which is far from the QTL identified in the present study.

QYld.dms-2D was the only QTL we found for grain yield that was located at 422.7-457.6 Mb on chromosome 2D. Grain yield is a complex trait affected by multiple agronomic and yield-related traits, environments, and genotype by environment (G×E) interactions, and QTL × QTL interactions (epistasis) (Wu et al. 2012, Xing et al. 2013). Chromosome 2D harbors multiple QTLs associated with spike number and agronomic traits (Perez-Lara et al. 2016, Zhang et al. 2015, Deng et al. 2019, Ma et al. 2020) as well as the photoperiodism response *Ppd-D1*gene. However, none of the previously reported QTLs are located within the same physical interval of the yield QTL identified in the present study.

We uncovered a minor effect QTL associated with leaf rust at 646.4-648.4 Mb on chromosome 4A (*QLr.dms-4A*), another minor effect QTL for stem rust at 536.8-543.6 Mb on 1A (*QSr.dms-1A*) and a major effect QTL for stem rust at 694.9-695.2 Mb on 2B (*QSr.dms-2B*) (Table 3). The leaf rust QTL on chromosome 4A (*QLr.dms-4A*) originated from 'HYAYT12-10' and was located between *Ra_c63534_581* and *RAC875_c6939_1042* at 646.4 and 648.4 Mb, respectively. Bemister et al. (2019) reported a minor effect leaf rust QTL on 4A at 602.7 Mb, which is 43.7 Mb far from the position of our QTL. Kertho et al. (2015) reported three QTLs on chromosome 4A for seedling leaf rust resistance at 93.5 cM, 151.3 cM, 198.8 cM. The closest QTL to *QLr.dms-4A* identified in the present study was flanked by marker *IWA7859* at 198.84 cM, which is physically located at 115.7 Mb; the two QTLs are over 530 Mb distant. The other QTL we detected for stem rust was mapped on chromosome 1A between *Excalibur_rep_c103592_955* at 536.8 Mb and *RAC875_rep_c69334_132* at 543.6 Mb. Other

studies have reported genes (e.g., *Sr1RS*) and QTLs associated with stem rust on 1A (Kumar et al. 2020, Leonova et al. 2020, and Megerssa et al. 2020), but direct comparisons across studies was difficult due to lack of physical information for most flanking markers.

Chromosome 2B harbors multiple genes, including *SrWeb, Sr28, Sr32, Sr39 Sr36, Sr40, Sr47* (Yu et al. 2014), *Sr9h, Sr16* (Kosgey et al. 2021, McCartney et al. 2005b, Venagas et al. 2007, Zurn et al. 2018). It also harbors several QTLs for stem rust resistance (Prins et al. 2016, Kosgey et al. 2021, Sharma et al. 2021), but all previously reported genes and QTLs are not within the physical confidence interval of the major effect stem rust QTL identified in the present study. For example, Kosgey et al. (2021) found a moderate effect stem rust QTL on 2B between markers BS00038820_51 and Tdurum_contig54704_176, which are located at 72.5 Mb and 658.6 Mb, respectively. Sharma et al. (2021) reported a major effect QTL that accounted for 33.3% of the phenotypic variation for stem rust on chromosome 2B between IWB7072 and IWB2380 and another moderate effect QTL (16.2%) between IWB71742 and IWB73196, which are located at 97.1 and 746.7 Mb, respectively.

We found moderate to high broad-sense heritability (0.41-0.78) and expected it to account for most of the phenotypic variance of each QTL. However, we were only able to account for <40% of the phenotypic variance of every QTL. Thus, most of the phenotypic variation remained unexplained by the identified QTL, which is consistent with several previous studies conducted in different Canadian spring wheat populations (Asif et al. 2015, Chen et al. 2015, Perez-Lara et al. 2016, Chen et al. 2020). Some of the factors that affect the probability of detecting QTL and the proportion of variance explained by each QTL include marker-density, mapping population type and size, trait heritability, the number of environments, and Genotype × Environment interactions (Semagn et al. 2010). DH populations are easy and quick to develop,

which makes them attractive for QTL mapping in various species, but they have poor resolution due to limited recombination. They have only gone through one round of recombination as compared with multiple rounds of recombination in RIL populations (Yan et al. 2017, Alqudah et al. 2020).

2.5 Conclusion

Using a DH population genotyped with 2,676 high quality SNPs and phenotyped for 5 traits at 3-5 environments, we found ten QTL linked to agronomic and disease qualities. Most of our QTL were of moderate effect and did not explain the majority of phenotypic variation. However, *QSr.dms-2B.2* explained 22% of stem rust response and its location (694.9-695.2 Mb) has been found by other researchers. These previous studies found a large interval on chromosome 2B responsible for stem rust reaction. Our QTL is in a 0.3 Mb interval which falls in this larger reported interval. Follow up studies are needed to validate and fine map the major effect *QSr.dms-2B.2* for stem rust.

2.6 Tables and Figures

2.6.1 Tables

Table 1

Table 1. Summary of descriptive statistics of observed maturity, lodging, grain yield, leaf and stem rusts of parents and the 'HYAYT12-10' × 'GP146' doubled haploid (DH) population over combined environments.

Trait	Parents			DH lines		F statistics		Broad-sense heritability
	HYAYT12-10	GP146	Difference*	Range	Mean ± Std	F-Value	p-value	
Maturity (days)	110.23	106.80	-3.43	104.78-111.60	108.26 ± 1.50	3.20	<0.0001	0.411
Lodging	1.52	4.84	3.32	1.17-5.01	2.59 ± 0.87	9.30	<0.0001	0.640
Yield (t ha^{-1})	6.26	5.52	-0.74	4.62-6.79	5.72 ± 0.36	2.50	<0.0001	0.470
Leaf rust	1.50	5.10	3.60	1.20-7.80	3.48 ± 1.80	23.70	<0.0001	0.779
Stem rust	1.50	7.00	5.50	1.20-8.80	3.79 ± 1.98	20.10	<0.0001	0.681

*Difference = Parent2 – Parent1

Table 2

Table 2. Summary of Single Nucleotide Polymorphism (SNP) marker number in the linkage groups and Quantitative Trait Loci (QTL) analysis in the 'HYAYT12-10' × 'GP146'doubled haploid (DH) mapping population.

Chromosome	No. of SNPs	Map length (cM)	Map length (Mb)
1A	75	2231.2	598.1
1B	142	3795.4	700.5
1D	49	1797.3	497.1
2A	164	5345.1	786.2
2B	109	4517.8	808.1
2D	54	2051.6	621.6
3A	122	3748.7	745.3
3B	379	3253.5	851.9
3D	56	2698.4	613.5
4A	169	4775.2	748.0
4B	156	2701.8	665.6
4D	39	2118.3	508.1
5A	148	2920.0	708.1
5B	332	2923.3	713.3
5D	61	1991.6	568.7
6A	244	3420.0	622.5
6B	56	3016.5	727.6
6D	35	1909.3	493.7
7A	145	3830.7	744.2
7B	96	3112.3	763.3
7D	45	2084.6	640.4
Total	**2,676**	**64,242.5**	**14,125.7**

Table 3

Table 3. Descriptive summary of identified quantitative trait loci (QTLs) associated with five traits in the 'HYAYT12-10' × 'GP146' DH mapping population over combined environments.

Trait	QTL	Chr‡	Position (cM)	Left CI (cM)	Right CI (cM)	Left CI (Mb)	Right CI (Mb)	LeftMarker	RightMarker	LOD∞	PVE§ (%)	Additive effect	Parent⸸
Maturity	*QMat.dms-4A*	4A	524.0	508.5	536.5	29.2	29.8	Kukri_c13639_1326	BS00065607_51	6.2	10.8	0.7	P1
Maturity	*QMat.dms-5B*	5B	2171.0	2143.5	2195.5	581.5	583.5	wsnp_Ex_c621_1230852	Excalibur_c9391_1016	3.1	12.0	-0.8	P2
Lodging	*QLdg.dms-4B*	4B	1517.0	1508.5	1519.5	505.3	512.8	wsnp_Ex_c4358_7854194	GENE-2331_126	9.7	12.2	-0.4	P1
Lodging	*QLdg.dms-5A*	5A	538.0	523.5	569.5	111.8	238.5	Excalibur_rep_c69159_392	Tdurum_contig67350_494	3.4	7.7	0.3	P2
Lodging	*QLdg.dms-5D*	5D	568.0	555.5	584.5	401.7	407.6	Tdurum_contig68472_115	Kukri_rep_c79943_189	4.2	9.6	-0.3	P1
Lodging	*QLdg.dms-7D*	7D	1102.0	1086.5	1130.5	372.1	391.5	Ra_c6845_1501	wsnp_cd454041D_Ta_2_1	3.7	8.7	-0.3	P1
Yield	*QYld.dms-2D*	2D	1221.0	1218.5	1256.5	422.7	457.6	BS00090129_51	Excalibur_c24307_739	3.9	8.6	0.1	P1
Leaf rust	*QLr.dms-4A*	4A	3127.0	3118.5	3139.5	646.4	648.4	Ra_c63534_581	RAC875_c6939_1042	12.1	9.0	-1.4	P1
Stem rust	*QSr.dms-1A*	1A	1305.0	1277.5	1307.5	536.8	543.6	Excalibur_rep_c103592_955	RAC875_rep_c69334_132	5.6	6.0	-0.7	P1
Stem rust	*QSr.dms-2B*	2B	3143.0	3132.5	3155.5	694.9	695.2	BobWhite_c3871_428	BS00065914_51	9.5	22.3	-1.3	P1

‡ Chr = Chromosome

∞ LOD = Logarithm of Odds

§ PVE = Phenotypic Variation Explained

⸸ P1 = HYAYT12-10, P2 = GP146

2.6.2 Figures

Figure 1

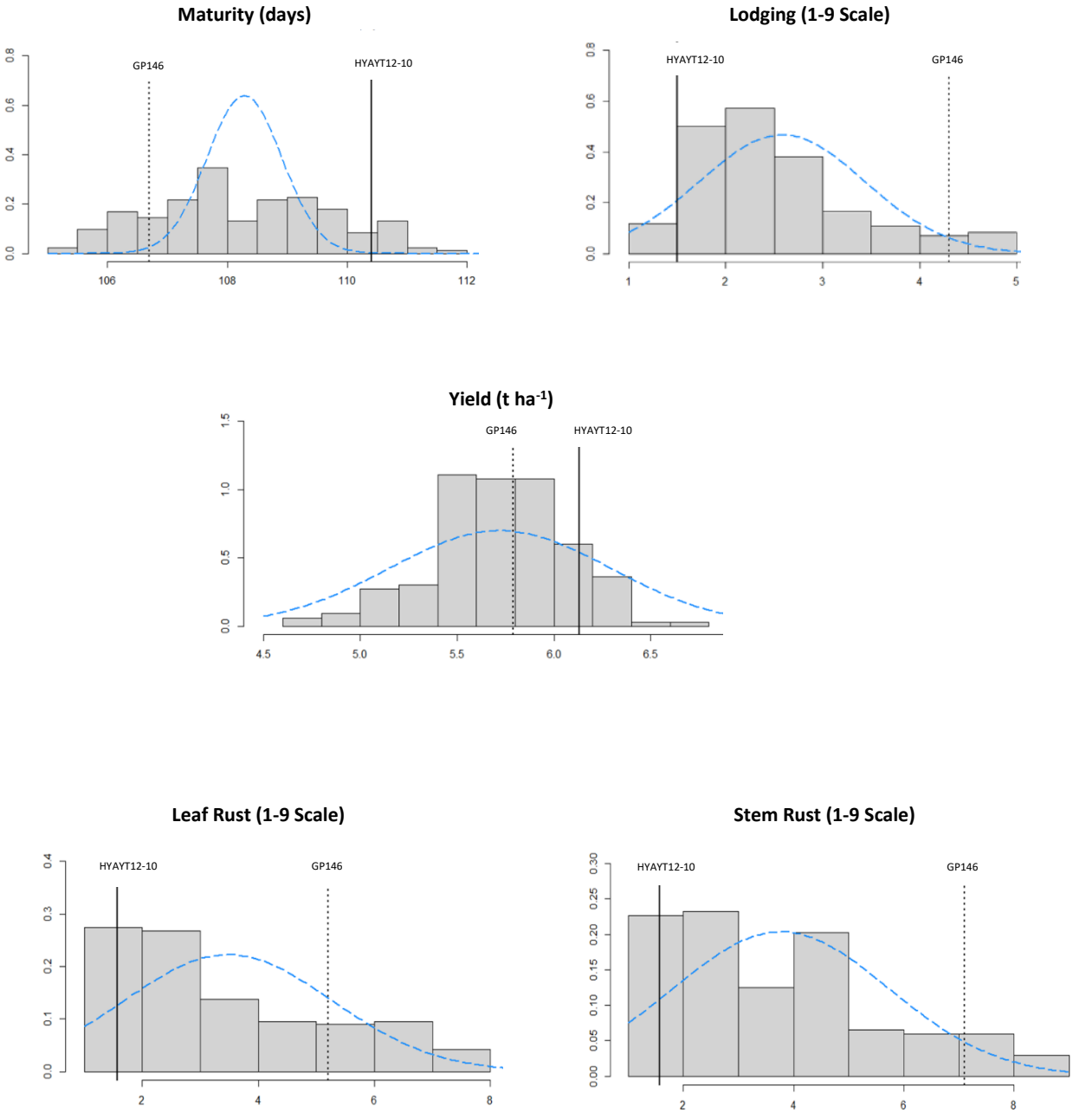
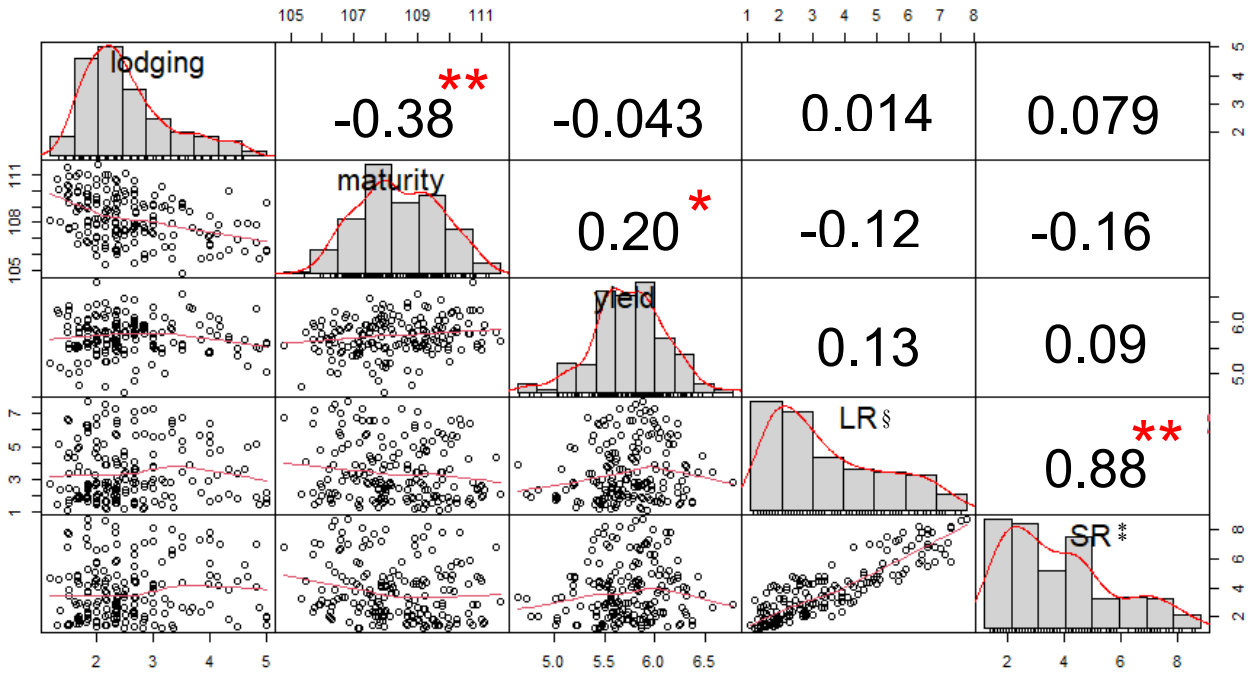


Figure 1. Frequency distributions of least square means of 167 entries in the doubled haploid (DH) mapping population 'HYAYT12-10' × 'GP146' by trait.

Figure 2

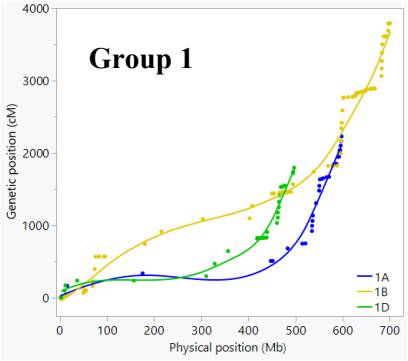


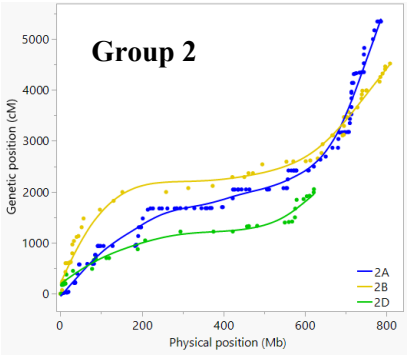
§ LR = Leaf Rust

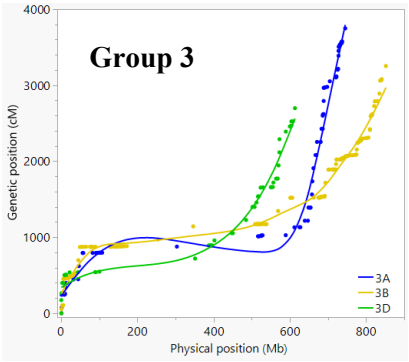
‡ SR = Stem Rust

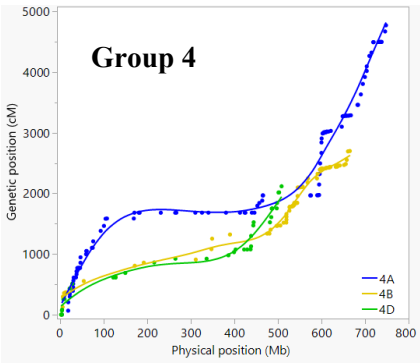
Figure 2. Correlogram of Pearson correlation coefficients for 5 phenotypic traits in 'HYAYT12-10' × 'GP146' mapping population in the combined environment analysis.

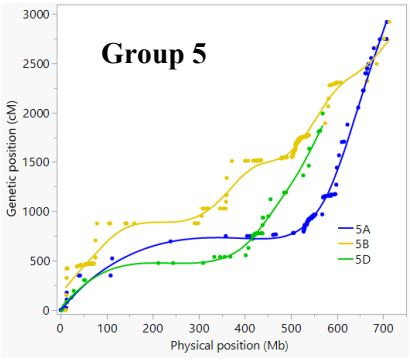
Figure 3

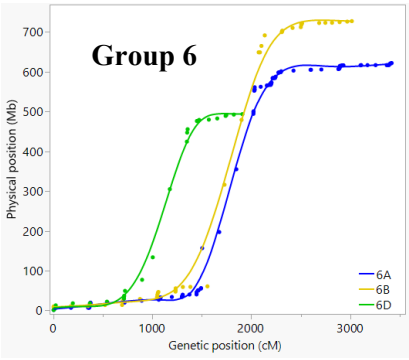












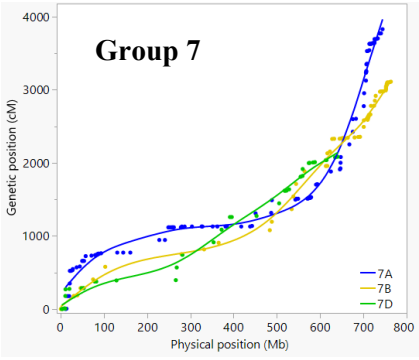


Figure 3. Scatter plot of genetic map in cM and physical map in Mb based on 2,676 SNPs. Pearson correlation between genetic map (cM) and IWGSC RefSeq v2.0 physical map (Mb) ranged from 0.785 on chromosome 1D to 0.987 on 7D.

Figure 4

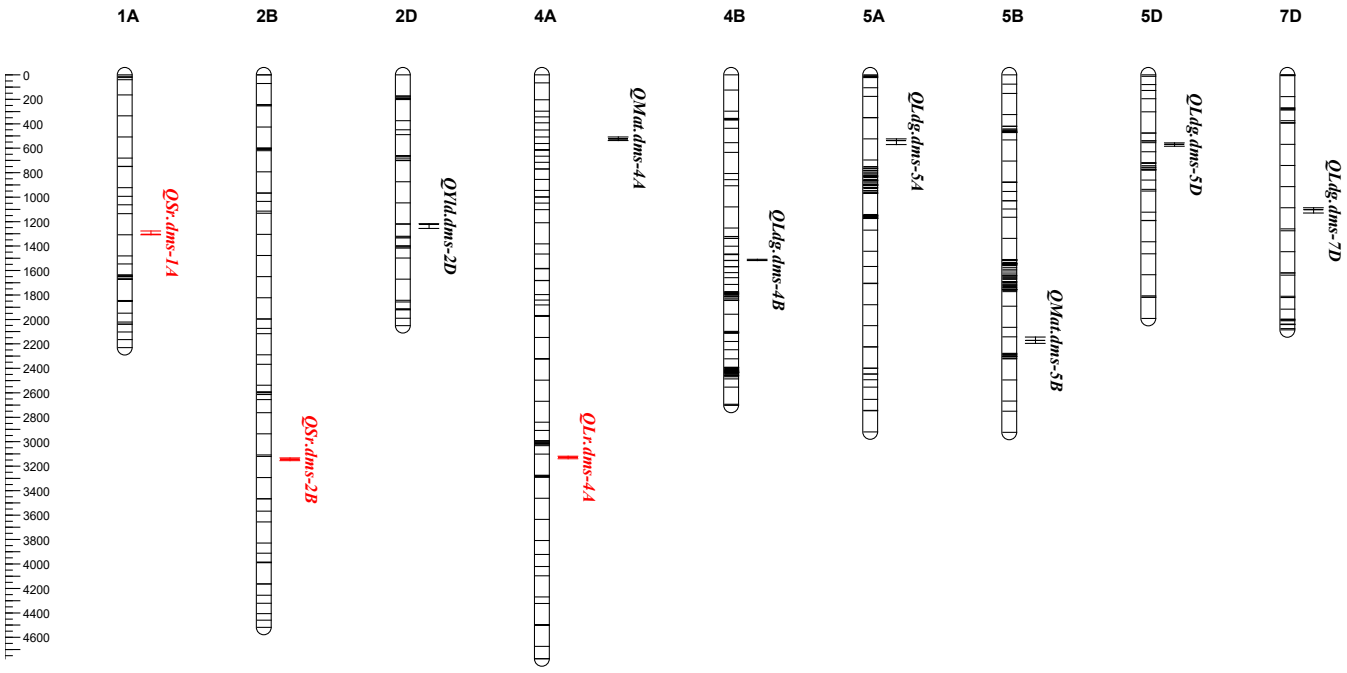


Figure 4. Genetic linkage and QTL maps of nine common wheat (*Triticum aestivum* L.) chromosomes with ten identified QTLs based on 167 DH lines genotyped with 2,676 SNPs. Map position (cM) is shown on the left, with each horizontal line on the chromosome representing a marker. QTL are shown on the right side of each chromosome, with bars indicating their confidence interval between two flanking markers. QTL associated with agronomic traits and rust resistance are in black and red fonts, respectively.

3. General Discussion

3.1 Introduction

The story of Canadian wheat (*Triticum aestivum* L.) began in 1842 in Ontario with Scottish immigrant David Fife and his grain he called Red Fife (Cuthbert 2006). This humble beginning has developed into wheat becoming a crop of economic importance, with over 35.2 million tonnes (Mt) harvested in 2020 across Canada and over 591 varieties registered in the past 60 years (Statistics Canada 2020b, https://inspection.canada.ca/active/netapp/regvar/regvar_lookupe.aspx). Varietal development relies on bringing together desirable characteristics into one grain. Wheat must satisfy the producer and the end user, which means that traits are analyzed from how the plant grows to what kind of seed it creates. It is assessed on maturity, height, lodging, yield, grain quality and the plant's natural ability to stave off diseases caused by biotic and abiotic stressors (McCallum and DePauw 2008, PGDC 2018, Randhawa et al. 2013). Disease resistance has become of particular importance since the beginning of the 1900s, when stem rust epidemics devasted harvests (McCallum et al. 2007). Now, it is mandatory to have moderate genetic resistance to at least some of Canada's top priority diseases: leaf rust (*Puccinia triticina* Erikss.), stem rust (*Puccinia graminis* f.sp. *tritici* Eriks. & E. Henn.T), stripe rust (*Puccinia striiformis* Westend.), fusarium head blight (*Fusarium graminerium* Schwabe) and bunt (*Tilletia tritici* and *T. laevis*) (PGDC 2018).

Traditional wheat breeding relies on phenotypic selection, the art of selecting a plant based on careful visual assessment (Acquaah 2012). In the past century, advances in technology have allowed us to finely select for known genes in the plant's genotype by using molecular marker assisted selection (MMAS). This gives breeders another method of screening if

environmental pressures are not sufficient for proper gene expression and can also cull lines in the early generations if it is confirmed that a certain line does not carry a trait of particular interest. However, it is important to remember that MMAS does not substitute phenotypic selection. A plant may carry a gene of interest but its response in the environment may be different and there may be other genes affecting its expression (Toth et al. 2018). Due to the fact that most agronomic traits are not controlled by just a single gene, studies discovering areas on the genome which contribute to these traits are of importance to breeders (Mackay 2001). These regions are known as quantitative trait loci (QTL) and the goal is to find what known markers they have tight linkage with so they can be selected in the lab. QTL analysis studies have become very important in uncovering new genes and their integration for trait improvement (Singh et al. 2014).

Our study looked at 167 doubled haploid lines from 'HYAYT12-10' × 'GP 146', which are unregistered lines that belong to the Canada Western Special Purpose wheat class known for its high yield and low protein profile (Canadian Grain Commission 2021). The objective of our study was to map genes and QTLs associated with agronomic and disease traits, specifically maturity, lodging, yield, and leaf and stem rusts.

3.2 Contribution to Knowledge

Overall, we uncovered ten QTLs of which seven were associated with agronomic traits and three were linked to disease responses. They ranged from moderate to high broad-sense heritability (0.41-0.78). There were two moderate maturity QTLs on chromosomes 4A and 5B which explained a total of 22.8% phenotypic variation. Previous studies have also found maturity QTLs on chromosomes 4A and 5B, although only Perez-Lara et al. (2016) reported an area

which physically overlaps with our finding on chromosome 4A. The vernalization and flowering gene *Vrn-B1* is located on chromosome 5B at 573.8 to 577.0 Mb which is about 6.5 Mb away from our maturity QTL.

A total of 38.2% was attributed to lodging by four QTLs on chromosomes 4B, 5A, 5D, and 7D. Most studies which also reported lodging QTLs on these chromosomes did not map to the same areas as our QTLs. Plant height gene *Rht-B1* is found on the short arm of chromosome 4B around 30 to 33 Mb and this is not in the same area as our locus on 4B at 505.3 to 512.8 Mb.

There was one minor (8.6% PVE) yield QTL found on chromosome 2D. This chromosome has been known to carry regions linked to agronomic qualities, including photoperiodism gene *Ppd-D1* (Deng et al. 2019, Ma et al. 2020, Perez-Lara et al. 2016, Zhang et al. 2015). No previous studies found QTLs reported in the same area and the *Ppd-D1* gene was also not in the same interval.

One minor leaf rust QTL was detected on chromosome 4A and a minor stem rust QTL was found on chromosome 1A. Other researchers also found QTL on these chromosomes which were linked to disease response, but none reported locations which matched those of the present study. The final QTL was on chromosome 2B for stem rust and it contributed 22.3% overall variation. Chromosome 2B has been reported to harbour stem rust QTLs (Kosgey et al. 2021, Prins et al. 2016, Sharma et al. 2021), and some notable stem rust genes, *SrWeb, Sr28, Sr32, Sr39 Sr36, Sr40, Sr47* (Yu et al. 2014), *Sr9h, Sr16* (Kosgey et al. 2021, McCartney et al. 2005b, Venagas et al. 2007, Zurn et al. 2018). Our QTL fell into a small interval between 694.9 and 695.2 Mb, and this is in the large interval that Sharma et al. (2012) found between 97.1 and 746.7 Mb that was responsible for 33.3% of the stem rust response.

These results indicate that there are minor loci that contribute to agronomic and disease responses and their integration into breeding material does contribute to significant differences. This was particularly true for the lines which carried more than one lodging QTL and also for lines which carried more than one disease QTL. These individuals showed full to moderate resistance to the abiotic (lodging) or biotic (fungal pathogen) stressors. The parents in this doubled haploid population were better for disease QTL analysis as they had markedly different responses to leaf and stem rusts. Agronomically, they were moderately different. 'HYAYT12-10' contributed all three disease QTLs, *QLr.dms-4A*, *QSr.dms-1A* and *QSr.dms-2B***.** It also gave five agronomic QTLs, *QLdg.dms-4B, QLdg.dms-5D, QLdg.dms-7D, QMat.dms-5,* and the only yield QTL *QYld.dms-2D.* 'GP146' contributed *QLdg.dms-5A* and *QMat.dms-5B.*

3.3 Conclusions

Most of the QTLs found in this study were not reported previously. There are the exceptions of the maturity QTL *QMat.dms-4A* on chromosome 4A which was also included in a QTL analysis by Perez-Lara et al. (2016) and *QSr.dms-2B* which falls into the interval reported on chromosome 2B by Sharma et al. (2012) for stem rust. *QMat.dms-4A* was a moderate QTL in this study at 10.8% PVE, but phenotypically it did not appear to show considerable improvement from the average days to maturity for this population. *QSr.dms-2B* was reported as a 0.3 Mb interval and was also the major QTL (22.3%) in our analysis. Sharma et al. (2012) described a larger QTL with over 33% variation explained; the location was imprecise spanning over 550 Mb, but it confirmed the present study's finding of a major stem rust resistance region. Chromosome 2B has confirmed genes and has formerly reported QTLs which are linked to stem rust response. Therefore, *QSr.dms-2B* should be validated with the flanking markers we

identified, BobWhite_c3871_428 and BS00065914_51, and the advanced breeding line 'HYAYT12-10' which it came from could be employed for gene pyramiding in other Canadian Western Special Purpose wheat class breeding lines.

3.4 Future Research

1. The parent 'HYAYT12-10', which was responsible for the three disease loci, *QLr.dms-4A*, *QSr.dms-1A* and *QSr.dms-2B*, and some individuals in this population could be used for pyramiding quantitative disease resistance genes in future research or breeding lines. In particular, entries 26, 90, and 141 were found to be high yielders (6.1 to 6.3 t ha^{-1}), and resistant to both rusts (1.5 to 1.6 scores for leaf rust and 1.3 to 1.7 for stem rust scores).
2. Further field and MMAS studies for confirmation of the location of *QSr.dms-2B*, its contribution and stability could prove useful as this was confirmed to be an area of interest by other researchers.

References

Abdelkader, B. (2014). *The history of wheat breeding in Algeria*. E. Porceddu, C. O. Qualset, & A. B. Damania (Eds.), Proceedings of the International Symposium on Genetics and breeding of durum wheat (Vol. 110, pp. 363-370). Bari : CIHEAM.

Aboukhaddour, R., Turkington, T., & Strelkov, S. (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

Acquaah, G. (2012). *Principles of Plant Genetics and Breeding* (2nd Edition ed.). John Wiley & Sons, Ltd. Hoboken, NJ, USA. Pages 3-10, 22, 33, 49, 54, 65, 85-86, 90-92, 101, 109-113, 306, 322, 385, 577-589.

Agriculture and Agri-Food Canada. (2020). *Canada: Outlook for Principal Field Crops*. Retrieved from https://publications.gc.ca/site/archivee-archived.html?url=https://publications.gc.ca/collections/collection_2021/aac-aafc/A77-1-2020-12-18-eng.pdf

Agriculture and Agri-Food Canada. (2013). *Crop Variety Registration in Canada Issues and Options*. Retrieved 2020-10-10 from publications.gc.ca/pub?id=9.698289&sl=0

Agrios, G. N. (2005). *Plant Pathology* (5th Edition ed.). Elsevier Academic Press. London, UK. Pages 133-144, 156-157, 562-571.

Aissaoui, M.R. & Fenni, M. (2021). Effect of supplemental irrigation on bread wheat genotypes yield under Mediterranean semi-arid conditions of north-eastern Algeria. R*evista Facultad Nacional de Agronomia Medellin, 74:* 9431-9440. doi:10.15446/rfnam.v74n1.89798.

Akond, Z., Alam, M.J., Hasan, M.N., Uddin, M.S., Alam, M., & Mollah, M.N.H. (2019). A comparison on some interval mapping approaches for QTL detection. *Bioinformation, 15*: 90-94. doi:10.6026/97320630015090.

Ali, S., & Bakkeren, G. (2011). Fungal and oomycete effectors - Strategies to subdue a host. *Canadian Journal of Plant Pathology*, *33*: 425-446. https://doi.org/10.1080/07060661.2011.625448

Alqudah, A. M., Sallam, A., Stephen Baenziger, P., & Börner, A. (2020). GWAS: Fast-forwarding gene identification and characterization in temperate Cereals: lessons from Barley – A review. *Journal of Advanced Research*, *22*: 119-135. https://doi.org/https://doi.org/10.1016/j.jare.2019.10.013

Araki, E., Miura, H., & Sawada, S. (1999). Identification of genetic loci affecting amylose content and agronomic traits on chromosome 4A of wheat. *Theoretical and Applied Genetics*, *98*(6-7): 977-984.

Asif, M., Yang, R.-C., Navabi, A., Iqbal, M., Kamran, A., Perez Lara, E., Randhawa, H., Pozniak, C. & 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*: 1129-1142. https://doi.org/10.2135/cropsci2014.01.0080

Bajgain, P., Rouse, M. N., Tsilo, T. J., Macharia, G. K., Bhavani, S., Jin, Y., & Anderson, J. A. (2016). Nested association mapping of stem rust resistance in wheat using genotyping by sequencing. *PloS ONE*, *11*(5):1-22. e0155760-e0155760. https://doi.org/10.1371/journal.pone.0155760

Beavis, W. (1994). *The power and deceit of QTL experiments: lessons from comparative QTL studies*, pp. 250–266 in 49th Annual Corn and Sorghum Industry Research Conference. American Seed Trade Association, Washington, DC.

Beavis, W. D. (2019). QTL analyses: power, precision, and accuracy. *Molecular Dissection of Complex Traits*, pp. 145-162. CRC press.

Bemister, D. H., Semagn, K., Iqbal, M., Randhawa, H., Strelkov, S. E., & Spaner, D. M. (2019). Mapping QTL associated with stripe rust, leaf rust, and leaf spotting in a Canadian spring wheat population. *Crop Science*, *59*(2): 650-658. https://doi.org/10.2135/cropsci2018.05.0348

Beres, B., Pozniak, C., Bressler, D., Gibreel, A., Eudes, F., Graf, R., Randhawa, H., Salmon, D., McLeod, G., Dion, Y., Irvine, B., Voldeng, H., Martin, R., Pageau, D., Comeau, A., DePauw, R., Phelps, S., & Spaner, D. (2013). A Canadian ethanol feedstock study to benchmark the relative performance of Triticale: II. Grain quality and ethanol production. *Agronomy Journal*, *105*: 1707-1720. https://doi.org/10.2134/agronj2013.0193

Bernardo, R. (2008). Molecular markers and selection for complex traits in plants: learning from the last 20 years. *Crop Science*, *48*(5): 1649-1664. https://doi.org/https://doi.org/10.2135/cropsci2008.03.0131

Biosearch Technologies. (2020). Genomic Analysis by LGC. How Does KASP Work? Retrieved 2021-11-10 from https://www.biosearchtech.com/support/education/kasp-genotyping-reagents/how-does-kasp-work

Bogard M., Jourdan M., Allard V., Martre P., Perretant M.R., Ravel C., Heumez E., Orford S., Snape J., Griffiths S., Gaju O., Foulkes J., & Le Gouis J. (2011). Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in a winter wheat population segregating for flowering time QTLs. *Journal of Experimental Botany*, *62*(10): 3621-3636. doi: 10.1093/jxb/err061

Bokore, F. E., Knox, R. E., Cuthbert, R. D., Pozniak, C. J., McCallum, B. D., N'Diaye, A., DePauw R.M., Campbell H.L., Munro C., Singh A., Hiebert C.W., McCartney C.A., Sharpe A.G., Singh A.K., Spaner D., Fowler D.B., Ruan Y., Berraies S., & Meyer, B. (2020). Mapping quantitative trait loci associated with leaf rust resistance in five spring wheat populations using single nucleotide polymorphism markers. *PLoS One*, *15*(4): 1-22. e0230855. https://doi.org/10.1371/journal.pone.0230855

Brar, G. S., Dhariwal, R., & Randhawa, H. S. (2018). Resistance evaluation of differentials and commercial wheat cultivars to stripe rust (*Puccinia striiformis*) infection in hot spot regions of Canada. *European Journal of Plant Pathology*, *152*(2): 493-502. https://doi.org/10.1007/s10658-018-1446-3

Brar, G. S., Fetch, T., McCallum, B. D., Hucl, P. J., & Kutcher, H. R. (2019). Virulence dynamics and breeding for resistance to stripe, stem, and leaf rust in Canada since 2000. *Plant Disease*, *103*(12): 2981-2995. https://doi.org/10.1094/pdis-04-19-0866-fe

Brenchley, R., Spannagl, M., Pfeifer, M., Barker, G. L. A., D'Amore, R., Allen, A. M., McKenzie N., Kramer M., Kerhornou A., Bolser D., Kay S., Waite D., Trick M., Bancroft I., Gu Y., Huo N., Luo M.C., Sehgal S., Gill B., Kianian S., Anderson O., Kersey P., Dvorak J., McCombie W.R., Hall A., Mayer K.F., Edwards K.J., Bevan M.W., & Hall, N. (2012). Analysis of the bread wheat genome using whole-genome shotgun sequencing. *Nature*, *491*(7426): 705-710. https://doi.org/10.1038/nature11650

Broman, K. W. (2001). Review of statistical methods for QTL mapping in experimental crosses. *Lab Animal*, *30*(7): 44-52.

Börner, A., Schumann, E., Fürste, A., Cöster, H., B, L., Röder, M., & Weber, E. (2002). Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, *105*: 921-936. https://doi.org/10.1007/s00122-002-0994-1

Canadian Grain Commission (2021). *Official Grading Guide*. https://www.grainscanada.gc.ca/en/grain-quality/official-grain-grading-guide/04-wheat/classes-varieties.html

Charcosset, A., & Gallais, A. (1996). Estimation of the contribution of quantitative trait loci (QTL) to the variance of a quantitative trait by means of genetic markers. *Theoretical and Applied Genetics*, *93*(8): 1193-1201. https://doi.org/10.1007/bf00223450

Che, M., Hiebert, C. W., McCartney, C. A., Zhang, Z., & McCallum, B. D. (2019). Mapping and DNA marker development for *Lr33* from the leaf rust resistant line KU168-2. *Euphytica*, *215*(29): 1-14. https://doi.org/10.1007/s10681-019-2343-3

Chen, H., Bemister, D., Iqbal, M., Strelkov, S., & Spaner, D. (2020). Mapping genomic regions controlling agronomic traits in spring wheat under conventional and organic managements. *Crop Science*, *60*: 2038-2052. https://doi.org/10.1002/csc2.20157

Chen, H., Iqbal, M., Perez Lara, E., Yang, R.-C., Pozniak, C., & Spaner, D. (2015). Earliness per se quantitative trait loci and their interaction with *Vrn-B1* locus in a spring wheat population. *Molecular Breeding*, *35*: 1-14. https://doi.org/10.1007/s11032-015-0373-7

Chen, X. M. (2005). Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat. *Canadian Journal of Plant Pathology*, *27*(3): 314-337. https://doi.org/10.1080/07060660509507230

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*(1): 169-196. https://doi.org/10.1007/s10681-005-1681-5

Conner, R. L., & Kuzyk, A. D. (1988). Effectiveness of fungicides in controlling stripe rust, leaf rust, and black point in soft white spring wheat. *Canadian Journal of Plant Pathology*, *10*(4): 321-326. https://doi.org/10.1080/07060668809501706

Coriton, O., Jahier, J., Leconte, M., Huteau, V., Trotoux, G., Dedryver, F., & Pope De Vallavieille, C. (2020). Double dose efficiency of the yellow rust resistance gene *Yr17* in bread wheat lines. *Plant Breeding*, *139*(2): 263-271. https://doi.org/10.1111/pbr.12768

Crossa, J., Burgueño, J., Dreisigacker, S., Vargas, M., Herrera-Foessel, S. A., Lillemo, M., Singh R.P., Trethowan, R., Warburton, M., Franco, J., Reynolds, M., Crouch, J.H. & Ortiz, R. (2007). Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. *Genetics*, *177*(3): 1889-1913. https://doi.org/10.1534/genetics.107.078659

Cuthbert, J. L., Somers, D. J., Brûlé-Babel, A. L., Brown, P. D., & Crow, G. H. (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. https://doi.org/10.1007/s00122-008-0804-5

Cuthbert, P. (2006). A bread from the history books. *Maclean's*, *119*(30): 1.

Dakouri, A., McCallum, B. D., & Cloutier, S. (2014). Haplotype diversity and evolutionary history of the *Lr34* locus of wheat. *Molecular Breeding*, *33*(3): 639-655. https://doi.org/10.1007/s11032-013-9981-2

Dakouri, A., McCallum, B. D., Radovanovic, N., & Cloutier, S. (2013). Molecular and phenotypic characterization of seedling and adult plant leaf rust resistance in a world wheat collection. *Molecular Breeding, 32*(3): 663-677. https://doi.org/10.1007/s11032-013-9899-8

Deng, M., Wu, F., Zhou, W., Li, J., Shi, H., Wang, Z., Lin, Y., Yang, X., Wei, Y., Zheng, Y. & Liu, Y. (2019). Mapping of QTL for total spikelet number per spike on chromosome 2D in wheat using a high-density genetic map. *Genetics and Molecular Biology, 42*(3): 603-610. https://doi.org/10.1590/1678-4685-GMB-2018-0122

DePauw, R. M., Knox, R. E., Clarke, F. R., Wang, H., Fernandez, M. R., Clarke, J. M., & McCaig, T. N. (2007). Shifting undesirable correlations. *Euphytica*, *157*(3): 409-415. https://doi.org/10.1007/s10681-007-9379-5

Distelfeld, A., Li, C., & Dubcovsky, J. (2009). Regulation of flowering in temperate cereals. *Current Opinion in Plant Biology*, *12*: 178-184. https://doi.org/10.1016/j.pbi.2008.12.010

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin, 19* (1): 11-15.

Dyck, P. L. (1987). The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. *Genome*, *29*(3): 467-469. https://doi.org/10.1139/g87-081

Dyck, P. L., Samborski, D. J., & Anderson, R. G. (1966). Inheritance of adult-plant leaf rust resistance derived from the common wheat varieties Exchange and Frontana. *Canadian Journal of Genetics and Cytology*, *8*(4): 665-671. https://doi.org/10.1139/g66-082

Ellis, H., Spielmeyer, W., Gale, R., Rebetzke, G., & Richards, A. (2002). "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. *Theoretical and Applied Genetics*, *105*: 1038-1042. https://doi.org/10.1007/s00122-002-1048-4

Ellis, J., Lagudah, E., Spielmeyer, W., & Dodds, P. (2014). The past, present and future of breeding rust resistant wheat. *Frontiers in Plant Science*, *5* (641): 1-13. https://doi.org/10.3389/fpls.2014.00641

Fetch, T. G., & Dunsmore, K. M. (2004). Physiologic specialization of *Puccinia graminis* on wheat, barley, and oat in Canada in 2001. *Canadian Journal of Plant Pathology, 26*(2): 148-155. https://doi.org/10.1080/07060660409507126

Fontaine, J. X., Ravel, C., Pageau, K., Heumez, E., Dubois, F., Hirel, B., & Le Gouis, J. (2009). A quantitative genetic study for elucidating the contribution of glutamine synthetase, glutamate dehydrogenase and other nitrogen-related physiological traits to the agronomic performance of common wheat. *Theoretical and Applied Genetics, 119*(4): 645-662. https://doi.org/10.1007/s00122-009-1076-4

Gao, L., Rouse, M., Mihalyov, P., Bulli, P., Pumphrey, M., & Anderson, J. A. (2017). Genetic characterization of stem rust resistance in a global spring wheat germplasm collection. *Crop Science*, *57*: 2576-2589. https://doi.org/10.2135/cropsci2017.03.0159

Garnica, D. P., Nemri, A., Upadhyaya, N. M., Rathjen, J. P., & Dodds, P. N. (2014). The ins and outs of rust haustoria. *PLoS pathogens, 10*(9), e1004329: 1-4. https://doi.org/10.1371/journal.ppat.1004329

Gaudet, D.A., Puchalski, B., Randhawa, H.S., Despins, T., and Laroche, A. (November 4-6, 2013). *Fungicide Control of Stripe Rust in Western Canada*. 34th Annual Meeting of the Plant Pathology Society of Alberta, Brooks, AB, Canada.

German, S. E., & Kolmer, J. A. (1992). Effect of gene *Lr34* in the enhancement of resistance to leaf rust of wheat. *Theoretical and Applied Genetics, 84*(1): 97-105. https://doi.org/10.1007/BF00223987

Ghazvini, H., Hiebert, C. W., Thomas, J. B., & Fetch, T. (2013). Development of a multiple bulked segregant analysis (MBSA) method used to locate a new stem rust resistance gene (*Sr54*) in the winter wheat cultivar Norin 40. *Theoretical and Applied Genetics, 126*(2): 443-449. https://doi.org/10.1007/s00122-012-1992-6

Graf, R., Beres, B., Laroche, A., Gaudet, D., Eudes, F., Pandeya, R., Badea, A. & Randhawa, H. (2013). Emerson hard red winter wheat. *Canadian Journal of Plant Science, 93*(4): 741-748. https://doi.org/10.4141/cjps2012-262

Guerrero-Chavez, R., Glover, K. D., Rouse, M. N., & Gonzalez-Hernandez, J. L. (2015). Mapping of two loci conferring resistance to wheat stem rust pathogen races TTKSK (Ug99) and TRTTF in the elite hard red spring wheat line SD4279. *Molecular Breeding, 35*(1): 8: 1-10. https://doi.org/10.1007/s11032-015-0198-4

Guizhi, Z., Yingying, W., Ying, G., Yan, Z., Fanmei, K., & Sishen, L. (2015). Characterization and mapping of QTLs on chromosome 2D for grain size and yield traits using a mutant line induced by EMS in wheat. *Crop Journal, 3*(2): 135-144. https://doi.org/10.1016/j.cj.2014.11.002

Gupta, P.K., Kulwal, P.L. & Jaiswal, V. (2019). Chapter Two - Association mapping in plants in the post-GWAS genomics era. *Advances in Genetics*, *104*: 75-154. doi: 10.1016/bs.adgen.2018.12.001

Hanocq, E., Laperche, A., Jaminon, O., Lainé, A. L., & Le Gouis, J. (2007). Most significant genome regions involved in the control of earliness traits in bread wheat, as revealed by QTL meta-analysis. *Theoretical and Applied Genetics, 114*(3): 569-584. https://doi.org/10.1007/s00122-006-0459-z

Hassan, M.A., Yang, M., Fu, L., Rasheed, A., Zheng, B., Xia, X., Xiao, Y., & He, Z. (2019). Accuracy assessment of plant height using an unmanned aerial vehicle for quantitative genomic analysis in bread wheat. *Plant Methods, 15*(37): 1-8. doi: 10.1186/s13007-019-0419-7

Heffelfinger, C., Fragoso, C. A., & 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

Helguera, M., Khan, I. A., Kolmer, J., Lijavetzky, D., Zhong-qi, L., & Dubcovsky, J. (2003). PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. *Crop Science, 43*(5): 1839-1847. https://doi.org/https://doi.org/10.2135/cropsci2003.1839

Herrera-Foessel, S. A., Lagudah, E. S., Huerta-Espino, J., Hayden, M. J., Bariana, H. S., Singh, D., & Singh, R. P. (2011). New slow-rusting leaf rust and stripe rust resistance genes *Lr67* and *Yr46* in wheat are pleiotropic or closely linked. *Theoretical and Applied Genetics, 122*(1): 239-249. https://doi.org/10.1007/s00122-010-1439-x

Hiebert, C., Thomas, J., & McCallum, B. (2005). Locating the broad-spectrum wheat leaf rust resistance gene Lr52 (LrW) to chromosome 5B by a new cytogenetic method. *Theoretical and Applied Genetics*, *110*(8): 1453-1457. https://doi.org/10.1007/s00122-005-1978-8

Hiebert, C. W., Fetch, T. G., Zegeye, T., Thomas, J. B., Somers, D. J., Humphreys, D. G., McCallum B.D., Cloutier S., Singh D., & Knott, D. R. (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. https://doi.org/10.1007/s00122-010-1430-6

Hiebert, C. W., Kassa, M. T., McCartney, C. A., You, F. M., Rouse, M. N., Fobert, P., & Fetch, T. G. (2016). Genetics and mapping of seedling resistance to Ug99 stem rust in winter wheat cultivar Triumph 64 and differentiation of *SrTmp, SrCad*, and *Sr42*. *Theoretical and Applied Genetics*, *129*(11): 2171-2177. https://doi.org/10.1007/s00122-016-2765-4

Hiebert, C. W., Kolmer, J. A., McCartney, C. A., Briggs, J., Fetch, T., Bariana, H., Choulet F., Rouse M.N., & Spielmeyer, W. (2016) major gene for field stem rust resistance co-locates with resistance gene *Sr12* in 'Thatcher' wheat. *PLoS One*, *11*(6): 1-16. doi: 10.1371/journal.pone.0157029

Hiebert, C. W., Rouse, M. N., Nirmala, J., & Fetch, T. (2017). Genetic Mapping of Stem Rust Resistance to *Puccinia graminis* f. sp. *tritici* Race TRTTF in the Canadian Wheat Cultivar Harvest. *Phytopathology*, *107*(2): 192-197. https://doi.org/10.1094/phyto-05-16-0186-r

Hiebert, C. W., Thomas, J. B., McCallum, B. D., Humphreys, D. G., DePauw, R. M., Hayden, M. J., Mago R., Schnippenkoetter W., & Spielmeyer, W. (2010). An introgression on wheat chromosome 4DL in RL6077 (Thatcher*6/PI 250413) confers adult plant resistance to stripe rust and leaf rust (*Lr67*). *Theoretical and Applied Genetics, 121*(6): 1083-1091. https://doi.org/10.1007/s00122-010-1373-y

Huang, L., Brooks, S. A., Li, W., Fellers, J. P., Trick, H. N., & Gill, B. S. (2003). Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. *Genetics, 164*(2): 655-664. https://doi.org/10.1093/genetics/164.2.655

Huang, X. Q., Cloutier, S., Lycar, L., Radovanovic, N., Humphreys, D. G., Noll, J. S., Somers D.J.,. & Brown, P. D. (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. https://doi.org/10.1007/s00122-006-0346-7

Hucl, P., & Baker, R. J. (1987). A study of ancestral and modern Canadian spring wheats. *Canadian Journal of Plant Science*, *67*(1): 87-97. https://doi.org/10.4141/cjps87-010

Jaganathan, D., Bohra, A., Thudi, M., & Varshney, R. K. (2020). Fine mapping and gene cloning in the post-NGS era: advances and prospects. *Theoretical and Applied Genetics, 133*(5): 1791-1810. https://doi.org/10.1007/s00122-020-03560-w

Jobson, E. M., Johnston, R. E., Oiestad, A. J., Martin, J. M., & Giroux, M. J. (2019). The impact of the wheat *Rht-B1b* semi-dwarfing allele on photosynthesis and seed development under field conditions. *Frontiers in Plant Science, 10*(51): 1-12. https://doi.org/10.3389/fpls.2019.00051

Kamran, A., Iqbal, M., Navabi, A., Randhawa, H., Pozniak, C., & Spaner, D. (2013). 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. https://doi.org/10.1007/s00122-013-2110-0

Kao, C.H., Z.B. Zeng and R.D. Teasdale. 1999. Multiple interval mapping for quantitative trait loci. *Genetics, 152*: 1203-1216.

Kassa, M. T., You, F. M., Hiebert, C. W., Pozniak, C. J., Fobert, P. R., Sharpe, A. G., Menzies, J., Humphreys, D., Harrison, N., Fellers, J., McCallum, B., & McCartney, C. A. (2017). Highly predictive SNP markers for efficient selection of the wheat leaf rust resistance gene *Lr16*. *BMC Plant Biology, 17*(1), 45: 1-9. https://doi.org/10.1186/s12870-017-0993-7

Keller, M., Karutz, C., Schmid, J., Stamp, P., Winzeler, M., Keller, B., & Messmer, M. (1999). Quantitative trait loci for lodging resistance in a segregating wheat spelt population. *Theoretical and Applied Genetics, 98*: 1171-1182. https://doi.org/10.1007/s001220051182

Kertesz, Z., Flintham, J. E., & Gale, M. D. (1991). Effects of *Rht* dwarfing genes on wheat grain yield and its components under eastern European conditions. *Cereal Research Communications, 19*(3): 297-304.

Kertho, A., Mamidi, S., Bonman, J. M., McClean, P. E., & Acevedo, M. (2015). Genome-wide association mapping for resistance to leaf and stripe rust in winter-habit hexaploid wheat landraces. *PLoS One, 10*(6): 1-18. https://doi.org/10.1371/journal.pone.0129580

Khan, R. R., Bariana, H. S., Dholakia, B. B., Naik, S. V., Lagu, M. D., Rathjen, A. J., Bhavani S., & Gupta, V. S. (2005). Molecular mapping of stem and leaf rust resistance in wheat. *Theoretical and Applied Genetics*, *111*(5): 846-850. https://doi.org/10.1007/s00122-005-0005-4

Khobra, R., Sareen, S., Meena, B. K., Kumar, A., Tiwari, V., & Singh, G. P. (2019). Exploring the traits for lodging tolerance in wheat genotypes: a review. *Physiology and Molecular Biology of Plants, 25*(3): 589-600. https://doi.org/10.1007/s12298-018-0629-x

Kilpatrick, R.A. (1975). New wheat cultivars and longevity of rust resistance, 1971-1975. USDA, *Agricultural Research Service* Northeast Reg (Rep.), ARS-NE 64. Pp.20.

Kislev, M. E. (1982). Stem rust of wheat 3300 years old found in Israel. *Science, 216*(4549): 993-994. https://doi.org/10.1126/science.216.4549.993

Knievel, D. C., Abdel-Aal, E. S. M., Rabalski, I., Nakamura, T., & Hucl, P. (2009). Grain color development and the inheritance of high anthocyanin blue aleurone and purple pericarp in spring wheat (*Triticum aestivum* L.). *Journal of Cereal Science, 50*(1): 113-120. https://doi.org/https://doi.org/10.1016/j.jcs.2009.03.007

Kolmer, J. A., Dyck, P. L., & Roelfs, A. P. (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, J. A., Garvin, D. F., & Jin, Y. (2011). Expression of a Thatcher wheat adult plant stem rust resistance QTL on chromosome arm 2BL is enhanced by *Lr34. Crop Science, 51*(2): 526-533. https://doi.org/https://doi.org/10.2135/cropsci2010.06.0381

Kolmer, J. A., & Liu, J. Q. (2002). Inheritance of leaf rust resistance in the wheat cultivars AC Majestic, AC Splendor, and AC Karma. *Canadian Journal of Plant Pathology, 24*: 327 - 331.

Korzun, V. (2002). Use of molecular markers in cereal breeding. *Cellular & Molecular Biology Letters, 7*(2b): 811-820.

Kosambi, D. D. (1943). The estimation of map distances from recombination values. *Annals of Eugenics, 12*(1): 172-175. https://doi.org/https://doi.org/10.1111/j.1469-1809.1943.tb02321.x

Kosgey, Z. C., Edae, E. A., Dill-Macky, R., Jin, Y., Bulbula, W. D., Gemechu, A., Macharia, G., Bhavani, S., Randhawa, M. S., & Rouse, M. N. (2021). Mapping and validation of stem rust resistance loci in spring wheat line CI 14275. *Frontiers in Plant Science, 11*(2147): 1-13. https://doi.org/10.3389/fpls.2020.609659

Krattinger, S. G., Lagudah, E. S., Spielmeyer, W., Singh, R. P., Huerta-Espino, J., McFadden, H., Bossolini E., Selter L.L., & Keller, B. (2009). A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science, 323*(5919): 1360-1363. https://doi.org/10.1126/science.1166453

Krattinger, S. G., Lagudah, E. S., Wicker, T., Risk, J. M., Ashton, A. R., Selter, L. L., Matsumoto T., & Keller, B. (2011). *Lr34* multi-pathogen resistance ABC transporter: molecular analysis of homoeologous and orthologous genes in hexaploid wheat and other grass species. *The Plant Journal, 65*(3): 392-403. https://doi.org/10.1111/j.1365-313X.2010.04430.x

Krattinger, S. G., Sucher, J., Selter, L. L., Chauhan, H., Zhou, B., Tang, M., Upadhyaya, N.M., Mieulet, D., Guiderdoni, E., Weidenbach, D., Schaffrath, U., Lagudah, E.S., & Keller, B. (2016). The wheat durable, multipathogen resistance gene *Lr34* confers partial blast resistance in rice. *Plant Biotechnology Journal, 14*(5): 1261-1268. https://doi.org/https://doi.org/10.1111/pbi.12491

Kumar, D., Kumar, A., Chhokar, V., Gangwar, O. P., Bhardwaj, S. C., Sivasamy, M., Prasad, S., Prakasha, T. L., Khan, H., Singh, R., Sharma, P., Sheoran, S., Iquebal, M. A., Jaiswal, S., Angadi, U. B., Singh, G., Rai, A., Singh, G. P., Kumar, D., & Tiwari, R. (2020). Genome-Wide Association Studies in Diverse Spring Wheat Panel for Stripe, Stem, and Leaf Rust Resistance. *Frontiers in Plant Science*, *11*, 748: 1-28. https://doi.org/10.3389/fpls.2020.00748

Lagudah, E. S. (2011). Molecular genetics of race non-specific rust resistance in wheat. *Euphytica, 179*(1): 81-91. https://doi.org/10.1007/s10681-010-0336-3

Lagudah, E. S., McFadden, H., Singh, R. P., Huerta-Espino, J., Bariana, H. S., & Spielmeyer, W. (2006). Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. *Theoretical and Applied Genetics, 114*(1): 21-30. https://doi.org/10.1007/s00122-006-0406-z

Lan, C., Randhawa, M. S., Huerta-Espino, J., & Singh, R. P. (2017). Genetic analysis of resistance to wheat rusts. *Wheat Rust Diseases*. pp. 137-149. Springer.

Lanning, S. P., Martin, J. M., Stougaard, R., Guillen-Portal, F., Blake, N., Sherman, J., Robbins, A.M., Kephart, K.D., Lamb, P., Carlson, G.R., Pumphrey, M., & Talbert, L. E. (2012). Evaluation of near-isogenic lines for three height-reducing genes in hard red spring wheat. *Crop Science, 52*: 1145-1152. https://doi.org/10.2135/cropsci2011.11.0625

Leonard, K. J., & Szabo, L. (2005). Stem rust of small grains and grasses caused by *Puccinia graminis*. *Molecular Plant Pathology, 6*: 99-111. https://doi.org/10.1111/J.1364.3703.2004.00273.X

Leonova, I.N., Skolotneva, E.S., Orlova, E.A., Orlovskaya, O.A., & Salina, E.A. (2020). Detection of genomic regions associated with resistance to stem rust in russian spring wheat varieties and breeding germplasm. *International Journal of Molecular Sciences, 21*(13),4706: 1-13. doi: 10.3390/ijms21134706

Li, H., Ren, B., Kang, Z., & Huang, L. (2016). Comparison of cell death and accumulation of reactive oxygen species in wheat lines with or without *Yr36* responding to *Puccinia striiformis* f. sp. *tritici* under low and high temperatures at seedling and adult-plant stages. *Protoplasma, 253*(3): 787-802. https://doi.org/10.1007/s00709-015-0833-2

Li, H., Ye, G., & 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., Hearne, S., Bänziger, M., Li, Z., & Wang, J. (2010). Statistical properties of QTL linkage mapping in biparental genetic populations. *Heredity, 105*: 257-267. doi:10.1038/hdy.2010.56.

Liu, J. Q., & Kolmer, J. A. (1997). Inheritance of leaf rust resistance in wheat cultivars Grandin and CDC Teal. *Plant Disease, 81*(5): 505-508. https://doi.org/10.1094/PDIS.1997.81.5.505

Liu, M., Rodrigue, N., & Kolmer, J. (2014). Population divergence in the wheat leaf rust fungus Puccinia triticina is correlated with wheat evolution. *Heredity, 112*(4): 443-453. https://doi.org/10.1038/hdy.2013.123

Liu, S., Cai, S., Graybosch, R., Chen, C., & Bai, G. (2008). Quantitative trait loci for resistance to pre-harvest sprouting in US hard white winter wheat Rio Blanco. *Theoretical and Applied Genetics*, *117*(5): 691-699. https://doi.org/10.1007/s00122-008-0810-7

Ma, J., Tu, Y., Zhu, J., Luo, W., Liu, H., Li, C., Li S., Liu J., Ding P., Habib A., Mu Y., Tang H., Liu Y., Jiang Q., Chen G., Wang J., Li W., Pu Z., Zheng Y., Wei Y., Kang H., Chen G., & Lan, X. (2020). Flag leaf size and posture of bread wheat: genetic dissection, QTL validation and their relationships with yield-related traits. *Theoretical and Applied Genetics, 133*(1): 297-315. https://doi.org/10.1007/s00122-019-03458-2

Manocha, M.S. (1975) Autoradiography and Fine Structure of Host-Parasite Interface in Temperature-Sensitive Combinations of Wheat Stem Rust. *Journal of Phytopathology*, *82*: 207-215. https://doi.org/10.1111/j.1439-0434.1975.tb03485.x

Martens, J. W., & Dyck, P. L. (1989). Genetics of Resistance to Rust in Cereals from a Canadian Perspective. *Canadian Journal of Plant Pathology, 11*(1): 78-85. https://doi.org/10.1080/07060668909501152

Marza, F., Bai, G. H., Carver, B. F., & Zhou, W. C. (2006). Quantitative trait loci for yield and related traits in the wheat population Ning7840 × Clark. *Theoretical and Applied Genetics, 112*(4): 688-698. https://doi.org/10.1007/s00122-005-0172-3

McCaig, T. N., DePauw, R. M., Clarke, J. M., McLeod, J. G., Fernandez, M. R., & Knox, R. E. (1996). AC Barrie hard red spring wheat. *Canadian Journal of Plant Science, 76*(2): 337-339.

McCallum, B., & DePauw, R. (2008). A review of wheat cultivars grown in the Canadian prairies. *Canadian Journal of Plant Science, 88*: 649-677. https://doi.org/10.4141/CJPS07159

McCallum, B., Reimer, E., McNabb, W., Foster, A., Barcellos Rosa, S., & Xue, A. (2021). Physiologic specialization of *Puccinia triticina*, the causal agent of wheat leaf rust, in Canada in 2015-2019. *Canadian Journal of Plant Pathology*: 1-14. https://doi.org/10.1080/07060661.2021.1888156

McCallum, B. D., Chen, X., Shorter, S., Sadasivaiah, R. S., & Tewari, J. P. (2007). Stripe rust reaction of 28 Canadian wheat cultivars. *Canadian Journal of Plant Pathology, 29*(4): 401-407. https://doi.org/10.1080/07060660709507486

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

McCallum, B. D., Hiebert, C. W., Cloutier, S., Bakkeren, G., Rosa, S. B., Humphreys, D. G., Marais, F., McCartney, C. A., Panwar, V., Rampitsch, C., Saville . B. J., & 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. https://doi.org/10.1080/07060661.2016.1145598

Mccallum, B. D., & Seto-Goh, P. (2006). Physiologic specialization of *Puccinia triticina*, the causal agent of wheat leaf rust, in Canada in 2003. *Canadian Journal of Plant Pathology, 28*: 208 - 213.

McCallum, B. D., Seto-Goh, P., & Xue, A. (2011). Physiologic specialization of *Puccinia triticina*, the causal agent of wheat leaf rust, in Canada in 2008. *Canadian Journal of Plant Pathology, 33*(4): 541-549. https://doi.org/10.1080/07060661.2011.627950

McCartney, C. A., Somers, D. J., Humphreys, D. G., Lukow, O., Ames, N., Noll, J., Cloutier S., & McCallum, B. D. (2005a). Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross RL4452 × 'AC Domain'. *Genome, 48*(5): 870-883. https://doi.org/10.1139/G05-055

McCartney, C. A., Somers, D. J., McCallum, B. D., Thomas, J., Humphreys, D. G., Menzies, J. G., & Brown, P. D. (2005b). Microsatellite tagging of the leaf rust resistance gene Lr16 on wheat chromosome 2BSc. *Molecular Breeding, 15*(4): 329-337. https://doi.org/10.1007/s11032-004-5948-7

Mcintosh, R., & Dyck, P. L. (1975). Cytogenetical Studies in Wheat. VII Gene *Lr23* for Reaction to *Puccinia recondita* in Gabo and Related Cultivars. *Australian Journal of Biological Sciences, 28*: 201-212.

Megerssa, S. H., Ammar, K., Acevedo, M., Brown-Guedira, G., Ward, B., Degete, A. G., Randhawa M.S., & Sorrells, M. E. (2020). Multiple-Race Stem Rust Resistance Loci Identified in Durum Wheat Using Genome-Wide Association Mapping. *Frontiers in Plant Science, 11*(1934): 1-18.. https://doi.org/10.3389/fpls.2020.598509

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

Merriam-Webster. (2021). Robigalia. In *Merriam-Webster.com dictionary.* Retrieved 2021-09-2021 from https://www.merriam-webster.com/dictionary/Robigalia

Miles, C., & Wayne, M. (2008). Quantitative trait locus (QTL) analysis. *Nature Education, 1*(1), 208.

Milus, E. A., Lee, K. D., & Brown-Guedira, G. (2015). Characterization of stripe rust resistance in wheat lines with resistance gene *Yr17* and implications for evaluating resistance and virulence. *Phytopathology, 105*(8): 1123-1130. https://doi.org/10.1094/PHYTO-11-14-0304-R

Moore, J., Herrera-Foessel, S.A., Lan, C., Schnippenkoetter, W., Ayliffe, M., Huerta-Espino, J., Lillemo, M., Viccars, L.A., Milne, R.J., Periyannan, S.K., Kong, X., Spielmeyer, W., Talbot, M.J., Bariana, H., Patrick, J.W., Dodds, P.N., Singh, R., & Lagudah, E.S. (2015). A recently evolved hexose transporter variant confers resistance to multiple pathogens in wheat. *Nature Genetics, 47*: 1494-1498. https://doi.org/10.1038/ng.3439

Olivera, P. D., Jin, Y., Rouse, M., Badebo, A., Fetch, T., Jr., Singh, R. P., & Yahyaoui, A. (2012). Races of *Puccinia graminis* f. sp. *tritici* with combined virulence to *Sr13* and *Sr9e* in a field stem rust screening nursery in Ethiopia. *Plant Disease, 96*(5): 623-628. https://doi.org/10.1094/pdis-09-11-0793

Olmstead, A. L., & Rhode, P. W. (2002). The red queen and the hard reds: Productivity growth in American wheat, 1800–1940. *The Journal of Economic History, 62*(4): 929-966. https://doi.org/10.1017/S0022050702001602

Ooijen, J.W., Ooijen, J.W., Verlaat, J.V., Ooijen, J.W., Tol, J., Dalén, J., Buren, J.B., Meer, J.V., Krieken, J.H., Ooijen, J.W., Kessel, J.S., Van, O., Voorrips, R.E., & Heuvel, L.P. (2006). JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations.

Peng, F. Y., & Yang, R.-C. (2017). Prediction and analysis of three gene families related to leaf rust (*Puccinia triticina*) resistance in wheat (*Triticum aestivum* L.). *BMC Plant Biology, 17*(1), 108: 1-17. https://doi.org/10.1186/s12870-017-1056-9

Peng, J., Richards, D. E., Hartley, N. M., Murphy, G. P., Devos, K. M., Flintham, J. E., Beales J., Fish L.J., Worland A.J., Pelica F., Sudhakar D., Christou P., Snape J.W., Gale M.D., & Harberd, N. P. (1999). 'Green revolution' genes encode mutant gibberellin response modulators. *Nature, 400*(6741): 256-261. https://doi.org/10.1038/22307

Perez-Lara, E., Fentaye, K., Tran, V., Ciechanowska, I., Chen, H., Iqbal, M., N'Diaye, A., Pozniak, C., Strelkov, S.E., Hucl, P.J., Graf, R.J., Randhawa, H., & Spaner, D. (2017). Population structure and genome-wide association analysis of resistance to wheat diseases and insensitivity to *Ptr* toxins in Canadian spring wheat using 90K SNP array. *Crop Science, 57*(3): 1522-1539. https://doi.org/10.2135/cropsci2016.10.0859

Perez-Lara, E., Semagn, K., Chen, H., Iqbal, M., N'Diaye, A., Kamran, A., Navabi, A., Pozniak, C., & 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),e0160623: 1-20. doi: 10.1371/journal.pone.0160623.

Peterson, R. F., Campbell, A. B., & Hannah, A. E. (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

Peturson, B. (1958). Wheat rust epidemics in western Canada in 1953, 1954 and 1955. *Canadian Journal of Plant Science, 38*(1):16-28. https://doi.org/10.4141/cjps58-004

PGDC. (2018). Prairie Recommending Committee for Wheat, Rye and Triticale. *Operating Procedures*. http://pgdc.ca/pdfs/wrt/CFIA_ACIA%20-%20_7730947%20-%20v2F%20-%20VRO-2018-19-RC-OP-PRCWRT_Prairie%20wheat%20committees%20operating%20procedures_March%2022%202018.pdf

Pilet-Nayel, M. L., Moury, B., Caffier, V., Montarry, J., Kerlan, M. C., Fournet, S., Durel C.E., & Delourme, R. (2017). Quantitative resistance to plant pathogens in pyramiding strategies for durable crop protection. *Frontiers in Plant Science, 8*, 1838: 1-9. https://doi.org/10.3389/fpls.2017.01838

Pinto da Silva, G. B., Zanella, C. M., Martinelli, J. A., Chaves, M. S., Hiebert, C. W., McCallum, B. D., & Boyd, L. A. (2018). Quantitative trait loci conferring leaf rust resistance in hexaploid wheat. *Phytopathology, 108*(12), 1344-1354. https://doi.org/10.1094/phyto-06-18-0208-rvw

Piñera-Chavez, F., Berry, P., Foulkes, M., Sukumaran, S., & Reynolds, M. (2021). Identifying QTLS for lodging-associated traits in the wheat doubled-haploid population Avalon × Cadenza. *Crop Science, 61*: 2371-2386. https://doi.org/10.1002/csc2.20485

Platten, J. D., Cobb, J. N., & Zantua, R. E. (2019). Criteria for evaluating molecular markers: Comprehensive quality metrics to improve marker-assisted selection. *PLoS One, 14*(1), e0210529: 1-20. https://doi.org/10.1371/journal.pone.0210529

Plotnikova, L. Y., & Stubei, T. Y. (2013). Effectiveness of the wheat *Lr22b, Lr34*, and *Lr37* genes for adult plant resistance to leaf rust in West Siberia and the cytophysiological basis of their action. *Russian Journal of Genetics: Applied Research, 3*(1): 47-53. https://doi.org/10.1134/S2079059713010115

Prasad, P., Savadi, S., Bhardwaj, S. C., & Gupta, P. K. (2020). The progress of leaf rust research in wheat. *Fungal Biology, 124*(6): 537-550. https://doi.org/10.1016/j.funbio.2020.02.013

Pretorius, Z. A., Singh, R. P., Wagoire, W. W., & Payne, T. S. (2000). Detection of Virulence to Wheat Stem Rust Resistance Gene *Sr31* in *Puccinia graminis.* f. sp. *tritici* in Uganda. *Plant Disease*, 84(2), 203. https://doi.org/10.1094/pdis.2000.84.2.203b

Prins, R., Dreisigacker, S., Pretorius, Z., van Schalkwyk, H., Wessels, E., Smit, C., Bender, C., Singh, D., & Boyd, L. A. (2016). Stem rust resistance in a geographically diverse collection of spring wheat lines collected from across Africa. *Frontiers in Plant Science*, *7*, 973: 1-15. https://doi.org/10.3389/fpls.2016.00973

Prins, R., Pretorius, Z. A., Bender, C. M., & Lehmensiek, A. (2011). QTL mapping of stripe, leaf and stem rust resistance genes in a Kariega × Avocet S doubled haploid wheat population. *Molecular Breeding, 27*(2): 259-270. https://doi.org/10.1007/s11032-010-9428-y

Puchalski, B., & Gaudet, D. (2010). Southern Alberta stripe rust survey. *Canadian Plant Disease Survey*, *91*: 69-70.

Qian, B., Gameda, S., Zhang, X., & Jong, R. (2012). Changing growing season observed in Canada. *Climatic Change, 112*: 339-353. https://doi.org/10.1007/s10584-011-0220-8

Rabbi, S., Kumar, A., Mohajeri Naraghi, S., Sapkota, S., Alamri, M. S., Elias, E. M., Kianian, S., Seetan, R., Missaoui, A., Solanki, S., & Mergoum, M. (2021). Identification of main-effect and environmental interaction QTL and their candidate genes for drought tolerance in a wheat RIL population between two elite spring cultivars. *Frontiers in Genetics*, *12*, 656037: 1-16. https://doi.org/10.3389/fgene.2021.656037

Randhawa, H., Puchalski, B. J., Frick, M., Goyal, A., Despins, T., Graf, R. J., Laroche, A., & Gaudet, D. A. (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, H. S., Asif, M., Pozniak, C., Clarke, J. M., Graf, R. J., Fox, S. L., Humphreys, D.G., Knox, R.E., DePauw, R.M., Singh, A.K., Cuthbert, R.D., Hucl, P., & Spaner, D. (2013). Application of molecular markers to wheat breeding in Canada. *Plant Breeding, 132*(5): 458-471. https://doi.org/https://doi.org/10.1111/pbr.12057

Randhawa, H.S., Sadasivaiah, R.S., Graf, R.J., & B.L. Beres. (2011). Bhishaj soft white spring wheat. *Canadian Journal of Plant Science, 91*(4): 805-810. https://doi.org/10.4141/cjps10144

Randhawa, M. S., Singh, R. P., Dreisigacker, S., Bhavani, S., Huerta-Espino, J., Rouse, M. N., Nirmala, J., & Sandoval-Sanchez, M. (2018). Identification and validation of a common stem rust resistance locus in two bi-parental populations. *Frontiers in Plant Science, 9*(1788): 1-11. https://doi.org/10.3389/fpls.2018.01788

Rawson, H. M., Zajac, M., & Penrose, L. D. J. (1998). Effect of seedling temperature and its duration on development of wheat cultivars differing in vernalization response. *Field Crops Research, 57*(3): 289-300. https://doi.org/https://doi.org/10.1016/S0378-4290(98)00073-2

Ridha, M. A., & Fenni, M. (2021). Effect of supplemental irrigation on bread wheat genotypes yield under Mediterranean semi-arid conditions of north-eastern Algeria. *Revista Facultad Nacional de Agronomía Medellín, 74*: 9431-9440. https://doi.org/10.15446/rfnam.v74n1.89798

Roelfs, A. P. (1982). Effects of Barberry Eradication on Stem Rust in the United States. *Plant Disease, 66*: 177-181.

Rollar, S., Serfling, A., Geyer, M., Hartl, L., Mohler, V., & Ordon, F. (2021). QTL mapping of adult plant and seedling resistance to leaf rust (*Puccinia triticina* Eriks.) in a multiparent advanced generation intercross (MAGIC) wheat population. *Theoretical and Applied Genetics, 134*(1): 37-51. https://doi.org/10.1007/s00122-020-03657-2

Sadasivaiah, R. S., Orshinsky, B. R., & Kozub, G. C. (1999). Production of wheat haploids using anther culture and wheat x maize hybridization techniques. *Cereal Research Communications, 27*(1): 33-40. https://doi.org/10.1007/BF03543916

Sadasivaiah, R. S., Perkovic, S. M., Pearson, D. C., Postman, B., & Beres, B. (2004). Registration of 'AC Andrew' Wheat. *Crop Science, 44*: 696-697. https://doi.org/10.2135/cropsci2004.0696

Samborski, D. J., & Dyck, P. L. (1982). Enhancement of resistance to *Puccinia recondita* by interactions of resistance genes in wheat. *Canadian Journal of Plant Pathology, 4*(2): 152-156. https://doi.org/10.1080/07060668209501317

Santra, D., Santra, M., Allan, R., Garland-Campbell, K., & Kidwell, K. (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*: 576-584. https://doi.org/10.1111/j.1439-0523.2009.01681.x

Schaid, D. J., Chen, W., & Larson, N. B. (2018). From genome-wide associations to candidate causal variants by statistical fine-mapping. *Nature Reviews Genetics, 19*(8): 491-504. https://doi.org/10.1038/s41576-018-0016-z

Segovia, V., Hubbard, A., Craze, M., Bowden, S., Wallington, E., Bryant, R., Greenland, A., Bayles, R., & Uauy, C. (2014). *Yr36* confers partial resistance at temperatures below 18°C to U.K. isolates of *Puccinia striiformis*. *Phytopathology, 104*(8): 871-878. https://doi.org/10.1094/phyto-10-13-0295-r

Semagn, K., Babu, R., Hearne, S., & Olsen, M. (2013). Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): Overview of the technology and its application in crop improvement. *Molecular Breeding, 33*: 1-14. https://doi.org/10.1007/s11032-013-9917-x

Semagn, K., Bjørnstad, Å., & Xu, Y. (2010). The genetic dissection of quantitative traits in crops. *Electronic Journal of Biotechnology, 13*: 16-17. https://doi.org/10.2225/vol13-issue5-fulltext-14

Semagn, K., Iqbal, M., Chen, H., Perez-Lara, E., Bemister, D. H., Xiang, R., Zou, J., Asif, M., Kamran, A., N'Diaye, A., Randhawa, H., Pozniak, C., & Spaner, D. (2021a). Physical mapping of QTL associated with agronomic and end-use quality traits in spring wheat under conventional and organic management systems. *Theoretical and Applied Genetics, 134*(11): 3699-3719. https://doi.org/10.1007/s00122-021-03923-x

Semagn, K., Iqbal, M., Chen, H., Perez-Lara, E., Bemister, D. H., Xiang, R., Zou, J., Asif, M., Kamran, A., N'Diaye, A., Randhawa, H., Pozniak, C., & Spaner, D. (2021b). Physical mapping of QTL in four spring wheat populations under conventional and organic management systems. I. Earliness. *Plants, 10*(5), 853: 1-23. https://doi.org/10.3390/plants10050853

Shamrai, S. N. (2014). Plant immune system: Basal immunity. *Cytology and Genetics, 48*(4): 258-271. https://doi.org/10.3103/S0095452714040057

Sharma, J. S., Overlander, M., Faris, J. D., Klindworth, D. L., Rouse, M. N., Kang, H., Long, Y., Jin, Y., Lagudah, E., & Xu, S. S. (2021). Characterization of synthetic wheat line Largo for resistance to stem rust. *G3 Genes Genomes Genetics, 11*(8): 1-9. https://doi.org/10.1093/g3journal/jkab193

Sharp, P. J., Johnston, S., Brown, G., McIntosh, R. A., Pallotta, M., Carter, M., Bariana, H. S. , Khatkar, S., Lagudah, E. S., Singh, R. P. , Khairallah, M. , Potter, R., & Jones, M. G. K. (2001). Validation of molecular markers for wheat breeding. *Australian Journal of Agricultural Research, 52*(12): 1357-1366. https://doi.org/https://doi.org/10.1071/AR01052

Sherman, J., Nash, D., Lanning, S., Martin, J., Blake, N., Morris, C. f., & Talbert, L. (2014). Genetics of end-use quality differences between a modern and historical spring wheat. *Crop Science, 54*: 1972-1980. https://doi.org/10.2135/cropsci2013.11.0749

Singh, A., Knox, R. E., DePauw, R. M., Singh, A. K., Cuthbert, R. D., Campbell, H. L., Shorter, S., & 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, R. N. P. (1993). Genetic association of gene *Bdv1* for tolerance to barley yellow dwarf virus with genes *Lr 34* and *Yr18* for adult plant resistance to rusts in bread wheat. *Plant Disease, 77*: 1103-1106.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Njau, P., Wanyera, R., Herrera-Foessel, S.A., & Ward, R. W. (2008). Will Stem Rust Destroy the World's Wheat Crop? *Advances in Agronomy*, *98*: 271-309. Academic Press. https://doi.org/https://doi.org/10.1016/S0065-2113(08)00205-8

Singh, R. P., Huerta-Espino, J., Bhavani, S., Herrera-Foessel, S. A., Singh, D., Singh, P. K., Velu, G., Mason, R.E., Jin, Y., Njau, P., & Crossa, J. (2011). Race non-specific resistance to rust diseases in CIMMYT spring wheats. *Euphytica, 179*(1): 175-186. https://doi.org/10.1007/s10681-010-0322-9

Singh, R. P., & McIntosh, R. A. (1986). Cytogenetical studies in wheat. XIV. *Sr8b* for resistance to *Puccinia graminis tritici*. *Canadian Journal of Genetics and Cytology, 28*(2): 189-197. https://doi.org/10.1139/g86-026

Song, P., Wang, X., Wang, X., Zhou, F., Xu, X., Wu, B., Yao , J., Lv, D., Yang, M., Song, X., & Sun, D. (2021). Application of 50K chip-based genetic map to QTL mapping of stem-related traits in wheat. *Crop and Pasture Science, 2*: 105-112. https://doi.org/10.1071/CP20326

Statistics Canada. (2020a). *Canadian Spring Wheat Varieties: Wheat, Canada Western Red Spring*. Retrieved 2020-10-09 from https://www.statcan.gc.ca/eng/statistical-programs/document/3401_D3_V1

Statistics Canada.(2020b). *Production of Principal Field Crops*, November 2020. Retrieved 2020-11-30 from https://www150.statcan.gc.ca/n1/daily-quotidien/201203/dq201203b-eng.htm <p class="MsoNormal" style="margin-bottom:0cm

Su, H., Conner, R. L., Graf, R. J., & Kuzyk, A. D. (2003). Virulence of *Puccinia striiformis* f. sp. *tritici*, cause of stripe rust on wheat in western Canada from 1984 to 2002. *Canadian Journal of Plant Pathology, 25*(3): 312-319. https://doi.org/10.1080/07060660309507084

Sukhikh, I. S., Vavilova, V. J., Blinov, A. G., & Goncharov, N. P. (2021). Diversity and phenotypical effect of allelic variants of *Rht* dwarfing genes in wheat. *Russian Journal of Genetics, 57*(2): 127-138. https://doi.org/10.1134/S1022795421020101

Thermo Scientific. (2010). *Full-Spectrum Spectrophotometer* [NanoDrop ND-1000 v3.8]. https://tools.thermofisher.com/content/sfs/manuals/nd-1000-v3.8-users-manual-8%205x11.pdf

Toth, J., Pandurangan, S., Burt, A., Mitchell Fetch, J., & Kumar, S. (2018). Marker-assisted breeding of hexaploid spring wheat in the Canadian prairies. *Canadian Journal of Plant Science, 99*(2): 111-127. https://doi.org/10.1139/cjps-2018-0183

Uauy, C., Brevis, J. C., Chen, X., Khan, I., Jackson, L., Chicaiza, O., Distelfeld, A., Fahima, T., & Dubcovsky, J. (2005). High-temperature adult-plant (HTAP) stripe rust resistance gene *Yr36* from *Triticum turgidum* ssp. *dicoccoides* is closely linked to the grain protein content locus *Gpc-B1*. *Theoretical and Applied Genetics, 112*(1): 97-105. https://doi.org/10.1007/s00122-005-0109-x

Vanegas, C. D. G. (2008). Genetics of stem rust resistance in the spring wheat cultivar Thatcher and the enhancement of stem rust resistance by *Lr34. Euphytica, 159*(3): 391-401-2008 v.2159 no.2003. https://doi.org/10.1007/s10681-007-9541-0

Verma, V., Worland, A. J., Sayers, E. J., Fish, L., Caligari, P. D. S., & Snape, J. W. (2005). Identification and characterization of quantitative trait loci related to lodging resistance and associated traits in bread wheat. *Plant Breeding, 124*(3): 234-241.

Visser, B., Herselman, L., Park, R. F., Karaoglu, H., Bender, C. M., & Pretorius, Z. A. (2011). Characterization of two new *Puccinia graminis* f. sp. *tritici* races within the Ug99 lineage in South Africa. *Euphytica, 179*(1): 119-127. https://doi.org/10.1007/s10681-010-0269-x

Voorrips, R. E. (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

Walker, J. M., & Rapley, R. (2008). *Molecular Biomethods Handbook* (2nd Edition ed.). Humana Press, Springer Science+Business Media LLC. https://doi.org/10.1007/978-1-60327-375-6.

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S., Milner, S.G., Cattivelli, L., Mastrangelo, A.M., Whan, A., Stephen, S., Barker, G., Wieseke, R., Plieske, J., International Wheat Genome Sequencing Consortium, Lillemo, M., Mather, D., Appels, R., Dolferus, R., Brown-Guedira, G., Korol, A., Akhunova, A.R., Feuillet, C., Salse, J., Morgante, M., Pozniak, C., Luo, M.C., Dvorak, J., Morell, M., Dubcovsky, J., Ganal, M., Tuberosa, R., Lawley, C., Mikoulitch, I., Cavanagh, C., Edwards, K.J., Hayden, M., & 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. https://doi.org/10.1111/pbi.12183

Wang, X., McCallum, B. D., Fetch, T., Bakkeren, G., & Saville, B. J. (2015). *Sr36*- and *Sr5*-Mediated resistance response to *Puccinia graminis* f. sp. *tritici* is associated with callose deposition in wheat guard cells. *Phytopathology, 105*(6): 728-737. https://doi.org/10.1094/phyto-08-14-0213-r

Wegulo, S. N., & Byamukama, E. (2012). *Rust Diseases of Wheat* (G2180). University of Nebraska. https://extensionpubs.unl.edu/publication/9000016368724/rust-diseases-of-wheat/

Wu, X., Chang, X., & Jing, R. (2012). Genetic insight into yield-associated traits of wheat grown in multiple rain-fed environments. *PLoS One, 7*: e31249: 1-14. doi:10.1371/journal.pone.0031249.

Würschum, T., Langer, S. M., & Longin, C. F. (2015). Genetic control of plant height in European winter wheat cultivars. *Theoretical and Applied Genetics, 128*(5): 865-874. https://doi.org/10.1007/s00122-015-2476-2

Xi, K., Kumar, K., Holtz, M. D., Turkington, T. K., & 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

Xiang, R., Semagn, K., Iqbal, M., Chen, H., Yang, R.-C., & Spaner, D. (2021). Phenotypic performance and associated QTL of 'Peace' × 'CDC Stanley' mapping population under conventional and organic management systems. *Crop Science, 61*: 3469-3483. https://doi.org/10.1002/csc2.20570

Xing, W., Zhao, H., & Zou, D. (2013). Detection of main-effect and epistatic QTL for yield-related traits in rice under drought stress and normal conditions. *Canadian Journal of Plant Science, 94*: 633-641. doi:10.4141/cjps2013-297.

Xu, Y., Li, P., Yang, Z., & Xu, C. (2017). Genetic mapping of quantitative trait loci in crops. *The Crop Journal, 5*(2): 175-184. https://doi.org/https://doi.org/10.1016/j.cj.2016.06.003

Yan, G., Liu, H., Wang, H., Lu, Z., Wang, Y., Mullan, D., Hamblin, J., & Liu, C. (2017). Accelerated generation of selfed pure line plants for gene identification and crop breeding. *Frontiers in Plant Science, 8,* 1786: 1-14. https://doi.org/10.3389/fpls.2017.01786

Yang, D., Liu, Y., Cheng, H., Chen, J., Li, M., Chang, L., & Chai, S. (2016). Genetic dissection of flag leaf morphology in wheat (*Triticum aestivum* L.) under diverse water regimes. *BMC Genetics, 17*(1): 1-15. https://doi.org/10.1186/s12863-016-0399-9

Yu, L.-X., Barbier, H., Rouse, M. N., Singh, S., Singh, R. P., Bhavani, S., Huerta-Espino, J., & Sorrells, M. E. (2014). A consensus map for Ug99 stem rust resistance loci in wheat. *Theoretical and Applied Genetics, 127*(7): 1561-1581. https://doi.org/10.1007/s00122-014-2326-7

Zhang, G., Wang, Y., Guo, Y., Zhao, Y., Kong, F., & Li, S. (2015). Characterization and mapping of QTLs on chromosome 2D for grain size and yield traits using a mutant line induced by EMS in wheat. *The Crop Journal, 3*(2): 135-144. https://doi.org/https://doi.org/10.1016/j.cj.2014.11.002

Zhang, P., Gebrewahid, T. W., Zhou, Y., Li, Q., Li, Z., & Liu, D. (2019). Seedling and adult plant resistance to leaf rust in 46 Chinese bread wheat landraces and 39 wheat lines with known Lr genes. *Journal of Integrative Agriculture, 18*(5): 1014-1023. https://doi.org/https://doi.org/10.1016/S2095-3119(19)62575-X

Zhang, Y. Wen, Y., Dunwell, J. M., & Zhang, Y. (2020). QTL.gCIMapping.GUI v2.0: An R software for detecting small-effect and linked QTLs for quantitative traits in bi-parental segregation populations. *Computational and Structural Biotechnology Journal, 18*: 59-65. https://doi.org/https://doi.org/10.1016/j.csbj.2019.11.005

Zou, J., Semagn, K., Chen, H., Iqbal, M., Asif, M., N'Diaye, A., Navabi, A., Perez-Lara, E., Pozniak, C., Yang, R., Graf, R., Randhawa, H., & Spaner, D. (2017). 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), 144: 1-14. https://doi.org/10.1007/s11032-017-0746-1

Zurn, J. D., Rouse, M. N., Chao, S., Aoun, M., Macharia, G., Hiebert, C. W., Pretorius, Z.A., Bonman, J.M., & Acevedo, M. (2018). Dissection of the multigenic wheat stem rust resistance present in the Montenegrin spring wheat accession PI 362698. *BMC Genomics, 19*(1), 67: 1-11. https://doi.org/10.1186/s12864-018-4438-y