Mapping of genomic regions associated with agronomic traits and resistance to diseases in Canadian spring wheat

by

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Abstract

Wheat breeders, in addition to phenotypic selection, employ molecular markers in their programs for different purposes, including parental selection, quality control, analysis of advanced lines (cultivars), on genetic purity and identity, and for markers assisted selection. In the first study of this thesis we evaluated 158 recombinant inbred lines (RILs) population for flowering, maturity, plant height and grain yield under field conditions. With a subset of 1809 single nucleotide polymorphisms (SNPs) and 2 functional markers (*Ppd-D1* and *Rht-D1*) we identified a total of 19 quantitative trait loci (QTLs) associated with flowering time under greenhouse (5) and field (6) conditions, maturity (5), grain yield (2) and plant height (1). These QTLs explained between 6.3 and 37.8% of the phenotypic variation. Only the QTLs on both 2D chromosome (adjacent to *Ppd-D1*) and 4D chromosome (adjacent *Rht-D1*) had major effects and, respectively reduced flowering and maturity time up to 5 days with a yield penalty of 436 kg ha^{-1} and reduced plant height by 13 cm, but increased maturity by 33 degree days. In the second study, we used genome-wide association analysis (GWAS) to identify markers associated with the wheat diseases leaf rust, stripe rust, tan spot, common bunt and three host selective toxins (HST) from *Pyrenophora tritici-repentis* (Ptr ToxA, B and C). We were able to identify 94 markers associated with all traits except Ptr ToxC sensitivity. Two major effect genomic regions on 5B and 1A were associated with Ptr ToxA sensitivity, of which the former coincided with the *Tsn1* gene. For Ptr ToxB, two other major effect regions on chromosomes 2B and 5B. The genomic regions associated with common bunt mapped on chromosomes 2B, 4B and 7A, while those associated with leaf rust mapped at two positions on 2B. A single marker-trait was associated each to tan spot on 7B and for yellow rust on 2A. Finally, we investigated the phenotypic effect of 50 markers associated with 16 genes for resistance to rust and tan spot, and Ptr toxin reaction

in a subset of 70 cultivars. We first report the marker makeup of the 70 cultivars to aid spring wheat breeders in parental choice for future crossing programs. We also identified 6-8 markers for yellow rust, 4-6 markers for leaf rust, 5-9 markers for tan spot resistance and 6-11 markers for Ptr ToxA insensitivity as the best predictors of the phenotypic variation observed across the 70 cultivars.

Preface

A version of Chapter 2 of this dissertation has been published as:

Perez-Lara E, Semagn K, Chen H, Iqbal M, N'Diaye A, Atif Kamran, Alireza Navabi, Curtis Pozniak, Dean Spaner (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. doi: 10.1371/journal.pone.0160623.

The population 'Cutler' x 'AC Barrie' presented in Chapter 2 was developed by Dr. Muhammad Iqbal and was phenotyped by Dr. Muhammad Iqbal, Dr. Atif Kamran, Dr. Alireza Navabi and Dean Spaner under field and greenhouse conditions from 2008 to 2011. I joined the project in 2012. I was partially responsible for collecting data in the field during seasons 2012 – 2013. I also participated in the extraction and shipping of the DNA samples to University of Saskatchewan, where they were genotyped using iSelect Illumina SNP Array. I carried out the genotyping of vernalization (*Vrn-A1, Vrn-B1 and Vrn-D1*), photoperiod (*Ppd-A1, Ppd-B1 and Ppd-D1*) and semi-dwarf genes (*Rht-B1* and *Rht-D1*) genes in the population and carried out the statistical analysis and the QTL mapping in conjunction with Dr. Kassa Semang and Dr. Hua Chen. I wrote a manuscript that was edited significantly by Dr. Kassa Semang as well as by the co-authors and editors.

A version of Chapter 3 has been submitted as a manuscript for publication to Crop Sciences as Enid Perez-Lara, Kassa Semagn, Van Anh Tran, Izabela Ciechanowska, Hua Chen, Muhammed Iqbal, Amidou N'Diaye, Curtis Pozniak, Stephen E. Strelkov, Pierre J. Hucl, Robert J. Graf, Harpinder Randhawa, D. Spaner. 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.

The wheat collection called Variety Composite used in Chapter 3 and 4 was originally put together by Dr. Curtis Pozniak and Pierre Hucl at the University of Saskatchewan. For chapter 3, I extracted the DNA samples and shipped them to the University of Saskatchewan where they were genotyped using an eSelect Illumina SNP Array. The disease scores for leaf rust, tan spot and common bunt in the diseases nursery in 2011 to 2015 were carried out by the wheat breeding group of the University of Alberta at the Edmonton Research Center, University of Alberta. Stripe rust scoring was done in Lethbridge and Creston and recorded by Dr. Harpinder Singh Randhawa and Dr. Dean Spaner. The infiltration and scoring of the Ptr ToxA, B and C was carried out by MSc. student Anh Van Tran in Dr. Stephen Strelkov's Lab. I was responsible for genotyping the genetic polymorphism for the rust markers (*Lr21, 34, 37, 46, 67* and *68*; *Yr10* and *Sr2*) as well as the tan spot markers *tsn1* and *tsc2*. In collaboration with Dr. Kassa Semang and Dr. Hua Chen we carried out the data analysis and the GWAS analysis. I wrote the manuscript that was edited by Dr. Kassa Semang according to feedback from the co-authors and editors.

For the experiment presented in Chapter 4, I carried out the genotyping of the population using 16 PCR-based markers. I did the DNA extraction, genotyping of the population, scoring of the results and wrote the manuscript. The data were analyzed in collaboration with Dr. Hua Chen and Dr. Kassa Semang who also participated in the manuscript edition for publication.

I was responsible for data analysis, interpretation and presentation of the results in the manuscripts of all the studies of presented in this thesis. Dr. Dean Spaner, Dr. Kassa Semang，Dr. Hua Chen, and Dr. Muhammad Iqbal provided considerable editorial and assistance over the duration of writing the thesis. All chapters and papers submitted and all other parts of the thesis have been edited extensively by Dr. Kassa Semang and Dr. Dean Spaner.

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Chapter 1 Introduction and literature review

1.1 Introduction

Cereals represent more than 50% of the world crop production with maize, rice and wheat in that order are the most produced. Wheat feeds almost a third of the world population (~30%) (Feuillet, 2016) and is the only source of macronutrients for almost 30% of the world's population (wheatgenome.org) and supplies 20% of the world total calories as estimated by the FAO (http://faostat.fao.org/). Canada is the sixth greatest producer of wheat in the world after China, India, USA, Russia and France (http://faostat.fao.org/) and more than 90% of the Canadian wheat crops are grown in the Prairie Provinces (Alberta, Saskatchewan and Manitoba) (Aboukhaddour et al., 2013). Wheat production in western Canada exceeded 28 million tons by 2016 (Statistics Canada, 2016).

It has been predicted that in the next half of a century there will be an increase of the world population from the current ~7 billion to ~10 billion people by 2050 (http://www.un.org/). This population will need to be fed with an efficient use of the already limited resources available for agriculture, meaning the same land area, limited water and other resources, while reducing pollutants. To achieve this goal, modern bread wheat (*Triticum aestivum* L.) breeding is facing a very major challenge: to develop high yielding varieties to meet the food production to feed a growing human population. New wheat varieties need to keep or increase the high yield and reduce loses due to biotic, abiotic stresses or even climate change.

The enhancement of bread wheat has been limited due to its large (~17Gb) (Paux et al., 2008a) and complex genome (hexaploid with three homeologous genomes termed A, B and D (Gill *et al.*, 2004) and a high proportion of repetitive DNA (~90%). This makes genomic analysis

challenging (Gill *et al.*, 2004). Efforts have been made to sequence the wheat genome and to establish reliable web-based databases to store the information that has been generated from various labs globally (http://www.wheatgenome.org). A draft of the genome was released by the IWGSC in July 2014. This physical map is based on isolation of single chromosome arms and it contains 124,201 genes evenly distributed throughout the 21 chromosomes (IWGSC, 2014). Wheat also has an incomplete database of putative full-length cDNAs. Triticeae full length coding sequence (CDS) database (TriFLDB) has released approximately 6,162 wheat full-length cDNA sequence (http://trifldb.psc.riken.jp/v3/index.pl) and contains 8,530 putative full-length coding sequences and their annotations (http://trifldb.psc.riken.jp/v3/index.pl).

In order to meet the current wheat breeding demands, a better understanding of the wheat genome structure and function is required. However, at present, there is a need to identify genes or effectors that play a role in stress or stimuli responses. With the development of the Next Generation Sequencing or High-throughput sequencing, Single Nucleotide Polymorphisms (SNPs) have rapidly became the molecular marker of choice for a number of genotyping applications to support gene discovery in wheat (Paris et al., 2003). SNPs are loci where the sequence of DNA differs by a single base pair, and they are abundant and stable in animal and plant genomes. The frequency of SNPs in plants has been estimated from around 1/21 to 1/8500 base pairs (Kanazin et al., 2002; Bundock and Henry, 2004; Rostoks et al., 2005), while in bread wheat is around 1SNP/540bp (Somers et al., 2003). Useful SNPs markers are mainly those located in promoters and expressed regions, which can directly affect gene function and for that reason can be useful to design 'perfect' molecular markers (Paris *et al.*, 2003).

Important tools as quantitative trait loci (QTL) analysis and genome-wide association (GWAS) help in the discovery of genomic areas in the wheat genome that can be used to develop molecular markers, which assist wheat breeders in obtaining high yields, disease resistance and high protein content, among other traits to do more efficient improvement work.

In higher latitudes like Canada, the regulation of flowering and maturing times using photoperiod response and vernalization genes offers the advantage of avoiding frost damage during the reproductive phase (Lantican et al. 2005). Our group recently reported three earliness *per se* QTL associated with flowering and maturity in a recombinant inbred lines (RILs) population derived from a cross between the spring wheat (*Triticum aestivum* L.) cultivars 'Cutler' and 'AC Barrie' using simple sequence repeat (SSR) and diversity arrays technology (DArT) markers (Kamran et al., 2013). Given the opportunity that the Illumina eSelect array represents, we are now able to use SNPs to report QTLs associated with flowering time, maturity, plant height, grain yield as well as leaf and stripe rust and common bunt resistance using high density SNP markers in the same population.

Genome wide association studies (GWAS) is other approach where SNPs are becoming useful. These studies give us the opportunity to associate traits with specific genome regions. Using different background cultivars we are able to find correlations between genome regions and relevant traits, due to the high frequency of SNPs compared with other markers the chances to reach the gene level are higher.

Leaf rust, yellow (stripe) rust, common bunt and tan spot are wheat diseases which cause significant economical loses in wheat producing areas in Canada. We assembled an association

mapping panel consisting of 81 historical and modern spring wheat cultivars released in western Canada over a century, evaluated their disease reaction in the field, their sensitivity to the three Ptr toxins in a greenhouse, and genotyped them with 19,639 polymorphic single nucleotide polymorphic markers (SNPs).

Also using an historical wheat collection, we evaluated the allelic variation of a subset of selected genes associated with grain quality and disease resistance to try to understand the phenotypic effect of the genes on grain yield, maturity and plant height.

The general objective of this project was to carry out the screening of wheat populations using high-throughput sequencing tools to identify genomics regions encoding for phenotypic traits of interest for Canadian wheat breeding programs, including disease resistance, flowering and maturing time.

My thesis specific objectives are:

1. Discover QTL associated with flowering time under greenhouse and field conditions, and maturity, plant height and grain yield under field conditions.
2. Identify genomic regions and SNP markers associated with resistance to four wheat diseases (leaf and stripe rusts, common bunt and tan spot) and insensitivity to three Ptr toxins in a Canadian western spring wheat collection.
3. Survey the allelic variation of a subset of selected genes associated with grain quality and resistance to diseases across a subset of spring wheat cultivars registered in western Canada, and understand the phenotypic effect of the genes on grain yield, maturity, plant height and disease resistance

4. Provide wheat breeders with favorable disease marker combinations to aid in the determination of parental material for crosses

Research hypothesis

Canadian wheat breeding programs have introduced a number of genes using local and foreign germplasm with the objective to reduce plant height, decrease the number of days to maturing, increase grain yield and enhance resistance to several wheat disease (McCallum and Depauw, 2008).

Therefore, this research was designed to test the hypotheses that:

1. Novel QTL for reduce plant height, decrease the number of days to maturity, increase grain yield and enhance resistance to several wheat diseases are present in local wheat germplasm populations.

2. It is possible identify novel genome regions associated with disease resistance in western Canadian wheat populations.

3. It is possible to survey the effect of genes in Canadian wheat collections to increase precision and efficiency for new cultivar development in Western Canada performance.

1.2 Literature review

1.2.1 Origin and taxonomy of wheat

Wheat is an important monocotyledonous grain plant of the genus *Triticum* family Gramineae (Poaceae) (also called true grasses). The complete taxonomy is as follows:

Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; BOP clade; Pooideae; Triticodae; Triticeae; Triticinae; *Triticum*; *Triticum aestivum* L. (http://www.ncbi.nlm.nih.gov/genome/11)

Wheat is thought to be one of the first domesticated crops in the history (Gill, 2004; Li et al., 2007) . Within the genus *Triticum*, the two most important species are *T. aestivum* (bread wheat) and *T. turgidum* (durum wheat), which are among the most important food staples for humans and animals (Paux et al., 2008b)

Diploid and tetraploid wheat center of origin and domestication is believed to be in the Fertile Crescent (modern Iraq, Iran, Syria, Turkey) around 10,000 years ago and they evolved under the influence of different evolutionary forces, including hybridization, mutation, natural and lately artificial selection. The hybridization that produced bread or hexaploid wheat is believed to have occurred about 8000 years ago (Feuillet and Muehlbauer, 2009). According to Jaret Diamonds in his book *Guns Germs and Steel* archeological findings have shown that bread wheat has been already grown in the Nile Valley, India, China, and England by about 6000 years ago (Brooks, 1999; Cooper, 2015)

Hypothetically, two diploid species (*T. uratu* (2n=2x= 14, A^UA^U), the donor of the A genome and one species (donor of the B genome) from the *Sitopsis* section, which remains unknown and might be closely related to *Aegilopus speltoides*) hybridized 0.23-1.3 MYA (Mori et al., 1995).

They formed a tetraploid variant that evolved on its own and became a new species that was domesticated more than 10,000 years ago. This species is known as emmer wheat or *Triticum turgidum* L. (emmer wheat *Triticum turgidum* subsp. *dicoccum* and durum or pasta wheat *T. turgidum* conv. *durum*) (Luo et al., 2007). *Triticum aestivum* L. (bread or common wheat) originated later as the result of another evolutionary process involving the hybridization of *T. turgidum* (2n=4x=28, AABB) with *Aegilopus tauschii* (2n=2x=14, DD), the donor of the D genome. This followed a duplication of chromosomes in the gametes or the offspring (Zohary and Hopf, 2012).

1.2.2 Production and uses of wheat

Wheat is a cereal with a high content of macronutrients, including carbohydrates, proteins, minerals, and vitamins. It is the only source of macronutrients for almost 30% of the world's population according to wheatgenome.org. Wheat supplies 20% of the world total calories as estimated by FAO (http://faostat.fao.org/) and it is the third largest crop produced worldwide after maize and rice (http://faostat.fao.org/). Canada is the sixth largest producer of wheat in the world after China, India, the USA, Russia and France (http://faostat.fao.org/).

More than 90% of the Canadian wheat crops are grown in the Prairie Provinces (Alberta, Saskatchewan and Manitoba) (Aboukhaddour et al., 2013). Wheat production in western Canada exceeded 28 million tons in 2016 (Statistics Canada, 2016). Different classes of wheat are currently planted in western Canada with a classification relying mainly on grain color and hardness. Classes are also defined based on the season they are planted, seed color, and end use. Different classes are used for different purposes as shown in Table 1-1 for western Canadian wheat (https://www.grainscanada.gc.ca/wheat-ble/classes/classes-eng.htm).

The earliest record of wheat cultivation in western Canada started with a group of Scottish settlers who arrived in Canada in 1812 and planted winter wheat in the area of the confluency of the Red and Assiniboine rivers. These same settlers started planting spring wheat a year later. Both types of wheat had been imported from Scotland (http://www.agr.gc.ca). After issues regarding weather and pests, they imported new seed from the USA in 1820. Around 1842, a cultivar known as 'Red Fife', was imported from Germany via Glasgow upon Mr. David Fife request (http://www.agr.gc.ca). The cultivar was named after it red color after full ripe and farmer Fife last name and 'Red Fife' is a major component in the pedigree of many current Canadian wheat cultivars such as: 'Marquis', 'Garnet', 'Thatcher' and 'Neepewa' (http://www.agr.gc.ca). Currently the main cultivars in western Canada are: 'Harvest', 'Lillian', 'Stettler', 'Unity', 'CDC Go', 'Glenn', 'CDC Utmost' and 'CDC Stanley' (http://www.grainscanada.gc.ca)

1.2.3 Wheat genetics

Wheat possesses the largest (~17Gb) (Paux et al., 2008b) and most complex genome among cultivated plants (hexaploid with three homeologous genomes termed A, B and D) (Gill, 2004). These genomes contain both homologous and homeologous chromosomes. Homologous chromosomes are similar in size and shape and contain the same genes in the same order, but may have different alleles. Homeologous (related) chromosomes can have similar gene content and order, but diverge in repetitive DNA content. Despite its genome complexity, hexaploid wheat behaves as a diploid at meiosis. This means that chromosome 1A only pairs with the homologous chromosome 1A but not with homeologous chromosomes 1B or 1D. This holds true for all the seven chromosomes groups (Martinez et al., 2001; Moore, 2002; Cifuentes et al., 2010). However, each gene usually occurs in at least three copies (Ganal and Röder, 2007). The

wheat genome is ~8 fold the size of the maize genome, ~35 fold the genome of rice (Arumuganathan and Earle, 1991) . In addition, 80% of its repetitive sequences are predominantly gene duplications (Saintenac et al., 2011) and retrotransposon amplification (Gill, 2004; Li et al., 2004), while just 2% of the genome is represented by genes (Brenchley et al., 2012). In the wheat genome, there are gene clusters or gene-rich regions (GRR) which are associated with low copy and miniature inverted repeat transposable elements (TEs). These GRRs are separated by long stretches of high copy TEs (gene poor regions (GPR) or gene free regions called intergenic space) (SanMiguel et al., 2002). In these gene rich regions, gene density and distribution are homogenous (Feuillet and Keller, 1999). So far, 48 GRRs have been described in wheat containing 94% of the gene markers and an average of ~7 GRRs per homeologous group. Different chromosomes groups differ in location and number of GRRs, in the short arms there is a total of 21 GRRs while 27 are located in the long arms, which account for the 25% and 59% of the wheat genes, respectively (Gupta et al., 2008). Eighteen GRRs are considered major GRRs containing 60% of the genes but covering just 11% of the genome suggesting a high density; also, the GRRs increase their density towards the distal part of the chromosome arms (Gupta et al., 2008). The recombination rate is also unequal through different chromosome regions; distal regions have higher recombination rates than proximal regions (Gupta et al., 2008).

Intensive work has been done to sequence the wheat genome and establish reliable web-based databases to maintain the huge amount of information generated from various laboratories around the world (http://www.wheatgenome.org). Wheat genome sequencing is taking a considerable amount of time due to the challenges that its genome poses. Nevertheless, a draft of

the genome was released by the IWGSC in July 2014 and a high quality reference genome is expected to be ready by 2019 (http://www.wheatgenome.org/-news/WI-Jan-2016).

A physical map based on isolation of single chromosome arms containing 124,201 genes evenly distributed through the 21 chromosomes was released in 2014 by the International Wheat Genome Sequencing Consortium (IWGSC, 2014). A Triticeae full length CDS database (TRIFLDB) was released, approximately 6,162 wheat full-length cDNA sequences (http://trifldb.psc.riken.jp/v3/index.pl) and which houses 8,530 putative full-length coding sequences and their annotations. However, we need a better understanding of the role that these resources will play in the identification of factors involved in the response of wheat to stress and other stimuli.

1.2.4 Wheat breeding

Since its origin, hexaploid wheat has been under human selection in order to improve yield, adaptation to different environmental conditions and agricultural practices. This wide potential of adaptation has been possible due to its complex genome, which provides great plasticity to the crop (Acevedo et al., 2007). Among the most significant features to be considered when improving this crop are its vernalization and photoperiod requirements, water-limiting conditions, low temperatures and soil toxicity, etc. (Yan et al., 2004; Wilhelm et al., 2009). To mention, the development of semi-dwarf (Ogbonnaya et al., 2006). The alien introgressions in wheat using these wild species have been extensively studied (Molnár-Láng et al., 2015). Some research groups including Kihara (1944) and McFadden and Sears (1944, 1946),(Ogbonnaya et al., 2006) have generated synthetic wheats using *Aegilops tauschii* (syn *Ae. squarrosa*, *Triticum tauschii*) as the progenitor of the D genome of hexaploid wheat (*T. aestivum*). Near Isogenic Lines (NILs), Recombinant Inbred Lines (RILs) and Doubled Haploid (DH) lines are among the

most extensively used in research and wheat breeding. Hybrids, generated by crossing two pure lines, have also been widely used (http://www.hybridwheat.net/HP/HomePageEN.aspx?idnode=551&).

1.2.5 Genetics of flowering and maturity

In plants, the transition from vegetative to reproductive stages is critical for the adaptation to different environments and for reproductive success (Greenup et al., 2009; Allard et al., 2012). Flowering time variability has evolved under the control of a large set of genes (Cockram et al., 2007). Due to the diversity of the three main genetic systems controlling flowering time in wheat (vernalization (*Vrn*), photoperiod (*Ppd*) and earliness per se (*Eps*)), this crop has been very flexible in its adaptation to different and extreme environmental conditions. This allows wheat to be planted in different regions of the world, generating satisfactory grain yield under very diverse temperatures photoperiods and soil conditions (Kamran et al., 2013).

Flowering and maturity are important traits in cereal selection and breeding programmes (Borràs-Gelonch et al., 2012) There are three groups of genes involved in wheat flowering and maturity: photoperiod (*Ppd*), vernalization (*Vrn*) and earliness per se (*Eps*). Vernalization is "the requirement for a prolonged exposure to cold temperatures" (Allard et al., 2012). Photoperiod sensitivity is "the need of long days to initiate floral transition" (Allard et al., 2012) and earliness *per se* genes "induce earlier flowering independent of *Vrn* and *Ppd* genes" (Van Beem et al., 2005; Kamran et al., 2013). Each of these gene systems is responsible for a part of the genetic variability in the heading time (Kamran et al., 2013) of bread wheat: *Vrn* about 70–75 %, *Ppd* for about 20–25 % and *Eps* for about 5 % (Stelmakh, 1998; Zare-kohan and Heidari, 2012)

The flowering time of spring wheat is shorter than winter wheat. Spring wheat does not require exposure to cold temperatures, while the winter wheat needs to be exposed to vernalization.

After the vernalization requirements are fulfilled, certain genotypes will required a specific day-length exposure (more than 10 h light) for many weeks before flowering. These genotypes are denominated photoperiod sensitive genotypes. The photoperiod insensitive (PI) genotypes will flower independently of the length of the day light (Lantican et al., 2005). Wheat is considered a long day plant which flower earlier when exposed to longer days, however PI genotypes which are mutants for *Ppd-1* genes are able to flower in short days (Dubcovsky et al., 2006; Shaw et al., 2012)

Early flowering time in spring wheat is controlled by vernalization-insensitivity (Pugsley, 1971). Vernalization sensitivity or insensitivity in hexaploid wheat is controlled by the major vernalization loci, *Vrn-1* (located on chromosome 5 of the three sub-genomes (A, B and D) of allo-hexaploid bread wheat (Iqbal et al., 2007; Kumar et al., 2012). Three other genes have been described: *Vrn-2* (located on subgenomes A and B in chromosome 5), *Vrn-3* (located on chromosome 7 in the 3 subgenomes) and *Vrn-4* (located on chromosome 5D) (Kato et al., 2003). Spring wheat genotypes carry one or more dominant alleles at *Vrn-1, Vrn-3* and *Vrn-4* loci, which confer partial or complete insensitivity to cold treatment (Iqbal et al., 2007; Kamran et al., 2014). On the other hand, winter wheat carries dominant alleles for *Vrn-2* and recessive alleles for the rest of the three loci (Kamran et al., 2014). *Vrn-1* and *Vrn-3* of wheat has been found to be orthologous to the Apetala 1 (*AP1*) (Chen and Dubcovsky, 2012) and Flowering locus T (*FT*) of *Arabidopsis thaliana* (Yan et al., 2006), respectively. Photoperiod sensitivity is controlled by a group of genes located on the group 2 chromosomes: *Ppd-A1*, *Ppd-B1*and *Ppd-D1* (Guo et al., 2010; Bentley et al., 2011; Kumar et al., 2012)

Earliness per se (*Eps*) (also termed ear emergence per se, earliness in the narrow sense, intrinsic earliness, or basic development rate) (Lewis et al., 2008) genes regulate flowering time

independently of environmental signals, vernalization and photoperiod genes (Kamran et al., 2013). *Eps* is responsible for fine-tuning flowering and for the adaptation of wheat to diverse environments (Lewis et al., 2008). The gene *Eps-A1* was mapped to within a 0.8 cM interval on chromosome 1AL of diploid wheat (*Triticum monococcum* L.) (Lewis et al., 2008). Three QTLs for *Eps* have been recently mapped on chromosomes 1B (Q*Eps*.dms1B1 and Q*Eps*.dms-1B2) and 5B (Q*Eps*.dms-5B1) (Kamran et al., 2013). *Eps* genes have also been found in related species, such as barley and rice (Cuesta-Marcos et al., 2008; Kolev et al., 2010; Gawronsky and Piotr, 2012; Tsehaye et al., 2012)

1.2.6 Genetics of plant height

Just before and during the "Green Revolution", a new era that changed agricultural methods allowing more people to be fed from the same planted area a group of changes were successfully introduced in wheat crops (Peng et al., 1999). Among these changes was improved lodging tolerance by selecting height reducing genes (dwarf and semi-dwarf) from Japanese and Korean cultivars, which were introduced into wheat lines in Europe and the Americas. Borojevic and Borojevic (Borojevic and Borojevic, 2005a) have described some features of this breeding process. According to these authors, the height reducing gene *Rht*8 and the daylight-insensitive gene *Ppd-D1* (both linked on chromosome 2D) were introduced from the Japanese cultivar 'Akakomugi' to European wheat cultivars and from there passed to South American cultivars (Borojevic and Borojevic, 2005b). These original cultivars had short straw, early maturity, and high yield potential (Lorenzetti, 2000). The gene Rht8 was introduced in Canada with a CIMMYT developed cultivar 'Pitic 62', which is part of a Canadian wheat historical collection (http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/fcd4238). Semi-dwarfing genes *Rht*-

B1 and *Rht-D1* (former *Rht1* and *Rht2*) were also introduced from a Japanese cultivar 'Norin10' to US cultivars.

Some of the American lines where passed to CIMMYT in Mexico, where Norman Bourlag (Nobel Peace Prize, 1970) developed these lines in the 1960s and 1970s, (Gale and Youssefion, 1985; Ellis et al., 2002). The lines were later introduced to many other countries such as Mexico, India, Pakistan, Turkey in order to increase yield and lodging tolerance (Ellis et al., 2002). The same lines were also introduced to Europe where they contributed to yield improvements (Borojevic and Borojevic, 2005a). The genes *Rht-B1* and *Rht-D1* encode a DELLA protein which represses GA-responsive growth; mutant types *Rht-B1b* and *Rht-D1b* make the plant GA-insensitive by producing more active form of the growth repressor, therefore making the plant unable to grow normally (Peng et al., 1999; Pearce et al., 2011)). *Rht*-B1 and *Rht*-D1, are homeologous, and have been isolated in wheat and discovered to be orthologous to the *Arabidopsis* GAI gene and maize dwarf8 (Peng and Harberd, 1993; Peng et al., 1997, 1999; Jaccoud et al., 2001), which is a de-repressible modulator of gibberellic acid (GA) response (Peng et al., 1999) Six alleles of *Rht-B* and *D1* have been identified, four on chromosome 4B and two on chromosome 4D (Ellis et al., 2002).

The effectors of dwarfism have been cloned and well-studied (Peng et al., 1999). Single nucleotide substitutions produce premature stop codons in the N-terminal coding region, therefore N-terminal truncated proteins are produced that increase repression of GA signaling. The exogenous application of the GA does not restore the wildtype (Rebetzke and Richards, 2000).

1.2.7 Genetics of resistance

There are two main classes of genetic resistance which are used to improve wheat and other crop plants. Resistance can be either qualitative or quantitative. Qualitative (or monogenic) resistance is often controlled by a major single gene (R) which is most frequently dominant (but can be recessive as in the case of tan spot resistance in wheat) or quantitative (or multigenic) which is conditioned by multiple genes with minor effects and are highly influenced by the environment (Poland et al., 2009). Resistance is considered "vertical" when a major gene confers a high level of resistance to a specific pathogen race and is called "horizontal" when few or many genes confers resistance to a wider spectrum of pathogens (Vale et al., 2001).

1.2.8 Qualitative resistance

According to Flor's gene for gene theory (Flor, 1956, 1971) for every R (resistance) gene in the host there is a corresponding *Avr* (avirulence) gene in the pathogen; a R gene confers resistance to a pathogen carrying a matching *Avr* gene. Therefore the resistance of the plant depends on the pathogen strain. Similarly, the ability of pathogen to overcome plant resistance relies completely on mutations in that specific *Avr* gene, which can change the topography of the effector and therefore, changing the recognition by the receptor protein coded by the R gene (Ellis et al., 2014). In other words, the resistance in plant-pathosystems is generally race-specific and is also very easy to neutralize by new races of the pathogen (Vale et al., 2001).

Resistance genes encode proteins that detect pathogen *Avr* genes products (McDowell and Woffenden, 2003). They are effective against a specific pathogen and at all of the plant's growth stages. There are eight groups of R genes described in the literature:

1. Cytoplasmic protein with a nucleotide binding site (NBS), a C-terminal Leucine rich repeat (LRR) and a putative coiled coil domain (CC) in the N-terminus [class I known as NBS-LRR-CC]

2. Cytoplasmic protein with LRR and NBS and, a N-terminal domain with homology to the mammalian toll-interleukin-1-receptr (TIL) domain [class II known as NBS-LRR-TIR]

3. Extra-cytoplasmic LRR (eLRR) attached to a transmembrane domain (TrD) [class III known as LRR-TrD]

4. Extracellular LLR domain, a TrD and an intracellular serine-threonine kinase (KIN) domain [class IV known as LRR-TrD-kinase]

5. Putative extracellular LLRs along with a (PEST (Pro-Glu-Ser-Thr) domain for protein degradation and short motifs (ECS) [class V known as LRR-TrD-PEST-ECS]

6. TrD fused to a putative CC [class VI known as TrD-CC]

7. TIR-NBS-LRR, the C-terminal extension with a putative nuclear localization signal (NLS) and a WRKY domain of 60 amino acids (aa) that is defined by a conserved aa sequence WRKYGQK at its terminal end [class VII known as TrD-CC]

8. Enzymatic with no LRR or NBS groups. For example, in barley stem rust gene encodes a receptor like kinase with two tandem kinase (kinase-kinase) and does not contain a strong membrane targeting motif or known receptor sequences [class VIII known as Enzymatic R-genes] (Brueggeman et al., 2002)

These are major genes which can confer near immunity against specific races. Resistance conferred by these genes, however, is easily overcome by pathogen evolution (Pink, 2002). Several R genes have been generally introduced in wheat from wild relatives (e.g. rye genes where the pathogen has not been previously exposed to selection when confronted with the R

gene under the new genetic background (Ellis et al., 2014). One example of such an introduction is *Sr24* gene from *Agropyron* sp. to wheat (Bariana and Mcintosh, 1993; McIntosh et al., 1995)

1.2.9 Quantitative resistance

Quantitative resistance is not as well understood as qualitative resistance and has not been utilized to its full potential in breeding (St.Clair, 2010). This resistance is defined as a resistance mediated by number of host genes, which reduce the incidence of infection but do not confer immunity (Poland et al., 2009). The phenotype in quantitative resistance varies in a continuous way in the host population, from nearly imperceptible to very strong. This resistance is also termed as partial, complex, residual, polygenic, oligogenic, horizontal, basal, field resistance and durable (Vale et al., 2001). This resistance is complex and has different levels (French et al., 2016).

Most commonly it is referred to as broad spectrum resistance; the resistance of the host plant to all pathogens of a specific species. This term has lately been challenged by Ellis et al.,(2014), who considers this to be misleading and believes it makes researchers think that such resistance will be durable (Ellis et al., 2014). According to the opinion of Ellis et al. (2014), it is the resistance to all known or test races at a specific time but not throughout the lifetime of the plant.

1.2.10 Plant defense mechanisms

Plant disease is defined as "any physiological abnormality or significant disruption in the 'normal' health of a plant" (Freeman, 2008). Disease can be caused by living (biotic) agents, including fungi and bacteria, or by environmental (abiotic) factors such as nutrient deficiency, drought, lack of oxygen, excessive temperature, ultraviolet radiation, or pollution (Freeman, 2008). Although plants lack the vertebrate immune system, they have developed extensive

structural, chemical, and protein-based defense mechanisms to contend pathogens before they are able to cause extensive damage (http://www.apsnet.org). Plants have two kinds of defense mechanisms: constitutive and inducible also called passive and active (Amil-Ruiz et al., 2011). Constitutive (continuous or passive) defenses mainly consist of barriers such as cell walls, waxy epidermal cuticles, bark, trichomes, etc. These barriers make it difficult for pathogens to access the cell interior or impede pathogen penetration (Underwood, 2012). Inducible defenses known as pattern-triggered immunity (PTI) (Jones and Dangl, 2006) involve the detection of elicitor molecules produced by the pathogen (microbe or pathogen associated molecular patterns (MAMPS or PAMPs) by transmembrane pattern recognition receptors (PRRs) (Jones and Dangl, 2006; Dodds and Rathjen, 2010). These receptors are generally product of the R genes. This recognition triggers the production of toxic chemicals, pathogen-degrading enzymes, and a cascade of chemical events including the oxidative burst (Lamb and Dixon, 1997) resulting in deliberate cell suicide or apoptosis (Kombrink and Schmelzer, 2001). This kind of response has been named as a hypersensitive response (HR) and can also be triggered by host derived elicitor known as damage-associated molecular patterns (DAMPs) (Underwood, 2012). The hypersensitive response is characterized by apoptosis, also called programmed cell death (PCD), which occurs around the site of pathogen invasion. The HR can lead to cell death or apoptosis, limiting the pathogen access to plant nutrients and thereby also limiting its survival. The HR is typically more pathogen-specific than basal resistance. The HR also triggers systemic acquired resistance (SAR) against a broad spectrum of other pathogens (viruses, bacteria, fungi, etc.) and enhances the resistance in distal organs of the plants that have yet not been infected (Conrath, 2006). Salicylic acid (SA), lipids, hydrogen peroxide, gaseous methyl salicylate, ethylene and other compounds. have been proposed as endogenous systemic signals that result in SAR (Shah

and Zeier, 2013). SAR has a complex nature suggesting that different signals might be involved depending on the plant species (Conrath, 2006). SAR is also characterized by the overexpression of a group of proteins known as pathogenesis-related (PR) proteins such as β-1,3-glucanases (BGL2) and chitinases (PR-3). Initially, these PR proteins were proposed to form the molecular basis of SAR. However, later studies disproved this theory and it was found that PRs have a minor role in SAR (Conrath, 2006). PR proteins were discovered to accumulate after infections (van Loon and van Strien, 1999; van Loon et al., 2006) and are classified in two groups: acidic PR proteins mainly found in intercellular spaces and basic PR proteins which are similar in function, diverse in molecular weights and amino acid sequences and are found in the vacuole (Heil and Bostock, 2002).

1.2.11 Principal diseases of wheat and their management

Similar to other crops, wheat has to face several environmental and biotic stresses. The major wheat diseases, their diagnosis and management are briefly described below:

1.2.11.1 Tan Spot

Many fungal diseases affect wheat plant development and yield. In western Canada, as well as in all major wheat growing areas around the world, *Pyrenophora tritici-repentis* (Died.) Dresh. (Anamorph: *Dreshlera tritici-repentis* (Died.) Shoem.) the causal agent of the wheat disease known as tan spot, causes potential yield losses that can reach 50% (Lamari and Strelkov, 2010)

An increased incidence of tan spot has occurred in recent decades (Lamari and Strelkov, 2010). This has happened mainly due to the newly adopted environmental friendly agricultural practices (such as zero tillage or stubble burning) that retain crop residues, allowing the pathogen survival overwintering in the stubble (Strelkov and Lamari, 2003)

The key diagnostic feature of tan spot is tan coloured lesions with a yellow margin. Mature tan spot lesions often have a dark area in the center. Lesions may merge as they expand, resulting in large sections of diseased leaf tissue. The fungus that causes tan spot survives in the debris of previous wheat crops and produces small, black reproductive structures (pseudothecia) in the spring. Tan spot can be managed by avoiding planting into wheat residue, tillage where appropriate and through genetic resistance and foliar fungicides.

The fungus causing tan spot is an ascomycete that is able to attack both durum (*T. turgidum* L. var. *durum*) and common wheat (*T. aestivum* L.) as well as other grasses(Strelkov and Lamari, 2003). The symptoms can appear at any stage of the wheat crop and depend on the aggressiveness of the inoculum and the weather conditions (Singh et al., 2012). The visible symptoms observed in a susceptible wheat cultivar are tan oval shaped lesions with a darker brown to black center, generally surrounded by a chlorotic edge (Strelkov and Lamari, 2003) Under favorable conditions the lesions can fuse in a big lesion producing the death of the leaf (Singh et al., 2012).

Several rating systems have been used to describe the response of wheat to tan spot, including percentage of infection, lesion size, percent of leaf area infected, and location of infected leaves. The most widely used is a 1-5 scale developed by (Lamari and Bernier, 1989a; Lamari; Bernier, 1991)

Older classification of Ptr isolates were based on the pathogen aggressiveness and in quantitative parameters such as lesion size, per cent leaf necrosis and number of lesions and percentage of infection (Strelkov and Lamari, 2003). Later, Lamari and Bernier (Lamari and Bernier, 1989b; Lamari; Bernier, 1991) revised and modified the classification this time according to the

interaction between wheat genotypes the toxins produced by the fungus and the ability to produce chlorosis, necrosis or both on the susceptible wheat genotypes . The new system was able to accommodate just four pathotype groups: 1 (necrosis^{+} chlorosis^{+}), 2 (necrosis^{+} chlorosis^{-}), 3 (necrosis^{-} chlorosis^{+}) and 4 (necrosis^{-} chlorosis^{-}). The need for a new classification came with the identification of isolates with new virulence patterns. A race-based classification system was created based on a differential wheat panel and in the toxins present in the isolates. The system is described in Table 1-2.

Tan spot disease cycle includes an overwintering stage in the crop residue as a dormant mycelium (Tran, 2014) or in surviving structures (pseudothecia) which produce the ascospores (Lamari and Strelkov, 2010). In spring the ascospores will be delivered and dispersed by blowing rain and wind and will be the primary source of inoculum for the next crop season in the field or neighboring fields. The new infected leaves produce conidia as a secondary inoculum.

The *Ptr* pathogen produces different host selective toxins (HTS) termed: Ptr ToxA, Ptr ToxB and Ptr ToxC (Strelkov and Lamari, 2003). Ptr ToxA is a protein of 13.2 KDa and causes necrosis in sensitive wheat lines. Ptr ToxB is a protein of 6.6 KDa and causes chlorosis in sensitive wheat genotypes. Ptr ToxC is a putative toxin which also produces chlorosis and it is considered a non-proteinaceous, non-ionic, polar, low molecular mass molecule (Strelkov and Lamari, 2003). As of 2010 only races 1, 2, 3, 4 and 5 were reported in North America (Lamari and Strelkov, 2010).

P. tritici-repentis is able to produce two necrosis inducing toxins and two that produce chlorosis. Ptr ToxA produces necrosis in susceptible cultivars but a second class of compounds named triticones (spirocyclic lactams) are able to induce necrosis as well (Singh et al., 2012). However

Ptr ToxA is a host selective toxin while triticones are non-specific (Singh et al, 2010). Ptr ToxA is produced by races 1, 2, 7 and 8 of *P. tritici-repentis*. Prt ToxA is codified by a single gene in *P. tritici-repentis*, which has been passed from *Stagonospora nodorum* by horizontal gene transfer (Friesen et al., 2006). The gene encoding Ptr ToxB occurs as multiple copies genes in the pathogens (Martinez et al., 2004). In the case of Ptr ToxC, further work is needed to evaluate its genetic nature as well as resistance

A single recessive gene *tsn1* controlling insensitivity to Ptr ToxA has been mapped to chromosome 5BL (Faris et al., 1996) and cloned (Faris et al., 2010). On the other hand, Ptr ToxB and Ptr ToxC are the toxins that produce chlorosis. Races 5,6,7 and 8 produce Ptr ToxB while races 3,6 and 8 produce Ptr ToxC (Strelkov and Lamari, 2003). The locus *tsc* 1 controlling insensitivity to toxin Ptr ToxC and *tsc2* controlling insensitivity to toxin Ptr ToxB, have been located on chromosomes 1BS and 2BS, respectively (Effertz et al., 2002; Abeysekara et al., 2010; Faris et al., 2013). Other race-specific toxin sensitivity genes as *tsn* 3 located on chromosome 3D (mapped in synthetic wheat line 'XX41' (Tadesse et al. 2006a) and *tsn* 4 mapped (in 'Salamouni' monosomic lines) (Tadesse et al. 2006b) Another group of resistance genes termed *Tsr* 1-6 have been mapped as well (Singh et al., 2012).

The wheat-*P.tritici-repentis* pathosystem follows the toxin or inverse gene-for-gene model, which is a mirror image of the classical gene-by-gene model (Strelkov and Lamari, 2003). Both quantitative and qualitative inherited genes have been found to control tan spot resistance (Singh et al., 2012). QTL and major genes associated with tan spot resistance have been mapped and cloned (Singh et al., 2012).

The disease affects the plant chloroplast affecting the photosystems and so decreases the photosynthesis (Lamari and Strelkov, 2010). Different sources of resistance to tan spot have been reported in wheat wild relatives (Lamari and Bernier, 1989a).

1.2.11.2 Canadian wheat and its reaction to tan spot

Most of the Canadian wheat cultivars are susceptible to tan spot (Lamari et al., 2005) The sensitivity or insensitivity of 86 current and historical CWRS wheat cultivars to Ptr ToxA and Ptr ToxB was examined in recent studies (Lamari et al., 2005; Tran, 2014). It has been found that most cultivars from the 1800s until the mid-20th century were sensitive to Ptr ToxA. Canadian cultivars released in the 1950s were resistant to this toxin, however the susceptibility was (inadvertently) reintroduced in the 1960s (Lamari et al., 2005; Tran, 2014). Sensitivity to Ptr ToxA first appeared in the cultivar 'Red Fife' and was then transferred through Canadian wheat lines by the extensive use of backcrossing to maintain the 'Marquis' and 'Thatcher' bread making quality. This sensitivity has remained present in most wheat cultivars ever since. This is of particular concern because in North America, races 1 and 2 of *P. tritici-repentis*, which are known to produce Ptr ToxA, are predominant among the eight races of the fungus (Lamari and Strelkov, 2010). Therefore, most of the wheat cultivars in Canada are susceptible to races 1 and 2 and sensitive to Ptr ToxA. Although Ptr ToxB-producing isolates are rarely found in Canada, the sensitivity of wheat cultivars to Ptr ToxB first appeared in the cultivar 'Thatcher' in 1934 and was subsequently transferred to many genotypes through backcross breeding programs. Sensitivity to Ptr ToxB has persisted in western Canadian cultivars for over 70 years (Lamari et al., 2005). This represents a potential cause for concern, particularly if Ptr ToxB-producing isolates of *P. tritici-repentis* were to become more widespread (Tran, 2014).

1.2.11.3 Rust diseases

Stem, leaf and stripe (also known as yellow rust) caused by the fungal species *Puccinia graminis f. sp. tritici*, *Puccinia triticina* Eriks and *Puccinia striiformis* Westend. f. sp. *tritici* (Pst), respectively, are three diseases that cause major losses in wheat production around the world (Ellis et al. 2014). Rust fungi are obligate parasites, meaning they require living host tissue for growth and reproduction and cannot survive as saprophytes (Schumann, 2000)

These diseases are managed mainly with foliar fungicides and genetic resistance (Singh 1998). However, due to the environmental and economic effect of the use of fungicides and due to the development of resistance in pathogens against chemicals (Oliver, 2014), genetic resistance has always been more suitable to control rusts. There are well known pathogen specific resistance genes (R genes) and adult plant resistance genes (APR genes) in wheat providing rust resistance.

Stem rust: Can be found worldwide in wheat growing areas. Losses can be severe (50 to 70%) over a large area and some fields can be totally destroyed (Singh et al., 2008). It is most important where dews are frequent during and after heading and when temperatures are warm (18-30°C). This pathogen has a complex life cycle. In spring, aeciospores (a major source of inoculum moved by the wind and rain) germinate on wheat leaves causing blister lesions (uredinia) on leaves, true stems and spikes (infection in the head is also possible), which produce brick-red urediniospores that are spread in the fields by the wind. Later in the cropping season, pustules (telia) which produce black thick walled teliospores begin to appear on infected grass species (Schumann, 2000). The pathogen is able to overwinter in the straw as teliospores. The teliospore (2n) is a dykaryotic spore and undergoes karyogamy (fusion of nuclei) and meiosis to form four haploid spores called basidiospores (n). Basidospores cannot infect cereals but infect

alternate hosts (barberry). They germinate to form a haploid mycelium that penetrates the leaf and form pycnia, resulting in two types of haploid gametes (Fetch et al., 2011). . Usually pycnia appears on the upper leaf surfaces and exude pycniospores in a sticky substance (honeydew). Five to 10 days later, cup-shaped structures filled with orange-yellow, powdery aeciospores break through the lower leaf surface. The aecial cups are yellow and sometimes elongate to extend up to 5 mm from the leaf surface. Microscopically, aeciospores have a slightly reticulated surface. There have not been reports of epidemics of wheat stem rust in Canada spring wheat since the 1950s; this was the result of programs of eradication of ornamental barberry (alternated host) in Canada and the USA, helped to reduce the pathogen inoculum and reducing the ability of the pathogen of produce new virulent races by recombination (Fetch et al., 2011). There is genetic resistance available for the disease and the use of breeding for stem rust in Canadian date back to 1939 with the cultivar 'Tatcher' which grew extensively until the 1970s with effective stem rust resistance. (McCallum et al, 2007). Other cultivars with genetic resistance carried *Sr2*, *Sr6*, *Sr7a* and *Sr9b* which had been reported to have adequate control of stem rust (McCallum et al, 2007).

Stem rust generally affects *Triticum* spp. but has also been found in *Secale cereale, Hordeum vulgaris, H. jubatum, H. pusillum and Elymus junceus*. Other formae specialis of *P. graminis* attack many cereals and related grasses, and many species are susceptible to more than one formae specialis (f.sp). The fungus uses volunteer wheat as source of the inoculum but *Berberis vulgaris* is an alternate host. Mature stem rust lesions are more elongated than those of leaf rust. There are about 60 genes for stem rust resistance (*Sr*), many of which are linked to or are likely to be the same as those for leaf rust.

Leaf rust: The disease frequently occurs during flowering. It produces small, orange-brownish lesions on the leaves, giving a rusty appearance. These blister-like lesions are most common on leaves but can occur on the leaf sheaths, which extend from the base of the leaf blade to the stem node. Lesions caused by leaf rust are normally smaller, more round, and cause less tearing of the leaf tissue than those caused by stem rust. Conditions favoring establishment are wet, cool temperatures, approximately 15-25° C, although the fungus can survive temperatures up to 40° C. The wind can disperse urediniospores between regions. The disease may be managed with foliar fungicides but genetic resistance is the most effective and environmentally friendly approach to control this disease. A large number of resistance genes exist for leaf rust. More than 60 different genes have been described and named *Lr* plus a number i.e *Lr1* to *Lr 68* (Draz et al., 2015). The most common leaf rust resistance genes in Canadian spring wheat are *Lr1, Lr10, Lr13, Lr14a, Lr16*, *Lr21, Lr22a and Lr34* (McCallum and Depauw, 2008). *Lr34* has been deployed in half of the western Canadian spring wheat cultivars registered since 1972, when was introduced in 'Glenlea' (CWES). The gene does not confers immunity, but when combined with other genes it confers a very high resistance (German and Kolmer, 1992; Kloppers and Pretorius, 1997)

Stripe (yellow) rust: *Puccinia striiformis* f.sp. *tritici* is a basidiomycete fungus and an obligate parasite of grass species and causes severe damage in most wheat areas with cool and moist weather (Wan and Chen, 2014). It produces blister-like lesions with yellow urediniospores that form a striped pattern on the leaves. Its yellow spore colour is different than that of leaf rust, as is the shape of the blisters (Fetch et al., 2011) . The disease is most common on leaves, but head tissue also can develop symptoms when disease is severe. Conditions favoring establishment include wet, cool temperatures (approximately 7-20°C), although it can survive temperatures up

to 40° C at which produces black teliospores attached to the leaves (Fetch et al., 2011). Barberry is alternate host as well (Jin et al., 2010). As with the other rusts diseases, genetic resistance is available to control this disease in addition to foliar fungicides. Stripe rust resistance genes are termed *Yr* and many of them have been found to be linked to *Sr* or *Lr* genes. There was not much information regarding the resistance genes carried by Canadian wheat cultivars before 2000 (Fetch et al., 2011), although the resistance gene *Yr18* (which is linked to Lr34) might have been introduced in 1972. A 2007 evaluation of cultivars found that a big majority of Canadian cultivars are either susceptible or moderately resistant to stripe rust (McCallum et al., 2007).

1.2.11.4 Common bunt or stinking smut

Common bunt, also known as stinking smut, is caused by one of two heterobasidiomycete fungal species *Tilletia caries* and *Tilletia foetida* (syn. *Tilletia laevis* Kühn and *Tilletia tritici* (Bjerk.)). They infect wheat kernels producing grain damage and yield loss (Cota et al., 2010). The kernels acquire a gray-greenish color and turn wider than healthy kernels. This disease has caused losses in major wheat producing areas throughout history. Presently, common bunt is not a major problem but yield losses are still reported and genetic resistance is a highly desirable trait in wheat cultivars suitable for organic growing (Liatukas and Ruzgas, 2008)

The host can be either bread or durum wheat. The spores remain on the seeds or in the soil and they develop during seed germination. The symptoms are visible only when the plants have reached maturity; the kernels of the infected plants have no “normal seeds” but the remnants of the seeds, just the intact coat with the inside stuffed with a black mass of spores, emanating a specific fishy odor of trymethilamine (Wilcoxson and Saari, 1996). Infected plants could be slightly shorter than healthy. After heading, the spikelets of infected plants tend to "flare-out"

and take on a greasy, off-green color. This "flaring out" of the spikelet is due to the expansion in size of the bunt infected seed that becomes filled with teliospores (Mathre, 2005).

Contaminated kernels can be seen in developing wheat heads but are often not detected until harvest. The outer layers of diseased kernels remain intact initially but are easily broken during grain harvest, releasing masses of black, powdery spores. The fungus produces “chemicals” with a fishy odor, which sometimes causes this disease to be referred to as “stinking smut.” Conditions favoring establishment are wet, cool temperatures (approximately 8-15° C), although the pathogen can survive temperatures up to 40° C. Genetic resistance, seed treatment with fungicides or using disease-free seeds are some approaches to manage common bunt. A number of common bunt resistance genes (named *Bt1* through *Bt15*) have been reported in wheat. Cultivars such as ‘Martin’ (carrying *Bt1*), ‘Turkey’ (carrying *Bt14*) and ‘Rio’ have been reported to have resistance to 25 known common bunt races. The Canadian cultivar ‘BW553’ has been reported as resistant and ‘Neepawa’ as susceptible (Gaudet et al., 2007).

1.2.11.5 Other important wheat diseases

Septoria tritici blotch: This fungal disease causes tan coloured, elongated lesions on wheat leaves. Lesions may have a yellow margin, but the degree of yellowing varies among cultivars. The dark, reproductive structures (pycnidia) produced by the fungus are key diagnostic features and can often be seen without magnification. This disease is also known as speckled leaf blotch and can be managed through genetic resistance, foliar fungicides or crop rotation (Ponomarenko et al., 2011)

Septoria/*Stagonospora nodorum* Blotch (SNB): caused by *Parastagonospora nodorum* (previously named *Septoria nodorum*, *Stagonospora nodorum*). The life cycle of the fungus is very similar to the causal agent of tan spot (Friskop and Liu, 2009) The lesions of SBN are normally brown or tan, surrounded by a thin, yellow halo. Lesions caused by SNB are more irregular in shape and often have a darker color than those of tan spot. The diagnosis of *P. nodorum* infection is made when small, honey-colored fungal reproductive structures (pycnidia) are detected; however, these structures are only visible with magnification that might require a stereoscopic microscope. This disease can be managed through genetic resistance, foliar fungicides, crop rotation or fungicide seed treatment. Contaminated seeds do not germinate or have reduce germination (Friskop and Liu, 2009) .

Loose smut: is caused by the fungus *Ustilago tritici* (Pers.) Rostr. Grains in infected spikes are completely replaced with dark masses of fungal spores having a black powdery appearance with no seed development in the infected heads (Gupta, 2016). The mycelium remains dormant in the seed in the season when they are infected and in the next season the pathogen will develop along with the plant if the conditions are good for the plant development. When the plant reaches its reproductive stage, the developing kernels are replaced by black teliospores. It is possible to see heads damaged by loose smut while much of the head is still inside the boot. Only the central stem of the head is left after the spores are released. Conditions favoring establishment include wet, cool temperatures. The disease is controlled by fungicide application in the seed or through the use of disease-free seed.

Fusarium head blight (FHB) or scab: is produced by *Fusarium* spp., including *Fusarium graminearum* (anamorph) *Gibberella zeae* (teleomorph)*; Fusarium avenaceum; Fusarium culmorum* and *Fusarium poae*. However, the predominant causal agent of FHB in most areas of the world is *F. graminearum*, which is an ascomycete and affects durum and bread wheat as well as other cereals and grasses (Schmale III and Bergstrom, 2003). The first symptom of FHB at flowering time when spikelets exhibit bleaching that spread throughout the heads as the pathogens grows. Its symptoms are tan or brown lesions encompassing one or more spikelet.

Some diseased spikelets may have a dark brown discoloration at the base and an orange fungal mass along the lower portion of the glume. Among others the fungus produces a mycotoxin known as deoxynivalenol (DON) that poses a significant threat to the health of domestic animals and humans. Conditions favoring establishment are precipitation or high humidity with temperature range from 16 to 30°C, with the optimum range for *F. graminearum* being 25 to 28°C. The spores can be spread by the wind and rain (Brennan et al., 2005). The disease is managed by avoiding planting susceptible cultivars, avoid planting into corn residue and application of foliar fungicides and crop rotation (Wegulo et al., 2015). The disease is difficult to manage as most cultivars are susceptible but genetic resistance is available. A major effect QTL had been reported in a mapping population derived from a cross between 'Sumai 3' (resistant) and 'Stoa' (moderately susceptible). The QTL *Qfhs.ndsu-3BS*, was mapped on chromosome arm 3BS between flanking markers *Xgwm493* and *Xgwm533* which explained 25 and 41% of the phenotypic variation respectively (Anderson et al., 2001). This QTL has been fine mapped and renamed *Fhb1* (Liu et al., 2006). It has been reported to be a type II resistance which retards the disease spread throughout the spike (Shen and Ohm, 2006) Another resistance gen denominated *Fhb 2* has been mapped in chromosome 6BS, between flanking markers *gwm133* and *gwm644* (Cuthbert et al., 2007).

Powdery mildew: is one of the most destructive wheat diseases worldwide (Huang et al., 2000) caused by the obligate, biotrophic fungi *Blumeria graminis* f. sp. *tritici*. It causes cottony white lesions on leaves but heads can be infected when disease is severe (Cowger et al., 2009). Mycelial growth is largely limited to outer plant surfaces and can be easily wiped away by rubbing a finger across affected areas. Mature lesions may have dark, reproductive structures (cleistothecia) mixed with the white mycelium. Conditions favoring establishment are wet, cool temperatures (ranging from 16-21° C), also cloudy weather conditions (Heffer et al., 2006). Fungal growth stops above 25°C. Disease development is favored by nitrogen application and

high canopy density. There is genetic resistance and foliar fungicides available to treat the disease.

1.2.12 Polymorphism detection and its uses

There are three broad classes of markers for polymorphism detection, those based on visually assessable traits (morphological and agronomic markers), those based on gene products (biochemical markers), and those relying on a DNA assay (molecular markers).

The main limitation of morphological markers (Van Beem et al., 2005) is the availability of mutants. But also, if a mutation is neutral it does not affect the phenotype. Although, phenotypic identification can be ambiguous due to complex genotype by environment interaction. On the other hand, dominant phenotypes expressed at a very low frequency makes their effective utilization in plant breeding difficult (Farooq and Azam, 2002)

A second group of markers is based on gene products; these are biochemical markers and are generally proteins. When the resulting protein is an enzyme with different isoforms they are denominated isozymes (Markert and Moller, 1959) . Isozymes are defined as different molecular forms of a specific enzyme with, qualitatively, the same catalytic function (Kumar et al., 2009). Medina-Filho (1980), established a genetic linkage between a nematode resistance gene and acid phosphatase locus (*Aps*-I) isozyme allele in tomato and the gene for resistance to root-knot nematodes (*Mi*) (Medina-Filho, 1980). This highlighted the opportunity of tagging genes of agronomic importance (Tanksley and Rick, 1980). The effect of isozymes and other proteins on plant phenotypes is usually neutral and is often expressed co-dominantly, making it possible to discriminate between homozygote and heterozygote. A major limitation of biochemical markers is the limited number of protein and isozyme markers available (small number of loci).

Therefore, the genetic information found is not representative of genes throughout the genome (White et al., 2007). However, the use of morphological and biochemical markers has limitations. First, they also have environmental effect and second they change with the stage from seedling to the adult stage of the plant (Delgado et al., 2006). Such changes do not happen with DNA molecular markers. For that reason, DNA molecular markers are much more desirable to carry out genetic studies.

1.2.12.1 DNA Markers

Molecular or DNA markers are not considered genes. They usually do not have a biological effect; they are identifiable DNA sequences that are transmitted from one generation to another by the standard laws of inheritance. They are defined as a "DNA sequence that is readily detected and whose inheritance can be easily monitored" (Kumar et al., 2009). Also, "Genetic markers are DNA sequences with known physical locations on chromosomes. They are points of variation that can be used to identify individuals or species, or may be used to associate an inherited disease with a gene through genetic linkage with nearby but possibly unidentified or uncharacterised genes" (http://www.nature.com/subjects/genetic-markers).

Two groups of DNA markers were used initially: restriction-based DNA markers and amplification-based DNA markers. Restriction-based markers involve digestion of genomic DNA with restriction enzymes followed by the hybridization of specific the DNA segment with probes for visualization. Restriction-fragment length polymorphism (RFLPs) is one such type of marker system. The major limitations of restriction-based marker systems are the need for base knowledge of the DNA segment to create the probes. The amplification-based marker systems make use of the polymerase chain reaction (PCR). The PCR technique was developed by Kary Mullis in 1983 and it involves amplification or reproduction of a copy of a DNA segment to

produce an exponential number of copies of the segment. The PCR uses a set of specific primers for the segment (generally a short segment of 18-22 base pairs), a mix of four deoxynucleotides (dNTPs), the sample (template) DNA and the enzyme DNA polymerase in a buffer mix. The enzyme DNA polymerase was first extracted from the thermophile bacteria *Thermus aquaticus* resulting in the generic name of Taq polymerase. PCR also needs specific cycles of a combination of different temperatures (thermocycling) to carry out the 3 main steps: denaturation, annealing, and amplification that are repeated for a number of cycles.

Among the markers developed using PCR are AP-PCR (arbitrary primers-PCR) and RAPDs (Random Amplified Polymorphic DNA) (Caetano-Anollés and Bassam, 1993); both are similar in nature as both use random or arbitrary primers to amplify random segments. An amplified fragment length polymorphism (AFLP) marker system (Vos et al., 1995) involves restriction digestion and selective amplification of the restricted segments with the use of the cut sequence and adapters. Inter-microsatellite sequence repeat (ISSR) (Zietkiewicz et al., 1994) and simple sequence repeats (SSRs or microsatellites) (Hearne et al., 1992) markers are based on the discovery of the microsatellites which are segments of repeated nucleotides in tandem and for which flanking regions were very well preserved during evolution. Other markers with good potential include inverse sequence-tagged repeats (ISTR), which are based on retro-transposon sequences (Rohde, 1996; Anzizar et al. 2011). Diversity array technology (DArT) markers are based on random DNA segments extracted from individuals belonging to the same species. The segments are cloned in bacteria and the polymorphic segments are then used to create an array. The array is used to genotype or evaluate the polymorphism of populations from the same species (Akbari et al., 2006). Segments are scored based on presence/absence to create a matrix that can be used in the same way as other markers (Jaccoud et al., 2001). Single nucleotide

polymorphisms or SNPs (Gu et al., 1998; Brookes, 1999) are single base differences between DNA sequences of individuals in a population.

1.2.12.2 Single nucleotide polymorphism

Single nucleotide polymorphisms or SNPs (Gu et al., 1998) are single base differences between DNA sequences of individuals of the same species. With the availability of next generation sequencing or high-throughput sequencing, single nucleotide polymorphisms (SNPs) have rapidly become the molecular marker of choice for a number of genotyping applications. Furthermore, SNPs are becoming more accessible due to the availability of genome sequence data (Batley and Edwards, 2009). SNPs are loci where the sequence of DNA differs by a single base pair. They are the most abundant markers, which makes them ideal for genotyping; further, these are stable markers in both animal and plant genomes. The frequency of SNP in plants has been estimated from around 1/21 to 1/8500 bases (Kanazin et al., 2002; Bundock and Henry, 2004; Rostoks et al., 2005) in bread wheat the frequency is around 1SNP/540bp (Somers et al., 2003). SNPs can be found in coding and non-coding regions. Not all SNPs are useful markers. Those found in promoters and expressed regions can directly affect the gene function and are, therefore, useful to create 'perfect' molecular markers (Paris et al., 2003). Therefore, the development of markers using high-throughput sequencing methods has become useful for finding markers related to important traits in plants.

Their abundance allows the construction of high-density genetic maps offering the potential to detect associations between allelic forms of a gene and a specific phenotype. Also, they are becoming more affordable and effective with the development of high throughput SNP arrays. SNPs are bi-allelic (Vignal et al., 2002), but are as or more informative than multi-allelic

markers (i.e., STS) due to their abundance, which compensates the bi-allelism. Another advantage of SNPs is that they have much lower mutation rate than STSs (Syvänen, 2001).

In general, DNA markers can be useful in population genetics, mapping, genetic diversity analysis, genetic distance, phylogenetic analysis and other uses. The discovery of the genetic basis of economically important quantitative traits in plants, such as flowering time, height and yield, has been a key motivation for breeders and geneticists aiming to improve crops and understand plant adaptation.

1.2.13 Genetic mapping

Genetic mapping is the process of determining the order of and relative distance between genetic markers (specific sequences or heritable elements that generate a phenotype) on a chromosome based on their pattern of inheritance. Genetic maps are tools used to identify phenotypes linked to specific DNA sequences and are useful tools for comparative mapping, high resolution mapping, map-based cloning of genes and marker assisted selection (Lehmensiek et al., 2009). The major use of genetic linkage maps has been to identify quantitative trait loci (QTL) which has contributed to our understanding of the genetic basis of quantitative traits (Mackay et al., 2009).

1.2.14 QTL discovery

Quantitative trait loci are "the regions of the genome underlying quantitative traits" (http://www.nature.com/scitable/definition/qtl-quantitative-trait-locus-quantitative-trait-loci-319). In plants as well as other organisms of agricultural importance, most economically important traits are quantitatively inherited, meaning they are polygenic or influenced by multiple genes or QTL (Tanksley, 1993). QTLs generally cover a segment of DNA measuring

few centimorgans (cM) that has been statistically correlated with a variation in the phenotype of a specific quantitative trait (e.g. height, weight, etc.). Therefore, the phenotype is controlled by a group of genes contained in this specific DNA sequence or by genes linked to this DNA segment.

The genetic linkage maps are constructed using molecular markers; the maps contain the position and distance calculated in cM between those markers which is calculated using the recombination frequency or crossover between those markers and obtaining a graphical representation of the markers on the chromosome (Collard et al., 2005; Lehmensiek et al., 2009). The main steps to constructing a map are:

i) Development of a mapping population
ii) Assessment of polymorphisms
iii) Genotyping with polymorphic markers
iv) Linkage analysis

A segregating population is developed by crossing "parents" showing extreme phenotypes for the traits of interest (Young, 1996) and displaying genetic polymorphism. Mapping populations like near isogenic lines (NIL), double haploid populations (DH), recombinant inbred lines (RIL) or F_2 have been used. The first 3 populations are the preferred as the individual genotypes in these populations are homozygous and can be tested in different environments, while the F_2 population can neither be replicated non planted in different environment or re-used for validation.

Linkage maps can be constructed manually but are generally generated with computer software such as MapMaker (Lander et al., 1987), JoinMap (Stam, 1993), PLABQTL (Utz and Melchinger,

1996), MapManager (Manly et al., 2001), Multipoint (Mester et al., 2003), QTLCatrographer (Basten et al., 2005) or IciMaping (Meng et al., 2015) also can be programmed in R with the package QTL (Broman et al., 2003).

The preferred markers for genotyping for a long time were simple sequence repeat (SSR) markers because of their abundance and distribution genome-wide, but RFLP, RAPD, AFLP, SNP, and DArT markers have also been used for QTL discovery (Lehmensiek et al., 2009)

There are different statistical methods to test if a trait is linked to a marker or not. Single marker analysis (SMA), also known as single point marker analysis, is the simplest QTL analysis. It involves ANOVA and linear regression (Hackett, 2002), likelihood analysis (Doerge et al., 1997) or t-tests (Collard et al., 2005). The markers are scored in two classes (one for each parental type) and the phenotypic means are calculated and analyzed. If significant differences are found, then the marker is linked to the trait. These do not require a linkage map and the main limitation is that if the marker is distant from the gene of interest, it will be harder to detect due to the recombination between marker and gene (Tanksley, 1993; Lehmensiek et al., 2009).

To overcome the disadvantage of SMA, a new method involving the creation of a linkage map called interval analysis or interval mapping (IM) was developed (Lander and Botstein, 1989). This method works well when the markers are far apart but yields similar results to SMA if the markers are closer (Tanksley, 1993).

Composite interval mapping (CIM) and inclusive composite interval mapping (ICIM) are other and more refined methods for QTL detection (Zeng, 1994; Wang et al., 2009; Chen, 2016a)

The main limitations of QTL analysis are:

- High environmental impact that quantitative traits have, therefore environmental change can bias or change the results for QTL.
- A long time and/or high cost to develop the biparental populations.
- The additive effect is detectable but does not provide information on the dominance relationship for any QTL (Haley and Andersson, 1997).
- Limited genomic resolution (generally few cM). This limitation can be overcome or improved by the use of high density and even distributions of markers through the species genome works better.

1.2.15 Genome-wide association study (GWAS)

Genome-wide association studies (GWASs) are unbiased genome screens of unrelated individuals and appropriately matched controls or affected parent-offspring trios to establish whether any genetic variant is associated with a trait. These studies typically focus on associations between single-nucleotide polymorphisms (SNPs) and major diseases (Nature 2016 http://www.nature.com/subjects/genome-wide-association-studies)

Genome-wide association (GWAS) overcomes some of the QTL analysis limitations by providing higher resolution, sometimes even at the gene level (Brachi et al., 2010). GWAS is able to use "well-studied populations in which commonly occurring genetic variations can be associated with phenotypic variation, allowing whole-genome scans to identify often small haplotype blocks that are significantly correlated with quantitative trait variation" (Brachi et al., 2010).

The use of single nucleotide polymorphism (SNP) markers, in conjunction with statistical approaches for association mapping (AM) based on ancient recombination (Gupta et al., 2005; Chen, 2016b)provides dense genome coverage, decreases genotypic errors, and allows the accurate identification of loci (Lorenz et al., 2012). The term is usually confounded with linkage disequilibrium (LD), which refers to non- random association between two markers (alleles at different loci), two genes, two QTLs, a gene and a QTL. Thus, association mapping is one of the uses of LD (Chen, 2016b).

Before quantitative genetics reached the current point, it was assumed that complex traits were controlled by a large number of genes with small and equal effects (Hill, 2010; Mäki-Tanila and Hill, 2014). Presently, it is believed that complex traits are controlled by a few to several loci, but the effects of those loci are not equal. Sometimes the genetic variation in a population can be explained by a few QTLs, each contributing moderately to large effects. It is also known that a plant phenotype is a modest predictor of its real or maximum genetic potential (Tanksley and Nelson, 1996).

Association mapping is divided into:

i) candidate- gene association mapping (relates polymorphism in selected candidates genes that are supposed to control phenotypic variation for specific traits)

ii) genome-wide association (GWAS), or genome scan which screen the genome to find signals of MTAs for traits of interest (Risch and Merikangas, 1996).

Similar to other techniques, association mapping also has limitations:

- local families, can be detected only when sampling is adequate at the local level

- allelic heterogeneity, the phenomenon in which multiple functional alleles of the same gene exist and are associated with different phenotypes, is common, especially in wide population samples
- single marker approaches suffer from genetic heterogeneity when multiple major loci are involved and in linkage disequilibrium (LD) with each other
- variation resulting from epistatic interactions between genes might go undiscovered because epistasis can only be investigated practically in a sequential scan of major common loci and the genome
- epigenetic variation, which requires sophisticated genotyping, is likely to be a source of missing heritability

Genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping are two powerful approaches allow to help discover genomic regions involved in controlling complex traits (Risch and Merikangas, 1996; Korte and Farlow, 2013). In wheat, the traditional QTL mapping approach might locate genomic regions with low resolution in bi-parental populations generally obtained with the intention to study specific traits, while GWAS takes advantage of historical recombination events to elucidate the loci encoding the traits (Zhu et al., 2008).

Table 1.1 Wheat classes in western Canada and their uses. (https://www.grainscanada.gc.ca/wheat-ble/classes/classes-eng.htm)*

Wheat Class	Uses
Canada Prairie Spring Red (CPSR**)**	Hearth bread, flat bread, steamed bread, noodles
Canada Prairie Spring White (CPSW**)**	Flat bread, noodles, chapatis
Canada Western Amber Durum (CWAD**)**	Semolina for pasta, couscous
Canada Western Extra Strong (CWES**)**	Ideal for blending, used in specialty products when high gluten strength is needed
Canada Western Hard White Spring (CWHWS**)**	Bread and noodle production
Canada Western Red Spring (CWRS**)**	Used for production of high volume pan bread , used alone or in blends with other wheat for hearth bread, steamed bread, noodles, flat bread, common wheat pasta
Canada Western Red Winter (CWRW**)**	French bread, flat bread, steamed bread, noodles
Canada Western Soft White Spring (CWSWS**)**	Cookies, cakes, pastry, flat bread, noodles, steamed bread, chapatis
Canada Northern Hard Red (CNHR**)**	Lower protein and gluten strength than CWRS but higher yields
Canada Western Special Purpose (CWSP**)**	High yielding; suited for ethanol production and livestock feed

*Canadian grain commission. Canadian wheat classes

Table 1.2 Races of Ptr based in the presence absence of Prt ToxA, B and C (Lamari and Strelkov, 2010) .

Ptr ToxA	Ptr ToxB	Putative Ptr ToxC	Ptr Race
A+	B -	C+	1
A+	B-	C-	2
A-	B-	C+	3
A-	B-	C-	4
A-	B+	C-	5
A-	B+	C+	6
A+	B+	C-	7
A+	B+	C+	8

Legend: + presence, - absence

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Chapter 2 QTLs associated with agronomic traits in the Cutler × AC Barrie spring wheat mapping population using single nucleotide polymorphic markers[1]

2.1 Introduction

Global hexaploid wheat (*Triticum aestivum* L.) production increased from 627 million t in 2005 to 729 million t in 2014 (http://faostat.fao.org). Canada is the seventh largest wheat-producing and the second largest wheat-exporting country. Average wheat yield in Canada has increased from 2.7 t ha^{-1} in 2005 to 3.1 t ha^{-1} in 2014, which is equivalent to an average yield increment of 35.7 kg ha^{-1} yr^{-1}. Diseases and drought contribute to substantial reductions in overall wheat yields in Canada. Wheat breeders there aim to develop short, early maturing cultivars, with high grain yield and protein content, combined with resistance to major diseases, leaf, stem and yellow rusts caused by *Puccinia* sp., fusarium head blight caused by *Fusarium graminearum* and common bunt caused by both *Tilletia tritici* and *T. laevis* (http://www.pgdc.ca).

Wheat breeders, in addition to phenotypic selection, employ molecular markers in their breeding programs for different purposes, including parental selection, quality control analysis of advanced lines (cultivars) on genetic purity and identity, and for marker-assisted selection (MAS) (Randhawa et al., 2013). The traditional method of exploiting molecular markers in MAS usually involves finding a subset of markers that are significantly associated with one or more genes or quantitative trait loci (QTLs) regulating the expression of a trait of interest. Both linkage analysis and association mapping can be used to identify significant marker-trait associations, with each method having its own strength and weaknesses (Collard et al., 2005; Semagn et al., 2010). Linkage-based QTL analysis depends on well-defined populations, such as

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F_2 or their derivatives, backcross (BC) , doubled haploid (DH), recombinant inbred lines (RILs), and near isogenic lines (NILs) (Collard et al., 2005). RILs, NILs and DHs are homozygous or 'true-breeding' lines that can be multiplied and used for multi-location phenotyping. Seeds from RILs, NILs and DHs can be exchanged between different collaborators to facilitate phenotyping and genotyping activities (Collard et al., 2005). The main limitations of NIL and RIL include (i) the long time and/or high cost required to develop these populations, and (ii) these populations only detect the additive component but provide no information on dominance relationships for any QTL (Haley and Andersson, 1997). Several mapping studies associated with grain yield and other agronomic traits have been conducted for many years (Huang et al., 2004; Huang et al., 2006; Kumar et al., 2007; Bennett et al., 2012; Kamran et al., 2013; Yu et al., 2014; Asif et al., 2015; Chen et al., 2015).

Recently, our group mapped QTLs associated with flowering, maturity, plant height and grain yield in a RIL population derived from the cross of two spring wheat cultivars, 'Cutler' and 'AC Barrie' (Kamran et al., 2013). The population was phenotyped in replicated field trials in four environments between 2007 and 2011, and genotyped with 488 microsatellite or simple sequence repeat (SSR) and diversity arrays technology (DArT) markers. The former study uncovered seven QTLs on chromosomes 1B, 1D, 4A and 5B, of which only three QTLs were associated with the phenotypic data combined across all four environments. This included *QEps.dms-1B1* for both flowering and maturity, *QEps.dms-1B2* for maturity and *QEps.dms-5B1* for flowering time. One drawback of that study was low marker density (low genome coverage), which varied from 2 on chromosomes 4D and 6D to 57 on chromosome 2B, with an average of 23 markers per chromosome. DArT markers enable the simultaneous typing of several hundred polymorphic loci spread over the genome (Jaccoud et al., 2001; Wenzl et al., 2004; Semagn et al., 2006), but the

dominant inheritance (present *vs.* absent variation) of DArT markers is one of the major drawbacks, as homozygous dominant and heterozygous individuals cannot be easily identified. SSR markers are commonly used by wheat researchers because they are widely available, co-dominant, multiallelic, highly polymorphic, generally repeatable and uniformly distributed in the genome (Gupta et al., 2002). As differences in SSR allele size are often difficult to resolve on agarose and polyacrylamide gels, high resolutions can be achieved through the use of DNA sequencers. However, one of the challenges of SSR markers is the difficulty in comparing data produced by different laboratories or the same lab at different times, due to the eventuality of inconsistencies in allele size calling. Such inconsistencies are mainly due to differences in SSR marker repeat length, and the large variety of automatic sequencing machines used for fragment analyses, each providing different migration, fluorescent dyes, and allele calling software (Vignal et al., 2002). Furthermore, SSR markers have low throughput that involve a high genotyping cost per marker, not easily amenable for automation and low cost genotyping.

Single nucleotide polymorphism (SNP) refers to a single base change in a DNA sequence, with a usual alternative of two possible nucleotides at a given position (Vignal et al., 2002). SNPs have emerged as powerful tools for many genetic applications because they have low assay cost, high genomic abundance, locus-specificity, co-dominant inheritance, simple documentation, potential for high throughput analysis and relatively low genotyping error rates (Rafalski, 2002; Schlotterer, 2004). High throughput SNP discovery and genotyping in wheat lags behind rice and maize due to the complex wheat genome and the lack of a reference genetic map. However, next generation sequencing technology used for the International Wheat Genome Sequencing Consortium (IWGSC) offers an alternative opportunity for conversional sequencing and amplification (www.wheatgenome.org). Currently, a total of 90,000 (90K) gene-associated SNPs

are available for wheat researchers through the iSelect platform. The consensus genetic position of 43,999 of the 90K SNPs was determined using eight mapping populations (Wang et al., 2014), which has provided a tremendous opportunity for wheat researchers conducting research requiring high marker density. The objectives of the present study were therefore to: 1) identify genomic regions associated with flowering time under greenhouse and field conditions, and maturity, plant height and grain yield under field conditions in the 'Cutler' × 'AC Barrie' RIL population using the 90K Illumina iSelect SNP array; and 2) compare the results with a previous study on the same population using 488 microsatellite and DArT markers

2.2 Materials and methods

2.2.1 Plant material and phenotyping

The present study was based on a subset of 158 of the 177 RILs used in our previous study (Kamran et al., 2013). The RILs were derived at the University of Alberta using a single seed descent approach from a cross between two spring wheat cultivars, 'Cutler' and 'AC Barrie' (Iqbal et al., 2006). 'AC Barrie' is characterized as having high protein content, late maturity (compared to 'Cutler') and resistance to some diseases (McCaig et al., 1996). 'Cutler' is an early maturing and semi-dwarf cultivar from the Canadian Prairie Spring class, and possesses dominant *VrnA1a*, recessive *vrn-B1* and *vrn-D1* vernalization alleles at *Vrn1* loci, and the photoperiod insensitive allele *Ppd-D1a*. 'AC Barrie' possesses the same vernalization genes as 'Cutler' with the photoperiod sensitive allele *PpdD1b*. 'Cutler' and 'AC Barrie' have the mutant *Rht-D1b and* wild type *Rht-D1a* alleles, respectively.

The 158 RILs and the two parents were evaluated five times for flowering time, maturity, plant height and grain yield under field conditions and twice for flowering time under greenhouse conditions (Kamran et al., 2013). Briefly, the RIL population and the parents were phenotyped

under field conditions in 2007, 2008 (early and late planting), 2011 and 2012 at the University of Alberta South Campus Crop Research facility (53°19'N, 113°35'W), Edmonton, Canada. Seeds from the $F_{6:7}$ were initially used for phenotyping in 2007; subsequent phenotyping trials were conducted using seeds multiplied from bulk harvest of typical heads of the previous year. Each field trial was conducted in a randomized incomplete block design with two to three replications depending on seed availability. All field trials were conducted in rain fed conditions using standard agronomic and cultural practices recommended for the station. Each RIL was evaluated for number of days to 50% flowering, maturity, plant height and grain yield. Days to flowering and maturity were converted into growing degree days by summing the average daily temperatures (over a base temperature of 0 ^{o}C) from the date of seeding to the date when flowering or maturity was recorded (Kamran et al., 2013). The RILs and the parents were also evaluated in a randomized incomplete block design with four replications under greenhouse conditions for flowering time in 2006 and 2008 as described in our previous study (Kamran et al., 2013). All except the 2012 phenotype data used in the present study are the same as our previous study.

2.2.2 DNA extraction and genotyping

Genomic DNA was extracted from three week old seedlings using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1987). DNA concentration was measured using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, USA), and normalized to about 100 ng/µL. The DNA samples were genotyped at the University of Saskatchewan Wheat Genomics Lab, Saskatoon, Canada, with a 90K Illumina iSelect SNP array (Wang et al., 2014). Alleles were called with the Illumina Genome Studio Polyploid Clustering version 1.0 software (Illumina, San Diego, USA). Because of the polyploidy nature of bread

wheat, all SNPs with more than three clusters (genotypes), those that exhibited ambiguity in discriminating the expected three genotypes (AA, AB, and BB) for a bi-allelic SNP, and those with very weak signal (weak amplification) were excluded from scoring.

We also genotyped the RIL population and the parents with two gene specific functional markers (*Ppd-D1* and *Rht-D1)* at the Agricultural Genomics and Proteomics Lab, University of Alberta, Edmonton, Canada. PCR analysis was performed in 96-well plates in a total reaction volume of 10 µL that consisted of 50 ng DNA, 1× magnesium-free PCR buffer, 1.5 mM $MgCl_2$, 0.50 µM of each of the forward and reverse primer, 0.20 mM of each dNTP, and 1 unit GoTaq® Flexi DNA polymerase. All PCR components were purchased from Promega, USA. PCR amplifications were performed using a Gene-Amp PCR System 9600 (PE-Applied Biosystems) as follows: 3 min initial denaturation at 94°C , followed by 40 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec, and a final extension of 7 min at 72°C. PCR fragments were separated with QIAxcel advanced (Qiagen, USA) as described in the user's manual using a fast analysis kit (with 50 bp to 1.5 kb QX DNA size marker, 15 bp to 3 kb QX alignment marker, and DM150 analysis method).

2.2.3 Statistical analyses

Least square means, F statistics and heritability were obtained using PROC MIXED and PROC IML in SAS version 9.3 (SAS Institute Inc. Cary, USA). We analysed each trial (environment) separately and then combined data across all environments. Genotypes were considered fixed, while replications, blocks and years were considered random. For each trait, both tests for normality and the frequency distribution were done using MiniTab v14. All SNPs that were monomorphic between the two parents and those with >20% missing data were excluded from linkage mapping. Linkage maps for the remaining SNPs were constructed in two steps. First,

'draft' linkage maps were generated using the minimum spanning tree map (MSTMap) software (Wu et al., 2008) using a stringent cut-off p-value of 1^{-10} and a maximum distance between markers of 15 cM. Second, the 'draft' maps were refined using the MapDisto version 1.7.5 software (Lorieux, 2012) using a cut off recombination value of 0.35, a minimum LOD score of 3.0 and a Kosambi mapping function (Kosambi, 1944). The best order of markers was generated using both "AutoCheckInversions" and "AutoRipple" commands. Linkage groups were assigned to chromosomes based on existing high density SNP maps of wheat (Cavanagh et al., 2013; Maccaferri et al., 2014; Wang et al., 2014).

Composite interval mapping (CIM) was performed on the least square means of each trait using PLABQTL version 1.2 (Utz and Melchinger, 2003) with the following parameters: a minimum LOD score of 3.0, automatic cofactor selection, walking speed of 1 cM, a model to determine additive effects at individual QTL and additive x additive epistatic interactions, and a F-to-Enter value of 10 (Semagn et al., 2007). QTL names were designated following the International Rules of Genetic Nomenclature (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm). In this study, QTLs that explained <10%, 10-20% and >20% of the total phenotypic variation (R^2) were arbitrarily classified into minor, moderate and major effect QTLs, respectively. Genetic maps and QTL graphs were drawn using MapChart v2.1 (Voorrips, 2002).

2.3 Results

2.3.1 Summary of the phenotypic traits and markers

'Cutler' flowered/matured 2.6 days earlier and was 12.9 cm shorter, but produced 154.9 kg ha^{-1} lower grain yield than 'AC Barrie'. The average plant height among the 158 RILs varied from 66 to 104 cm, and required between 49 and 58 days for flowering and between 91 and 101 days for maturity. Mean grain yield of the RILs varied between 4.6 and 7.4 t ha^{-1} (Appendix 1). Broad sense heritability was 0.27 for grain yield, 0.43 for number of days to flowering, 0.48 for degree days to flowering, 0.50 for number of days to maturity, 0.46 for maturity in degree days, and 0.80 for plant height. Analysis of variance showed highly significant ($p < 0.001$) differences among genotypes for all traits (Appendix 1). The distribution of least square means estimated from the combined phenotype data of all environments was normal or approximately normal ($P \geq 0.073$) for flowering time, maturity, and grain yield. However, the Shapiro-Wilk test rejected the hypothesis of normality ($P = 0.010$) for plant height, which showed an approximately bimodal distribution (Figure 2-1) than a more quantitative frequency distribution.

Among the 90K SNPs used for genotyping the RIL population, approximately 91% (81,587 SNPs) were scored, but 87.3% of the scored SNPs (71,245 out of the 81,587 SNPs) were discarded for a number of reasons, including a lack of polymorphisms between the two parents, heterozygosity in one or both parents, high amount (>20%) of missing data, very high segregation distortion, and lack of linkage with other markers. The remaining 10,342 SNPs (12.7%) were incorporated into the genetic linkage maps of the 21 chromosomes. However, many SNPs mapped at exactly the same position, so they were excluded from the final dataset. Hence, only 1,809 of the 81,587 scored SNPs (2.2%) and two gene-based functional markers (*Rht-D1b* and *Ppd-D1a*) were used for QTL analyses, which are summarized in Table 2-1. The

number of markers retained for QTL mapping varied from 9 on 5D to 221 on 5B, with an average of 86 markers per chromosome. The total map length across the 21 chromosomes was 3996 cM, with each chromosome varying in length from 22.3 cM on 5D to 373.7 cM on 5A. Map distance between adjacent markers (inter-marker interval) varied from 0.1 to 32.5 cM (Figure 2-2) and the overall average was 2.2 cM.

2.3.2 QTL analyses

Composite interval mapping (CIM) was performed on the least square means estimated for individual environments and also combined across two environments for flowering time under greenhouse and five environments for flowering time, maturity, plant height and grain yield under field conditions. Table 2-2 and Appendix 2 show summary of the QTL results for the combined and individual environments, respectively. We found 5 QTLs associated with the two years combined flowering time data under greenhouse, which altogether explained 73.1% of the phenotypic variance. The five QTLs for flowering time under greenhouse conditions mapped at the proximal end of chromosome 2D (*QFlt.dms-2D*), at 187 cM on 5A (*QFlt.dms-5A.1*), at 44 cM on 5B (*QFlt.dms-5B*), at 59 cM on 6B (*QFlt.dms-6B.1*) and at 5 cM on 7A (*QFlt.dms-7A.1*). The proportion of phenotypic variance explained by each flowering time QTL in the greenhouse varied from 6.9% for *QFlt.dms-5A.1* to 36.6% for *QFlt.dms-2D*. *QFlt.dms-2D* is the only major effect QTL for flowering time under greenhouse, flanked by the known photoperiod insensitive allele *Ppd-D1a* and a SNP marker (wsnp_CAP11_c3842_1829821) (Table 2-2). For the combined phenotype data across all five environments conducted under field conditions, there were 4-6 QTLs for flowering time, 4-5 QTLs for maturity, one QTL for plant height, and two QTLs for grain yield (Table 2-2). We found four QTLs (*QFlt.dms-2D*, *QFlt.dms-3B*, *QFlt.dms-*

6B.2 and *QFlt.dms-7A.1*) associated with days to flowering (the original) and six QTLs (*QFlt.dms-2D*, *QFlt.dms-3B*, *QFlt.dms-6B.2*, *QFlt.dms-7A.1*, *QFlt.dms-4A.1* and *QFlt.dms-5A.2*) for degree days to flowering. Four of the QTLs for flowering time under field conditions (*QFlt.dms-2D*, *QFlt.dms-3B*, *QFlt.dms-6B.2* and *QFlt.dms-7A*) were common between the number of days and degree days. For maturity, we found four QTLs (*QMat.dms-2D*, *QMat.dms-4A.2*, *QMat.dms-4D.1* and *QMat.dms-7A.2*) for the number of days to maturity and five QTLs (*QMat.dms-2D*, *QMat.dms-4A.1*, *QMat.dms-4D.2*, *QMat.dms-7A.1* and *QMat.dms-7A.2*) for maturity in degree days, but only two QTLs (*QMat.dms-2D* and *QMat.dms-7A.2*) were common between the two datasets. To account for the difference in temperature across years, therefore, we only present details for the QTLs associated with flowering time and maturity in growing degree days.

The total phenotypic variance explained by all QTLs associated with flowering time and maturity in degree days, plant height and grain yield across the combined data of the five environments was 74.8, 63.7, 37.8 and 16.3%, respectively. All QTLs associated with each trait exhibited mainly additive effects and QTL by QTL interactions were negligible ($R^2 < 1.5\%$). The six QTLs associated with flowering time (in degree days) mapped at the proximal tip of 2D (*QFlt.dms-2D*), at 94 cM on 3B (*QFlt.dms-3B*), at 41 cM on 4A (*QFlt.dms-4A.1*), at 253 cM on 5A (*QFlt.dms-5A.2*), at 118 cM on 6B (*QFlt.dms-6B.2*) and at 9 cM on 7A (*QFlt.dms-7A.1*). Each QTL individually explained between 6.3 and 25.4% of the phenotypic variance across the five environments (Table 2-2); with *QFlt.dms-2D* as the only major effect QTL for flowering time under field conditions. The favorable alleles for all flowering QTLs except *QFlt.dms-3B* and *QFlt.dms-5A.2* originated from 'Cutler'. Lines that were homozygous to the favorable alleles at the two flanking markers of each flowering QTL showed a reduction of 13.8 to 21.1 degree

days in flowering time compared with those lines that were homozygous to the unfavorable alleles. When individual environments were considered (Appendix 2), *QFlt.dms-2D* was consistently detected in four of the five environments, followed by *QFlt.dms-7A.1* in two environments. The other QTLs were identified only in a single environment.

The five QTLs associated with maturity in degree days across the combined data were located at the proximal tip of 2D (*QMat.dms-2D*), at 41 cM on 4A (*QMat.dms-4A.1*), at 37 cM on 4D (*QMat.dms-4D.2*) plus at both 13 and 42 cM on 7A (*QMat.dms-7A.1* and *QMat.dms-7A.2*). Each maturity QTL explained between 9.1 and 16.2% of the phenotypic variance across all combined environments (Table 2-2). The favorable alleles for *QMat.dms-4D.2 and QMat.dms-7A.2* originated from 'AC Barrie', while those for *QMat.dms-2D*, *QMat.dms-4A.1* and *QMat.dms-7A.1* originated from 'Cutler'. RILs that were homozygous for the favorable alleles at the two flanking markers of each QTL showed a reduction in maturity from 11.2 to 33.0 degree days compared with those lines that were homozygous for the unfavorable alleles. When results from individual environments were considered, only *QMat.dms-2D* and *QMat.dms-4D.2* were detected in two and three environments, respectively (Appendix 2); the other QTLs were detected either in a single environment or only in the combined environments (but not in any of the individual environments).

For plant height, we found a single major effect QTL that mapped at 37 cM on chromosome 4D (*QPht.dms-4D*), flanked by a height reducing *Rht-D1b* gene and *wsnp_CAP11_c356_280910*. This QTL had a LOD score of 16.2 and accounted for 37.8% of the phenotypic variance for plant height across the combined data of the five environments. RILs that were homozygous to the 'Cutler' alleles at the two flanking markers were on average 13.2 cm shorter than those lines that were homozygous to the 'AC Barrie' allele. When individual environments were considered,

QPht.dms-4D1 was consistently detected within the same confidence interval in all five individual environments (Appendix 2). The LOD score and phenotypic variance explained by this QTL on individual environments varied from 13.2 to 18.8% and from 30.8 to 38.5%, respectively, which is equivalent to a reduction in plant height of 10.7 to 14.3 cm. For grain yield, we found two QTLs at the proximal tip on 2D (*QYld.dms-2D*) and at 34 cM on 5B (*QYld.dms-5B*), which explained 7.7 to 8.6% of the phenotypic variance for grain yield across five environments. RILs that were homozygous to the 'AC Barrie' alleles at the two flanking markers of *QYld.dms-2D* and *QYld.dms-5B* produced on average 436.0 and 321.8 kg ha^{-1} more grain yield than those RILs that were homozygous to the 'Cutler' allele. When individual environments were considered, each QTL was detected only in a single environment (Appendix 2).

2.3.3 Coincident QTLs

Four of the 19 QTLs associated with the combined phenotype data of the four traits were common (coincident) for two or three traits. The first coincident QTL is the one that mapped at the proximal tip on chromosome 2D, which is associated with flowering time both under greenhouse and field conditions (*QFlt.dms-2D*), maturity (*QMat.dms-2D*) and grain yield (*QYld.dms-2D*). As in our previous study (Kamran et al., 2013), coincident QTLs for both flowering and maturity time belong to earliness *per se* QTL; hence, both *QFlt.dms-2D* vs *QMat.dms-2D* are named *QEps.dms-2D*. *QEps.dms-2D* explained 36.6% and 25.4% for flowering time under greenhouse and field conditions, respectively, 10.4% for maturity, and 8.6% for grain yield. RILs carrying the 'Cutler' alleles at the two flanking markers for *QEps.dms-2D* have differed from those containing 'AC Barrie' alleles for flowering time ($p < 0.012$), maturity ($p < 0.050$) and grain yield ($p < 0.001$), but not for plant height (Appendix 3).

On average, therefore, RILs homozygous to the 'Cutler' allele at the two flanking markers of the *QEps.dms-2D* flowered/matured 1.1-5.4 days earlier, but suffered a yield penalty of 436 kg ha^{-1} relative to those RILs that were homozygous to the 'AC Barrie' allele (Table 2-2). The second coincident QTL mapped at 41 cM on 4A and it was associated with both flowering time (*QFlt.dms-4A.1*) and maturity (*QMat.dms-4A.1*), here referred as *QEps.dms-4A.1*. RILs carrying 'Cutler' alleles at the two flanking markers for *QEps.dms-4A.1* differed ($p < 0.005$) from those containing 'AC Barrie' alleles for flowering time under field conditions and maturity, but not for plant height or grain yield (Appendix 3). Homozygous RILS carrying the 'Cutler' allele at the two flanking markers for *QEps.dms-4A.1*flowered/matured 17.6-22.7 degree days earlier than those RILs that were homozygous to the 'AC Barrie' allele (Table 2-2). The third coincident QTL was on 4D and it was associated with both maturity (*QMat.dms-4D.2*) and plant height (*QPht.dms-4D*). RILs carrying the 'Çutler' alleles at the two flanking markers of the coincident QTL on 4D showed significant differences ($p < 0.001$) with those containing 'AC Barrie' alleles for maturity and plant height, but not for flowering time and grain yield (Appendix 3). RILs that were homozygous to the 'Cutler' allele at the two flanking markers of *QMat.dms-4D.2* and *QPht.dms-4D* were 13.2 cm shorter, but took 33 degree days longer to mature than those RILs that were homozygous to the 'AC Barrie' allele (Table 2-2). Finally, the QTL that mapped between 8 and 16 cM on 7A was the third earliness *per se* QTL (*QEps.dms-7A*), associated with both flowering time (*QFlt.dms-7A.1*) and maturity (*QMat.dms-7A.1*), with RILs homozygous to the 'Cutler' allele at the two flanking markers showing a reduction in flowering/maturity by 11.2-15.9 degree days related to those RILs that were homozygous with the 'AC Barrie' allele (Table 2-2). However, RILs carrying the 'Çutler' alleles at the two flanking markers for *QEps.dms-7A* were different from those containing 'AC Barrie' alleles only for flowering time

under field conditions ($p < 0.001$), but not for maturity, plant height and grain yield (Appendix 3).

2.4 Discussion

2.4.1 Comparison with our previous study

Based on 488 SSR and DArT markers, we previously reported three QTLs associated with the combined phenotypic data across four environments (Kamran et al., 2013), which includes one coincident QTL for both flowering time and maturity at 31-33 cM on 1B (*QEps.dms-1B1*), one QTL for maturity at 36 cM on 1B (*QEps.dms-1B2*) and one QTL for flowering time at 76 cM on 5B (*QEps.dms-5B1*). That study failed to identify any QTL for both plant height and grain yield across the combined phenotypic data of the four environments; only two environment specific QTLs were reported for grain yield. Our previous study was based on a total map length of 2,279 cM, with individual chromosomes varying from 36 to 229 cM; the overall average map distance among adjacent markers (inter-marker interval) was 4.7 cM. We thought that the low marker density and dominant inheritance of the DArT marker might have restricted our ability to identify more QTLs with larger phenotypic effects. The use of larger number of polymorphic markers provides a more accurate overview of informative recombinations and greater saturation of genetic linkage maps. The denser the genetic maps, the lower the chance of missing true QTLs (Zych et al., 2015). Our present study was based on 1809 polymorphic SNPs and two known gene-based functional markers (*Ppd-D1a* and *Rht-D1b*), which resulted in a total map length of 3996 cM and an overall average inter-marker interval of 2.2 cM. As compared with our previous study, therefore, the genome coverage in the present study increased by 78%, while average inter-marker interval decreased over two fold. Based on such higher genome coverage and reduction in map distance among adjacent markers, we expected to narrow down the confidence

interval of the QTLs that we reported in our previous study and also uncover additional QTLs that may have been missed in our previous study. In the present study, we uncovered a total of nineteen QTLs associated with the combined phenotypic data, which includes five for flowering time in the greenhouse, six for flowering time in the field, five for maturity, one for plant height, and two for grain yield (Table 2-2). However, we only identified one environment specific QTL for grain yield on 1B (*QYld.dms-1B*) and one QTL for flowering time under greenhouse on 5B, but we were not sure whether these two QTLs mapped at the same confidence interval as the three QTLs (*QEps.dms-1B1, QEps.dms-1B2* and *QEps.dms-5B1*) reported in our previous study.

In order to verify the position of the QTLs identified on 1B and 5B in the two studies, we conducted QTL analyses using a genetic map constructed by combining DArT, SSR and SNP markers on chromosomes 1B and 5B. Only 131 out of the 158 RILs had a complete DArT, SSR and SNP genotypic data. The analyses conducted on genotypic and phenotypic data of 131 RILs and combined map of the three types of markers (DArT, SSRs and SNPs) identified one of the QTLs for maturity on 1B between 74.5 and 80.5 cM interval, which accounted for 9.7-13.1% of the phenotypic variance for maturity in degree days across the combined data plus the 2007 and 2011 environments (Appendix 4). Although the genetic position for *QEps.dms-1B2* was different between the two studies (which is expected with addition of large number of SNPs into DArT and SSRs), one of the flanking DArT markers (wPt-2694) remained the same. However, the position of the QTL associated with the combined grain yield data across 5 environments (*QYld.dms-1B*) was 52 cM distal to wPt-2694, which suggests that the QTL for maturity is different from that of the QTL for grain yield. For the QTL on 5B, the analysis using combined DArT, SSR and SNP markers identified *QEps.dms-5B1*, which was reported in our previous study (Kamran et al., 2013). In the present study, *QEps.dms-5B1* was associated with flowering

time in the 2007 and maturity in the 2008 early planting environments (Appendix 4). This QTL was flanked by two DArT markers (wPt-1304 and wPt-666939), and explained between 7.6 and 11.8% of the phenotypic variance for flowering time and maturity in the individual environments. In both the previous and present studies, wPt-666939 is one of the flanking markers for *QEps.dms-5B1*. Therefore, the inclusion of the DArT markers on both 1B and 5B allowed us to identify the QTLs for earliness *per se* that we failed to detect using the SNP markers alone. In addition, the inclusion of DArT markers has also helped us to uncover three additional QTLs on 5B, which includes one coincident QTL for grain yield and plant height at 194-204 cM interval and one QTL for plant height (Appendix 4).

However, the integration of the SSR and DArT markers with the SNPs had two limitations. First, it reduced the number of RILs with complete genotypic and phenotypic data from 158 to 131. Secondly, the SSR and DArT markers affected the locus order for most of the SNPs and slightly inflated the map length. Hence, we suspected an error in the DArT genotypic data, either mislabeling and/or data coding errors during linkage map construction and QTL analyses. The second possible reason may be the use of large numbers of DArT markers in our previous study, which are primarily dominant in inheritance (Jaccoud et al., 2001; Akbari et al., 2006). The proportion of dominant DArT markers used for genotyping the 'Cutler' x 'AC Barrie' RIL population was 90.6% on 1B and 83.3% on 5B. Dominant markers produce fragments from homozygous dominant and heterozygous alleles (fragment present), but no fragment is produced from homozygous recessive alleles (fragment absent). There are several factors that could affect the absence of a DNA fragment and it is difficult for the users to be certain whether such absence of a fragment is biological or a genotyping error. Genotyping errors can be generated for all types of molecular markers at every step of the genotyping process (sampling, DNA extraction,

PCR amplification, fragment detection, scoring, and data analysis) and by a variety of factors (chance, human error, and technical artefacts) (Bonin et al., 2004). However, the genotyping error could be much higher for dominant markers, such as DArTs that have exhibited frequent errors, especially for low-grade markers (Akbari et al., 2006). We therefore present only QTL results obtained using the SNPs and the two functional markers (*Ppd-D1a* and *Rht-D1b)*.

2.4.2 Comparison with other studies

The QTLs for flowering time in the combined environments both under greenhouse and field conditions mapped on chromosomes 2D, 3B, 4A, 5A, 5B, 6B and 7A, each explaining between 6.3 and 36.6% of the phenotypic variance (Table 2-2). For maturity, we found QTLs on 2D, 4A, 4D and 7A, each explaining between 9.1 and 16.2% of the phenotypic variance across five environments. In a study conducted on four European winter wheat DH populations (Griffiths et al., 2009), the authors reported QTLs for flowering time on almost all the wheat chromosomes. In another Canadian western red spring wheat RIL population derived from a cross between 'CDC Teal' and 'CDC Go', our group has also recently reported a QTL associated with heading, flowering and maturity on chromosome 4A that accounted for 8.9-20.2% of the phenotypic variance across three environments (Chen et al., 2015). Several previous studies have reported genes and/or QTLs for both flowering time and maturity on both homeologous group 5 (Law and Worland, 1997; Yan et al., 2003) and group 2 (Worland et al., 1998; Tanio and Kato, 2007; Wilhelm et al., 2009) chromosomes. The vernalization response in bread wheat is controlled by 3 distinct *Vrn* loci (*Vrn-1*, *Vrn-2* and *Vrn-3*); *Vrn-1* genes mapped on the long arm of chromosomes 5A, 5B and 5D (Preston and Kellogg, 2008) and directly influence both flowering and maturity (Galiba et al., 1995; Dubcovsky et al., 1998). However, we are not sure whether the QTLs for flowering time that we mapped on both 5A and 5B are in the same positions as the

Vrn-1 genes, because (i) direct comparison of the genetic map positions across different studies is not possible without having either a common set of markers or physical positions; (ii) "Cutler' and 'AC Barrie' were monomorphic for the *VRN1* loci, both having the dominant *VrnA1a* and recessive *vrn-B1* and *vrn-D1* alleles (Iqbal et al., 2006).

In the present study, we found a major effect and coincident QTL on 2D for flowering time under both greenhouse and field conditions (*QFlt.dms-2D*), maturity (*QMat.dms-2D*) and grain yield (*QYld.dms-2D*). This coincident QTL is flanked by the well-known photoperiod response *Ppd-D1a* locus, and accounted from 19.6 to 36.6% for flowering time, from 10.4 to 11.2% for maturity, and 8.6% for grain yield (Table 2-2). In wheat, photoperiod response is another important factor that influences flowering time and maturity in wheat (Chen et al., 2013) and is mainly controlled by the *Ppd-1* loci on the short arms of chromosomes 2D, 2B, and 2A (Wilhelm et al., 2009). In general, the *Ppd-D1* allele for photoperiod insensitivity is considered the most potent, followed by *Ppd-B1* and *Ppd-A1* (Worland et al., 1998), but there are conflicting reports that suggests that *Ppd-B1a* could be as strong as *Ppd-D1* (Tanio and Kato, 2007). The favorable alleles for the flowering time/maturity and grain yield QTL on 2D originated from 'Cutler' and 'AC Barrie', respectively. If selection were to be made for the 'Cutler' alleles at all three traits, RILs carrying the 'Çutler' alleles at the two flanking markers showed a significant ($p < 0.05$) reduction on flowering/maturity, but suffered highly significant ($p < 0.001$) yield penalty (i.e., reduction in grain yield of 436 kg ha^{-1}). Identification of such types of coincident QTLs has been reported in several other studies (Babu et al., 2003; Lanceras et al., 2004; Quarrie et al., 2006; Pushpendra et al., 2007; Bai et al., 2013), which could be due to (i) tight linkages between genes or QTLs that regulate the expression of separate traits, but the statistical method failed to discriminate them; or (ii) pleiotropic effect, the same gene or QTL may have an effect on two or

more traits simultaneously (Tuberosa et al., 2002). In the present study, the genetic distance between the two flanking markers (*Ppd-D1a* and wsnp_CAP11_c3842_1829821) for the coincident QTL on 2D (*QFlt.dms-2D*, *QMat.dms-2D* and *QYld.dms-2D*) is 22.6 cM, which is too large. It is, therefore, highly likely that the chromosomal segments associated with this coincident QTL on 2D carry two or more genes or QTLs, which could be determined by screening larger numbers of recombinants to break up the linkage (Kolb et al., 2001).

Although the 'Cutler' and 'AC Barrie' RIL population was primarily developed to study flowering time, maturity and photoperiodism (Iqbal et al., 2006), results from our studies showed that 'Cutler' matured 2.6 days earlier and 12.9 cm shorter, but produced 154.9 kg ha^{-1} lower yield than 'AC Barrie', which clearly suggests that the same population could also be used for mapping genomic regions associated with plant height and grain yield. Our previous study, however, failed to uncover QTLs for the combined plant height and grain yield data across four environments (Kamran et al., 2013).

The present study identified a major QTL for plant height (*QPht.dms-4D*) and medium effect QTL for maturity (*QMat.dms-4D.2*) on chromosome 4D. *QPht.dms-4D* was consistently detected at the same confidence interval in all five individual environments and also combined across all environments, while *QMat.dms-4D.2* has been detected in the 2008 (both early and late planting), 2012 and combined environments. RILs carrying the 'Çutler' alleles at the two flanking markers of this coincident QTL on 4D showed highly significant differences ($p < 0.005$) with those containing 'AC Barrie' alleles for both maturity and plant height, but not for flowering time and grain yield. Depending on the data used for analyses (individual or combined environments), this coincident QTL explained from 30.9 to 38.5% and from 13.8 to 19.3% of the phenotypic variance for plant height and maturity, respectively. RILs that were homozygous for

the ‘Cutler’ alleles at the two flanking markers were on average 10.7 to 14.3 cm shorter, but required from 30.5 to 82.3 more degree days to mature than those RILs that were homozygous for the ‘AC Barrie’ alleles. Traits that showed more quantitative frequency distributions with a single peak are believed to be controlled by several QTLs, each with moderate to small individual effects, as compared to a bimodal distribution that is predominantly controlled by a single gene, clusters of tightly linked genes or few major effect QTLs (Chee et al., 2001; Buerstmayr et al., 2002). The least squares means of plant height across the five environments showed bimodal distribution (Figure 2-1). It is not, therefore unexpected to uncover a large effect genomic region associated with plant height with bimodal frequency distribution. One of the flanking markers for this coincident QTL on 4D is *Rht-D1b*, a well-known semi-dwarfing gene (Ellis et al., 2002; Pearce et al., 2011). In the combined data of the five environments, the *Rht-D1b* mutant allele was present in 54 RILs and absent in 78 RILs. In hexaploid wheat, dwarfing has been achieved mainly through the introduction of *Rht-B1b* on 4B and *Rht-D1b* on 4D (Ellis et al., 2002; Pearce et al., 2011), which have been introduced in many cultivars grown worldwide (Evans, 1998).

The QTL on 4D that reduced plant height also increased days to maturity. As discussed above for the coincident QTL on 2D, coincident QTLs on 4D could also be due to either tight linkages between genes or QTLs or pleiotropic effect (Tuberosa et al, 2002). For example, one study fine mapped phenotypic effects segregating within a 1 cM chromosome interval in *Arabidopsis thaliana* for which lines with recombination breakpoints were available (Kroymann and Mitchell-Olds, 2005). The authors found that the 1 cM chromosome interval contained two growth rate QTLs within 210 kb which showed epistasis. In the present study, the two flanking markers (*Rht-D1b* and wsnp_CAP11_c356_280910) for the coincident QTL on 4D (*QMat.dms-*

4D.2 and *QPht.dms-4D*) are 5 cM apart, which possibly contain two or more tightly linked genes or QTLs. Additional study is needed to explore whether such major effect coincident genomic region is due to tight linkage or pleotropic effect.

2.5 Conclusions

There were discrepancies between our QTL results from the present and previous studies. First, we were not able to clearly confirm the flowering time and maturity QTLs on both 1B and 5B that were identified in our previous study using DArT and SSR markers. Second, the SNP and two functional markers provided us a better opportunity to uncover eight moderate effect and two major effect QTLs along with several other minor effect QTLs that were not identified in our previous study using SSR and DArT markers. The two major effect QTLs mapped on both chromosomes 2D and 4D. The QTL on 2D mapped adjacent to a well-known photoperiod response *Ppd-D1* gene and reduced flowering and maturity time up to 5 days but showed yield penalty by 436 kg ha^{-1}. The QTL on 4D mapped adjacent to a well-known height reducing *Rht-D1* gene and reduced plant height on average by 13 cm, but increased maturity 33 degree days. The coincident nature of the QTLs on 2D and 4D is very likely due to linkage, which may be determined by screening large numbers of recombinants to break up the linkage.

2.7 Figures and Tables

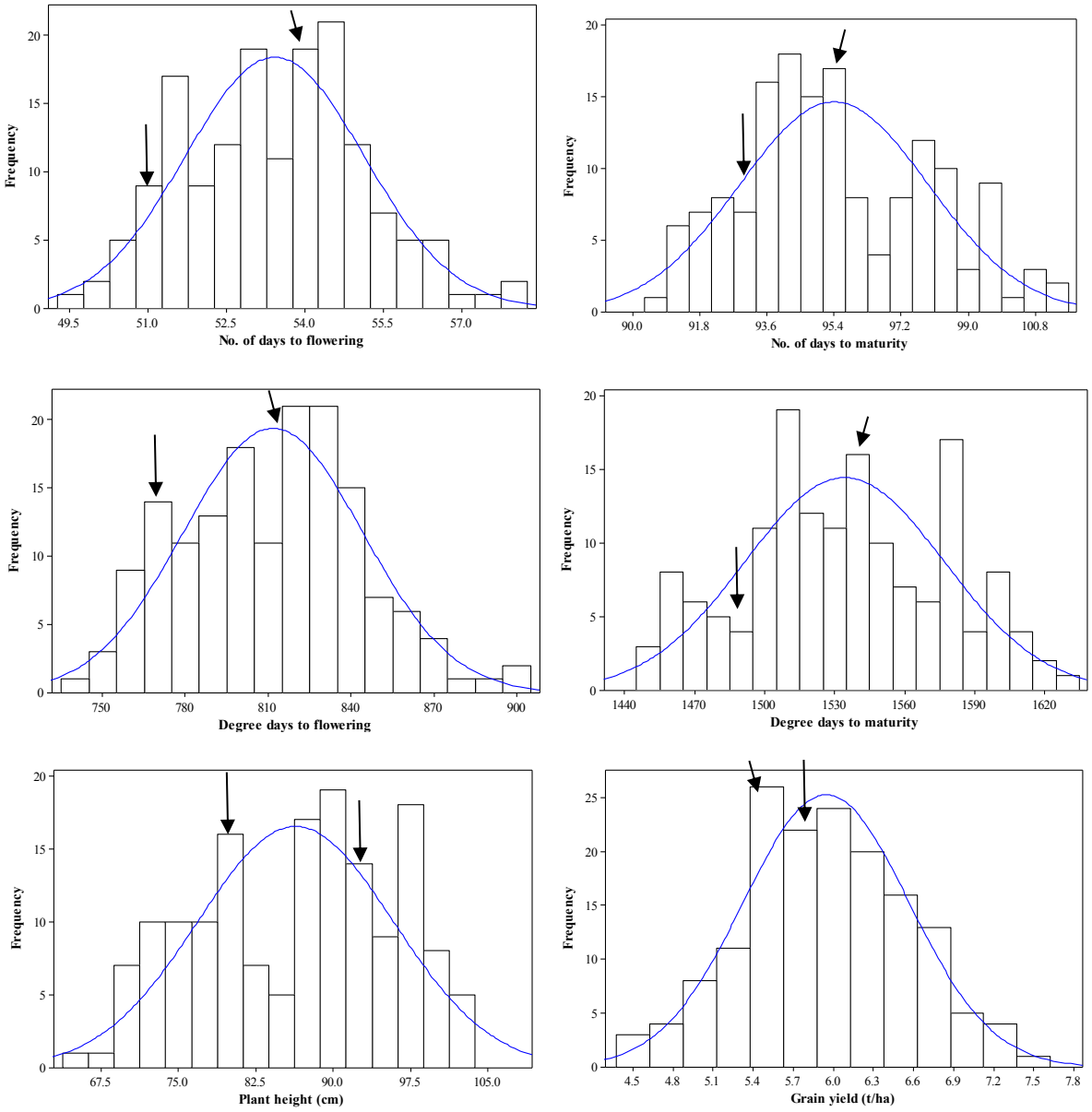

Figure 2.1 Frequency distribution of least square means computed from the combined data of five environments. 'Cutler' flowered and matured 2.6 days earlier, 12.9 cm shorter but produced 154.9 kg ha-1 lower grain yield than 'AC Barrie'.

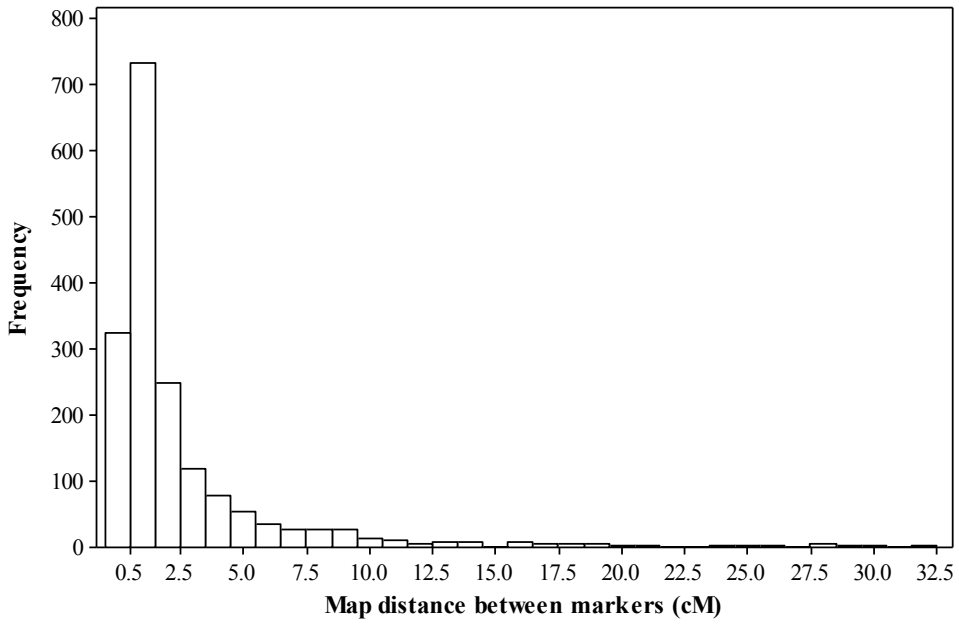

Figure 2.2 Observed frequency distribution of linkage map distances between adjacent loci based on the 1811 markers mapped to the 21 hexaploid wheat chromosomes.

1B
2A
2D
3B
4A
4D
QGyl.dms-1B
QGyl.dms-2A
QFlt.dms-2D
QMat.dms-2D
QYld.dms-2D
QMat.dms-3B
QFlt.dms-3B
QPht.dms-4D
QYld.dms-4D
QMat.dms-4D.1
QMat.dms-4D.2
QMat.dms-4A.1
QMat.dms-4A.2
QMat.dms-4A.3
QFlt.dms-4A.1
QFlt.dms-4A.2
QFlt.dms-4A.3
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
270
280

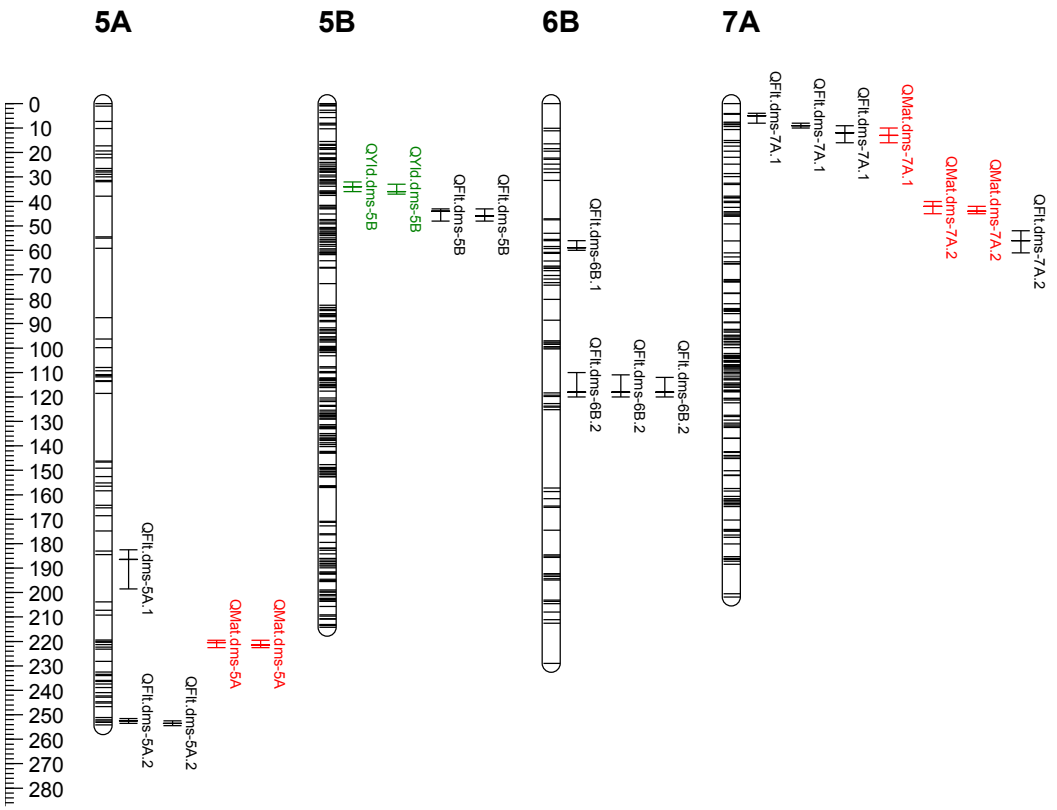

Figure 2.3 Linkage map of the 10 wheat chromosomes that have at least one QTL associated with flowering time, maturity, plant height and/or grain yield. Map position in centiMorgans (cM) is shown on the left side of the chromosomes, with each horizontal line representing a marker. QTLs associated with individual and/or combined environments are shown on the right side of each linkage group, with bars indicating their genetic confidence interval. For each trait, QTLs with exactly the same confidence interval are plotted only once. QTLs for flowering, maturity, plant height and grain yield are in black, red, pink and green font, respectively. See Table 2-2 for details.

Table 2.1 Summary of 1809 polymorphic SNP and 2 functional markers (*Ppd-D1* and *Rht-D1*) used for genotyping 158 recombinant inbred lines derived from a cross between 'Cutler' x 'AC Barrie'.

Chromosome	No. of markers	Map length (cM)	Mean map distance/Marker
1A	101	178.2	1.8
1B	124	212.7	1.7
1D	46	118.4	2.6
2A	118	299.3	2.5
2B	173	229.3	1.3
2D	15	97.4	6.5
3A	106	326.6	3.1
3B	129	291.0	2.3
3D	30	96.4	3.2
4A	121	237.2	2.0
4B	91	244.2	2.7
4D	18	74.8	4.2
5A	108	373.7	3.5
5B	221	214.2	1.0
5D	9	22.3	2.5
6A	73	200.2	2.7
6B	67	229.0	3.4
6D	9	43.0	4.8
7A	145	201.8	1.4
7B	73	168.5	2.3
7D	34	137.7	4.1

Table 2.2 Summary of the QTLs associated with the flowering time, maturity, plant height and grain yield on 158 recombinant inbred lines derived from a cross between spring wheat cultivars 'Cutler' and 'AC Barrie'. The population was phenotyped twice for flowering time under greenhouse conditions and five times for flowering time, maturity, plant height and grain yield.

QTL	Trait*	Chrom	Position (cM)	Confidence interval (cM)	Left marker	Right marker	LOD	R^2 (%)	Additive effect	Difference**
QFld.dms-2D	Flowering (GH)	2D	0	0-3	*Ppd-D1a*	wsnp_CAP11_c3842_1829821	16.3	36.6	2.6	-5.4
QFld.dms-5A.1	Flowering (GH)	5A	187	183-199	Kukri_c20258_143	JD_c3525_1503	3.3	6.9	-0.9	2.0
QFld.dms-5B	Flowering (GH)	5B	44	43-48	BS00063785_51	IACX5818	5.0	12.3	1.1	-1.8
QFld.dms-6B.1	Flowering (GH)	6B	59	56-60	Tdurum_contig11700_1247	wsnp_Ra_c2730_5190365	4.2	10.0	1.1	-2.0
QFld.dms-7A.1	Flowering (GH)	7A	5	4-8	Excalibur_c16355_712	RAC875_c18446_521	3.7	7.3	1.0	-1.4
QFld.dms-2D	Flowering	2D	0	0-5	*Ppd-D1a*	wsnp_CAP11_c3842_1829821	7.2	19.6	0.8	-1.1
QFld.dms-3B	Flowering	3B	94	91-95	Excalibur_c45968_83	CAP12_rep_c7901_114	5.8	13.5	-0.6	1.0
QFld.dms-6B.2	Flowering	6B	118	110-120	wsnp_Ex_c4124_7455225	Kukri_c49331_77	3.1	6.7	0.4	-0.7
QFld.dms-7A.1	Flowering	7A	9	8-10	Tdurum_contig11613_329	wsnp_Ex_c30239_39179460	4.7	12.7	0.5	-0.9
QFld.dms-2D	Flowering (DD)	2D	0	0-5	*Ppd-D1a*	wsnp_CAP11_c3842_1829821	7.8	25.4	14.2	-21.0
QFld.dms-3B	Flowering (DD)	3B	94	91-95	Excalibur_c45968_83	CAP12_rep_c7901_114	6.7	10.7	-10.6	19.7
QFld.dms-4A.1	Flowering (DD)	4A	41	38-42	CAP12_rep_c4000_432	wsnp_Ex_c54453_57331510	3.7	6.3	7.6	-17.6
QFld.dms-5A.2	Flowering (DD)	5A	253	252-254	Tdurum_contig86202_175	wsnp_Ra_c10915_17838202	3.7	7.5	-7.5	16.1
QFld.dms-6B.2	Flowering (DD)	6B	118	111-120	wsnp_Ex_c4124_7455225	Kukri_c49331_77	3.4	9.3	7.4	-13.8
QFld.dms-7A.1	Flowering (DD)	7A	9	8-10	Tdurum_contig11613_329	wsnp_Ex_c30239_39179460	6.4	15.6	10.3	-15.9
QMat.dms-2D	Maturity	2D	0	0-7	*Ppd-D1a*	wsnp_CAP11_c3842_1829821	3.1	11.2	0.7	-1.3
QMat.dms-4A.2	Maturity	4A	53	51-56	Ra_c7973_1185	wsnp_Ex_c10390_17007929	3.5	6.5	0.7	-1.2
QMat.dms-4D.1	Maturity	4D	26	19-29	Excalibur_c5010_1336	Kukri_rep_c68594_530	4.9	13.4	-0.8	1.6
QMat.dms-7A.2	Maturity	7A	44	42-45	RAC875_c14982_577	Tdurum_contig20214_279	3.1	8.8	-0.6	1.2
QMat.dms-2D	Maturity (DD)	2D	0	0-8	*Ppd-D1a*	wsnp_CAP11_c3842_1829821	3.9	10.4	14.2	-21.2
QMat.dms-4A.1	Maturity (DD)	4A	41	35-42	CAP12_rep_c4000_432	wsnp_Ex_c54453_57331510	3.1	12.0	10.7	-22.7
QMat.dms-4D.2	Maturity (DD)	4D	37	34-43	*Rht-D1b*	wsnp_CAP11_c356_280910	5.7	16.0	-15.7	33.0
QMat.dms-7A.1	Maturity (DD)	7A	13	10-16	wsnp_Ra_c63822_63288359	wsnp_BG313770A_Ta_2_3	3.0	9.1	11.3	-11.2
QMat.dms-7A.2	Maturity (DD)	7A	42	40-45	Tdurum_contig37154_190	RAC875_c14982_577	5.7	16.2	-15.7	20.8
QPht.dms-4D	Plant height	4D	37	35-39	*Rht-D1b*	wsnp_CAP11_c356_280910	16.2	37.8	6.3	-13.2
QYld.dms-2D	Grain yield	2D	2	0-12	*Ppd-D1a*	wsnp_CAP11_c3842_1829821	4.3	8.6	248.2	-436.0
QYld.dms-5B	Grain yield	5B	34	32-36	Excalibur_c30667_102	Ku_c6193_821	3.7	7.7	184.0	-321.8

* Flowering (GH) = number of days to 50% flowering under greenhouse; Flowering = days to 50% flowering under field conditions; Maturity = number of days to maturity under field condition; Flowering (DD) = flowering time in degree days; Maturity (DD) = maturity in degree days. **Difference = the difference between all RILs that were homozygous for the 'Cutler' and 'AC Barrie' alleles at the two flanking markers of each QTL. The units for differences are number of days for flowering (GH), flowering and maturity; degree days for Flowering (DD) and Maturity (DD), cm for plant height and kg ha^{-1} for grain yield.

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Chapter 3 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[2]

3.1 Introduction

Wheat is Canada's largest crop, and Canada is the sixth largest wheat producing and the second largest wheat exporting country in the world. Most wheat is produced in the western Canadian prairie provinces of Manitoba, Saskatchewan and Alberta. Wheat production in the region has diversified over time for different reasons, including the development of new cultivars with good agronomic characteristics and good end-use quality (McCallum and DePauw, 2008). The most important agronomic improvements have been improved disease resistances, higher yield potential, better lodging resistance, earlier maturity, and shorter plant type, with good end-use quality, such as increased grain protein concentration, better gluten strength, increased milling yield, enhanced bread-making quality, and reduced susceptibility to pre-harvest sprouting. Canadian wheat breeding programs released hundreds of cultivars, of which more than 100 of them are spring wheat cultivars adapted to the western Canada growing conditions. Molecular diversity analyses have been conducted to assess changes in allelic frequency across 74 cultivars registered between 1845 and 2004 using simple sequence repeat (SSR) markers (Fu and Somers, 2009), but the genetic variation and patterns of relationship of cultivars released over a century have not been investigated using high density single nucleotide polymorphisms (SNP). Currently, the wheat 90K Illumina iSelect array that consists of 81,587 gene-associated SNPs (Wang et al., 2014) is the most high-throughput genotyping platform available to wheat researchers.

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New cultivars to be registered in western Canada must be at least intermediately resistant to five priority diseases, such as stem rust (*Puccinia graminis* f. sp. *tritici*), leaf rust (*Puccinia triticina*), yellow or stripe rust (*Puccinia striiformis* f. sp. *tritici*), common bunt (*Tilletia tritici* and *Tilletia. laevis*) and fusarium head blight (http://www.pgdc.ca). At present, more than 70 leaf rust resistance (*Lr*) and 65 yellow rust resistance (*Yr*) genes have been reported in the literature (McIntosh et al., 2012), but only a few genes and gene combinations have provided a good level of resistance to rusts in many of the cultivars grown in western Canada (McCallum et al., 2007). In western Canada, common bunt, also known as stinking smut and covered smut, is caused by two very closely related fungi, *Tilletia tritici* (syn. *Tilletia caries*) and *T. laevis* (syn. *T. foetida*) (Gaudet and Puchalski, 1989). It reduces both grain yield and quality through the formation of bunt balls that replace the grain with brown black teliospores, resulting in unpleasant smelling spores (Martens et al., 1984). Both the incidence and severity of common bunt have been controlled in the Canadian prairies largely by introgressing resistance genes, such as *Bt10* and *Bt8* (Menzies et al., 2006; McCallum and DePauw, 2008; Hiebert et al., 2011). The resistance has remained generally stable against shifts in virulence in the pathogen, but the vulnerability of such major genes through intense selection pressure on the pathogen is always a concern (Wang et al., 2009). Quantitative trait loci (QTL) associated with resistance to common bunt have also been reported on chromosomes 1B and 7A (Galaev et al., 2006; Fofana et al., 2008; Wang et al., 2009).

Fungal leaf spot diseases are caused by three species (*Pyrenophora tritici-repentis*, *Stagonospora nodorum* blotch and *Zymoseptoria tritici* blotch) and can cause major reductions in test weights and grain yield. Tan spot, caused by *P. tritici-repentis*, is the most destructive leaf spotting disease of wheat in Canada and other major wheat growing countries (Faris et al., 1997; Friesen

and Faris, 2004). Isolates of *P. tritici-repentis* (Ptr) are classified into 8 races based on their ability to cause necrosis and/or chlorosis in differential wheat lines, which is determined by the production of host specific toxins (HSTs) (Lamari et al., 2003). The three HSTs produced by Ptr have been designated as Ptr ToxA, Ptr ToxB, and Ptr ToxC (Ciuffetti et al., 1998; Ciuffetti et al., 2010). Fungal isolates producing both Ptr ToxA and Ptr ToxC are highly abundant in the Canadian prairies, while those producing Ptr ToxB are extremely rare in this region (Lamari et al., 1998; Lamari et al., 2003; Aboukhaddour et al., 2013).

The identification of new sources of disease resistance and their introgression into commercially grown wheat cultivars is the most cost effective and environmentally safe means to manage wheat diseases (Singh et al., 2012). However, breeding for disease resistance is often challenging for at least two reasons. First, breeders often need to pyramid resistance to multiple diseases into the same genetic background. Second, inheritance of resistance to yellow rust, leaf rust, tan spot and common bunt diseases of wheat is both qualitative and quantitative (Faris et al., 1996, 1997; Faris and Friesen, 2005; Singh et al., 2007; Chu et al., 2008; Chu et al., 2010; Singh et al., 2016b). Qualitative resistance is controlled by a single gene with a major effect, but most single genes lose their effectiveness over time due to changes in pathogen populations (i.e., they have proven to be non-durable). On the other hand, quantitative resistance is controlled by minor genes or QTLs with small additive effects, and are more durable (Singh et al., 2008) but require the introgression of multiple genes or QTLs for each disease. Given these challenges, the identification of new resistance genes and major effect QTLs would facilitate the use of molecular marker-assisted selection (MAS) in pyramiding disease resistance in a given wheat germplasm.

The availability of well-validated and fine mapped genes or major effect QTLs and user friendly molecular markers closely linked to resistance genes or QTLs offers alternative methods to complement phenotypic selection, facilitates effective pyramiding of multiple resistance genes and QTLs, and offers the possibility of selecting resistance genotypes in the absence of the pathogens (Singh et al., 2007; Lin and Chen, 2009). Both linkage analysis and association mapping have been extensively used to identify genes and QTLs associated with traits of economic importance. In contrast with linkage-based QTL analysis that depends on bi-parental populations, association mapping is a population-based survey that capitalizes on historical recombination to identify candidate genes affecting complex traits (Falconer et al., 1996). Unlike linkage analysis, where familial relationships are used to predict correlations between phenotype and genotype in biparental populations, association mapping relies on gametic phase disequilibrium to identify population-wide marker-trait associations (Kruglyak, 1999; Ewens and Spielman, 2001; Jannink et al., 2001). In wheat, association studies have been used to map and characterize genomic regions associated with a wide range of traits, including resistance to leaf and yellow rust (Zegeye et al., 2014; Jighly et al., 2015; Kertho et al., 2015; Maccaferri et al., 2015; Naruoka et al., 2015), tan spot (Kollers et al., 2014; Singh et al., 2016b) and common bunt (Singh et al., 2012). These studies reported different numbers of significant marker-trait associations that generally explained a significant proportion of the phenotypic variance. However, there is still a need for an extensive survey for genes or QTLs associated with wheat diseases and insensitivity to Ptr toxins in Canadian spring wheat cultivars. The objectives of our study were, therefore, to (i) evaluate the genetic relationship and population structure of historical and modern Canadian western spring wheat cultivars released over a century using the

90K SNP array, and (ii) identify genomic regions associated with resistance to four wheat diseases (leaf and stripe rusts, common bunt and tan spot) and insensitivity to three Ptr toxins.

3.2 Materials and methods

3.2.1 Phenotypic evaluation

The present study was conducted in an association mapping panel (population) that consisted of 81 historical and modern Canadian spring wheat cultivars (Table 3-1) released between 1885 and 2011 (Chen et al., 2016). The association mapping panel was evaluated at eight environments (trials) for reaction to yellow rust under field conditions at Creston, British Columbia (49.06° N, 116.31° W) between 2013 and 2015, at Lethbridge, Alberta (49.7° N, 112.83° W) between 2012 and 2015, and at St. Albert, Alberta (53.63 N, 113.63°W) in 2015. The association mapping panel also was evaluated at four environments from 2012 to 2015 for its reaction to leaf rust, common bunt and tan spot at the Crop Research Facility of the University of Alberta, South Campus (53°19'N, 113°35'W), Alberta, Canada. For all trials, the mapping population was evaluated in a randomized incomplete block design with two replications in disease screening nurseries. The following cultivars were used as checks: (i) yellow rust nurseries: 'AC Barrie' and 'AC Crystal' as susceptible, and 'Lillian' and 'Carberry' as resistant checks; (ii) leaf rust nurseries: 'AC Barrie' and 'Park' as moderately susceptible to susceptible checks, and 'Peace' and 'Carberry' as moderately resistant to resistant checks; (ii) tan spot nurseries: 'AC Barrie', 'Unity' and 'Glenlea' as moderately susceptible checks, and 'Neepawa' as moderately resistant check; and (iii) for common bunt nurseries: 'Glenlea' and 'Neepawa' as moderately susceptible checks; 'AC Barrie' and 'Unity' as moderately resistant and resistant checks, respectively. All susceptible checks were used as spreader rows. Ten seeds of each cultivar were planted per hill with a spacing of 25 cm between hills or rows. To create homogeneous disease epidemics within

each trial, spreader rows of susceptible checks were planted every three rows. For leaf and yellow rust epidemic initiation, spreader rows were sprayed with an equal mixture of urediniospores of the prevalent races in the region using a hand sprayer; the urediniospores were collected from spreader rows of the previous year in the nursery and suspended in mineral oil. Both leaf and yellow rust severity ratings were recorded based on a modified Cobb scale (Peterson et al., 1948), when the spreader rows reached maximum infection. Visual disease assessment was done on a scale of 1 (no visible sign or symptom = resistant) to 9 (leaf area totally covered with spores = highly susceptible) on each hill plot basis.

Tan spot evaluation was conducted by spraying spore suspension consisting of an equal mixture of two isolates (AB7-2 and AB50-2) belonging to race 1 of *P. tritici-repentis* (Ptr), which is predominant in Alberta (Aboukhaddour et al., 2013). When wheat grains were at the milk-stage, disease reaction was recorded on a scale of 1 to 9 in the same manner as described for the leaf and yellow rusts. For common bunt screening, 10 seeds of each cultivar were mixed with common bunt spores in an envelope (Sukhwinder et al., 2003), which consisted of an even mixture of race L16 of *T. laevis* and race T19 of *T. tritici*. At the dough stage, all heads of each cultivar in a hill plot were examined for common bunt infection and scored in percentages according to the number of infected and healthy heads. These percentages were converted to a scale of 1 to 9 in the same manner as rusts and tan spot. For all diseases, plants with mean disease scores $\leq$ 3.0 were considered resistant, 3.1–5.0 moderately resistant, 5.1-7.0 moderately susceptible, and 7.1–9.0 susceptible.

The virulence of *P. tritici-repentis* depends on the production of Ptr ToxA, Ptr ToxB and/or Ptr ToxC by the different races of the fungus (Lamari and Strelkov, 2010). The sensitivity testing of the association mapping panel was conducted separately for each Ptr toxin in the greenhouse

using established protocols (Lamari and Bernier, 1989a; Aboukhaddour et al., 2013). Each cultivar was evaluated for a particular toxin twice, each in three replications. Briefly, seeds of each cultivar were sown in 10-cm-diameter plastic pots at a density of 6 seeds per pot. The seedlings were maintained for 2 weeks in a growth cabinet at 20°C (day) and 18°C (night) with a 16 hour photoperiod, and were watered and fertilized as required (Lamari and Bernier, 1989a; Aboukhaddour et al., 2013). Four healthy seedlings from each cultivar were then infiltrated with each toxin provided by the Plant Pathology Lab (S.E. Strelkov), University of Alberta, at the two- to three-leaf stage (~14 days old). The cultivars 'Salamouni' and '5700PR' were used as an insensitive check for the three Ptr toxins, while 'Glenlea', '6B662' and '6B365' were used as sensitive checks for Ptr ToxA, Ptr ToxB and Ptr ToxC, respectively. The symptoms caused by each Ptr toxin were assessed every 24 h until the necrosis or chlorosis was severe on the sensitive cultivars. The toxin reactions of the cultivar was then recorded as either 0 (no toxin reaction or insensitive) or 1 (toxin reaction or sensitive) at 3, 8, and 17 days post-infiltration with Ptr ToxA, Ptr ToxB and Ptr ToxC, respectively.

3.2.2 Genotyping

The 81 cultivars were genotyped with the Wheat 90K Illumina iSelect SNP array that consisted of 81,587 SNPs (Wang et al., 2014) as described in our previous study (Perez-Lara et al., 2016). Alleles were called with the Illumina Genome Studio Polyploid Clustering 1.0 software (Illumina, San Diego, USA) using default clustering parameters. We also genotyped the 81 cultivars with 11 gene specific markers, which included *Lr21* (Huang and Gill, 2001), *Lr22* (Raupp et al., 2001; Schnurbusch et al., 2004), *Lr34/Yr18* (Lagudah et al., 2009), *Lr46/Yr29* (Singh et al., 1998), *Lr37/Yr17* (Robert et al., 1999; Seah et al., 2001), *Lr67/Yr46* (Herrera-Foessel et al., 2014), *Lr68* (Herrera-Foessel et al., 2012), *Yr10* (Wang et al., 2002), XBE444541

for the *Tsc2* gene associated with Ptr ToxB (Abeysekara et al., 2010), and both Xfcp1 and Xfcp394 for the *Tsn1*gene associated with Ptr ToxA (Lu et al., 2006; Zhang et al., 2009). For all 11 gene specific markers, PCR amplification and fragment separation was done at the Agricultural Genomics and Proteomics Lab, University of Alberta, Edmonton, Canada as described in a previous study (Perez-Lara et al., 2016).

3.2.3 Statistical analyses

For each trait, least square means, variance statistics, and heritability were obtained using PROC MIXED and PROC IML in SAS 9.3 (SAS Institute Inc. Cary, USA). Each cultivar was considered as a fixed effect, while replications, blocks and environments were considered as random effects. The phenotypic analyses were conducted by combining all environments (trials). The Pozniak Laboratory (University of Saskatchewan) provided genotypic data for 28,639 out of 81,587 SNPs (Wang et al., 2014). Additional filtering was done as described in one of our previous studied (Perez-Lara et al., 2016) to select only SNPs that segregated in a biallelic pattern based on data from multiple mapping populations available to our programs and those with a minor allele frequency $\geq$ 0.05. From this, 19,919 SNPs (24.4%) and 11 gene specific markers remained and were used for all statistical analyses. The extent of pairwise linkage disequilibrium (LD) among markers was evaluated by computing the squared allele-frequency correlations (r^2) values between pairs of markers with TASSEL v5.2.30 (Bradbury et al., 2007). LD decay was estimated at the point where a second degree LOESS curve intersects the threshold of the critical LD using R for Windows (Team, 2014). Background LD was estimated as the 95th percentile of the distribution of r^2 values for unlinked SNP loci (Breseghello and Sorrells, 2006).

Genetic distance was calculated between each pair of cultivars using the identity by descent (IBS) method implemented in TASSEL v.5.2.30. A dendrogram was constructed from the distance matrix using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm implemented in molecular evolutionary genetics analysis (MEGA) v.6.0 (Tamura et al., 2013). Population structure (Q-matrix) was evaluated via principal components analysis (PCA) implemented in TASSEL v5.2.30. The first two principal components from PCA were plotted for visual examination of the clustering pattern of cultivars. As described in other studies (Gurung et al., 2014; Kertho et al., 2015), the first nine PCs that accounted for 51% of the variation were used to correct for population structure. An identity-by-state (IBS) relative kinship matrix (K-matrix) was estimated between pairs of cultivars as a measure of relatedness using TASSEL v5.2.30. Genome-wide association studies (GWAS) were conducted using weighted mixed linear model (MLM) implemented in TASSEL v5.2.30 on the following datasets: (a) 19,919 SN and 11 gene specific markers distributed across the 21 wheat chromosomes (Table 3-2) with a minor allele frequency of $\geq$ 0.05; (b) the K-matrix from kinship; (c) the Q-matrix that consisted of the first nine PCs from PCA as covariates to account for population structure; and d) the least square means estimated for each trait averaged across all environments. The optimum compression level, with re-estimates after each marker option, was used in the analysis. Using the GWAS results from weighted MLM analysis, the threshold Bonferroni correction value (Benjamini and Hochberg, 1995) was calculated by dividing α=0.05 to the total number of markers with $p \leq 0.05$ and used to declare significant-marker trait associations. Genome-wide p values, indicating the strength of the marker-trait association, were visualized as quantile-quantile and Manhattan plots using a graphical tool for SNP effect viewing and graphing (SNPevg) (Wang et al., 2012).

3.3 Results

3.3.1 Reaction to diseases and insensitivity to the Ptr toxins

In order to better understand the magnitude of leaf rust, yellow rust, tan spot and common bunt pressure in our trials, we first examined the mean disease scores for the susceptible and resistant checks in combined environments. For all four wheat diseases, mean disease scores varied from 1.1 to 2.8 for the resistant checks and from 5.3 to 7.4 for the susceptible checks. On average, tan spot, common bunt, leaf rust and yellow rust scores in the population varied from 2.8 to 7.3, from 1.0 to 7.7, from 1.8 to 8.2 and from 1.6 to 6.7, respectively. Fig. 1-3 summarizes the least square means for the association mapping panel evaluated across all environments. A total of 11, 20, 29 and 54 cultivars were found to be resistant with a disease score rating of $\leq$3.0 for tan spot, leaf rust, yellow rust and common bunt, respectively. When two or more diseases were considered, only 1, 16 and 18 cultivars were resistant to a combination of four, three and two diseases, respectively, which clearly demonstrates the challenge in developing cultivars with good resistance to all the four traits. Of the 81 genotypes evaluated for sensitivity to the host-selective Ptr toxins, approximately 69%, 27% and 69% were sensitive to Ptr ToxA, ToxB and ToxC, respectively. The correlation among the three Ptr toxins was very low, ranging between 0 and 0.15. When reactions to two to three toxins were considered, (i) only 7% and 16% of the cultivars were insensitive and sensitive to all the three toxins, respectively; (ii) 26% were insensitive to both Ptr ToxA and Ptr ToxB, 23% were insensitive to both Ptr ToxA and Ptr ToxC, and 10% were insensitive to both Ptr ToxB and Ptr ToxC. The distribution of disease scores averaged across all environments was normal for all traits, except common bunt which deviated ($p < 0.010$) from normality (data not shown). Cultivars differed ($p < 0.0001$) for their reactions to

all diseases and Ptr toxins. Broad-sense heritability varied from 0.23 for tan spot to 0.53 for common bunt.

3.3.2 Markers and germplasm characterization

Among the 81,587 SNPs used for genotyping the 81 diverse spring wheat cultivars, approximately 78% of the markers were discarded either during scoring or prior to data analyses for different reasons, including lack of polymorphism, lack of genetic positions or had minor allele frequency < 0.05. Thus, only 19,919 SNPs (24.4%) and 11 gene-specific markers were used for all statistical analyses. The number of markers retained for analyses varied from 58 on chromosome 4D to 2148 on chromosome 2B (Table 3-2) and the overall average per chromosome was 949. The proportion of SNPs that belonged to the A, B and D genome was 38.2%, 53.8% and 8.0%, respectively. The proportion of missing data per marker varied from 0 to 19.8%, but only 132 SNPs had >10% missing data. Minor allele frequency per SNP varied from 0.05 to 0.50 and the overall average was 0.26. Approximately 60% of the SNPs had a MAF >0.20 (Fig. 3-2). Significant LD was observed for 27.8% of pairs of markers. The average significant ($p < 0.01$) intra-chromosomal LD was 23 cM and the median value was 13 cM. The highest average significant LD was observed in the A genome, followed by the B and D genomes, respectively. The estimates of median LD showed the same trend as mean LD value. The threshold r^2 in the population was 0.16. LD declined to 50% of its initial value at about 23 cM for the A genome, at 22 cM for the B genome and at 34 cM for the D genome. For the whole genome, LD declined to 50% of its initial value at about 24 cM (Fig.3-3).

The genetic distance between pairwise comparisons of the 81 cultivars ranged from 0.012 to 0.504 (Appendix 5) and the overall average was 0.354. Only 6 pairs of cultivars showed genetic differences lower than 0.05, including ‘McKenzie’ vs. ‘Unity’ (0.049), ‘Superb’ vs. ‘CDC

Abound' (0.023), 'Neepawa' vs. 'Katepwa' (0.012), 'Glenlea' vs. 'Burnside' (0.032), 'Minnedosa' vs. 'AC Vista' (0.048), and 'AC Crystal' vs. 'AC Taber' (0.029). Approximately 76% of the pairs of cultivars had a genetic distance ranging between 0.3 and 0.5 (Fig. 3-4). We also looked at the genetic relationship among the 81 cultivars using cluster and principal components analysis, which revealed three clear groups (Fig. 3-5). The grouping of the cultivars showed some pattern based on Western Canadian wheat classes (Table 3- 1), but this was not distinct. The first group consisted of 20 cultivars belonging to the Canada Western Red Spring wheat (18 cultivars) and Canadian Western Hard White Spring wheat (2 cultivars) classes. The second group consisted of 22 cultivars belonging to the Canada Prairie Spring Red (9 cultivars), Canada Western Extra Strong (6 cultivars), Canada Western General Purpose (2 cultivars), Canada Western Soft White Spring (4 cultivars) and Canada Western Red Spring (1 cultivar) classes. Group 3 was the largest cluster with 39 cultivars that all belong to the Canada Western Red Spring wheat class (Table 3-1, Fig. 3-5).

3.3.3 Genome-wide association analysis

The threshold p value for Bonferroni correction for multiple testing in our data was 5×10^{-5}, which is equivalent to a $Log_{10}(1/p)$ value of 4.3. Using this threshold p value, GWAS identified a total of 94 significant marker-trait associations, which included one each for tan spot and yellow rust, 5 for leaf rust, 10 for common bunt, 28 for Ptr ToxA and 49 for Ptr ToxB (Fig. 3-6, Table 3-3), but none for Ptr ToxC. In order to verify the reliability of our results, we first examined the markers significantly associated with Ptr ToxA, which has been reported to be regulated by a single dominant gene, the *Tsn1* gene that maps between Xfcp1 and Xfcp394 on the long arm of chromosome 5B (Lamari and Bernier, 1989b; Faris et al., 1996; Gamba and Lamari, 1998). We found clusters of 9 and 19 SNPs associated with Ptr ToxA at 52-53 cM on

chromosome 1A and at 71-74 cM on chromosome 5B, respectively (Table 3-3). The genomic region on 1A and 5B explained on average 25.2 and 45.9% of the phenotypic variance, respectively. Pairwise LD values between Xfcp1 and 1,964 SNPs on chromosome 5B ranged from 0 to 0.82, with SNPs that mapped between 71 and 74 cM interval showing LD values ranging between 0.67 and 0.82 (Fig. 3-7). Such high LD values between Xfcp1 and other SNP markers clearly confirmed the *Tsn1* gene and demonstrates the quality of the genotypic and phenotypic data in our study. Pairwise LD values between the Xfcp394 and the 1,964 SNPs on chromosome 5B ranged from 0 to 0.50. All SNPs that mapped between 71 and 74 cM on 5B had LD > 0.40, with the highest LD observed between Xfcp394 and wsnp_Ku_c40334_48581010; the latter SNP showed the highest $Log_{10}(1/p)$ value of 26.8 from the GWAS (Table 3-3).

The 49 significant marker-trait associations for Ptr ToxB included one SNP at 122 cM on 1A, clusters of 30 SNPs that mapped between 17 and 39 cM on 2B and another cluster of 18 SNPs that mapped between 123-124 cM on 5B (Table 3-3). The genomic regions associated with Ptr ToxB on chromosomes 2B and 5A explained on average 28.9 and 25.8% of the phenotypic variance, respectively. Ptr ToxB induces chlorosis (Strelkov et al., 1999; Lamari et al., 2003) in wheat lines harboring the dominant *Tsc2* gene, which mapped 0.6 cM proximal to XBE444541 on the short arm of chromosome 2B (Friesen and Faris, 2004; Abeysekara et al., 2010). Pairwise LD values between XBE444541 and the 2,147 SNPs that mapped on chromosome 2B ranged from 0 to 0.13 (data not shown), which suggest that the genomic region associated with Ptr ToxB on 2B is different from the *Tsc2* gene.

The 10 significant markers associated with common bunt mapped at 162 cM on 2B (4 SNPs), at 43 and 83 cM on 4B (1 SNP each), and at 36, 48 and 192 cM on 7A (2, 1 and 1 SNPs, respectively). Each significant marker explained between 8.7 and 20.5% of the phenotypic

variance (Table 3-3). For leaf rust, there were five significant marker-trait associations that mapped at 123-127 cM (3 SNPs) and 167 cM (2 SNPs) on chromosome 2B, which explained on average 18.7-19.2% of the phenotypic variance. The single significant marker-trait association for yellow rust and tan spot were mapped at 21 cM on 2A and at 77 cM on 7B, respectively, and accounted for 19.1 to 20.9% of the phenotypic variance (Table 3-3). Pairwise LD values between *Lr37/Yr17/Sr38* gene specific marker and 1,197 SNPs that mapped on 2A were very low, ranging from 0 to 0.10 (data not shown), which suggests that the genomic region associated with yellow rust on 2A is different from the *Lr37/Yr17/Sr38* gene. For 7B, pairwise LD values between the *Lr68* gene specific marker and 1,248 SNPs varied from 0 to 0.58, but the highest LD values were observed between *Lr68* and most SNPs that mapped between 144 and 152 cM (Fig. 3-7), which is different from the genomic region associated with tan spot.

3.4 Discussion

3.4.1 Marker LD and population structure

The decay of LD over genetic or physical distances among all pairs of markers within a chromosome or across the genome of a given population determines the marker density needed to perform an association analysis (Flint-Garcia and Thornsberry, 2003; Gaut and Long, 2003). The extent of LD and LD decay estimated using SNP loci from all three wheat genomes is generally in agreement with several other studies (Chao et al., 2010; Edae et al., 2014; Lopes et al., 2014). The higher average extent of LD observed in the present study than other studies may be due to the relatively short evolutionary and breeding history of Canadian western spring wheat cultivars.

Our results revealed that only six pairs of cultivars had low genetic differences ($\leq$ 5% of the total number of markers), which was due to shared pedigree. For instance, 'McKenzie' is the

backcross parent of 'Unity' (Fox et al., 2010), 'Superb' is one of the parents of 'CDC Abound' (McCallum and DePauw, 2008), while 'AC Crystal' and 'AC Taber' are sibs (Fernandez et al., 1998). Although a substantial number of cultivars shared one or more common parents, approximately 94% of the pairwise comparisons of the genetic distance among the 81 cultivars varied between 0.20 and 0.50, which suggests the presence of large genetic variation among the Canadian western spring wheat cultivars released. Our results also showed the presence of a clear population structure, with three distinct clusters or subpopulations (Fig. 3-5). Group 3 consisted of 29 cultivars that all belong to the Canadian Western Red Spring wheat class, while group 1 consisted of 20 cultivars that belong to the Canadian Western Red Spring wheat (18 cultivars) and Canada western hard white spring (2 cultivars). Several cultivars in the Canadian Western Red Spring wheat class have 'Red Fife' (registered in 1885) as one of their parents (McCallum and DePauw, 2008). However, we were not able to explain why the Canadian Western Red Spring wheat class was divided into two groups (groups 1 and 3) and why 'Red Fife' did not belong to either group (Table 3-1). The second group consisted of a mixture of cultivars that belongs to five different spring wheat classes, which are characterized by the presence of large variation in agronomic traits and disease reactions.

3.4.2 Resistance to diseases and insensitivity to the Ptr toxins

In the present study, approximately 86% of the cultivars were susceptible to Ptr race 1 isolates, while the remaining 14% (11 out of the 81 cultivars) were resistant to race 1, with a disease rating $\leq$3. The majority (69%) of cultivars were also sensitive to both Ptr ToxA and ToxC as compared with the 27% that were sensitive to Ptr ToxB. The susceptibility of a majority of cultivars to the race 1 isolates and their sensitivity to both Ptr ToxA and ToxC may be due to two reasons. First, most Ptr isolates collected in Alberta belonged to race 1 (62%), followed by race 2

(36%) and race 3 (2%) (Lamari et al., 1998; Lamari et al., 2003; Aboukhaddour et al., 2013). Fungal isolates of races 2, 3, and 5 produce Ptr ToxA, Ptr ToxC, and Ptr ToxB, respectively (Strelkov and Lamari, 2003; Lamari and Strelkov, 2010). Isolates of races 1, 6, and 7 each produce two HSTs, with race 1 producing Ptr ToxA and Ptr ToxC, race 6 producing Ptr ToxB and Ptr ToxC, and race 7 producing Ptr ToxA and Ptr ToxB. Race 8 isolates produce all of the three HSTs (Lamari et al., 2003, Faris et al., 2013). When infiltrated into sensitive genotypes, both Ptr ToxB and Ptr ToxC induce chlorosis, while Ptr ToxA induces necrosis. Hence, the susceptibility and sensitivity of the majority of the wheat cultivars used in our study to the tan spot fungus, Ptr ToxA and Ptr ToxC seems due to the prevalence of races 1 and 2 isolates in the province. In contrast, the Ptr ToxB-producing race 5 is extremely rare in western Canada, with only a single weakly virulent isolate reported in the prairie provinces (Strelkov et al., 2002). It is, therefore, not surprising to find that a majority of the cultivars are sensitive to Ptr ToxA and ToxC, but insensitive to Ptr ToxB. Secondly, many of the modern spring wheat cultivars in western Canada were developed using a few common parent cultivars (e.g., 'Red Fife', 'Thatcher', 'Neepawa', 'Katepwa' and 'AC Barrie') that possess good agronomic characteristics and good end-use quality (Lamari et al., 2005; McCallum and DePauw, 2008), but susceptible to tan spot. Results from our study showed the susceptibility of all these key wheat cultivars to race 1 isolates, with disease ratings $\geq$ 5 (data not shown). The repeated use of such popular but tan spot susceptible cultivars as parents by breeders has resulted in the unintentional release of new cultivars with susceptibility to tan spot and sensitivity to the Ptr toxins.

For the tan spot pathogen, we found a single SNP at 77 cM on chromosome 7B that explained 20.9% of the phenotypic variance for this disease (Table 3-3). Previous genome-wide association studies conducted on a set of historical CIMMYT bread wheat germplasm identified 9 genomic

regions on chromosomes 1A, 4A, 6B and 7B that conferred resistance to tan spot(Singh et al., 2016b). In the present study, one of the two genomic regions associated with Ptr ToxA mapped at 52-53 cM on chromosome 1A and explained 25.2% of the phenotypic variance for Ptr ToxA (Table 3-3). In wheat, reaction to each of the three Ptr toxins is regulated by a single gene (Lamari and Bernier, 1989b; Faris et al., 1996; Gamba and Lamari, 1998), with the dominant and recessive genotypes showing sensitivity and insensitivity to the toxins, respectively. The dominant *Tsc1* gene on the short arm of chromosome 1A confers sensitivity to Ptr ToxC (Effertz et al., 2002), but we are not aware of single genes associated with Ptr ToxA on chromosome 1A. However, a major QTL on the short arm of chromosome 1A has been found to be associated with resistance to chlorosis induced by race 1 isolates in a RIL population derived from a cross between synthetic wheat 'W-7984' and 'Opata 85' (Faris et al., 1997). In a different study conducted using a RIL population derived from a cross between the Chinese landrace 'Wangshuibai' (resistant) and Chinese breeding line 'Ning7840' (highly susceptible), a QTL associated with resistance to race 1 has been reported on the short arm of chromosomes 1A (*QTs.ksu-1AS*) that accounted for 39% of the phenotypic variation (Sun et al., 2010). These results, together with ours, provide evidence that there is possibly a common genomic region on chromosome 1A associated with resistance to race 1 isolates, irrespective of the type of toxins produced by the fungus (Ptr ToxA or ToxC or both).

We also identified a second major genomic region associated with Ptr ToxA at 71-74 cM on chromosome 5B (Table 3-3). The dominant *Tsn1* gene, which maps between Xfcp1 and Xfcp394 on the long arm of chromosome 5B (Zhang et al., 2009) confers sensitivity to Ptr ToxA (Faris et al., 1996). We think that the significant marker-trait association identified between 71 and 74 cM on chromosome 5B corresponds with the *Tsn1* gene for two reasons. First, this genomic region

explained approximately 46% of the phenotypic variation for Ptr ToxA; such a high proportion of phenotypic variance is often associated with a single gene or major effect QTLs. Second, pairwise LD values between Xfcp1 (the closest flanking marker for the *Tsn1* gene) and SNPs that mapped between 71 and 74 cM on chromosome 5B ranged between 0.67 and 0.82 (Fig. 7), which is an indirect evidence for the strong association between the closest *Tsn1* flanking marker and the SNPs that mapped between 71 and 74 cM on 5B. A major genomic region associated with sensitivity to Ptr ToxA has also been reported on the long arm of chromosome 5B (Cheong et al., 2004).

In the case of Ptr ToxB, one of the genomic regions mapped on chromosomes 2B (17-39 cM) and explained 28.9% of the phenotypic variance (Table 3-3). This region was much broader than other regions (Table 3-3), which may be due to the insensitivity of a majority of the western Canadian spring wheat cultivars to Ptr ToxB. Ptr ToxB is a proteinaceous host-selective toxin that induces chlorosis (Strelkov et al., 1999; Lamari et al., 2003) in wheat lines harboring the dominant *Tsc2* gene, which mapped on the short arm of chromosome 2B (Friesen and Faris, 2004; Abeysekara et al., 2010). The *Tsc2* gene accounted for 69% of the phenotypic variation for race 5 isolates in the international *Triticeae* mapping initiative (ITMI) population (Friesen and Faris, 2004). In addition, the same authors have also reported three minor effect QTLs on the short arm of 2A, the long arms of both 2B and 4A. Therefore, the sensitivity of wheat genotypes to Ptr ToxB depends not only on the *Tsc2* gene on chromosome 2B, but also the additional minor effect QTLs on 2A, 2B and 4A. Based on the high LOD score that we observed in our Manhattan plot (Fig. 6), we expected the 17-39 cM on chromosome 2B to either be the *Tsc2* gene or one of the major effects QTL reported adjacent to the *Tsc2* gene. However, pairwise LD values between XBE444541, the diagnostic marker for the *Tsc2* gene (Abeysekara et al., 2010) and SNPs that

mapped between 17 and 39 cM on chromosome 2B was very low, ranging from 0 to 0.13 (data not shown). The genomic region identified for Ptr ToxB on chromosome 2B is, therefore, different from the *Tsc2* gene.

The second genomic region associated with ToxB mapped at 123-124 cM on chromosome 5B, which is 49 cM distal to the interval associated with the Ptr ToxA. This region accounted for 25.8% of the phenotypic variance for Ptr ToxB (Table3-3). In previous studies, five QTLs associated with both races 1 and 2 were reported in a doubled haploid (DH) wheat population derived from a cross between TA4152-60 and ND495, which includes *QTs.fcu-5BL.1* on 5B between markers *Xbarc138* and *Xgwm260* and *QTs.fcu-5Bl.2* on 5B between markers *Xfcp615* and *Xbarc142*; each QTL explained between 9 and 22% of the phenotypic variance (Chu et al., 2008). Although direct comparisons among different studies was not possible due to differences on the types of markers used and lack of physical positions, results of our and previous studies suggest the presence of hot spot regions on chromosome 5B that are associated with multiple Ptr toxins.

For common bunt, we found significant marker-trait association on chromosomes 2B, 4B and 7A, each explaining between 8.7 and 20.5% of the phenotypic variance (Table 3-3). At least 13 monogenic and race-specific genes (*Bt1* to *Bt13*) that conferred resistance to common bunt have been reported in wheat, of which *Bt1* was mapped on chromosome 2B (Sears et al., 1960). Six QTLs associated with resistance to common bunt have been reported in a double haploid population derived from a cross between ‘Carberry’ and ‘AC Cadillac’, which includes a QTL on 4B that explained 7.6% of the phenotypic variance (Singh et al., 2016a). Four QTLs associated with resistance to common bunt have been reported in a DH population derived from a cross between ‘Trintella’ x ‘Pikoon’, which includes a QTL on chromosome 7A (Dumalasová

et al., 2012). In a Canadian spring wheat DH population derived from a cross between 'RL 4452' x 'AC Domain', two QTLs for common bunt were also reported on chromosomes 1B and 7A (Fofana et al., 2008). However, direct comparisons among the different studies was not possible due to differences on the types of markers used and lack of physical positions of the flanking markers reported in the different studies.

The current study identified a SNP marker associated with yellow rust on chromosome 2A that explained 19.1% of the phenotypic variance (Table 3-3). *Yr1*, Yr17, *Yr32*, *YrR61* and several QTLs associated with yellow rust have been reported on chromosome 2A using different bi-parental and association mapping populations (Eriksen et al., 2004; Sharma-Poudyal et al., 2013; Naruoka et al., 2015). The QTLs reported for yellow rust resistance include *Qyr.wpg-2A.1, Qyr.wpg-2A.3, Qyr.wpg-2A.4, Qyr.wpg-2A.5* and *Qyr.wpg-2A.6* (Naruoka et al., 2015), *QYrst.orr-2AS* (Vazquez et al., 2012)*, QYr.sun-2A* (Bansal et al., 2014), *QYR2* (Boukhatem et al., 2002) and *QYr.inra-2AL* (Dedryver et al., 2009). *QRYr2A.1* was reported in seven studies and appeared to be a gene rich region containing clusters of several genes associated with stripe rust resistance at the seedling stage (Zegeye et al., 2014). Some of these QTLs mapped in the vicinity of the *Yr32* and *YrR61*, while others mapped far apart from these genes. In a DH population derived from two Canadian spring wheat cultivars, AC Cadillac and Carberry (Singh et al., 2014), the authors reported QTLs associated with yellow rust resistance on both chromosomes 2A and 4B that were detected across multiple environments. A genome-wide association analysis conducted to map genomic regions associated with leaf and yellow rust resistance in 170 wheat lines genotyped with 813 DArT markers identified a total of 212 significant marker-trait associations (90 for leaf rust and 122 for yellow rust), which were distributed across all wheat chromosomes except 6D.

The number of cultivars used in the present studies is somewhat greater than the 70 winter wheat lines used for genome-wide association mapping of resistance to both tan spot and *Stagonospora nodorum* Blotch (Liu et al., 2015), and comparable to the 80 inbred lines in maize, 80 recombinant inbred lines in barley (Inostroza et al., 2009), and 88 clones in sugarcane (Racedo et al., 2016). Our population size was nevertheless smaller than that used in several other studies, which may have resulted in a decreased in power of QTL detection (Yu et al., 2008). However, we think that the results presented in the present study are reliable for a number of different reasons. First, the 81 cultivars used in the present study were registered for commercial production in western Canada between 1885 and 2011, and consisted of a highly diverse set of germplasm, which forms a representative set for studying the genetic variation and population structure. The presence of a high level of genetic variation is evident from the molecular data, which showed a genetic distance ranging between 0.10 and 0.50 for over 94% of the pairs of cultivars (Appendix 5). Second, the cultivars also showed highly significant differences for all seven phenotypic traits used in our study. Third, we first used nine principal components that accounted for 51% of the variation to account for population structure, followed by a high threshold p-value (5×10^{-5}) for declaring significant marker-trait associations. Finally, our genome-wide association mapping unambiguously identified the *Tsn1* gene associated with Ptr ToxA on chromosome 5B, which is an indication of the quality of the genotype and phenotype data used in the present study.

3.5 Figures and Tables

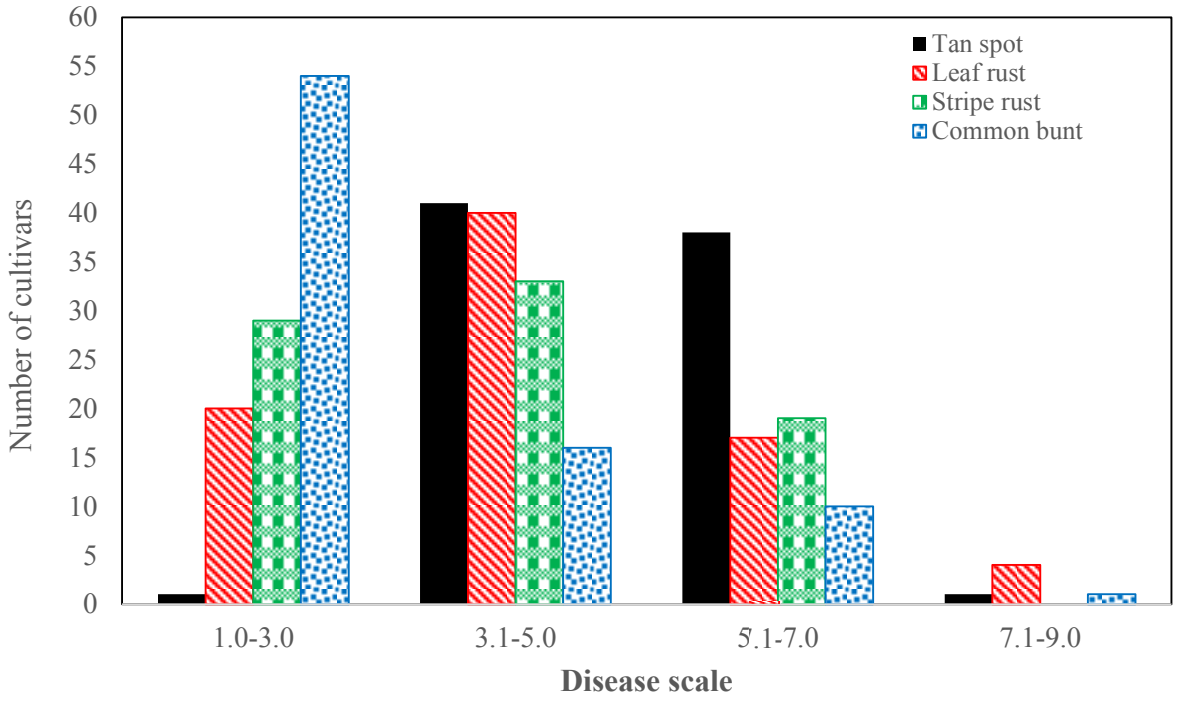


Figure 3.1 Summary of the least square means of disease scores for 81 spring wheat cultivars evaluated for reaction to four wheat diseases across multiple environments.

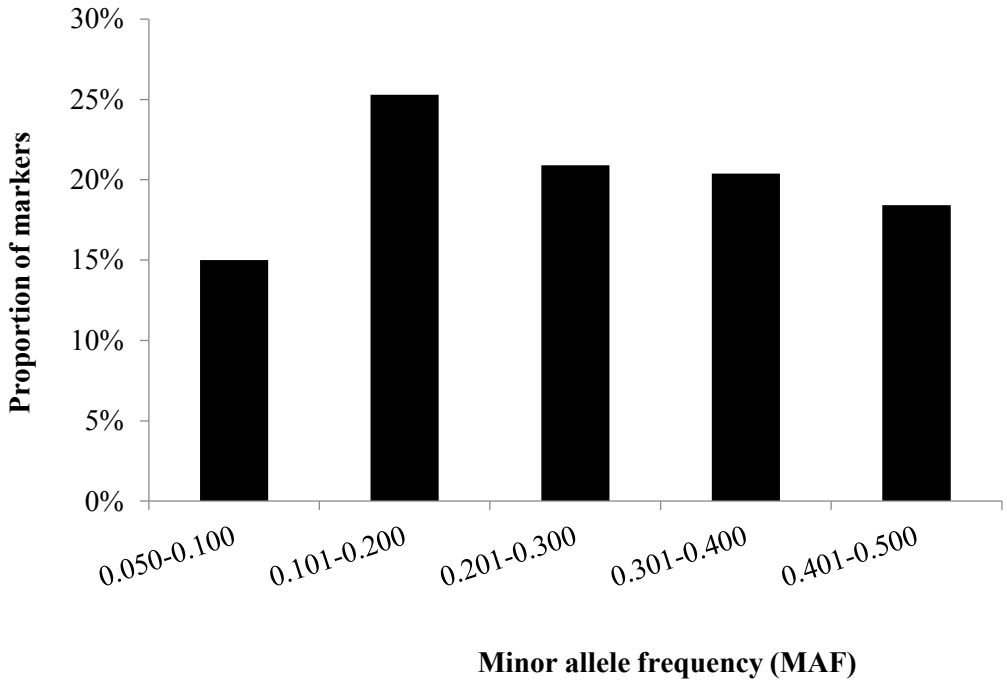


Figure 3.2 Summary of minor allele frequency (MAF) for 19,930 markers used for genotyping the 81 spring wheat cultivars.

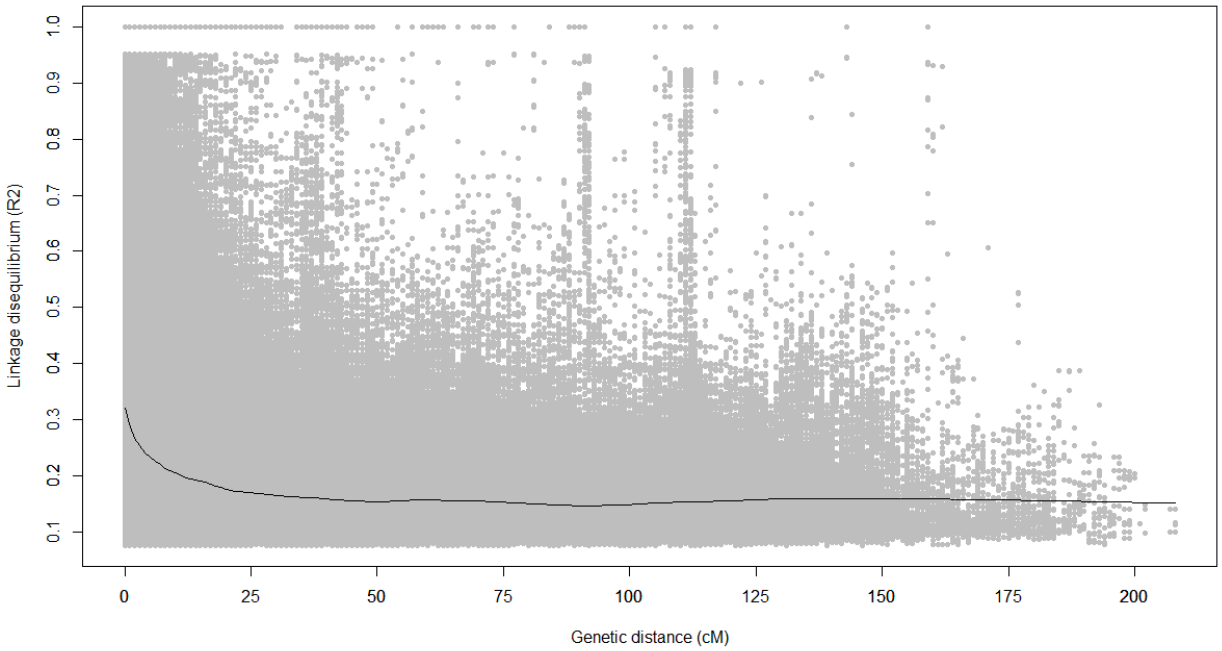


Figure 3.3 Relationship between r^2 values (y-axis) as an estimate of whole genome linkage disequilibrium among pairs of markers and map distance in centiMorgan (cM) (x-axis). The horizontal dotted line indicates the 95^{th} percentiles of the distribution that is used as a threshold r^2 value to declare for linkage.

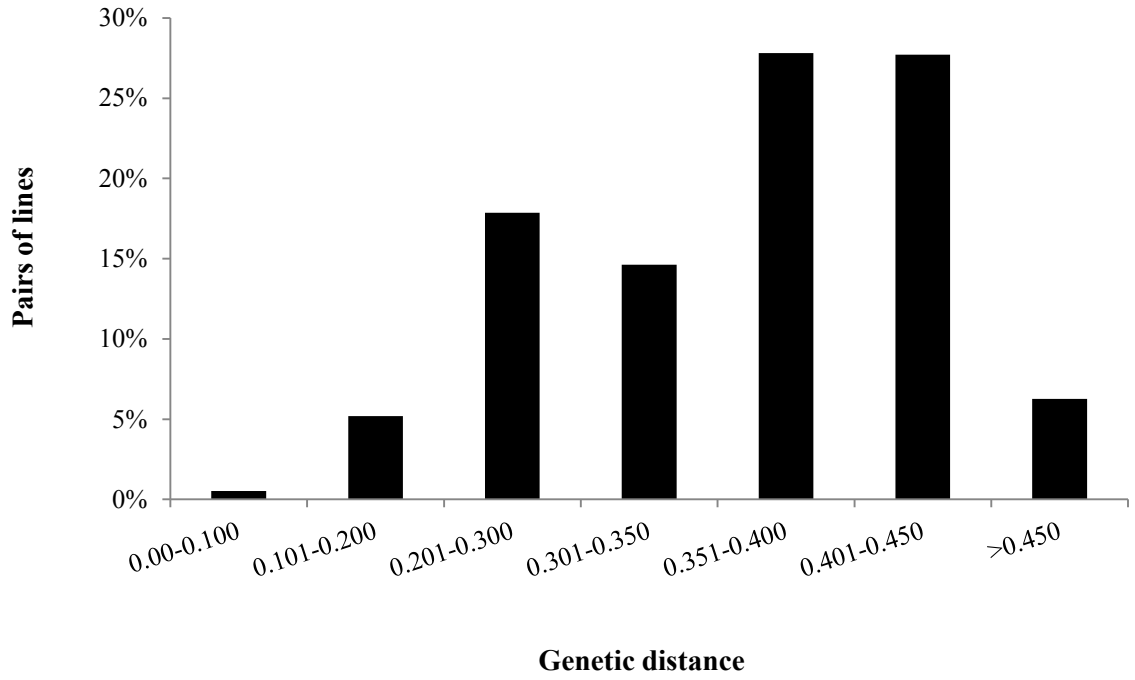


Figure 3.4 Frequency distribution of pairwise genetic distance among 81 spring wheat cultivars based on 19,930 polymorphic markers.

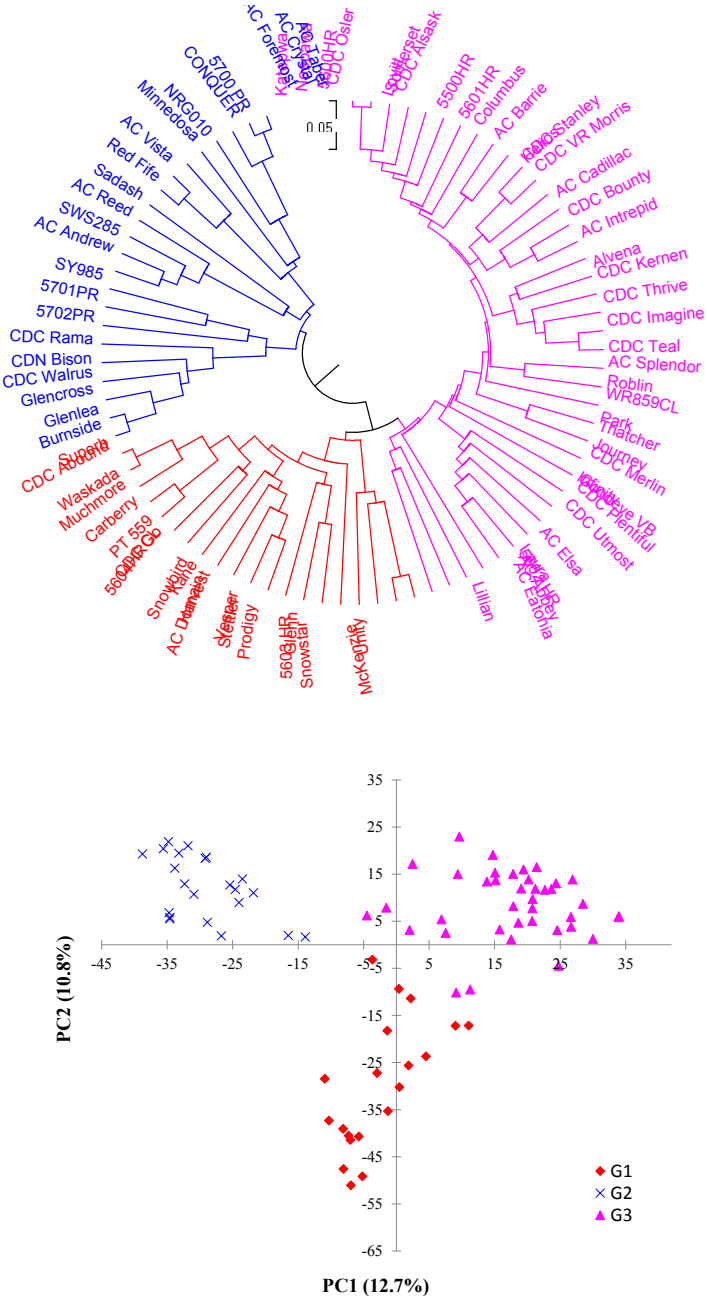


Figure 3.5 Cluster (top) and principal component (bottom) analyses of 81 spring wheat cultivars based on 19,930 markers. Cultivars that belong to groups 1, 2 and 3 (G1, G2 G3) are shown in red, blue and pink font, respectively. Detailed membership of each cultivar and pairwise genetic distance are gown in Table 3- 1 and Supplementary material 3-1, respectively.

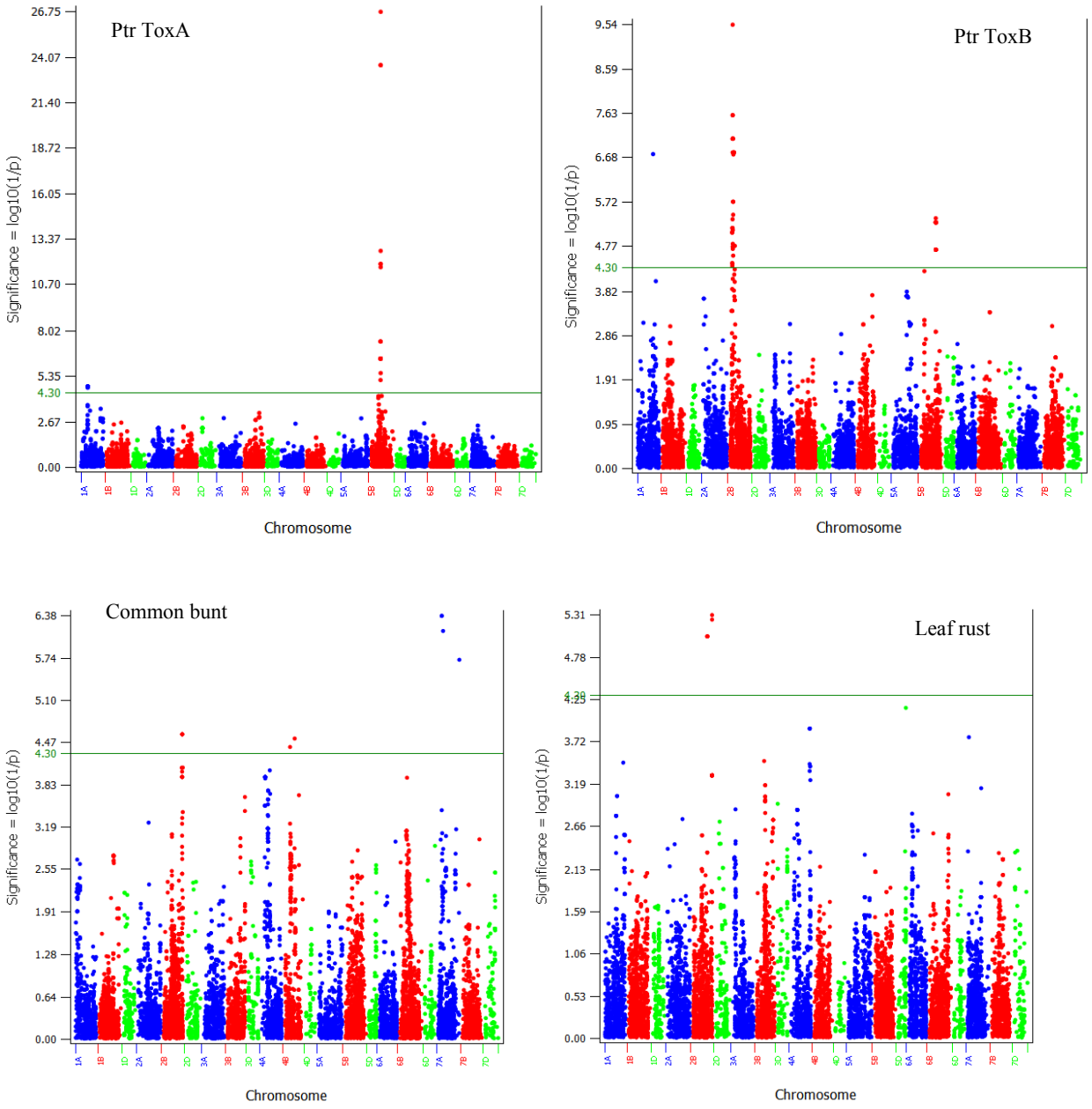


Figure 3.6 Manhattan plots for 6 traits based on mixed linear model obtained for 81 spring wheat cultivars genotyped with 19,930 markers. The horizontal line shows the genome-wide significance threshold Log_{10} (1/p) value of 4.3. The A, B and D genomes are in blue, red and green color, respectively.

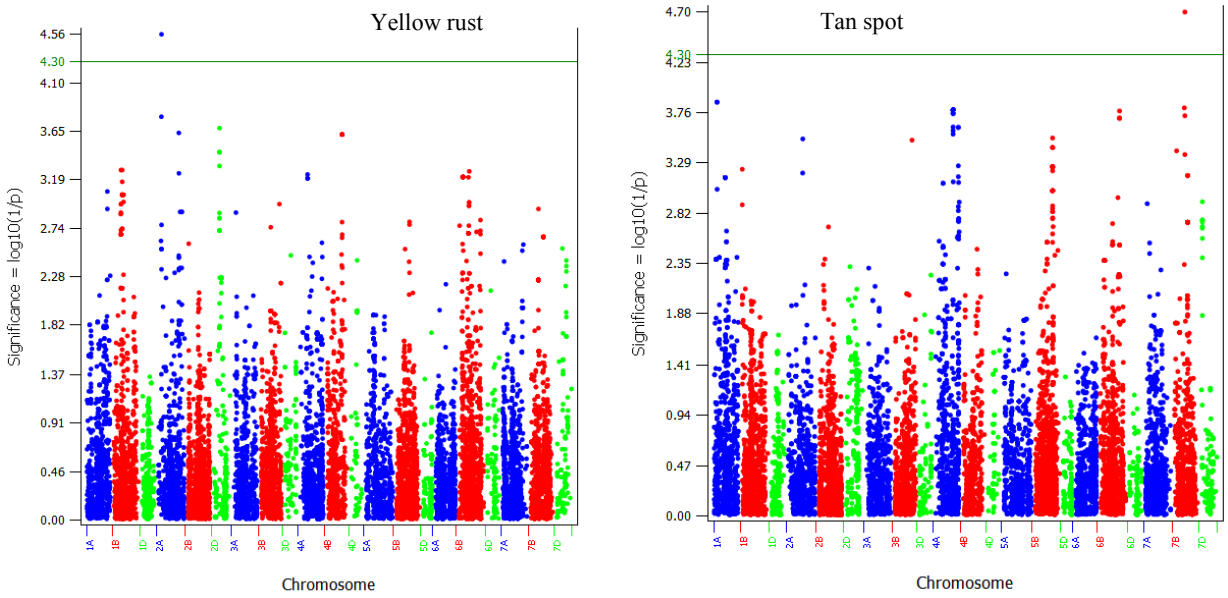
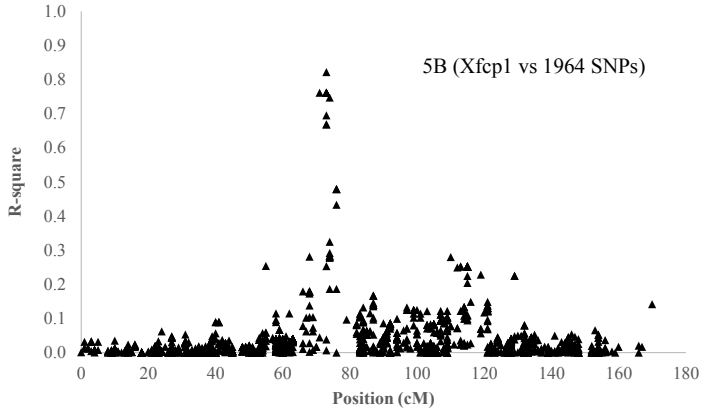
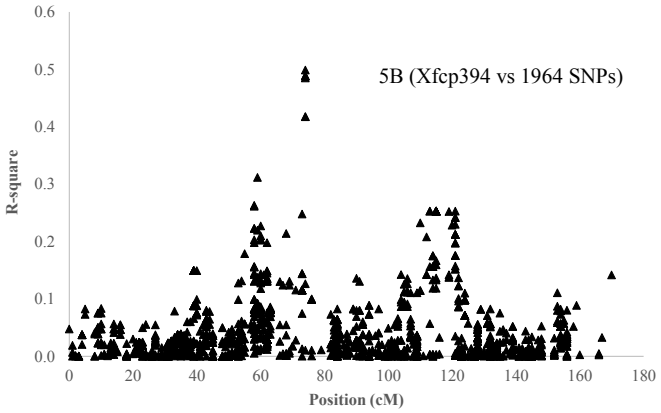


Figure 3.6 Manhattan plots for 6 traits (Cont.)





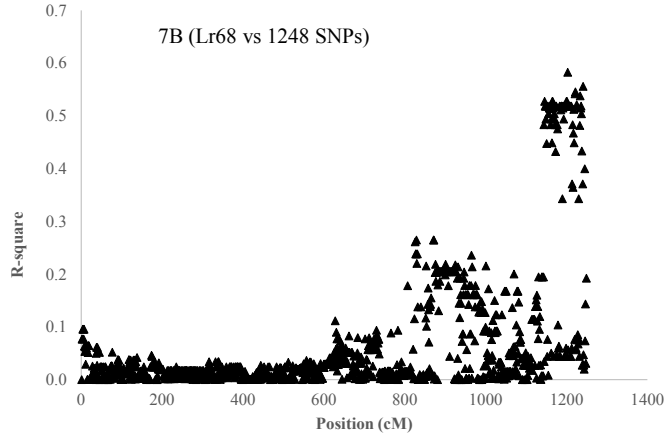


Figure 3.7 Pairwise linkage disequilibrium (LD) between two *Tsn1* flanking markers (Xfcp1 and Xfcp394) and 1,964 single nucleotide polymorphic markers on chromosome 5B, and between Lr68 and 1,248 SNPs on 7B.

Table 3.1 Summary of the 81 hexaploid spring wheat cultivars used in the present study.

Cultivar name	Year of release	Wheat class*	Group membership based on PCA	Group membership based on cluster analysis
Red Fife	1885	CWRS	2	2
Thatcher	1935	CWRS	3	3
Park	1963	CWRS	3	3
Neepawa	1969	CWRS	3	3
Glenlea	1972	CWES	2	2
Columbus	1980	CWRS	3	3
Katepwa	1981	CWRS	3	3
Laura	1986	CWRS	3	3
Roblin	1986	CWRS	3	3
AC Reed	1991	CWSWS	2	2
AC Taber	1991	CPSR	2	2
CDC Teal	1991	CWRS	3	3
CDC Merlin	1992	CWRS	3	3
AC Domain	1993	CWRS	1	1
AC Eatonia	1993	CWRS	3	3
AC Barrie	1994	CWRS	3	3
AC Foremost	1995	CPSR	2	2
AC Cadillac	1996	CWRS	3	3
AC Crystal	1996	CPSR	2	2
AC Elsa	1996	CWRS	3	3
AC Vista	1996	CPSR	2	2
AC Intrepid	1997	CWRS	3	3
AC Splendor	1997	CWRS	3	3
McKenzie	1997	CWRS	1	1
AC Abbey	1998	CWRS	3	3
Prodigy	1998	CWRS	1	1
5600HR	1999	CWRS	3	3
CDC Bounty	1999	CWRS	3	3
5500HR	2000	CWRS	3	3
5700 PR	2000	CPSR	2	2
AC Andrew	2000	CWSWS	2	2
Snowbird	2000	CWHWS	1	1
5601HR	2001	CWRS	3	3
5701PR	2001	CPSR	2	2
CDC Rama	2001	CWES	2	2
Superb	2001	CWRS	1	1
CDC Imagine	2002	CWRS	3	3
Journey	2002	CWRS	3	3
Lovitt	2002	CWRS	3	3
CDC Go	2003	CWRS	1	1
CDC Osler	2003	CWRS	3	3
CDC Walrus	2003	CWES	2	2
PT 559	2003	CWRS	1	1
SWS285 (Bhishaj)	2003	CWSWS	2	2
5602 HR	2004	CWRS	3	3

Burnside	2004	CWES	2	2
CDC Alsask	2004	CWRS	3	3
Harvest	2004	CWRS	1	1
Infinity	2004	CWRS	3	3
Lillian	2004	CWRS	3	3
Somerset	2005	CWRS	3	3
Alvena	2006	CWRS	3	3
CDC Abound	2006	CWRS	1	1
Helios	2006	CWRS	3	3
Kane	2006	CWRS	1	1
Snowstar	2006	CWHWS	1	1
5702PR	2007	CPSR	2	2
Glencross	2007	CWES	2	2
Goodeve VB	2007	CWRS	3	3
Sadash	2007	CWSWS	2	2
Unity	2007	CWRS	1	1
Waskada	2007	CWRS	1	1
5603 HR	2008	CWRS	1	1
CDN Bison	2008	CWES	2	2
Minnedosa	2008	CWGP	2	2
Stettler	2008	CWRS	1	1
WR859CL	2008	CWRS	3	3
5604HRCL	2009	CWRS	1	1
CDC Kernen	2009	CWRS	3	3
CDC Thrive	2009	CWRS	3	3
CDC Utmost	2009	CWRS	3	3
CONQUER=HY 682	2009	CPSR	2	2
Carberry	2009	CWRS	1	1
Glenn	2009	CWRS	1	1
Muchmore	2009	CWRS	1	1
NRG010=GP 010	2009	CWGP	2	2
CDC Stanley	2009	CWRS	3	3
CDC VR Morris	2010	CWRS	3	3
SY985	2010	CPSR	2	2
Vesper	2010	CWRS	1	1
CDC Plentiful	2011	CWRS	3	3

*CPSR: Canada Prairie Spring Red; CWES: Canada western extra strong; CWGP: Canada western general purpose; CWHWS: Canada western hard white spring; CWRS: Canada Western Red Spring; CWSWS: Canada western soft white spring.

Table 3.2 The chromosomal distribution of the 19,930 markers used in the present study and map length across 21 wheat chromosomes.

Chromosome	No. of markers	Map length (cM)	No. of markers per cM
1A	1052	168	6.3
1B	1534	171	9.00
1D	355	83	4.3
2A	1198	195	6.1
2B	2148	169	12.7
2D	523	112	4.7
3A	972	180	5.4
3B	1418	156	9.1
3D	141	87	1.6
4A	953	174	5.5
4B	869	153	5.7
4D	58	66	0.9
5A	954	211	4.5
5B	1966	167	11.8
5D	244	51	4.8
6A	1101	149	7.4
6B	1542	197	7.8
6D	134	79	1.7
7A	1386	192	7.2
7B	1249	161	7.8
7D	133	82	1.6
A-genome	7616	1269	6.1
B-genome	10726	1174	9.1
D-genome	1588	560	2.8

Table 3.3 Chromosome location, minor allele frequency (MAF), p-value and R^2 of the 94 markers significantly associated with six phenotypic traits evalauted in 81 western Canada spring wheat cultivars. Markers were declared significant at a minimum threshold p-value of 5×10^{-5} or Log_{10} (1/p) value $\geq$ 4.3.

Trait	Marker	Chrom	Position (cM)	P-value	Log_{10} (1/p)	R^2	Minor allele frequency	Proportion of missing
Ptr ToxA	BS00064197_51	1A	52	1.99E-05	4.7	25.2%	0.44	0.0%
Ptr ToxA	Kukri_c23985_229	1A	52	1.99E-05	4.7	25.2%	0.44	0.0%
Ptr ToxA	BobWhite_c4646_119	1A	53	1.99E-05	4.7	25.2%	0.44	0.0%
Ptr ToxA	Excalibur_c47654_70	1A	53	1.99E-05	4.7	25.2%	0.44	0.0%
Ptr ToxA	Excalibur_c77035_156	1A	53	2.33E-05	4.6	25.1%	0.44	1.2%
Ptr ToxA	IACX5982	1A	53	1.99E-05	4.7	25.2%	0.44	0.0%
Ptr ToxA	Kukri_c23985_166	1A	53	1.99E-05	4.7	25.2%	0.44	0.0%
Ptr ToxA	Kukri_rep_c104386_273	1A	53	1.99E-05	4.7	25.2%	0.44	0.0%
Ptr ToxA	RAC875_c5544_4156	1A	53	1.99E-05	4.7	25.2%	0.44	0.0%
Ptr ToxA	Excalibur_c37642_1416	5B	71	4.56E-07	6.3	33.4%	0.20	0.0%
Ptr ToxA	Ex_c13277_2025	5B	73	4.56E-07	6.3	33.4%	0.20	0.0%
Ptr ToxA	Kukri_c17396_2448	5B	73	4.56E-07	6.3	33.4%	0.20	0.0%
Ptr ToxA	Kukri_c54078_114	5B	73	8.22E-06	5.1	27.5%	0.18	1.2%
Ptr ToxA	Kukri_c90424_72	5B	73	4.56E-07	6.3	33.4%	0.20	0.0%
Ptr ToxA	Kukri_rep_c113115_261	5B	73	4.56E-07	6.3	33.4%	0.20	0.0%
Ptr ToxA	Kukri_rep_c113115_424	5B	73	4.56E-07	6.3	33.4%	0.20	0.0%
Ptr ToxA	Ra_c38583_333	5B	73	4.56E-07	6.3	33.4%	0.20	0.0%
Ptr ToxA	wsnp_Ex_c13277_20936069	5B	73	4.56E-07	6.3	33.4%	0.20	0.0%
Ptr ToxA	wsnp_Ku_c3102_5810751	5B	73	4.46E-08	7.4	38.0%	0.16	0.0%
Ptr ToxA	wsnp_Ku_c3102_5811860	5B	73	4.46E-08	7.4	38.0%	0.16	0.0%
Ptr ToxA	BS00010590_51	5B	74	2.32E-24	23.6	80.8%	0.37	0.0%
Ptr ToxA	BobWhite_c48435_165	5B	74	2.04E-13	12.7	57.7%	0.42	0.0%
Ptr ToxA	IACX9261	5B	74	2.32E-24	23.6	80.8%	0.37	0.0%
Ptr ToxA	Tdurum_contig25513_123	5B	74	1.21E-12	11.9	55.8%	0.41	1.2%
Ptr ToxA	tplb0027f13_1493	5B	74	1.86E-12	11.7	55.6%	0.42	2.5%
Ptr ToxA	tplb0027f13_452	5B	74	1.21E-12	11.9	55.8%	0.41	1.2%
Ptr ToxA	wsnp_Ku_c17396_26488733	5B	74	3.23E-06	5.5	29.9%	0.19	2.5%
Ptr ToxA	wsnp_Ku_c40334_48581010	5B	74	1.68E-27	26.8	86.0%	0.39	4.9%
Ptr ToxB	Excalibur_c23598_1632	1A	122	1.78E-07	6.8	32.9%	0.08	1.2%
Ptr ToxB	Kukri_rep_c103261_918	2B	17	8.63E-06	5.1	25.0%	0.27	0.0%
Ptr ToxB	Kukri_rep_c69177_180	2B	17	8.63E-06	5.1	25.0%	0.27	0.0%
Ptr ToxB	RAC875_c2698_132	2B	17	3.93E-05	4.4	22.1%	0.28	0.0%
Ptr ToxB	RAC875_c62831_255	2B	17	8.63E-06	5.1	25.0%	0.27	0.0%
Ptr ToxB	Excalibur_c14396_1629	2B	18	4.56E-05	4.3	21.3%	0.27	0.0%
Ptr ToxB	Kukri_c148_1484	2B	18	6.91E-06	5.2	26.1%	0.17	0.0%
Ptr ToxB	wsnp_Ku_c9883_16462146	2B	19	4.11E-05	4.4	22.4%	0.25	0.0%
Ptr ToxB	Kukri_c148_1346	2B	20	6.91E-06	5.2	26.1%	0.17	0.0%
Ptr ToxB	Kukri_c148_1512	2B	20	4.49E-06	5.3	27.3%	0.18	1.2%
Ptr ToxB	BS00010318_51	2B	21	2.90E-10	9.5	45.0%	0.22	3.7%
Ptr ToxB	BS00070050_51	2B	21	2.57E-08	7.6	36.9%	0.20	1.2%
Ptr ToxB	BS00070051_51	2B	21	8.25E-08	7.1	34.5%	0.20	0.0%
Ptr ToxB	BS00072619_51	2B	21	8.25E-08	7.1	34.5%	0.20	0.0%
Ptr ToxB	BS00072620_51	2B	21	2.57E-08	7.6	36.9%	0.20	1.2%

Ptr ToxB	BS00075303_51	2B	21	8.25E-08	7.1	34.5%	0.20	0.0%
Ptr ToxB	RAC875_c38003_164	2B	21	1.78E-05	4.7	24.3%	0.31	0.0%
Ptr ToxB	wsnp_Ra_c4321_7860456	2B	21	1.64E-07	6.8	31.0%	0.42	0.0%
Ptr ToxB	Ku_c63748_1264	2B	22	7.71E-06	5.1	25.4%	0.37	0.0%
Ptr ToxB	RAC875_c19575_84	2B	22	1.53E-05	4.8	23.9%	0.36	0.0%
Ptr ToxB	wsnp_Ex_rep_c66551_64836327	2B	22	1.96E-05	4.7	23.9%	0.34	2.5%
Ptr ToxB	Kukri_c45103_371	2B	24	1.87E-06	5.7	29.9%	0.37	2.5%
Ptr ToxB	Excalibur_c1986_439	2B	25	1.87E-06	5.7	29.9%	0.37	2.5%
Ptr ToxB	Excalibur_rep_c112367_293	2B	25	3.57E-06	5.4	28.1%	0.36	1.2%
Ptr ToxB	GENE-1343_556	2B	25	8.25E-08	7.1	34.5%	0.20	0.0%
Ptr ToxB	Kukri_c3067_398	2B	25	2.73E-05	4.6	23.6%	0.36	1.2%
Ptr ToxB	BS00011149_51	2B	27	1.80E-07	6.7	33.9%	0.33	1.2%
Ptr ToxB	IAAV5802	2B	31	1.64E-07	6.8	31.0%	0.42	0.0%
Ptr ToxB	wsnp_Ex_c19371_28311667	2B	31	1.64E-07	6.8	31.0%	0.42	0.0%
Ptr ToxB	wsnp_Ex_rep_c105401_89840110	2B	31	1.64E-07	6.8	31.0%	0.42	0.0%
Ptr ToxB	IACX8278	2B	39	1.69E-05	4.8	24.4%	0.47	3.7%
Ptr ToxB	BS00064274_51	5B	123	5.27E-06	5.3	26.8%	0.16	0.0%
Ptr ToxB	BobWhite_c14360_420	5B	123	5.27E-06	5.3	26.8%	0.16	0.0%
Ptr ToxB	Excalibur_c23661_1712	5B	123	5.27E-06	5.3	26.8%	0.16	0.0%
Ptr ToxB	Excalibur_c32979_1152	5B	123	5.27E-06	5.3	26.8%	0.16	0.0%
Ptr ToxB	GENE-2855_121	5B	123	5.27E-06	5.3	26.8%	0.16	0.0%
Ptr ToxB	IAAV5779	5B	123	5.27E-06	5.3	26.8%	0.16	0.0%
Ptr ToxB	IACX11346	5B	123	5.27E-06	5.3	26.8%	0.16	0.0%
Ptr ToxB	Kukri_c11272_486	5B	123	4.29E-06	5.4	27.3%	0.14	0.0%
Ptr ToxB	wsnp_Ex_c23661_32900048	5B	123	5.27E-06	5.3	26.8%	0.16	0.0%
Ptr ToxB	wsnp_RFL_Contig3139_3096141	5B	123	5.27E-06	5.3	26.8%	0.16	0.0%
Ptr ToxB	wsnp_RFL_Contig3238_3265410	5B	123	5.27E-06	5.3	26.8%	0.16	0.0%
Ptr ToxB	BS00097105_51	5B	124	2.03E-05	4.7	24.0%	0.35	0.0%
Ptr ToxB	Kukri_rep_c112425_506	5B	124	2.03E-05	4.7	24.0%	0.35	0.0%
Ptr ToxB	Kukri_rep_c112425_98	5B	124	2.03E-05	4.7	24.0%	0.35	0.0%
Ptr ToxB	RAC875_c6890_150	5B	124	2.03E-05	4.7	24.0%	0.35	0.0%
Ptr ToxB	Tdurum_contig8171_1548	5B	124	2.03E-05	4.7	24.0%	0.35	0.0%
Ptr ToxB	Tdurum_contig8171_1712	5B	124	2.03E-05	4.7	24.0%	0.35	0.0%
Ptr ToxB	Tdurum_contig8171_1772	5B	124	2.03E-05	4.7	24.0%	0.35	0.0%
Common bunt	BS00032381_51	2B	162	2.58E-05	4.6	18.7%	0.49	0.0%
Common bunt	Excalibur_c48404_59	2B	162	2.58E-05	4.6	18.7%	0.49	0.0%
Common bunt	Kukri_c38413_121	2B	162	2.58E-05	4.6	18.7%	0.49	0.0%
Common bunt	wsnp_Ex_c15646_23969140	2B	162	2.58E-05	4.6	18.7%	0.49	0.0%
Common bunt	Excalibur_c36630_2194	4B	43	3.80E-05	4.4	8.7%	0.40	0.0%
Common bunt	RAC875_c104178_425	4B	83	3.02E-05	4.5	17.5%	0.24	6.3%
Common bunt	BS00065648_51	7A	36	4.39E-07	6.4	11.5%	0.19	1.2%
Common bunt	BS00074110_51	7A	36	4.39E-07	6.4	11.5%	0.19	1.2%
Common bunt	Kukri_c106476_87	7A	48	8.06E-07	6.1	20.5%	0.43	7.3%
Common bunt	Excalibur_c9109_428	7A	192	1.91E-06	5.7	10.3%	0.48	4.9%
Leaf rust	BobWhite_c18540_97	2B	123	8.97E-06	5.0	19.2%	0.10	0.0%
Leaf rust	BobWhite_c18540_351	2B	127	8.97E-06	5.0	19.2%	0.10	0.0%
Leaf rust	wsnp_Ra_rep_c74497_72390803	2B	127	8.97E-06	5.0	19.2%	0.10	0.0%
Leaf rust	BS00079941_51	2B	167	4.85E-06	5.3	18.1%	0.05	4.9%
Leaf rust	RAC875_c52856_250	2B	167	5.55E-06	5.3	19.3%	0.05	1.2%
Tan spot	BobWhite_rep_c66630_331	7B	77	1.98E-05	4.7	21.0%	0.47	0.0%
Yellow rust	wsnp_Ex_c997_1906900	2A	21	2.77E-05	4.6	19.1%	0.29	1.2%

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Chapter 4 Allelic variation and effect of 16 candidate genes on disease reaction in western Canadian spring wheat

4.1 Introduction

Over 85% of Canadian wheat is produced in the western Prairie Provinces of Manitoba, Saskatchewan and Alberta (McCallum and DePauw, 2008). In the Prairie Provinces where the growing season is short and days are long, the development of early maturing cultivars avoids frost damage, which directly affects both grain yield and grain quality (Iqbal et al., 2007; Randhawa et al., 2013). Several studies have reported the direct influence of both the vernalization (*Vrn*) and photoperiod (*Ppd*) response genes on flowering time and maturity, which mapped on homeologous group 5 (Law and Worland, 1997; Yan et al., 2003) and group 2 (Worland et al., 1998; Tanio and Kato, 2007; Wilhelm et al., 2009; Zanke et al., 2014) chromosomes. In bread wheat, vernalization response is controlled by three *Vrn* loci (*Vrn-1*, *Vrn-2* and *Vrn-3*) of which *Vrn-A1*, *Vrn-B1 and Vrn-D1* mapped on the long arm of chromosomes 5A, 5B and 5D, respectively (Preston and Kellogg, 2008; Chen et al., 2013). Photoperiod response is primarily controlled by the *Ppd-1* loci that mapped on the short arms of chromosomes 2D, 2B, and 2A (Law et al., 1978; Scarth and Law, 1983; Beales et al., 2007; Wilhelm et al., 2009; Chen et al., 2013). In general, the *Ppd-D1* gene has been considered the strongest photoperiod insensitivity allele, followed by *Ppd-B1* and *Ppd-A1* (Worland et al., 1998; Guo et al., 2010; Chen et al., 2013), but there are conflicting reports that suggests that *Ppd-B1a* could be as strong as *Ppd-D1* (Tanio and Kato, 2007).

Dwarfing has been achieved mainly through the introduction of the favorable alleles of two height reducing (*Rht*) genes, such as the *Rht-B1b* on chromosome 4BS and *Rht-D1b* on chromosome 4DS (Ellis et al., 2002; Pearce et al., 2011). These two alleles have been introduced in many cultivars grown worldwide, which resulted in increased grain yield through reduced lodging, improved harvest index, and greater grain biomass assimilation potential (Evenson and Gollin, 2003; Borojevic and Borojevic, 2005). Both alleles are present in a significant proportion of globally registered wheat cultivars. For example, *Rht-D1b* was reported in 44 % of all registered wheat cultivars in Germany (Knopf et al., 2008), 46% of Chinese wheat (Zhang et al., 2006), and over 90% of modern wheat cultivars in the USA (Guedira et al., 2010). Recently, our wheat breeding group at the University of Alberta investigated the effect of eight genes (*Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Ppd-A1*, *Ppd-B1, Ppd-D1, Rht-B1* and *Rht-D1*) on a subset of 82 spring wheat cultivars registered in western Canada over a century (Chen et al., 2016b). That study revealed the presence of genetic variation for all genes, except *Ppd-A1* and *Ppd-B1*: (i) approximately 40% of the cultivars carried both *Vrn-A1a* and *Vrn-B1;* (ii) a quarter of the cultivars consisted of the semi-dwarfing *Rht-B1b* or *Rht-D1b*, but none carried both alleles; (iii) cultivars that consisted of the *Vrn-B1*, *Rht-1b* and *Ppd-D1a* haplotype were shorter and produced the highest yielded than all other combination of these three genes; (iv) cultivars with *vrn-B1*, *Rht-1a* and *Ppd-D1b* haplotypes yielded the lowest grain, but they were 16 cm taller than those having *Vrn-B1*, *Rht-1b* and *Ppd-D1a* in combination (Chen et al., 2016b). Both *Vrn-A1a* and *Vrn-B1* existed in Canadian Spring wheat germplasm registered over a century, but *Ppd-D1a* and *Rht-B1*/*Rht-D1* were detected only in cultivars registered after 1986 and 1990, respectively.

The target breeding traits in the Prairie Provinces are not only higher yield potential, early maturity and short stature, but also good end-use quality and improved resistance to diseases.

Currently, cultivars to be registered in western Canada must be at least intermediately resistant to stem rust (*Puccinia graminis* f. sp. *tritici*), leaf rust (*Puccinia triticina*), yellow (stripe) rust (*Puccinia striiformis* f. sp. *tritici*), common bunt (caused by two very closely related fungi, *Tilletia tritici* and *Tilletia laevis*) and fusarium head blight (http://www.pgdc.ca). Tan spot, caused by *Pyrenophora tritici-repentis* (Ptr), is another economically important leaf spotting disease of wheat in Canada and other major wheat growing countries (Strelkov and Lamari, 2003; Lamari and Strelkov, 2010; Faris et al., 1997; Friesen and Faris, 2004). Ptr isolates produce three host specific toxins (HSTs) which have been designated as Ptr ToxA, Ptr ToxB, and Ptr ToxC (Ciuffetti et al., 1998; Ciuffetti et al., 2010; Lamari and Strelkov, 2010). Fungal isolates producing both Ptr ToxA and Ptr ToxC are highly abundant in the Prairie Provinces, while those producing Ptr ToxB are extremely rare in this region (Lamari et al., 1998; Lamari et al., 2003; Aboukhaddour et al., 2013).

In one of our previous studies, we studied the genetic relationship and population structure of 81 historical and modern Canadian western spring wheat cultivars released between 1885 and 2011, and identified genomic regions associated with resistance to four wheat diseases (yellow rust, leaf rust, tan spot and common bunt) and insensitivity to three Ptr toxins (Ptr ToxA, Ptr ToxB and Ptr ToxC). Based on disease scores from multi-location field experiments, Ptr toxins reactions under greenhouse experiments and genome-wide single nucleotide polymorphic (SNP) markers from a Wheat 90K iSelect array, we identified 94 markers associated with four diseases and two toxins (Perez-Lara et al., 2016b). In that study, we did not use any of the known gene-specific markers associated with resistance to the three rusts, tan spot, and Ptr ToxA. The objectives of the present study were therefore to: (i) report on the total allelic makeup of 70 cultivars for 50 disease resistance markers to aid spring wheat breeders in parental choice for future crossing programs,

(ii) survey allelic variation of a subset of selected genes associated with grain quality and resistance to diseases on a subset of spring wheat cultivars registered in western Canada in the last 5 decades; and (iii) understand the phenotypic effect of the genes on maturity, plant height, grain yield, and four wheat diseases.

4.2 Material and methods

4.2.1 Plant material and phenotyping

We initially used 82 historical and modern spring wheat cultivars registered in western Canada between 1885 and 2011. The 82 cultivars, along with susceptible and resistant checks, were evaluated for yellow rust at eight environments (trials) under field conditions at Creston, British Columbia (49.06° N, 116.31° W) between 2013 and 2015, at Lethbridge, Alberta (49.7° N, 112.83° W) between 2012 and 2015, and at St. Albert, Alberta (53.63 N, 113.63°W) in 2015. They also were evaluated at four environments (2012 to 2015) for their reaction to leaf rust and tan spot at the Crop Research Facility of the University of Alberta, Edmonton. In all trials, the cultivars were evaluated in a randomized complete block design with two replications in disease screening nurseries. Each cultivar was also evaluated for Ptr ToxA reaction twice under greenhouse conditions, each in three replications. The detailed phenotyping protocols for diseases and toxin reactions have been described in our previous study (Perez-Lara et al., 2016b). Visual disease assessment was done on a scale of 1 (no visible sign or symptom = resistant) to 9 (leaf area totally covered with spores or spore pustules depending on the disease = highly susceptible) on each hill plot basis. Reactions of the cultivar to Ptr ToxA was recorded as either 0 (no toxin reaction or insensitive) or 1 (toxin reaction or sensitive) three days post-infiltration.

As described in another study (Chen et al., 2016b), the cultivars also were evaluated at 6 field environments for 7 agronomic traits in randomized incomplete block design with two to three replications at the University of Alberta South Campus Crop Research facility in Edmonton, Canada in 2011, 2012 and 2013; at the University of Alberta St. Albert Research Center, Canada, in 2013, and at the University of Saskatchewan Kernen Crop Research Center in Saskatoon, Canada, in 2011 and 2012. Of the 7 traits that were evaluated for every cultivar, we only selected maturity, plant height, and grain yield in order to assess the effect of a subset of selected genes associated with resistance to diseases and toxin reactions on these three agronomic traits.

4.2.2 Genotyping

Genomic DNA was extracted from 3-4 week old greenhouse grown seedlings using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1987). DNA concentration was measured with a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, USA), and normalized to about 100 ng/µL. The 82 cultivars were genotyped using 50 gene specific markers using three genotyping platforms (Table 4.1). First, the cultivars were genotyped with markers associated with flowering/maturity (*Vrn-A1, Vrn-B1 and Ppd-D1)* and plant height (*Rht-B1*, *Rht-D1*) at the Agricultural Genomics and Proteomics lab, University of Alberta, Edmonton, as described in one of our previous studies (Chen et al., 2016b). Second, the cultivars were genotyped with 12 markers associated with resistance to rusts (*Sr2/Yr30*, *Sr26*, *Lr34/Yr18*, *Lr46/Yr29*, *Yr5*, *Yr10*, *Yr26*, *Yr36* and *Yr68*), *Tsn1* which regulates Ptr ToxA sensitivity, and *Rht8* that using our in-house genotyping system as described in a recent study (Perez-Lara et al., 2016a). Third, the cultivars were genotyped with 4 single nucleotide

polymorphic (SNP) markers that detect point mutations at the *Lr34/Yr18* (wMAS000003 and wMAS000004), *Rht-B1* (wMAS000001) and *Rht-D1* (wMAS000002) loci using the Kompetitive Allele Specific PCR (KASP) platform (Semagn et al., 2014; Grogan et al., 2016) at the LGC Genomics Lab, USA (http://www.lgcgroup.com). Detailed information about KASP-based SNPs recommended for marker-assisted selection in wheat is available at http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/kasp_download.php. Finally, the cultivars were genotyped with the Wheat 90K iSelect SNP array (Wang et al., 2014) as described in a previous study (Perez-Lara et al., 2016b).

4.2.3 Statistical analyses

Linkage disequilibrium (LD) among the gene-specific markers and the Wheat 90K SNPs array that mapped on the same chromosome was evaluated by computing the squared correlation r^2 values between pairs of markers using TASSEL v5.2.30 (Bradbury et al., 2007). A total of 29 SNPs that had > 0.70 LD with the *Yr10* locus on chromosome 1B (11 SNPs), the *Tsn1* locus on 5B (6 SNPs) and the *Rht8* locus on 2D (12 SNPs) were selected and used in the present study. After excluding cultivars that had >20% missing genotype data, the final dataset used for all statistical analyses consisted of 70 cultivars, 50 gene-specific markers and 4 phenotypic traits (yellow rust, leaf rust, tan spot and Ptr ToxA), which are given in Appendix 6. In order to understand the effect of allelic variants that increased resistance to diseases or insensitivity to Ptr ToxA, we also included maturity, plant height and grain yield for comparison purposes only in the discussion section.

We performed five different univariate and multivariate analyses. First, Spearman rank correlation coefficients between markers and the four phenotypic traits were computed using XLSTAT 2012. Second, we performed partial least squares regression (PLSR) analysis to select the best markers that predict each of the phenotypic traits used in our study. PLS is a multivariate regression analysis technique that models both the predictor (X) and response (Y) matrices simultaneously to find the latent (or hidden) variables in X that best predict the latent variables in *Y*. Details on the application and methodology of PLSR for exploring the relationship between genotypic and phenotypic data are provided in other studies (Høy et al., 1998; Bjørnstad et al., 2004; Colombani et al., 2012). PLSR was performed using the genotype data as predictors against each of the phenotypic traits as a response variable using 'THE UNSCRAMBLER' software, v9 (http://www.camo.com). For every phenotypic trait, PLSR analysis was performed by standardizing the genotypic and phenotypic variables using a standard deviation weighting option (centered to a mean of 0, and scaled to a standard deviation of 1). The first two components were plotted for visual examination of the clustering pattern of the markers (X-axis) and phenotypic traits (Y- axis).

Third, we ran stepwise discriminant analysis to select a subset of molecular markers that best reveal the difference among categorical variables (phenotypic classes of each trait). For each phenotypic trait, the categorical variables were obtained by assigning each cultivar into resistant, moderately susceptible or susceptible categories based on disease scores, and into sensitive or insensitive groups based on Ptr toxin reaction. For all three diseases, cultivars with mean disease scores $\leq$ 3.0, 3.1-5.0 and >5.0 were considered resistant, moderately susceptible and susceptible, respectively. We performed stepwise discriminant analysis using the PROC STEPDISC procedure of SAS v9.4 as described elsewhere (Semagn et al., 2000). For each trait, markers

were chosen to enter or leave the discrimination model among groups based on the significance level of an F test ($p = 0.15$) from analysis of covariance, where the markers already chosen act as covariates and markers under consideration as dependent variable. Fourth, classificatory discriminant analysis was performed for each categorical variable (phenotypic class) using the software JMP version 7.0 (SAS Institute Inc., Cary, NC, USA). For each categorical variable, the first two canonical components were plotted for visual examination of the clustering pattern of the cultivars. Finally, analysis of variance (ANOVA) was performed on a subset of markers selected by PLSR, STEPDISC and/or correlation analysis in order to: (i) determine if there was a significant difference on phenotypic effect of the allelic variants of each marker, and (ii) identify the favorable allele(s) that increased resistance to rusts and tan spot or Ptr ToxA insensitivity.

4.3 Results

4.3.1 Allele frequency and correlation analysis

Each of the 50 gene-specific markers was fixed (homozygous) in nearly all 70 spring wheat cultivars, with allele frequency varying from 0.06 to 0.94. Major allele frequency in 27 markers associated with 8 genes (*Lr46/Yr29, Sr26*, *Yr5*, *Tsn1*, *Vrn-A1*, *Rht-D1*, *Rht-B1* and *Rht8*) varied from 0.75 to 0.94. For the remaining 23 markers, major and minor allele frequencies varied from 0.51 to 0.74 and from 0.27 to 0.49, respectively (Table 4.1). We computed Spearman correlation coefficients to identify the markers that were significantly associated with each of the four phenotypic traits. A total of 15 of the 50 markers showed significant ($p < 0.05$) positive or negative correlations with one or more of the four phenotypic traits used in the present study (Table 4.2). The significant correlations included 6 markers (cssfr5, wMAS000003 and

wMAS000004 for *Lr34/Yr18* plus Kukri_c54078_114, Tdurum_contig57027_347 and wsnp_Ku_c17396_26488733 for *Tsn1*) with yellow rust, one marker (*Vrn-A1*) with leaf rust, 6 markers (cssfr5, wMAS000003 and wMAS000004 for *Lr34/Yr18*, csGS for *Lr68* plus Tdurum_contig57027_347 and wsnp_Ku_c17396_26488733 for *Tsn1*) with tan spot, and 11 markers (Xfcp1, Xfcp394, Kukri_c54078_114, Tdurum_contig25513_123, Tdurum_contig57027_347, wsnp_Ku_c17396_26488733, wsnp_Ku_c3102_5810751 and wsnp_Ku_c3102_5811860 for *Tsn1; Vrn-A1*; *Ppd-D1*; S23M41 for *Yr5*) with Ptr ToxA (Table 4.2). All markers that were significantly correlated with yellow rust with the exception of Kukri_c54078_114, were also correlated with Ptr ToxA. Similarly, two markers were also significantly correlated with both tan spot and Ptr ToxA.

4.3.2 Partial least square regression (PLSR)

Partial least square regression identified 8 markers, which included (cssfr5, wMAS000003 and wMAS000004 for *Lr34/Yr18*; Xfcp1, Kukri_c54078_114, Tdurum_contig25513_123, Tdurum_contig57027_347, wsnp_Ku_c17396_26488733 and wsnp_Ku_c3102_5810751 for *Tsn1*) as the main predictor of yellow rust disease scores (Figure 4.1). The two flanking SNP markers for *Lr34/Yr18* (wMAS000003 and wMAS000004) were better predictors for yellow rust than any of the other markers (Figure 4.1). Approximately 39% of the cultivars consisted of the T:T and C:C haplotypes at the wMAS000003 and wMAS000004 markers, respectively, which on average had a 0.6 lower yellow rust score than those cultivars with the A:A and T:T haplotypes (Table 4.3).

For leaf rust, we found 6 markers associated with three genes, *Vrn-A1*; cssfr5, wMAS000003 and wMAS000004 for *Lr34/Yr18*; csGS for *Lr68*, and Xbarc181 for *Yr26*, as the main predictor for leaf rust disease scores (Figure 4.1). However, both *Lr34/Yr18* and *Vrn-A1* appear to be the best predictor for leaf rust. Approximately 39% of the cultivars consisted of the T:T and C:C haplotypes at wMAS000003 and wMAS000004 markers, respectively, and had on average a leaf rust disease score 1.3 lower than those cultivars with the A:A and T:T haplotypes (Table 4.4). For the *Vrn-A1* gene, approximately 90% of the cultivars had the dominant *Vrn-A1a* allele and an average leaf rust score 2.0 lower than those cultivars that had the recessive *vrn-A1* allele.

For tan spot, a group of 9 markers associated with both *Lr34/Yr18* (cssfr5, wMAS000003 and wMAS000004) and *Tsn1* (Xfcp1, Kukri_c54078_114, Tdurum_contig25513_123, Tdurum_contig57027_347, wsnp_Ku_c17396_26488733 and wsnp_Ku_c3102_5810751) were identified by PLSR as the main predictors (Figure 4.2), but the predictive ability of every marker was not that strong (Appendix 6). As indicated above, all markers identified as the predictor of tan spot disease resistance, except Tdurum_contig25513_123, were also identified as the main predictor of yellow rust resistance. When haplotypes of the *Lr34/Yr18* and *Tsn1* gene-specific markers were considered separately, (i) 14 out of the 70 cultivars possessed the favorable haplotype combinations for the *Tsn1* gene and had 0.7 lower tan spot disease score than those cultivars that possessed the unfavorable haplotypes; (ii) 26 out of the 70 cultivars possessed the favorable haplotype combinations at the three *Lr34/Yr18* markers and had 0.6 lower tan spot disease score than those cultivars that possessed the unfavorable haplotypes. PLSR identified 6 markers associated with the *Tsn1* gene (Xfcp1, Xfcp394, Kukri_c54078_114, Tdurum_contig25513_123, Tdurum_contig57027_347, wsnp_Ku_c17396_26488733 and wsnp_Ku_c3102_5810751) as the main predictor of Ptr ToxA reaction; all the 6 markers were

also selected for tan spot. However, Tdurum_contig25513_123 and wsnp_Ku_c3102_5810751 were the most discriminatory markers for Ptr ToxA reaction (Appendix 6).

4.3.3 Discriminant analyses

Stepwise discriminant analysis was used to identify a subset of linear combinations of the gene specific markers that best reveals the variation among *a priori* defined groups (classes) for disease reaction (susceptible, moderate or resistant) and Ptr ToxA reaction (sensitive vs insensitive). The stepwise discrimination model identified a total of 17 markers associated with one or more of the four phenotypic traits (Table 4.5), which included 4 markers for leaf rust, 6 markers for yellow rust, 5 markers for tan spot and 6 markers for Ptr ToxA. Sixteen of the 17 markers identified by the stepwise discriminant analyses individually explained between 5.4 and 21.7, while one marker associated with Ptr ToxA (Tdurum_contig25513_123) explained a very high proportion (69.6%) of the variation for Ptr ToxA reaction. Tdurum_contig25513_123 also had the highest correlation value with Ptr ToxA ($r = 0.79$) and was also identified as one of the best predictors for Ptr ToxA by PLSR. We used classificatory discriminant analysis to evaluate the usefulness of the 50 markers in predicting the most likely group membership of each cultivar into resistant, moderately susceptible or susceptible categories and into sensitive or insensitive to Ptr ToxA reactions. The classificatory discriminant analysis assigned 90%, 80%, 94%, and 97% of the cultivars into their respective predefined group based on yellow rust, leaf rust, tan spot and Ptr ToxA reactions (Figures 4.3 and 4.4). These results suggest good prediction ability of the gene-specific markers for yellow rust, tan spot and toxin reaction, but not so good for leaf rust.

4.3.4 Analysis of variance

For all markers that were significantly correlated with one or more of the phenotypic traits or those markers that were identified as the best predictor (explanatory variable) either by PLSR and/or stepwise discriminant analyses, we compared differences in allelic frequency and the effect of the variant alleles on each trait using ANOVA. Overall, we found a total of 9 markers for leaf rust, 13 markers for tan spot and Ptr ToxA and 12 markers for yellow rust. Excluding markers that were identified two or more times by the different univariate or multivariate methods, 23 of the 50 markers were either significantly correlated with the phenotypic traits or they were selected as the best predictor of the phenotypic traits by PLSR and/or stepwise discriminant analyses (Table 4.5). Of the 23 markers identified by the different methods as predictors of one or more of the three phenotypic traits, ANOVA revealed significant differences between allelic variants of all markers except four markers (Xwmc44, S23M41, *Vrn-B1* and Xfcp394) (Table 4.3). This includes 16 markers for yellow rust, 8 markers for leaf rust and 11 markers for tan spot, of which 6 markers were common between yellow and leaf rust, 10 markers were common between yellow rust and tan spot, and 4 markers were common for all three traits. The same alleles from four markers (*Vrn-A1* plus cssfr5, wMAS000003 and wMAS000004 for *Lr34/Yr18*) significantly increased both leaf and yellow rust resistance by decreasing disease scores up to 1.6 (in 1 to 9 scale). Cultivars with the resistance *Lr34/Yr18* allele at the STS cssfr5 marker and the two KASP-based SNPs (wMAS000003 and wMAS000004) had 0.5-1.3 lower yellow rust, leaf rust and tan spot, disease scores, produced 49-195 kg ha^{-1} more grain yield, and were 2-3 cm taller than those that possessed the susceptible allele (Table 4.3); cultivars with and without the *Lr34/Yr18* resistance allele did not differ in maturity. Cultivars with the *Vrn-A1a* allele showed a reduction in both yellow rust and leaf rust disease scores by 0.9-2.0, matured 4

days earlier, but had a 0.7 higher tan spot score, produced 418 kg ha^{-1} lower grain yield and were 9 cm taller than those cultivars with the *vrn-A1* allele (Table 4.3).The number of cultivars with the *Vrn-A1a* allele was 62, which was 12 fold greater than those with *vrn-A1*. Ptr ToxA reaction was scored as qualitative data (sensitive vs. insensitive) and we were not able to perform ANOVA.

4.4 Discussion

Targeted breeding traits in the prairie provinces of Canada are early maturity, short plant height, higher yield potential with good end-use quality, and improved resistance to priority wheat diseases such as yellow rust, stem rust, and leaf rust, common bunt and fusarium head blight. Using conventional breeding methods and marker-assisted selection, breeders in the region have spent much time and resources in developing various improved spring wheat cultivars (McCallum and DePauw, 2008), which possess combinations of alleles of well-known candidate genes which contribute to shortened flowering/maturity time and plant height and increased resistance to leaf, stem and yellow rusts.

Our group has recently investigated the genetic variation of 6 candidate genes for flowering/maturity time (*Vrn-A1*, *Vrn-B1, Vrn-D1* and *Ppd-D1*) and plant height (*Rht-B1* and *Rht-D1*) on a set of 82 cultivars evaluated for different agronomic and quality traits (Chen et al., 2016b). That study demonstrated the presence of large differences in allelic frequencies of each candidate gene and its effect: (i) cultivars with the *Vrn-B1*, *Rht-1b* and *Ppd-D1a* haplotype produced the highest yielded and were shorter than all other combinations of these three genes; (iv) cultivars with *vrn-B1*, *Rht-1a* and *Ppd-D1b* in combination yielded the lowest and were 16

cm taller than those having *Vrn-B1*, *Rht-1b* and *Ppd-D1a* in combination. In the present study, we advanced the work further using 50 markers associated with 16 candidate genes that are associated with stem, leaf and yellow rust (9 genes), tan spot or Ptr ToxA reaction (1 gene), flowering/maturity time (3 genes) and plant height (3 genes).

Our results revealed the presence of high variation in allele frequency. For some markers, the minor and major allele frequencies were fairly comparable, ranging between 0.3 and 0.5, while for others the differences between minor and major alleles were very large, which varied from 0.06 to 0.94 (Table 4.1). Of the 50 markers used in the present study, a total of 15 markers associated with 6 candidate genes (*Lr34/Yr18*, *Lr68*, *Yr5*, *Tsn1*, *Ppd-D1* and *Vrn-A1*) showed significant positive or negative correlations with the leaf rust, yellow rust, tan spot and Ptr ToxA reactions (Table 4.2). Although a significant positive or negative correlation coefficient does not necessarily imply a cause-and-effect relationship, we found it very useful in understanding the relationship among the markers and the phenotypic traits. Approximately 93% of the markers that were found to be significantly correlated with one or more of the four phenotypic traits were also identified as the best predictors or explanatory variables by the multivariate PLSR and/or STEPDISC methods. The 23 markers (associated with 13 candidate genes) were identified as the best predictor/explanatory variables for one or more of the four phenotypic traits (Appendix 7). Using ANOVA we uncovered significant differences between pairs of allelic variants for 19 of the 23 markers (Table 4.3). Overall, between 8 and 16 markers were associated with each of the phenotypic traits.

Over 70 leaf rust resistance (*Lr*) and 65 yellow rust resistance (*Yr*) genes have been reported in the literature (McIntosh et al., 2012), but only some genes and gene combinations have provided a good level of resistance to rusts in many of the cultivars grown in the region (McCallum et al.,

2007), which includes the slow-rusting *Lr34*/*Yr18* on chromosome 7DS (Suenaga et al., 2003), *Lr46*/*Yr29* on 1BL (William et al., 2003), *Sr2*/*Yr30* on 3BS (Singh et al., 2005), and *Lr67/Yr46* on 4DL (Herrera-Foessel et al., 2014). *Lr34/Yr18* is one of the few non-race-specific loci that confers resistance against multiple diseases, including stem rust, leaf rust, yellow rust, powdery mildew and leaf tip necrosis (Dyck, 1987; Spielmeyer et al., 2005; Lagudah et al., 2009; Lillemo et al., 2013). Five allele-specific sequence-tagged site (STS) (cssfr1–cssfr5) and one SNP (cssfr6) marker were developed for *Lr34/Yr18* (Lagudah et al., 2009). However, cssfr5 and cssfr6 have been recommended by the authors as the best markers for selecting the resistance allele from *Lr34/Yr18* using low throughput agarose gel electrophoresis and capillary electrophoresis, respectively (Lagudah et al., 2009).

Using the cssfr5 marker, our group recently investigated the effect of *Lr34/Yr18* allelic variants on diseases and agronomic traits on a recombinant inbred lines (RIL) population derived from 'CDC Teal' x 'CDC Go' spring wheat cultivars (Chen et al., 2016a). In that study, lines carrying the resistance allele from the cssfr5 matured earlier, but were taller and produced lower grain yield than those lines with the susceptible allele. In the present study, we used the cssfr5 marker along with two additional SNPs (wMAS000003 and wMAS000004) that have been recommended for selecting *Lr34/Yr18* resistance alleles.(http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/kasp_download.php). Cultivars with the resistance allele of cssfr5 exhibited 0.5-1.1 lower yellow rust, leaf rust and tan spot scores and produced 49 kg ha^{-1} less grain yield (Table 4.3). In a recombinant inbred line population, our group has recently reported an increased resistance to rusts due to *Lr34/Yr18* allele with reduced grain yield of 99 kg ha^{-1} using the same marker (Chen et al., 2016a).

Cultivars with the TT genotype at wMAS000003 (an additional *Lr34/Yr18* marker) had a 1.3 lower yellow rust score, 0.6 lower leaf rust and tan spot scores, and produced 196 kg ha^{-1} more grain yield, but were 3 cm taller than those with the alternative alleles (Table 4.3); cultivars TT with and without the resistance allele did not differ in maturity. The *Lr34/Yr18* allele was a minor allele with a frequency of 39% (Table 4.1), which is different from another study that reported that over 50% of cultivars registered in western Canadian carry the *Lr34/Yr18* resistance allele (McCallum et al., 2012).

One SNP marker for the *Rht-B1* (wMAS000001) and three SNPs for *Rht8* (BS00090678_51, JD_c63957_1176 and Ku_c19185_1569) resulted in a reduction in yellow rust score by 0.5-1.0, a reduction in plant height by 1-5 cm, increased grain yield by 505-833 kg ha^{-1}, but delayed maturity by 1-2 days (Table 4.3). For two markers (BS00090678_51 and JD_c63957_1176), however, the alleles that increased resistance to yellow rust increased leaf rust scores by 0.7. Although the relationship between the height reducing genes and rust resistance is not clear, there are three possibilities. In all four markers, cultivars that had a lower yellow rust score were up to 5 cm shorter than those with higher scores. The *Rht* genes reduce plant stature and improve lodging resistance, which indirectly increases resistance to disease because lodging creates an ideal environment for the spread of diseases (i.e., lodged plants are often more affected by disease than erect plants). In both rice and *Brachypodium distachyon*, have been reported clusters of adjacent protein kinases (PKs) which contain each a leucine-rich repeat (LRR)-RLK which are putative *Rht8* candidates. These have been found to play several roles, including in disease resistance (Song et al., 1995; Gasperini et al., 2012). Finally, the proportion of cultivars that showed lower yellow rust scores was much lower (5.7 to 13.2%) as compared with those cultivars that had higher disease scores (Table 4.3). Thus, our results may be influenced by sample size. Similarly, cultivars with the *Vrn-A1a* allele had 1-2 lower yellow rust and leaf rust scores, matured 4 days earlier, were 9 cm taller and produced 418 kg ha^{-1} lower grain yield than those cultivars that possessed the *vrn-A1* allele (Table 4.3). The indirect effect of the *Vrn-A1* gene on reduction in leaf and yellow rust scores may be associated with differences in plant maturity, which may help the plants to partly escape infection by rusts. The proportion of cultivars with the *Vrn-A1a* and *vrn-A1* alleles was 92.5 and 7.5%, respectively, which might have also biased our results.

The dominant *Tsn1* gene mapped between Xfcp1 and Xfcp394 on the long arm of chromosome 5B (Zhang et al., 2009) and confers sensitivity to Ptr ToxA (Lamari and Bernier, 1989; Faris et al., 1996; Gamba and Lamari, 1998). In another study using genome-wide association mapping, we unambiguously identified clusters of SNPs on chromosome 5B that were highly associated with the *Tsn1* gene (Perez-Lara et al., 2016b). In the present study, we found six markers associated with *Tsn1* that decreased both yellow rust and tan spot scores by 0.5 to 1.0, but only two markers (Xfcp1 and Tdurum_contig57027_347) seem the best for predicting disease resistance and Ptr ToxA sensitivity. Cultivars that consisted of a 350 bp fragment from Xfcp1 accounted for 18.8%, had up to 1.0 lower yellow rust and tan spot scores, were primarily insensitive to the Ptr ToxA reaction, produced 93 kg ha^{-1} higher grain yield, but were 4 cm taller than those cultivars that had the 395 bp fragment (Tables 4.3, 4.4). Similarly, cultivars that consisted of the CC genotype at Tdurum_contig57027_347 accounted for 15.2%, had up to 1.0 lower yellow rust and tan spot scores, were insensitive to the Ptr ToxA reaction, produced 62 kg ha^{-1} higher grain yield, but were 2 cm taller than those cultivars that had the AA genotype (Table 4.3). In approximately 77% of the cases, the two markers correctly assigned the cultivars into insensitive or sensitive groups in a manner consistent with the Ptr ToxA reactions observed under greenhouse conditions with the toxin infiltration. Therefore, the use of one or both of these markers would provide highly useful information for wheat breeders in developing Ptr ToxA insensitive cultivars.

4.5 Conclusions

Results from the present study clearly demonstrated the presence of large allelic variation at the 16 candidate genes. Some genes and gene combinations increased resistance to yellow rust, tan spot and grain yield without significantly affecting both maturity and plant height, while others increased resistance but decreased grain yield and increased maturity and/or plant height. The different markers for *Lr34/Yr18* were consistently identified as the best markers associated not only with leaf and yellow rust, but also other traits. Results from the present study demonstrate

the complexity in developing wheat cultivars that are resistant to diseases without significant yield penalties, delays in maturity or increase plant height.

4.6 Figures and Tables

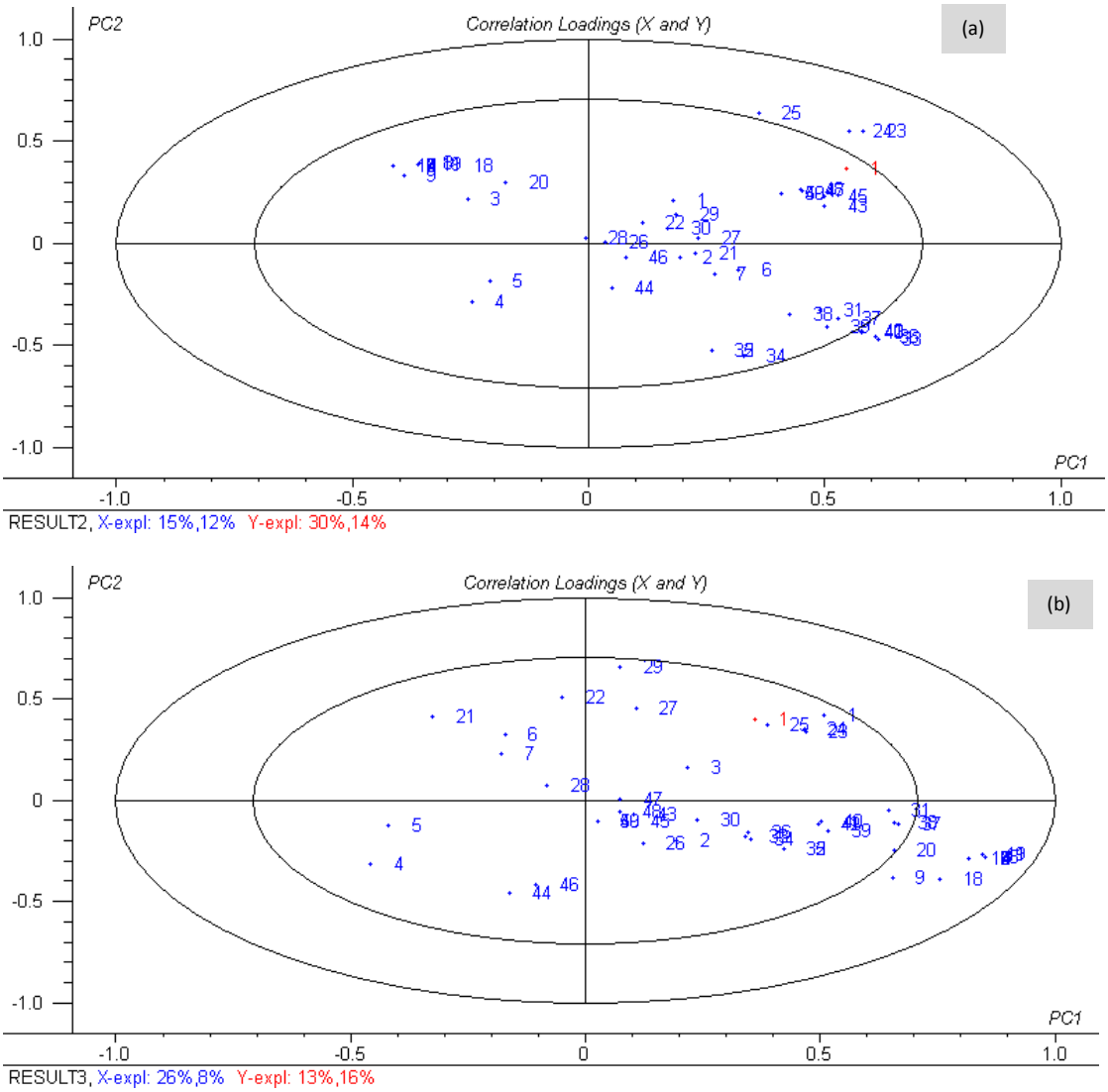

Figure 4.1 A correlation loadings plot of PC1 and PC2 from partial leas square regression conducted using 50 gene specific markers as predictor (X) variables and (a) yellow rust and (b) leaf rust disease scores as response (Y) variables. For each trait, the inner and outer ellipse indicates 50 and 100% explained variance, respectively. Markers are in blue font and are numbered from 1 to 50 as in Table 1, while the phenotypic traits are in red font. PC1 and PC2 explained 30 and 14% of the phenotypic variance for yellow rust and 13 and 16% of the phenotypic variance for leaf rust.

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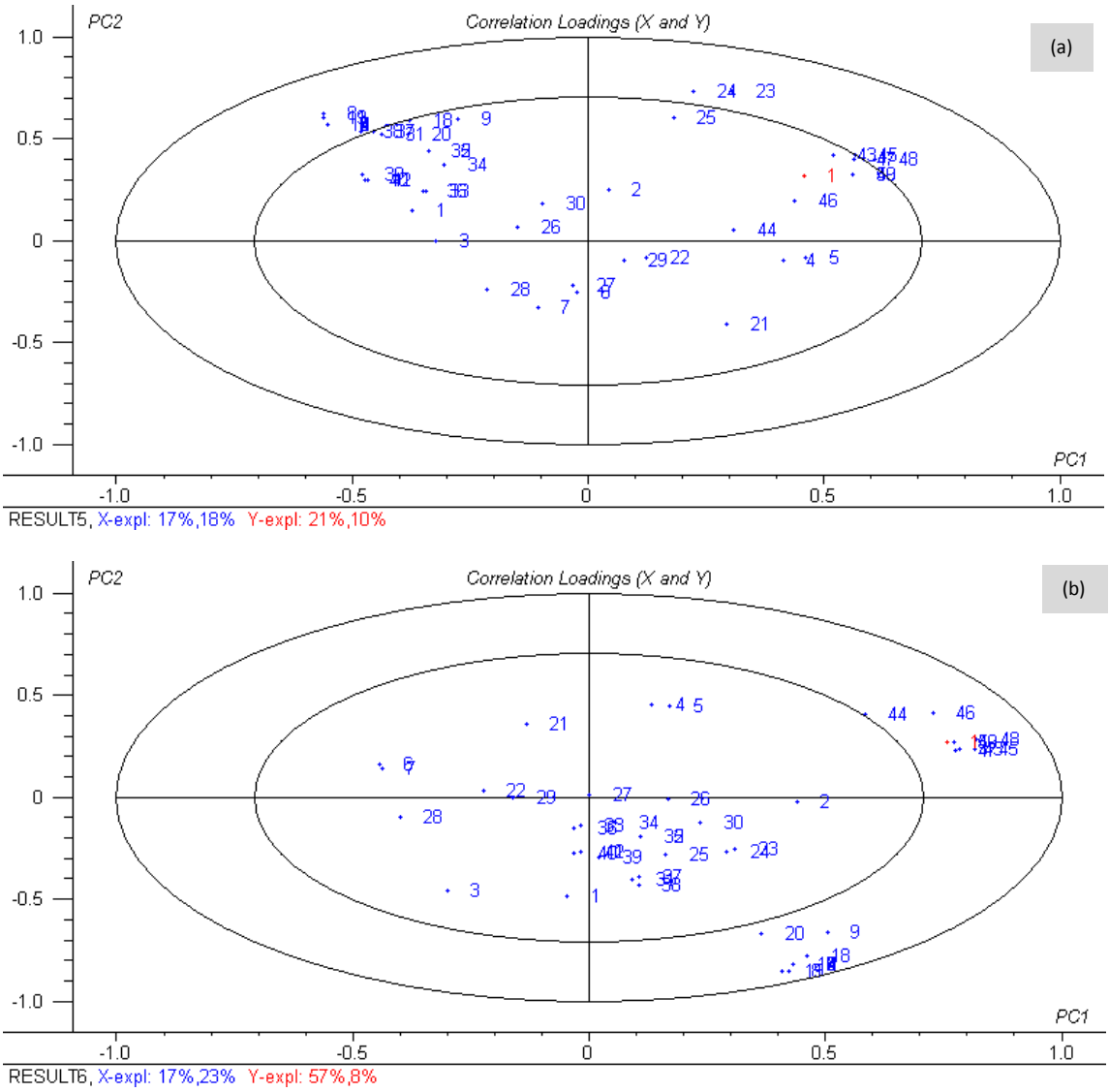

Figure 4.2 A correlation loadings plot of PC1 and PC2 from partial leas square regression conducted using 50 gene specific markers as predictor (X) variables and (a) tan spot disease scores and (b) reaction to Ptr ToxA as response (Y) variables. For each trait, the inner and outer ellipse indicates 50 and 100% explained variance, respectively. Markers are in blue font and are numbered from 1 to 50 as in Table 1, while the phenotypic traits are in red font. PC1 and PC2 explained 21 and 10% of the phenotypic variance for tan spot and 57 and 8% of the phenotypic variance for Ptr ToxA reaction.

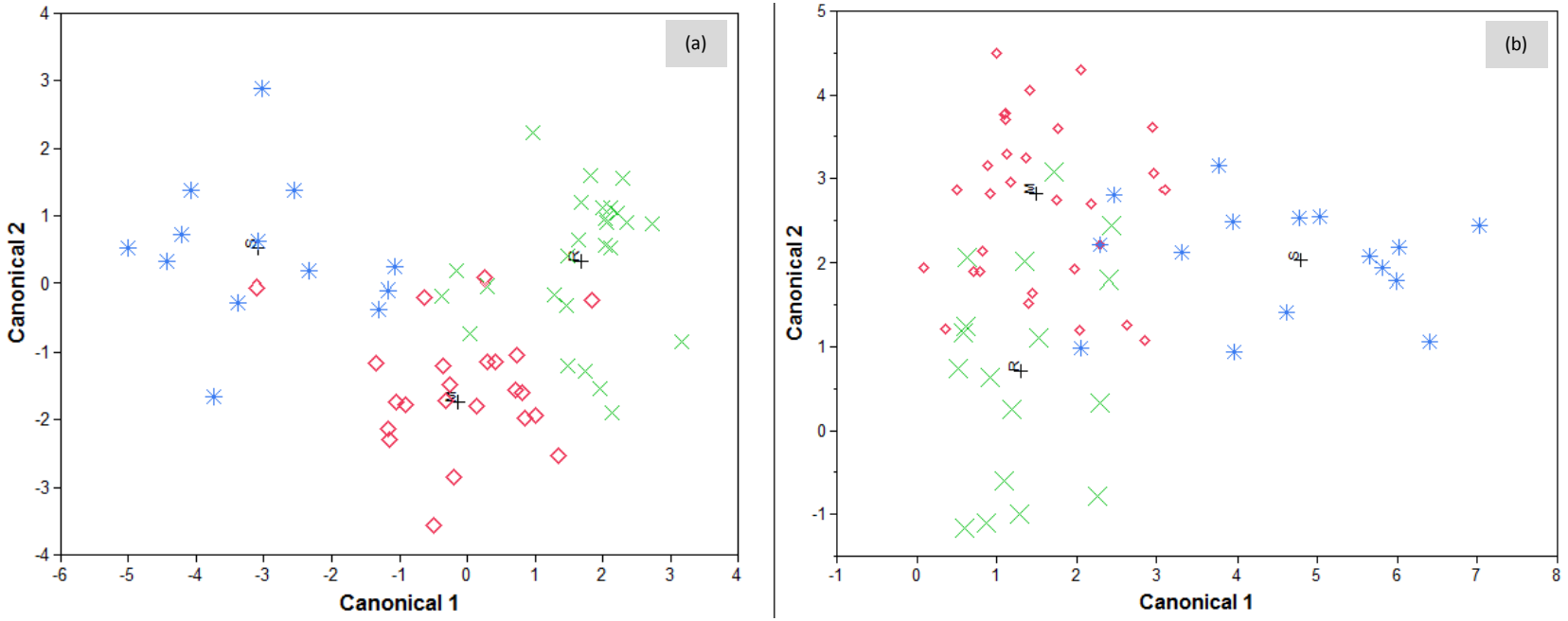

Figure 4.3 Plot of the first two canonical axes from canonical discriminant analysis using: (a) yellow rust score as categorical variable and markers as explanatory variables; (b) leaf rust as categorical variable and markers as explanatory variables. Cultivars with disease score of <3.0, 3.1-5.0 and >5.0 were assigned in to resistant (green), moderately resistant (red) and susceptible (blue), respectively. Of the 70 cultivars, 10% in yellow rust and 20% in leaf rust were misclassified.

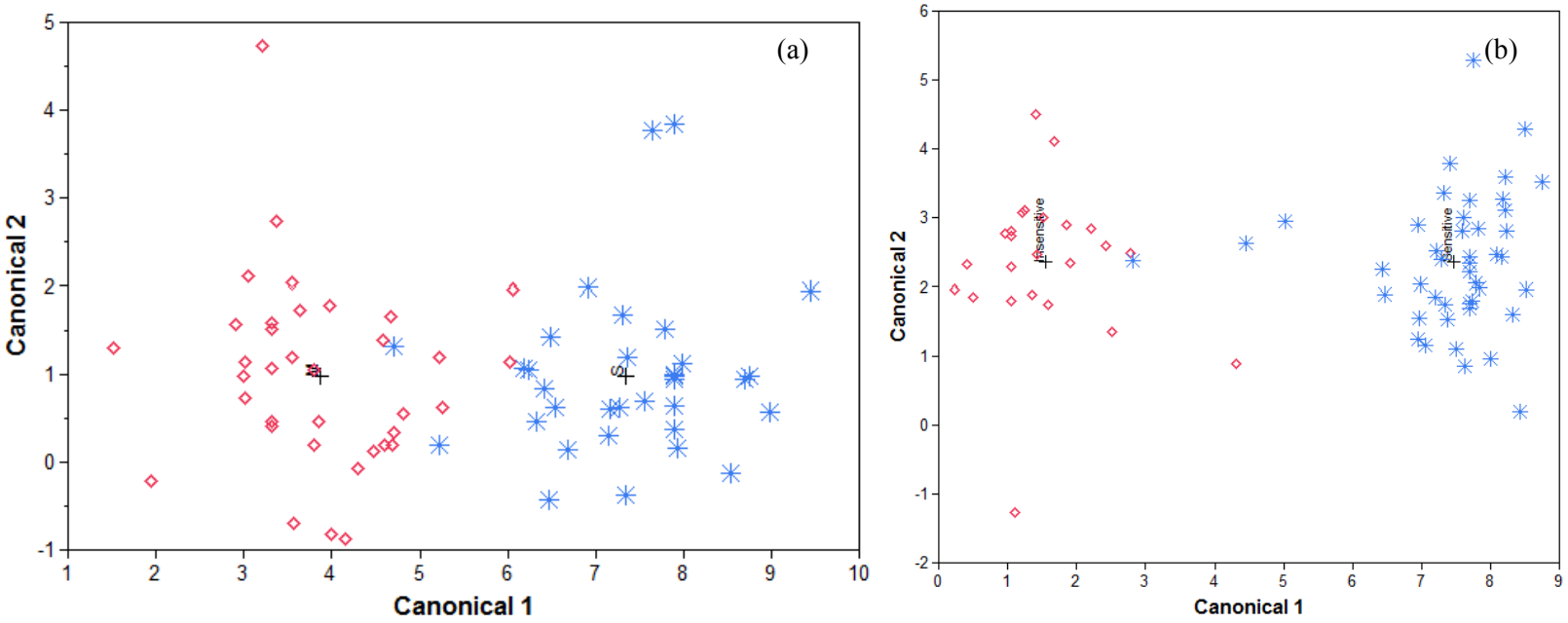

Figure4.4 Plot of the first two canonical axes from canonical discriminant analysis using (a) tan spot disease score and (b) Ptr ToxA reaction as categorical variables and the 50 markers as explanatory variables. For tan spot, cultivars with disease score of <3.0, 3.1-5.0 and >5.0 were assigned into resistant (green), moderately resistant (red) and susceptible (blue) groups respectively. Among the 70 cultivars, 2.9% of the cultivars evaluated for Ptr ToxA class and 5.7% of cultivars evaluated for tan spot were misclassified.

Table 4.1 Summary of the 50 markers used for genotyping 70 spring wheat cultivars registered in western Canada between 1963 and 2011.

Marker ID	Marker	Locus	Chrom	Type of marker	Major allele	Major allele frequency	Minor Allele
1	Vrn-A1	*Vrn-A1*	5A	STS	Vrn-A1a	0.925	vrn-A1
2	Vrn-B1	*Vrn-B1*	5B	STS	vrn-b1	0.507	Vrn-B1a
3	Ppd-D1	*Ppd-D1*	2D	STS	Ppd-D1b	0.627	Ppd-D1a
4	Rht-D1	*Rht-D1*	4D	STS	Rht-D1a	0.851	Rht-D1b
5	Rht-D1	*Rht-D1*	4D	KASP SNP	C	0.879	A
6	Rht-B1	*Rht-B1*	4B	STS	Rht-B1a	0.881	Rht-B1b
7	Rht-B1	*Rht-B1*	4B	KASP SNP	G	0.868	A
8	WMS261	*Rht8*	2D	SSR	150 bp	0.892	192 bp
9	wsnp_CAP12_c455_248396	*Rht8*	2D	90K SNP	C	0.941	A
10	BS00090678_51	*Rht8*	2D	90K SNP	A	0.886	C
11	JD_c63957_1176	*Rht8*	2D	90K SNP	A	0.900	C
12	Kukri_c51992_290	*Rht8*	2D	90K SNP	C	0.886	A
13	Kukri_rep_c106786_230	*Rht8*	2D	90K SNP	C	0.886	A
14	Kukri_rep_c113120_104	*Rht8*	2D	90K SNP	C	0.886	A
15	wsnp_Ex_c14779_22892053	*Rht8*	2D	90K SNP	A	0.886	C
16	wsnp_JD_rep_c63957_40798083	*Rht8*	2D	90K SNP	A	0.886	C
17	wsnp_JD_rep_c63957_40798121	*Rht8*	2D	90K SNP	C	0.886	A
18	RAC875_c74_204	*Rht8*	2D	90K SNP	A	0.940	C
19	tplb0053n05_793	*Rht8*	2D	90K SNP	A	0.900	C
20	Ku_c19185_1569	*Rht8*	2D	90K SNP	A	0.943	C
21	stm559	*Sr2*	3B	SSR	237 bp	0.708	260 bp
22	Sr26#43	*Sr26*	6A	SSR	207 bp	0.754	303 bp
23	wMAS000003	*Lr34/Yr18*	7D	KASP SNP	A	0.606	T
24	wMAS000004	*Lr34/Yr18*	7D	KASP SNP	T	0.612	C
25	cssfr5	*Lr34/Yr18*	7D	SSR	730 bp	0.657	520 bp
26	Xwmc44	*Lr46/Yr29*	1B	SSR	242 bp	0.836	290 bp
27	csGS	*Lr68*	7B	STS	385 bp (+)	0.667	385 bp (-)
28	S23M41	*Yr5*	2B	STS	150 bp	0.886	275 bp
29	Xbarc181	*Yr26*	1B	SSR	185 bp	0.696	170 bp
30	Xbarc101b	*Yr36*	6B	SSR	160 bp	0.729	120 bp
31	psp3000	*Yr10*	1B	SSR	240 bp	0.582	270 bp
32	Kukri_c37738_417	*Yr10*	1B	90K SNP	C	0.657	A
33	RAC875_c30138_595	*Yr10*	1B	90K SNP	A	0.686	C
34	Ra_c78638_309	*Yr10*	1B	90K SNP	A	0.671	C
35	TA001594-0748	*Yr10*	1B	90K SNP	A	0.657	C
36	Tdurum_contig28703_293	*Yr10*	1B	90K SNP	A	0.696	C
37	Excalibur_c30569_384	*Yr10*	1B	90K SNP	A	0.612	C
38	Kukri_c8390_1515	*Yr10*	1B	90K SNP	C	0.586	A
39	BS00066271_51	*Yr10*	1B	90K SNP	A	0.671	C
40	Excalibur_c43567_633	*Yr10*	1B	90K SNP	C	0.676	A
41	Kukri_c8390_547	*Yr10*	1B	90K SNP	C	0.681	A
42	RAC875_c8282_644	*Yr10*	1B	90K SNP	C	0.681	A
43	Xfcp1	*Tsn1*	5B	SSR	395 bp	0.813	350 bp
44	Xfcp394	*Tsn1*	5B	SSR	390 bp	0.621	340 bp
45	Kukri_c54078_114	*Tsn1*	5B	90K SNP	A	0.826	C
46	Tdurum_contig25513_123	*Tsn1*	5B	90K SNP	A	0.571	C
47	Tdurum_contig57027_347	*Tsn1*	5B	90K SNP	A	0.864	C
48	wsnp_Ku_c17396_26488733	*Tsn1*	5B	90K SNP	A	0.797	C
49	wsnp_Ku_c3102_5810751	*Tsn1*	5B	90K SNP	C	0.843	A
50	wsnp_Ku_c3102_5811860	*Tsn1*	5B	90K SNP	C	0.843	A

Table 4.2 Summary of Spearmank correlation coefficients between markers and phenotypic traits. Only markers that showed significant ($p < 0.05$) positive or negative correlations with at least one of the four phenotypic traits were included in the table.

Marker	Gene	Trait	Correlation
wMAS000003	*Lr34/Yr18*	Yellow rust	0.485
wMAS000004	*Lr34/Yr18*	Yellow rust	0.456
cssfr5	*Lr34/Yr18*	Yellow rust	0.398
Kukri_c54078_114	*Tsn1*	Yellow rust	0.251
Tdurum_contig57027_347	*Tsn1*	Yellow rust	0.249
wsnp_Ku_c17396_26488733	*Tsn1*	Yellow rust	0.269
Vrn-A1	*Vrn-A1*	Leaf rust	0.323
wMAS000003	*Vrn-A1*	Tan spot	0.321
wMAS000004	*Lr34/Yr18*	Tan spot	0.289
cssfr5	*Lr34/Yr18*	Tan spot	0.383
csGS	*Lr68*	Tan spot	-0.246
Tdurum_contig57027_347	*Tsn1*	Tan spot	0.288
wsnp_Ku_c17396_26488733	*Tsn1*	Tan spot	0.278
Vrn-A1	*Vrn-A1*	Ptr ToxA	-0.315
Ppd-D1	*Ppd-D1*	Ptr ToxA	-0.364
S23M41	*Yr5*	Ptr ToxA	-0.282
Xfcp1	*Tsn1*	Ptr ToxA	0.305
Xfcp394	*Tsn1*	Ptr ToxA	0.527
Kukri_c54078_114	*Tsn1*	Ptr ToxA	0.514
Tdurum_contig25513_123	*Tsn1*	Ptr ToxA	0.791
Tdurum_contig57027_347	*Tsn1*	Ptr ToxA	0.547
wsnp_Ku_c17396_26488733	*Tsn1*	Ptr ToxA	0.525
wsnp_Ku_c3102_5810751	*Tsn1*	Ptr ToxA	0.556
wsnp_Ku_c3102_5811860	*Tsn1*	Ptr ToxA	0.556

Table 4.3 Comparison of the effects of allelic variants of 23 markers associated with 12 genes on disease scores and agronomic traits. P values were obtained from analysis of variance. Differences between alleles are diseases scores, days to maturity, cm for plant height and kg ha-1 for grain yield.

Marker	Locus	Allele	Proportion of cultivars (%)	Yellow rust	Leaf rust	Tan spot	Maturity	Height	Yield
cssfr5	*Lr34/Yr18*	520 bp	65.7	4.0	4.5	5.4	99.4	96.9	4828.8
		730 bp	34.3	2.9	4.0	4.7	99.6	98.9	4877.8
		Difference		-1.1	-0.5	-0.7	0.2	2.0	48.9
		P value		0.0	0.0	0.0	0.4	0.0	0.4
wMAS000003	*Lr34/Yr18*	AA	59.7	4.2	4.4	5.5	99.1	96.8	4727.4
		TT	40.3	2.9	3.8	4.9	99.4	99.3	4923.0
		Difference		-1.3	-0.6	-0.6	0.3	2.5	195.6
		P value		0.0	0.0	0.0	0.0	0.0	0.0
wMAS000004	*Lr34/Yr18*	CC	39.7	2.9	3.8	4.9	99.4	99.3	4923.2
		TT	60.2	4.2	4.5	5.4	99.3	96.8	4755.4
		Difference		-1.3	-0.7	-0.5	0.1	2.5	167.8
		P value		0.0	0.0	0.0	0.0	0.0	0.1
Xwmc44	*Lr46/Yr29*	242 bp	83.6	3.6	4.3	5.1	99.6	98.1	4814.4
		290 bp	16.4	3.3	4.2	5.3	98.7	94.8	4947.5
		Difference		-0.3	-0.1	0.2	-1.0	-3.3	133.1
		P value		0.1	0.7	0.4	0.0	0.0	0.1
csGS	*Lr68*	385 bp (-)	66.7	3.5	4.2	5.3	99.4	98.5	4856.9
		385 bp (+)	33.3	3.6	4.5	4.8	99.6	96.1	4853.5
		Difference		0.1	0.3	-0.5	0.2	-2.4	-3.4
		P value		0.6	0.1	0.0	0.5	0.0	1.0
S23M41	*Yr5*	150 bp	88.6	3.6	4.3	5.2	99.2	98.2	4860.9
		275 bp	11.4	3.7	4.1	4.7	100.6	93.8	4749.5
		Difference		-0.1	0.2	0.5	-1.3	4.4	111.4
		P value		0.6	0.4	0.1	0.0	0.0	0.2
Excalibur_c43567_633	*Yr10*	AA	32.4	3.9	4.5	5.0	100.1	97.1	4820.9
		CC	67.6	3.5	4.2	5.2	99.1	97.9	4872.4
		Difference		-0.4	-0.3	0.3	-1.0	0.8	51.5
		P value		0.0	0.2	0.1	0.0	0.1	0.4
Xbarc181	*Yr26*	170 bp	30.9	3.5	3.9	5.2	99.1	99.6	4796.2
		185 bp	69.1	3.6	4.5	5.2	99.5	96.5	4861.2
		Difference		-0.1	-0.6	0.0	-0.4	3.0	-65.0
		P value		0.4	0.0	0.9	0.1	0.0	0.2
Xfcp1	*Tsn1*	395 bp	81.3	3.8	4.3	5.2	99.4	96.9	4802.9
		350 bp	18.8	3.0	4.2	4.7	99.4	101.0	4896.3
		Difference		-0.8	0.0	-0.5	0.1	4.1	93.4
		P value		0.0	0.8	0.0	0.8	0.0	0.2
Xfcp394	*Tsn1*	390 bp	62.1	3.5	4.1	5.1	99.3	97.6	4837.9
		340 bp	37.9	3.7	4.5	5.1	99.4	97.2	4829.0
		Difference		-0.3	-0.4	0.0	-0.1	0.4	9.0
		P value		0.1	0.1	1.0	0.6	0.5	0.9
Kukri_c54078_114	*Tsn1*	AA	82.6	3.7	4.3	5.3	99.4	97.1	4846.5
		CC	17.4	2.7	4.2	4.7	99.3	100.0	4850.4
		Difference		-1.0	-0.1	-0.6	-0.1	2.8	3.9
		P value		0.0	0.7	0.0	0.8	0.0	1.0
Tdurum_contig25513_123	*Tsn1*	AA	57.1	3.5	4.1	5.3	99.2	98.1	4843.6
		CC	42.9	3.7	4.6	5.1	99.6	97.2	4854.3
		Difference		-0.2	-0.5	0.2	-0.4	0.9	-10.6
		P value		0.2	0.0	0.3	0.1	0.1	0.8
Tdurum_contig57027_347	*Tsn1*	AA	84.8	3.8	4.3	5.3	99.5	97.1	4846.6
		CC	15.2	2.8	4.4	4.6	99.3	98.9	4908.2

		Difference		-0.9	0.0	-0.8	-0.2	1.8	61.6
		P value		0.0	1.0	0.0	0.7	0.0	0.4
wsnp_Ku_c17396_26488733	*Tsn1*	AA	79.7	3.8	4.4	5.3	99.4	97.3	4843.1
		CC	20.3	2.9	4.1	4.6	99.2	100.1	4854.8
		Difference		-0.9	-0.2	-0.7	-0.2	2.8	11.7
		P value		0.0	0.3	0.0	0.5	0.0	0.9
wsnp_Ku_c3102_5810751	*Tsn1*	AA	15.7	3.0	4.3	4.7	99.0	100.9	4784.1
		CC	84.3	3.7	4.3	5.3	99.5	97.1	4860.1
		Difference		-0.7	0.0	-0.6	-0.4	3.9	-75.9
		P value		0.0	0.9	0.0	0.2	0.0	0.3
wsnp_Ku_c3102_5811860	*Tsn1*	AA	15.7	3.0	4.3	4.7	99.0	100.9	4784.1
		CC	84.3	3.7	4.3	5.3	99.5	97.1	4860.1
		Difference		-0.7	0.0	-0.6	-0.4	3.9	-75.9
		P value		0.0	0.9	0.0	0.2	0.0	0.3
BS00090678_51	*Rht8*	AA	88.6	3.6	4.2	5.2	99.3	97.8	4790.3
		CC	11.4	3.1	5.0	4.8	99.9	96.6	5295.4
		Difference		-0.5	0.8	-0.4	0.6	-1.2	505.1
		P value		0.0	0.0	0.2	0.1	0.2	0.0
JD_c63957_1176	*Rht8*	AA	90.0	3.6	4.2	5.2	99.3	97.8	4792.4
		CC	10.0	3.0	4.9	4.7	100.4	96.8	5348.8
		Difference		-0.6	0.6	-0.5	1.1	-1.0	556.5
		P value		0.0	0.0	0.1	0.0	0.3	0.0
Ku_c19185_1569	*Rht8*	AA	9.3	3.6	4.3	5.2	99.3	98.0	4800.4
		CC	5.7	2.7	4.7	4.9	101.3	92.9	5633.8
		Difference		-1.0	0.5	-0.3	2.0	-5.1	833.4
		P value		0.0	0.2	0.4	0.0	0.0	0.0
wMAS000001	*Rht-B1*	AA	13.2	2.9	4.1	5.3	100.6	95.4	5309.2
		GG	86.8	3.7	4.3	5.2	99.2	98.0	4780.9
		Difference		-0.8	-0.2	0.1	1.4	-2.6	528.2
		P value		0.0	0.5	0.6	0.0	0.0	0.0
Vrn-A1	*Vrn-A1*	vrn-A1	7.5	4.5	6.1	4.5	103.4	89.5	5209.5
		Vrn-A1a	92.5	3.6	4.1	5.2	99.0	98.6	4791.2
		Difference		-0.9	-2.0	0.7	-4.4	9.1	-418.3
		P value		0.0	0.0	0.0	0.0	0.0	0.0
Vrn-B1	*Vrn-B1*	vrn-b1	49.3	3.5	4.3	5.1	99.1	97.6	4829.4
		Vrn-B1a	50.7	3.7	4.2	5.2	99.6	98.2	4823.7
		Difference		-0.2	0.1	-0.1	-0.5	-0.6	5.7
		P value		0.1	0.6	0.7	0.1	0.2	0.9
Ppd-D1	*Ppd-D1*	Ppd-D1a	37.3	3.3	4.3	5.0	99.6	95.9	4926.5
		Ppd-D1b	62.7	3.7	4.3	5.2	99.2	99.1	4766.9
		Difference		-0.4	0.0	-0.2	0.5	-3.1	159.6
		P value		0.0	0.9	0.2	0.1	0.0	0.0

Table 4.4 Phenotypic and genotypic data of 70 spring wheat cultivars. The genotypic data consists of three markers (wMAS000003, wMAS000004 and cssfr5) for Lr34/Yr18 and two (Xfcp1 and Tdurum_contig57027_347) for Tsn1 gene. For complete genotype data of the 70 cultivars, refer to Supplementary material 1. The wheat classes are as follows: CPSR: Canada Prairie Spring Red; CWES: Canada western extra strong; CWGP: Canada western general purpose; CWHWS: Canada western hard white spring; CWRS: Canada Western Red Spring; CWSWS: Canada western soft white spring.

Cultivar	Year of release	Wheat class*	wMAS000003	wMAS000004	cssfr5	Xfcp1	Tdurum_ contig57027_347	Yellow rust	Leaf rust	Tan spot	Reaction to Ptr ToxA	Maturity (d)	Height (cm)	Gr yi ha
Park	1963	CNHR	AA	TT	520 bp	350 bp	CC	5.8	7.4	7	Sensitive	97.5	102.1	42
Neepawa	1969	CNHR	AA	TT	520 bp	395 bp	AA	4.5	7.2	6	Sensitive	97.9	104.3	40
Glenlea	1972	CWES	TT	CC	730 bp	395 bp	AA	3.2	4	3.8	Sensitive	101.1	113.9	53
Columbus	1980	CNHR	AA	TT	520 bp	395 bp	AA	4.3	5.6	4.5	Sensitive	99.9	111.2	43
Katepwa	1981	CNHR	AA	TT	520 bp	395 bp	AA	4.4	4.4	6	Sensitive	96.4	102.9	42
Laura	1986	CWRS	TT	CC	520 bp	350 bp	CC	3	2.6	6	Insensitive	100	103.6	41
Roblin	1986	CWRS	TT	CC	730 bp	395 bp	AA	3.6	5.6	6.5	Insensitive	95.8	98	42
AC Reed	1991	SWSW	AA	TT	520 bp	395 bp	AA	4.9	6.2	7.3	sensitive	100	83.2	54
AC Taber	1991	CNHR	AA	TT	520 bp	395 bp	AA	5.7	6.2	6	Insensitive	104.4	88.8	44
CDC Teal	1991	CWRS	TT	CC	730 bp	395 bp	AA	3.5	4.6	4.8	Sensitive	98.2	101.1	49
CDC Merlin	1992	CWRS	AA	TT	520 bp	-	-	3.7	5.8	6.5	Insensitive	100.9	106.9	45
AC Domain	1993	CWRS	AA	TT	520 bp	395 bp	AA	2.5	4.4	5.8	Sensitive	98.5	94.4	40
AC Eatonia	1993	CNHR	TT	CC	730 bp	350 bp	CC	2.4	6	5	Insensitive	99.9	103.3	40
AC Barrie	1994	CWRS	AA	TT	520 bp	395 bp	AA	6.1	5.2	5.5	Insensitive	98.5	99.1	45
AC Foremost	1995	CNHR	AA	TT	520 bp	395 bp	AA	6.1	6	4.8	Insensitive	102	80.2	50
AC Cadillac	1996	CWRS	TT	CC	730 bp	395 bp	AA	2.9	4	4.5	Insensitive	97.8	106.5	47
AC Crystal	1996	CNHR	AA	TT	520 bp	395 bp	AA	6.1	6.2	5.3	Insensitive	103.4	88.9	41
AC Elsa	1996	CWRS	TT	CC	730 bp	350 bp	CC	2	3.6	4.5	Insensitive	98.8	99.1	45
AC Vista	1996	CPSR	AA	TT	520 bp	395 bp	AA	4.1	5.8	4.5	Sensitive	100.5	90.4	56
AC Intrepid	1997	CWRS	AA	TT	520 bp	395 bp	AA	2	2.4	4.8	Sensitive	95.3	101.6	48
AC Splendor	1997	CWRS	AA	TT	520 bp	395 bp	AA	4.3	4.2	6	Insensitive	93.2	103.4	42
McKenzie	1997	CNHR	AA	TT	520 bp	395 bp	AA	4.6	3.8	6.5	Sensitive	97.1	99.3	46
AC Abbey	1998	CNHR	-	-	520 bp	395 bp	AA	2.9	5.2	5.8	Insensitive	98.6	93	49

Prodigy	1998	CWRS	AA	TT	520 bp	395 bp	AA	4.1	4.6	5	Sensitive	98.1	106.2	44
CDC Bounty	1999	CWRS	TT	CC	730 bp	-	AA	3.7	5.8	5	Sensitive	98.4	104.2	44
5700 HR	2000	CPSR	AA	TT	520 bp	395 bp	AA	6.7	4.2	4.8	Insensitive	103	83.2	48
AC Andrew	2000	CWSWS	AA	TT	520 bp	395 bp	AA	2.6	5.4	3.8	Sensitive	102.7	90.9	64
Snowbird	2000	CWHWS	AA	TT	520 bp	395 bp	AA	2.6	3.4	5.5	Sensitive	100.3	102	46
5701 HR	2001	CPSR	TT	CC	520 bp	350 bp	CC	2.2	2.4	4.8	Sensitive	101.3	86.4	53
CDC Rama	2001	CWES	TT	CC	730 bp	395 bp	AA	1.6	4.8	5	Sensitive	103.1	109.4	52
Superb	2001	CWRS	AA	TT	520 bp	-	AA	5.4	5	5	Sensitive	101	92.8	50
Journey	2002	CWRS	AA	TT	520 bp	395 bp	AA	4.4	4	5	Sensitive	98.5	96.6	43
Lovitt	2002	CWRS	AA	TT	520 bp	395 bp	AA	5.6	4.4	5.5	Sensitive	96.8	101.6	39
CDC Go	2003	CWRS	AA	TT	520 bp	-	AA	3.8	3.8	6.3	Sensitive	100.9	87.6	53
CDC Osler	2003	CNHR	TT	CC	730 bp	395 bp	AA	3.7	3	7	Sensitive	96.5	98.4	45
CDC Walrus	2003	CWES	TT	CC	730 bp	395 bp	AA	2.6	4	3.3	Sensitive	100.7	105.3	53
Bhishaj	2003	CWSWS	AA	TT	520 bp	-	AA	2	5.2	6	Sensitive	101.7	90.5	61
5602 HR	2004	CWRS	TT	CC	730 bp	395 bp	AA	2.8	1.8	4	Sensitive	99.1	97.5	49
Burnside	2004	CWES	TT	CC	730 bp	395 bp	AA	1.6	2.8	4	Sensitive	98.1	108.6	53
CDC Alsask	2004	CWRS	TT	CC	730 bp	350 bp	-	2.6	1.8	4.3	Insensitive	98.1	103.7	49
Harvest	2004	CNHR	AA	TT	520 bp	395 bp	AA	3.8	4.2	6.5	Sensitive	96.9	94.1	47
Infinity	2004	CWRS	AA	TT	520 bp	395 bp	AA	4.4	5	5.5	Insensitive	96.2	99.2	44
Lillian	2004	CNHR	TT	CC	730 bp	395 bp	AA	2	4.4	4.5	Insensitive	98	100.1	46
Somerset	2005	CWRS	AA	TT	520 bp	395 bp	AA	3	2.5	5	Sensitive	97.8	108.3	46
Alvena	2006	CNHR	AA	TT	520 bp	395 bp	AA	3	4.2	4	Sensitive	97	99.5	48
CDC Abound	2006	CWRS	TT	CC	520 bp	395 bp	AA	5.1	4	6	Sensitive	101.4	89.9	50
Helios	2006	CWRS	AA	TT	520 bp	395 bp	AA	5.3	4.8	5.5	Insensitive	97.8	99	46
Kane	2006	CNHR	AA	TT	730 bp	395 bp	AA	3.4	2.8	5.8	Sensitive	99.2	93.7	49
Snowstar	2006	CWHWS	AA	TT	520 bp	395 bp	AA	5.8	4.6	6.3	Sensitive	96.6	92.2	40
5702 HR	2007	CPSR	TT	CC	730 bp	395 bp	AA	3.7	4.4	4.8	Sensitive	102.4	91.9	56
Glencross	2007	CWES	TT	CC	730 bp	395 bp	AA	2.6	4.2	5.3	Sensitive	96.7	110.1	54
Goodeve	2007	CWRS	AA	TT	-	395 bp	AA	3.6	2.7	5.8	Sensitive	99.2	98.4	42

VB														
Sadash	2007	CWSWS	-	-	520 bp	395 bp	AA	2.1	4.6	5.3	Sensitive	104.7	89.7	62
Waskada	2007	CWRS	AA	TT	-	395 bp	AA	3.3	2.8	4.5	Sensitive	98.9	102	47
5603 HR	2008	CNHR	AA	TT	520 bp	350 bp	-	5.2	3.6	4.8	Insensitive	98.9	101.1	49
Minnedosa	2008	CWSP	AA	TT	520 bp	395 bp	AA	2.4	3.4	5.3	Sensitive	99.4	100.2	45
Stettler	2008	CWRS	AA	TT	520 bp	395 bp	AA	2	3	5	Sensitive	101.1	94.3	48
WR859CL	2008	CWRS	AA	TT	520 bp	395 bp	AA	3.2	2.2	5.3	Sensitive	101.2	91.9	49
5604 HRCL	2009	CWRS	AA	TT	520 bp	395 bp	AA	5.3	3.4	6.8	Sensitive	97.6	94.9	44
CDC Kernen	2009	CWRS	TT	CC	520 bp	395 bp	AA	4.1	4	6	Sensitive	101.3	101.7	51
CDC Thrive	2009	CWRS	TT	CC	730 bp	395 bp	AA	4.2	6	5.8	Sensitive	97	101.4	47
CDC Utmost	2009	CWRS	TT	CC	730 bp	350 bp	CC	3.3	6.4	4.8	Insensitive	96.5	94.8	47
CONQUER	2009	CNHR	-	TT	520 bp	350 bp	CC	1.8	6.8	2.8	Insensitive	104.5	98.9	59
Carberry	2009	CWRS	TT	CC	730 bp	395 bp	AA	1.7	2.2	4.3	Sensitive	103.8	85.1	46
Muchmore	2009	CWRS	TT	CC	730 bp	-	AA	2.5	2.6	5	Sensitive	103.1	83.2	50
CDC Stanley	2009	CWRS	AA	TT	730 bp	395 bp	AA	3.2	3.2	3.3	Insensitive	99.3	96.4	52
CDC VR Morris	2010	CWRS	TT	CC	-	350 bp	CC	2.3	4	4.8	Insensitive	97.9	98.1	51
SY985	2010	CPSR	TT	CC	730 bp	350 bp	CC	3.9	2.8	4.3	Insensitive	102.5	90.3	54
Vesper	2010	CWRS	AA	TT	520 bp	395 bp	AA	6.3	3.6	5.3	Sensitive	99.4	97	46
CDC Plentiful	2011	CWRS	TT	CC	520 bp	350 bp	-	1.9	2	3.5	Insensitive	97.1	96	48

Table 4.5 Summary of the stepwise discriminant anayses conducted on 50 markers and 4 phenotypic traits.

Trait	Step	Entered marker(s)	Locus	Partial R^2	F Value	Pr > F	Wilks' Lambda	Pr < Lambda	Average squared canonical correlation	Pr > ASCC
Yellow rust	1	wMAS000003	*Lr34/Yr18*	16.5%	6.6	0.002	0.835	0.002	0.082	0.002
Yellow rust	2	Vrn-B1	*Vrn-B1*	12.4%	4.7	0.013	0.732	0.000	0.142	0.000
Yellow rust	3	Xfcp394	*Tsn1*	10.0%	3.6	0.033	0.659	0.000	0.185	0.000
Yellow rust	4	JD_c63957_1176	*Rht8*	9.8%	3.5	0.038	0.595	<.0001	0.226	<.0001
Yellow rust	5	Xwmc44	*Lr46/Yr29*	8.8%	3.1	0.055	0.543	<.0001	0.260	<.0001
Yellow rust	6	cssfr5	*Lr34/Yr18*	6.9%	2.3	0.111	0.505	<.0001	0.286	<.0001
Leaf rust	1	Vrn-A1	*Vrn-A1*	21.7%	9.3	0.000	0.783	0.000	0.109	0.000
Leaf rust	2	Tdurum_contig25513_123	*Tsn1*	6.5%	2.3	0.110	0.732	0.000	0.134	0.001
Leaf rust	3	Kukri_c54078_114	*Tsn1*	6.8%	2.4	0.102	0.682	0.000	0.167	0.000
Leaf rust	4	BS00090678_51	*Rht8*	6.9%	2.4	0.100	0.635	0.000	0.193	0.000
Tan spot	1	cssfr5	*Lr34/Yr18*	16.2%	13.1	0.001	0.838	0.001	0.162	0.001
Tan spot	2	S23M41	*Yr5*	10.9%	8.2	0.006	0.747	<.0001	0.253	<.0001
Tan spot	3	csGS	*Lr68*	14.1%	10.8	0.002	0.642	<.0001	0.358	<.0001
Tan spot	4	Vrn-B1	*Vrn-B1*	8.2%	5.8	0.019	0.589	<.0001	0.411	<.0001
Tan spot	5	wMAS000001	*Rht-B1*	5.4%	3.6	0.061	0.558	<.0001	0.442	<.0001
Ptr ToxA	1	Tdurum_contig25513_123	*Tsn1*	69.6%	155.4	<.0001	0.304	<.0001	0.696	<.0001
Ptr ToxA	2	wsnp_Ku_c3102_5810751	*Tsn1*	14.5%	11.3	0.001	0.260	<.0001	0.740	<.0001
Ptr ToxA	3	Ku_c19185_1569	*Rht8*	12.4%	9.3	0.003	0.228	<.0001	0.772	<.0001
Ptr ToxA	4	Xfcp1	*Xfcp1*	11.1%	8.1	0.006	0.203	<.0001	0.797	<.0001
Ptr ToxA	5	Vrn-A1	*Vrn-A1*	8.3%	5.8	0.019	0.186	<.0001	0.814	<.0001
Ptr ToxA	6	Excalibur_c43567_633	*Yr10*	7.1%	4.8	0.033	0.173	<.0001	0.827	<.0001

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Chapter 5 General Discussion and Conclusions

5.1 Introduction

Modern wheat faces several challenges to feed a fast growing world population that will be 9.1 billion by 2050. Wheat breeders will need, among other goals, to create high-yielding cultivars, assure yield stability, create cultivars locally adapted and with increased stress-tolerance (Feuillet, 2016). The first challenge is the large and complex wheat genome (Gill, 2004; Paux et al., 2008c) and interaction among its genes. Molecular markers are powerful tools to use in wheat breeding; they are free of environmental influence and relatively cheap, fast and accurate if compared with long-term and extensive field trials. At present it is possible for breeders to undertake marker assisted selection to detect the presence of genes in specific developing populations in the earliest filial generations. This may save time and resources, and may allow the breeder to be more efficient and to focus on those lines carrying the alleles or genes of interest. Large efforts have been made in the last two or three decades to produce readily usable markers to evaluate disease resistance and quality from early stages, and a reasonable set of them is now available to wheat breeders to enhance their programs (i.e. http://maswheat.ucdavis.edu/). Early maturity, disease resistance and quality are among the main traits wheat breeders are trying to incorporate. Gene mapping (QTL, GWAS, etc.) is a tool used by the breeders to detect genome regions relevant to these traits and it is the first step in the creation of functional markers.

My work in the present thesis aimed to:

1) Discover QTL associated with flowering time under greenhouse and field conditions, and maturity, plant height and grain yield under field conditions.
2) Identify genomic regions and SNP markers associated with resistance to four wheat diseases (leaf and stripe rusts, common bunt and tan spot) and insensitivity to three Ptr toxins in historical and modern Canadian western spring wheat cultivars released over a century.
3) Survey the allelic variation of a subset of selected genes associated with grain quality and resistance to diseases across a subset of spring wheat cultivars registered in western

Canada over a century; and understand the phenotypic effect of the genes on grain yield, maturity, plant height and disease resistance.

4) Provide wheat breeders with favorable disease marker combinations to aid in the determination of parental material for crosses

5.2 Contribution to Knowledge

The contribution of the first study is the presentation of 19 QTLs mapped on 8 chromosomes that explained between 6.3 and 37.8% of the phenotypic variation. Four QTLs were found to be associated with multiple traits. Only two QTLs have major effects, and they mapped adjacent to well-known photoperiod response *Ppd-D1* and height reducing *Rht-D1* genes, located on chromosome 2D and chromosome 4D, respectively. The QTL on 2D reduced flowering and maturity time up to 5 days with a yield penalty of 436 kg ha^{-1}, while the QTL on 4D reduced plant height by 13 cm, but increased maturity by 33 degree days. Results from this study provide information to wheat researchers developing early maturing and short stature spring wheat cultivars.

In the second study, we identified 94 markers from 7 chromosomes associated with all traits except Ptr ToxC. Two major effect genomic regions on 5B (71-74 cM) and 1A (52-53 cM) were associated with Ptr ToxA, of which the former coincided with the *Tsn1* gene. For Ptr ToxB, we identified two other major effect regions on chromosomes 2B and 5B. The genomic regions associated with common bunt mapped on 2B, 4B and 7A, while those associated with leaf rust mapped at two positions on 2B. We were only able to uncover a single marker-trait association for tan spot on 7B and for yellow rust on 2A.

In the third study we identified disease marker combinations of 70 Canadian wheat cultivars to aid wheat breeders in the determination of parental material for cross combinations. Further, using stepwise discriminant analysis and partial least square regression, we identified 6-8 markers for yellow rust, 4-6 markers for leaf rust, 5-9 markers for tan spot and 6-11 markers for Ptr ToxA as the best predictor of the phenotypic variation observed across the 70 cultivars.

5.3 General discussion

The mapping population of 158 RILs and its two parents 'Cutler' x 'AC Barrie' was genotyped with a subset of 1809 SNPs and 2 functional markers (*Ppd-D1* and Rht-D1). Using composite interval mapping on the combined phenotype data across all environments, we identified a total of 19 QTLs on 8 chromosomes and they individually explained between 6.3 and 37.8% of the phenotypic variation. The QTL on 2D reduced flowering and maturity time up to 5 days with a yield penalty of 436 kg ha-1, while the QTL on 4D reduced plant height by 13 cm, but increased maturity by 33 degree days. Previously in this population were reported three QTLs associated with the combined phenotypic data across four environments (Kamran et al., 2013) , which includes one coincident QTL for both flowering time and maturity at 31-33 cM on 1B (*QEps.dms-1B1*), one QTL for maturity at 36 cM on 1B (*QEps.dms-1B2*) and one QTL for flowering time at 76 cM on 5B (*QEps.dms-5B1*). Our study failed to identify any QTL for both plant height and grain yield across the combined phenotypic data of the four environments; only two environment specific QTLs were reported for grain yield. Low marker density and dominant inheritance of the DArT marker might have restricted the ability to identify more QTLs with larger phenotypic effect. The use of a larger number of polymorphic markers provides a more accurate overview of informative recombination and greater saturation of genetic linkage maps (Zych et al., 2015). The denser the genetic maps, the lower the chance of missing true QTLs

Leaf rust, yellow (stripe) rust, common bunt and tan spot are economically significant diseases affecting wheat production in Canada. In the second study, we investigated the genetic relationship and population structure of 81 historical and modern Canadian western spring wheat cultivars released between 1885 and 2011, and identified genomic regions associated with resistance to the above four diseases and insensitivity to three Ptr toxins (Ptr ToxA, Ptr ToxB and Ptr ToxC). There were large genetic and phenotypic differences among pairwise comparisons of cultivars,

except six pairs that showed <0.05 genetic distance. For instance, 'McKenzie' is the backcross parent of 'Unity'(Fox et al., 2010) 'Superb' is one of the parents of 'CDC Abound' (McCallum and Depauw, 2008) , while 'AC Crystal' and 'AC Taber' are sibs (Fernandez et al., 1998). Although a substantial number of cultivars shared one or more common parents, approximately 94% of the pairwise comparisons of the genetic distance among the 81 cultivars varied between 0.20 and 0.50, which suggests the presence of large genetic variation among the Canadian western spring wheat cultivars released. The cultivars exhibited a clear population structure, generally in agreement with the major western Canadian spring wheat classes. Using a threshold of $p < 5\times10^{-5}$ and a weighted mixed linear model, we identified 94 markers from 7 chromosomes associated with all traits except Ptr ToxC. Two major effect genomic regions on 5B and 1A were associated with Ptr ToxA, the region in 5B explained approximately 46% of the phenotypic variation, and coincided with the *Tsn1* gene (Zhang et al., 2009), other authors have reported a major genomic region on 5BL associated with sensitivity to Ptr ToxA (Cheong et al., 2004). For Ptr ToxB, we identified two other major effect regions on chromosomes 2B and 5B. In this case the region in 2B is harboring the dominant *Tsc2* gene, which mapped on the short arm of chromosome 2B (Friesen and Faris, 2004; Abeysekara et al., 2010). The genomic regions associated with common bunt were mapped on 2B, 4B and 7A, Six QTLs associated with resistance to common bunt have been reported in a double haploid population derived from a cross 'Carberry' x 'AC Cadillac', which includes a QTL on 4B that explained 7.6% of the phenotypic variance (Singh et al., 2016). In a Canadian spring wheat DH population derived from a cross between 'RL 4452' x 'AC Domain', two QTLs for common bunt were also reported on chromosomes 1B and 7A (Fofana et al., 2008) . Those associated with leaf rust mapped at two positions on 2B. We were only able to uncover a single marker-trait association for tan spot on

7B and for yellow rust on 2A. Pairwise LD values between *Lr37/Yr17/Sr38* gene specific marker and 1,197 SNPs that mapped on 2A were very low, which suggests that the genomic region associated with yellow rust on 2A is different from the *Lr37/Yr17/Sr38* gene. For 7B, pairwise LD values between *Lr68* gene specific marker and 1,248 SNPs varied from 0 to 0.58, but the highest LD values were observed between Lr68 and most SNPs that mapped between 144 and 152 cM, which is different from the genomic region associated with tan spot. We also confirm the susceptibility of 86% of Western Canadian wheat cultivars to tan spot race 1 isolates and their sensitivity to both Ptr ToxA and ToxC. This may have to two possible explanations: most *Ptr* isolates collected in Alberta belonged to race 1 (62%) versus race 2 (36%) and race 3 (2%) (Aboukhaddour et al., 2013) and many of the modern spring wheat cultivars in western Canada were developed using few common parent cultivars (e.g., 'Red Fife', 'Thatcher', 'Neepawa', 'Katepwa' and 'AC Barrie') that possess good agronomic characteristics and good end-use quality (Lamari et al., 2005; McCallum and DePauw, 2008), but are susceptible to tan spot. Direct comparisons among the different studies was not possible due to differences on the types of markers used and lack of physical positions of the flanking markers reported in the different studies.

In the third and last study we identified disease marker combinations of 70 Canadian wheat cultivars to aid wheat breeders in the determination of parental material for cross combinations. Using correlation, stepwise discriminant analysis and partial least square regression, we identified 6-8 markers for yellow rust, 4-6 markers for leaf rust, 5-9 markers for tan spot and 6-11 markers for Ptr ToxA as the best predictor of the phenotypic variation observed across the 70 cultivars. Most markers identified as the best predictor of each trait also showed significant correlation with one or more traits. Overall, 23 of the 50 gene specific markers were strongly

associated with one or more of the four phenotypic traits. ANOVA showed significant differences between pair of alleles of 22 of the 23 markers. In most analyses, the markers for *Lr34*, *Vrn-A1* and *Tsn1* were consistently identified as the best predictor of the three diseases and Ptr ToxA.

5.4 Future Research

To better understand the genetic background of Canadian western spring wheat, further work is needed. I list below a number of recommendations arising from my thesis work:

i. Due to the importance of marker density and distribution to carry out linkage mapping, I believe that the 90K SNPs marker is an excellent choice to conduct further work. The use of the same array would allow us to combine studies in the future and to create more reliable linkage maps.
ii. In western Canada, the frequency of *Rht-8*, *Lr22a*, *Lr37, Lr 68* are relatively low in the germplasm collection. It would be interesting to see these genes in Canadian backgrounds and evaluate their effect in early maturity, plant height, grain yield and also evaluate the resistance genes for durable resistance.
iii. Increase or intensify the breeding for tan spot resistance especially to races carrying Ptr ToxA and ToxC.
iv. Explore the polymorphism of quality genes as glutein (*Glu*), polyphenoloxidase activity (*Ppo*), waxi (*Wx*), etc. as well as resistance genes at *tsc2* (Ptr ToxB resistance) in Canada western spring wheat cultivars and increase the size of the germplasm collection in study.
v. Develop a novel marker for *tsc2* also could be a good addition to the current set of markers available to breeders.
vi. An increase in the number of accessions in the historical collection used to do association mapping studies is paramount to reach sufficient statistical power
vii. To carry out a fine mapping of the *Eps* genes in chromosome 2B and 4A where QTL associated with days to heading, flowering and maturity has been detected in the ‘Cutler’ x ‘Barrie’ population.

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Appendices

Appendix 1. Descriptive statistics and F statistics

Trait*	Parents		RILs (n = 158)			F statistics			
	AC Barrie mean	Cutler mean	Min	Max	Mean	DF (genotypes)	DF (all environments and replications)	F Value	Pr > F
Flowering (Field)-days	53.8	51.2	49.4	58.0	53.4	157	1550	8.13	<.0001
Flowering (degree days)	815.5	770.5	735.7	898.8	811.7	157	1551	10.12	<.0001
Maturity (days)	95.2	92.6	90.8	101.3	95.4	157	1548	10.67	<.0001
Maturity (degree days)	1538.9	1491.9	1452.2	1626.4	1534.2	157	1546	9.62	<.0001
Plant height (cm)	92.4	79.5	65.7	103.5	86.3	157	1545	40.85	<.0001
Grain yield (kg)	5760.4	5605.5	4.6	7.4	5.9	157	1535	5.02	<.0001

* Summary of the flowering time data under greenhouse is provided in our previous study (Kamran et al. 2013)

Appendix 2. Summary of QTLs for individual and combined environments. Note that QTLs for flowering under field and maturity are given both for the number of days and degree days.

QTL	Trait*	Condition	Environment	Chrom	Position (cM)	Left marker	Right marker	Confidence interval (cM)	LOD	Partial R^2 (%)	Additive effect	RILs with Cutler alleles	RILs with AC Barrier alleles	Difference (Cutler-AC Barrie)
QFlt.dms-2D	Flowering (days)	GH	Combined	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-3	16.3	36.6	2.6	38.9	44.3	-5.4
QFlt.dms-2D	Flowering (days)	Field	Combined	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-5	7.2	19.6	0.8	53.0	54.1	-1.1
QFlt.dms-2D	Flowering (days)	GH	2006	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-4	10.7	24.2	1.6	37.3	39.8	-2.5
QFlt.dms-2D	Flowering (days)	GH	2008	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-0	5.0	37.6	46.7	40.5	49.4	-8.9
QFlt.dms-2D	Flowering (days)	Field	2008 Early	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-7	8.2	24.4	0.8	54.2	55.4	-1.2
QFlt.dms-2D	Flowering (days)	Field	2008 Late	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-4	7.1	15.8	1.1	44.9	46.5	-1.6
QFlt.dms-2D	Flowering (days)	Field	2011	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-6	4.7	10.8	0.9			
QFlt.dms-2D	Flowering (days)	Field	2012	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-8	5.9	12.6	0.6			
QFlt.dms-2D	Flowering (DD)	Field	Combined	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-5	7.8	25.4	14.2	804.4	825.4	-21.0
QFlt.dms-2D	Flowering (DD)	Field	2008 Early	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-6	8.3	22.3	17.2	806.4	833.7	-27.3
QFlt.dms-2D	Flowering (DD)	Field	2008 Late	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-4	8.2	25.0	20.9	744.9	775.8	-30.9
QFlt.dms-2D	Flowering (DD)	Field	2011	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-6	4.7	10.8	15.0			
QFlt.dms-2D	Flowering (DD)	Field	2012	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-10	4.9	12.8	11.5			
QFlt.dms-3B	Flowering (days)	Field	Combined	3B	94	Excalibur_c45968_83	CAP12_rep_c7901_114	91-95	5.8	13.5	-0.6	53.9	52.9	1.0
QFlt.dms-3B	Flowering (DD)	Field	Combined	3B	94	Excalibur_c45968_83	CAP12_rep_c7901_114	91-95	6.7	10.7	-10.6	821.9	802.2	19.7
QFlt.dms-3B	Flowering (DD)	Field	2008 Late	3B	94	Excalibur_c45968_83	CAP12_rep_c7901_114	92-95	5.4	8.7	-13.7			
QFlt.dms-4A.1	Flowering (DD)	Field	2008 Early	4A	38	CAP12_rep_c4000_432	wsnp_Ex_c54453_57331510	30-42	3.8	7.3	9.3			
QFlt.dms-4A.1	Flowering (days)	Field	2008 Early	4A	39	CAP12_rep_c4000_432	wsnp_Ex_c54453_57331510	30-42	4.3	7.8	0.4			
QFlt.dms-4A	Flowering (DD)	Field	Combined	4A	41	CAP12_rep_c4000_432	wsnp_Ex_c54453_57331510	38-42	3.7	6.3	7.6	803.7	821.3	-17.6
QFlt.dms-4A.2	Flowering (days)	Field	2007	4A	53	Ra_c7973_1185	wsnp_Ex_c10390_17007929	51-56	3.6	10.0	0.7			
QFlt.dms-4A.2	Flowering (DD)	Field	2007	4A	53	Ra_c7973_1185	wsnp_Ex_c10390_17007929	51-56	3.7	10.1	13.9			
QFlt.dms-4A.3	Flowering (days)	Field	2008 Early	4A	168	BobWhite_c22176_295	RAC875_c59673_500	166-171	6.1	14.6	-0.5			
QFlt.dms-4A.3	Flowering (DD)	Field	2008 Early	4A	168	BobWhite_c22176_295	RAC875_c59673_500	166-171	5.4	14.8	-10.9			
QFlt.dms-5A.1	Flowering (days)	GH	Combined	5A	187	Kukri_c20258_143	JD_c3525_1503	183-199	3.3	6.9	-0.9	43.1	41.1	2.0
QFlt.dms-5A.2	Flowering (DD)	Field	Combined	5A	253	Tdurum_contig86202_175	wsnp_Ra_c10915_17838202	252-254	3.7	7.5	-7.5	819.3	803.2	16.1
QFlt.dms-5A.2	Flowering (DD)	Field	2008 Late	5A	254	wsnp_Ra_c12183_19587379	wsnp_Ra_c3414_6378271	253-254	3.5	8.7	-10.8			
QFlt.dms-5B	Flowering (days)	GH	Combined	5B	44	BS00063785_51	IACX5818	43-48	5.0	12.3	1.1	41.0	42.8	-1.8
QFlt.dms-5B	Flowering (days)	GH	2008	5B	46	IACX5818	wsnp_Ku_c17875_27051169	43-48	3.6	10.9	1.7			
QFlt.dms-6B.1	Flowering (days)	GH	Combined	6B	59	Tdurum_contig11700_1247	wsnp_Ra_c2730_5190365	56-60	4.2	10.0	1.1	41.0	43.0	-2.0
QFlt.dms-6B.1	Flowering (days)	GH	2008	6B	59	Tdurum_contig11700_1247	wsnp_Ra_c2730_5190365	56-60	3.6	11.1	1.7			
QFlt.dms-6B.2	Flowering (days)	Field	Combined	6B	118	wsnp_Ex_c4124_7455225	Kukri_c49331_77	110-120	3.1	6.7	0.4	53.1	53.8	-0.7
QFlt.dms-6B.2	Flowering (DD)	Field	Combined	6B	118	wsnp_Ex_c4124_7455225	Kukri_c49331_77	111-120	3.4	9.3	7.4	805.5	819.3	-13.8
QFlt.dms-6B.2	Flowering (DD)	Field	2008 Late	6B	118	wsnp_Ex_c4124_7455225	Kukri_c49331_77	112-120	3.3	8.5	10.8			
QFlt.dms-7A	Flowering (days)	GH	Combined	7A	5	Excalibur_c16355_712	RAC875_c18446_521	4-8	3.7	7.3	1.0	41.2	42.6	-1.4
QFlt.dms-7A.1	Flowering (days)	GH	2006	7A	9	Tdurum_contig11613_329	wsnp_Ex_c30239_39179460	8-10	4.0	8.2	0.8			
QFlt.dms-7A.1	Flowering (days)	Field	2011	7A	9	Tdurum_contig11613_329	wsnp_Ex_c30239_39179460	8-10	4.0	8.2	0.7			
QFlt.dms-7A	Flowering (days)	Field	Combined	7A	9	Tdurum_contig11613_329	wsnp_Ex_c30239_39179460	8-10	4.7	12.7	0.5	53.0	53.9	-0.9
QFlt.dms-7A	Flowering (DD)	Field	Combined	7A	9	Tdurum_contig11613_329	wsnp_Ex_c30239_39179460	8-10	6.4	15.6	10.3	803.8	819.7	-15.9
QFlt.dms-7A.1	Flowering (DD)	Field	2008 Late	7A	9	Tdurum_contig11613_329	wsnp_Ex_c30239_39179460	8-10	5.6	13.0	13.9			
QFlt.dms-7A.1	Flowering (DD)	Field	2011	7A	9	Tdurum_contig11613_329	wsnp_Ex_c30239_39179460	8-10	4.0	8.0	11.4			
QFlt.dms-7A.1	Flowering (days)	Field	2008 Early	7A	12	wsnp_Ra_c63822_63288359	wsnp_BG313770A_Ta_2_3	9-16	3.5	6.8	0.4			

* Flowering (days) = number of days to 50% flowering under greenhouse or field; Maturity (days) = number of days to maturity under field condition; Flowering (DD) and Maturity (DD) = flowering and maturity time in degree days, respectively.

Appendix 2. Summary of QTLs for individual and combined environments (CONT.).

QTL	Trait*	Condition	Environment	Chrom	Position (cM)	Left marker	Right marker	Confidence interval (cM)	LOD	Partial R^2 (%)	Additive effect	RILs with Cutler alleles	RILs with AC Barrier alleles	Difference (Cutler-AC Barrie)
QFlt.dms-7A.2	Flowering (DD)	Field	2008 Late	7A	56	IACX4711	wsnp_Ku_c7873_13486065	52-61	3.8	10.9	-11.5			
QMat.dms-2D	Maturity (days)	Field	2008 Early	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-11	3.1	7.0	0.7			
QMat.dms-2D	Maturity (days)	Field	2008 Late	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-6	4.3	10.0	1.5			
QMat.dms-2D	Maturity (days)	Field	Combined	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-7	3.1	11.2	0.7	94.8	96.1	-1.3
QMat.dms-2D	Maturity (DD)	Field	Combined	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-8	3.9	10.4	14.2	1525.3	1546.5	-21.2
QMat.dms-2D	Maturity (DD)	Field	2008 Early	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-11	3.1	6.8	13.5			
QMat.dms-2D	Maturity (DD)	Field	2008 Late	2D	0	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-6	3.6	7.7	32.1			
QMat.dms-3B	Maturity (days)	Field	2012	3B	91	wsnp_Ku_c210_413608	Excalibur_c45968_83	88-95	3.4	7.0	-0.6			
QMat.dms-4A.1	Maturity (days)	Field	2008 Early	4A	41	CAP12_rep_c4000_432	wsnp_Ex_c54453_57331510	35-42	3.8	12.5	0.7			
QMat.dms-4A.1	Maturity (days)	Field	2008 Late	4A	41	CAP12_rep_c4000_432	wsnp_Ex_c54453_57331510	36-42	4.4	11.1	1.3			
QMat.dms-4A.1	Maturity (DD)	Field	Combined	4A	41	CAP12_rep_c4000_432	wsnp_Ex_c54453_57331510	35-42	3.1	12.0	10.7	1524.0	1546.7	-22.7
QMat.dms-4A.1	Maturity (DD)	Field	2008 Early	4A	41	CAP12_rep_c4000_432	wsnp_Ex_c54453_57331510	35-42	3.9	12.6	12.9			
QMat.dms-4A.2	Maturity (days)	Field	2007	4A	50	wsnp_Ex_c5690_9994305	wsnp_Ex_rep_c67799_664887	46-51	4.7	10.3	1.2			
QMat.dms-4A.2	Maturity (DD)	Field	2007	4A	50	wsnp_Ex_c5690_9994305	wsnp_Ex_rep_c67799_664887	46-51	4.5	9.9	15.2			
QMat.dms-4A.2	Maturity (days)	Field	Combined	4A	53	Ra_c7973_1185	wsnp_Ex_c10390_17007929	51-56	3.5	6.5	0.7	94.9	96.1	-1.2
QMat.dms-4A.3	Maturity (days)	Field	2008 Early	4A	162	GENE-2307_140	RAC875_c95150_286	161-163	5.1	11.4	-0.8			
QMat.dms-4A.3	Maturity (DD)	Field	2008 Early	4A	162	GENE-2307_140	RAC875_c95150_286	161-163	5.0	11.2	-14.6			
QMat.dms-4D.1	Maturity (days)	Field	Combined	4D	26	Excalibur_c5010_1336	Kukri_rep_c68594_530	19-29	4.9	13.4	-0.8	96.3	94.7	1.6
QMat.dms-4D.2	Maturity (days)	Field	2012	4D	34	Kukri_rep_c68594_530	Rht-D1b	31-38	3.5	13.3	-0.6	1474.7	1444.2	30.5
QMat.dms-4D.2	Maturity (DD)	Field	2012	4D	34	Kukri_rep_c68594_530	Rht-D1b	32-37	5.5	16.7	-13.4	1474.7	1444.2	30.5
QMat.dms-4D.2	Maturity (days)	Field	2008 Early	4D	36	Rht-D1b	wsnp_CAP11_c356_280910	34-39	9.7	20.5	-1.1	96.9	94.4	2.5
QMat.dms-4D.2	Maturity (DD)	Field	2008 Early	4D	36	Rht-D1b	wsnp_CAP11_c356_280910	34-39	9.0	19.3	-21.5	1565.7	1518.5	47.2
QMat.dms-4D.2	Maturity (days)	Field	2008 Late	4D	37	Rht-D1b	wsnp_CAP11_c356_280910	34-43	7.0	15.8	-1.8	92.8	89.2	3.6
QMat.dms-4D.2	Maturity (DD)	Field	Combined	4D	37	Rht-D1b	wsnp_CAP11_c356_280910	34-43	5.7	16.0	-15.7	1554.0	1521.0	33.0
QMat.dms-4D.2	Maturity (DD)	Field	2008 Late	4D	38	Rht-D1b	wsnp_CAP11_c356_280910	35-42	4.4	13.8	-32.3	1677.3	1595.0	82.3
QMat.dms-5A	Maturity (DD)	Field	2011	5A	221	IACX3911	BS00077858_51	220-223	3.1	7.9	-10.6			
QMat.dms-5A	Maturity (days)	Field	2011	5A	222	BS00077858_51	BS00067209_51	220-223	3.2	8.0	-0.8			
QMat.dms-7A.1	Maturity (DD)	Field	Combined	7A	13	wsnp_Ra_c63822_63288359	wsnp_BG313770A_Ta_2_3	10-16	3.0	9.1	11.3	1529.3	1540.5	-11.2
QMat.dms-7A.2	Maturity (DD)	Field	Combined	7A	42	Tdurum_contig37154_190	RAC875_c14982_577	40-45	5.7	16.2	-15.7	1545.9	1525.1	20.8
QMat.dms-7A.2	Maturity (days)	Field	2008 Late	7A	44	RAC875_c14982_577	Tdurum_contig20214_279	42-45	5.4	10.3	-1.4			
QMat.dms-7A.2	Maturity (days)	Field	Combined	7A	44	RAC875_c14982_577	Tdurum_contig20214_279	42-45	3.1	8.8	-0.6	96.0	94.8	1.2
QPht.dms-4D	Plant height	Field	2008 Early	4D	36	Rht-D1b	wsnp_CAP11_c356_280910	34-38	18.8	38.5	7.1	80.8	94.9	-14.1
QPht.dms-4D	Plant height	Field	Combined	4D	37	Rht-D1b	wsnp_CAP11_c356_280910	35-39	16.2	37.8	6.3	78.4	91.6	-13.2
QPht.dms-4D	Plant height	Field	2007	4D	37	Rht-D1b	wsnp_CAP11_c356_280910	35-39	16.6	30.9	5.7	67.8	78.5	-10.7
QPht.dms-4D	Plant height	Field	2008 Late	4D	37	Rht-D1b	wsnp_CAP11_c356_280910	35-39	14.6	34.6	6.3	81.8	95.1	-13.3
QPht.dms-4D	Plant height	Field	2011	4D	37	Rht-D1b	wsnp_CAP11_c356_280910	35-39	13.2	32.0	6.9	85.6	99.9	-14.3
QPht.dms-4D	Plant height	Field	2012	4D	41	wsnp_CAP11_c356_280910	BS00036421_51	35-43	14.7	32.9	6.3	79.8	93.3	-13.5
QYld.dms-1B	Grain yield	Field	2007	1B	155	Tdurum_contig50988_500	wsnp_Ex_c13878_21738866	152-156	4.9	8.1	-280.2			
QYld.dms-2A	Grain yield	Field	2007	2A	271	Tdurum_contig86243_288	BS00063368_51	268-272	5.6	11.0	-304.3			
QYld.dms-2D	Grain yield	Field	Combined	2D	2	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-12	4.3	8.6	248.2	5698.9	6134.9	-436.0
QYld.dms-2D	Grain yield	Field	2011	2D	2	Ppd-D1a	wsnp_CAP11_c3842_1829821	0-11	5.2	12.1	448.4			
QYld.dms-4D	Grain yield	Field	2008 Early	4D	71	D_GDEEGVY01C7BQU_446	BobWhite_c20689_427	68-72	5.3	6.8	-794.9			
QYld.dms-5B	Grain yield	Field	Combined	5B	34	Excalibur_c30667_102	Ku_c6193_821	32-36	3.7	7.7	184.0	5767.3	6089.1	-321.8
QYld.dms-5B	Grain yield	Field	2011	5B	36	Ku_c6193_821	Tdurum_contig31131_198	35-37	4.5	10.7	327.3			

* Flowering (days) = number of days to 50% flowering under greenhouse or field; Maturity (days) = number of days to maturity under field condition; Flowering (DD) and Maturity (DD) = flowering and maturity time in degree days, respectively.

Appendix 3. Comparison of recombinant inbred lines that were homozygous with 'Cutler' or 'AC Barrie' alleles at the two flanking markers of each coincident QTL.

All RILs with recombinant genotype at the flanking markers of each coincident QTL were excluded from analysis.

Coincident QTL	Trait	Source od variation	DF*	Mean square	F	P
QFlt.dms-2D	Flowering (days un	Genotypes	1	473.4	43.4	0.001
QMat.dms-2D		Error	74	10.9		
QYld.dms-2D						
	Flowering (days un	Genotypes	1	15.1	6.6	0.012
		Error	74	2.3		
	Flowering (degree	Genotypes	1	5555.9	6.8	0.011
		Error	74	821.3		
	Maturity (days)	Genotypes	1	28.9	4.4	0.039
		Error	74	6.5		
	Maturity (degree d	Genotypes	1	6787.0	3.8	0.045
		Error	74	1811.0		
	Plant height (cm)	Genotypes	1	144.7	1.6	0.207
		Error	74	89.5		
	Grain yield (kg)	Genotypes	1	3455603.0	11.5	0.001
		Error	74	301074.0		
QFlt.dms-4A.1	Flowering (days un	Genotypes	1	7.0	0.4	0.513
QMat.dms-4A.1		Error	133	16.2		
	Flowering (days un	Genotypes	1	20.8	8.3	0.005
		Error	133	2.5		
	Flowering (degree	Genotypes	1	8009.5	8.9	0.003
		Error	133	901.5		
	Maturity (days)	Genotypes	1	64.9	11.3	0.001
		Error	133	5.7		
	Maturity (degree d	Genotypes	1	17407.0	10.5	0.002
		Error	133	1664.0		
	Plant height (cm)	Genotypes	1	71.7	0.8	0.386
		Error	133	94.6		
	Grain yield (kg)	Genotypes	1	11015.0	0.0	0.864
		Error	133	374685.0		

QMat.dms-4D.2	Flowering (days un	Genotypes	1	10.2	0.7	0.422
QPht.dms-4D		Error	121	15.7		
	Flowering (days un	Genotypes	1	1.6	0.6	0.451
		Error	121	2.7		
	Flowering (degree	Genotypes	1	832.2	0.8	0.362
		Error	121	993.8		
	Maturity (days)	Genotypes	1	109.0	20.1	0.001
		Error	121	5.4		
	Maturity (degree d	Genotypes	1	36324.0	24.1	0.001
		Error	121	1509.0		
	Plant height (cm)	Genotypes	1	5210.8	103.1	0.001
		Error	121	50.5		
	Grain yield (kg)	Genotypes	1	615678.0	1.6	0.215
		Error	121	3970019.0		
QFlt.dms-7A.1	Flowering (days un	Genotypes	1	47.9	3.0	0.086
QMat.dms-7A.1		Error	142	16.0		
	Flowering (days un	Genotypes	1	29.0	12.0	0.001
		Error	142	2.4		
	Flowering (degree	Genotypes	1	9568.0	10.9	0.001
		Error	142	875.0		
	Maturity (days)	Genotypes	1	9.0	1.5	0.23
		Error	142	6.2		
	Maturity (degree d	Genotypes	1	1882.0	1.1	0.308
		Error	142	1798.0		
	Plant height (cm)	Genotypes	1	4.8	0.1	0.816
		Error	142	88.3		
	Grain yield (kg)	Genotypes	1	118.0	0.0	0.986
		Error	142	389330.0		

Appendix 4. QTL results for chromosome 1B and 5B based on combined genotype and linkage maps of SSR, DArT and SNP markers from 131 recombinant inbred lines

QTL*	Trait	Experiment	Chrom	Position (cM)	Confidence interval (cM)	LeftMarker	RightMarker	LOD	R^2 (%)	Additive effect
QEps.dms-1B2	Maturity	Combined	1B	76	74.5-79.5	wPt-2694	wPt-7242	4.4	11.0	-1.0
QEps.dms-1B2	Maturity (degree days)	2011	1B	78	75.5-79.5	wPt-7242	wPt-5562	4.6	13.6	-13.3
QEps.dms-1B2	Maturity (degree days)	Combined	1B	78	75.5-79.5	wPt-7242	wPt-5562	4.4	9.7	-15.8
QEps.dms-1B2	Maturity	2011	1B	79	78.5-79.5	wPt-7242	wPt-5562	4.5	13.1	-1.0
QEps.dms-1B2	Maturity	2007	1B	80	79.5-80.5	wPt-2744	wsnp_Ex_c25782_35041189	4.1	9.9	-1.2
QEps.dms-1B2	Maturity (degree days)	2007	1B	80	79.5-80.5	wPt-2744	wsnp_Ex_c25782_35041189	4.0	9.7	-15.5
QPht.dms-5B1	Plant height	2008L late	5B	70	69.5-71.5	wPt-2607	wPt-3457	5.1	8.4	-2.8
QEps.dms-5B1	Maturity	2007	5B	180	176.5-182.5	wPt-1304	wPt-666939	5.4	11.4	1.3
QEps.dms-5B1	Maturity (degree days)	2007	5B	180	176.5-182.5	wPt-1304	wPt-666939	5.5	11.8	16.9
QEps.dms-5B1	Flowering	2008 early	5B	181	174.5-182.5	wPt-666939	wPt-5092	3.4	7.6	0.5
QPht.dms-5B2	Plant height	Combined	5B	199	194.5-203.5	wPt-5514	wPt-1457	5.0	13.1	270.4
QPht.dms-5B2	Plant height	2011	5B	201	194.5-203.5	wPt-5514	wPt-1457	3.1	7.5	3.1
QGyl.dms-5B1	Grain yield	2011	5B	201	193.5-203.5	wPt-5514	wPt-1457	3.4	10.0	322.2

*Eps: Earliness per se QTL associated with both flowering and maturity time

Appendix 5. Pairwise genetic distance matrix and other categorical variables for 81 spring wheat cultivars based on 19,930 markers

S/N	Name in SNPs	Corrected name SNP data	Year of release	Wheat class*	Group membership based on PCA	Group membership based on cluster anaysis	5500HR	5600HR	5601HR	5602HR	5603HR	5604HRCL	5700PR
1	5500HR	5500HR	2000	CWRS	3	3							
2	5600HR	5600HR	1999	CWRS	3	3	0.1891						
3	5601HR	5601HR	2001	CWRS	3	3	0.2243	0.1866					
4	5602HR	5602_HR	2004	CWRS	3	3	0.3232	0.3240	0.3390				
5	5603HR	5603_HR	2008	CWRS	1	1	0.3545	0.3221	0.3218	0.3614			
6	5604HRCL	5604HRCL	2009	CWRS	1	1	0.3268	0.2836	0.3294	0.3199	0.3367		
7	5700PR	5700_PR	2000	CPSR	2	2	0.4002	0.4538	0.3952	0.3941	0.4128	0.4214	
8	5701PR	5701PR	2001	CPSR	2	2	0.3712	0.3957	0.3612	0.3357	0.3817	0.3698	0.3059
9	5702PR	5702PR	2007	CPSR	2	2	0.3806	0.3835	0.4137	0.3350	0.3848	0.4162	0.3750
10	ACAbbey	AC_Abbey	1998	CWRS	3	3	0.3208	0.3001	0.3628	0.3973	0.3886	0.3860	0.4062
11	ACAndrew	AC_Andrew	2000	CWSWS	2	2	0.4497	0.4496	0.4660	0.4235	0.4298	0.4360	0.4112
12	ACBarrie	AC_Barrie	1994	CWRS	3	3	0.1995	0.1677	0.2348	0.2501	0.3803	0.2992	0.4102
13	ACCadillac	AC_Cadillac	1996	CWRS	3	3	0.1965	0.2152	0.2779	0.2761	0.3749	0.3198	0.4129
14	ACCrystal	AC_Crystal	1996	CPSR	2	2	0.3903	0.4485	0.4299	0.3843	0.4509	0.4510	0.2161
15	ACDomain	AC_Domain	1993	CWRS	1	1	0.3635	0.3185	0.3481	0.3577	0.3852	0.1913	0.4267
16	ACEatonia	AC_Eatonia	1993	CWRS	3	3	0.2986	0.3033	0.3355	0.4097	0.3625	0.3575	0.4053
17	ACElsa	AC_Elsa	1996	CWRS	3	3	0.3269	0.3183	0.3629	0.3621	0.3808	0.3662	0.4082
18	ACForemost	AC_Foremost	1995	CPSR	2	2	0.4088	0.4710	0.4498	0.4018	0.4658	0.4648	0.1746
19	ACIntrepid	AC_Intrepid	1997	CWRS	3	3	0.2655	0.2290	0.2772	0.3461	0.3681	0.3583	0.4282
20	ACReed	AC_Reed	1991	CWSWS	2	2	0.4300	0.4524	0.4541	0.4321	0.4314	0.4335	0.3992
21	ACSplendor	AC_Splendor	1997	CWRS	3	3	0.2455	0.1602	0.2280	0.2956	0.3294	0.3131	0.4133
22	ACTaber	AC_Taber	1991	CPSR	2	2	0.3751	0.4269	0.4187	0.3757	0.4385	0.4327	0.2252
23	ACVista	AC_Vista	1996	CPSR	2	2	0.3678	0.4143	0.3886	0.3850	0.3953	0.4180	0.3242
24	Alvena	Alvena	2006	CWRS	3	3	0.2383	0.2158	0.2676	0.3196	0.3829	0.3581	0.4117
25	Burnside	Burnside	2004	CWES	2	2	0.3989	0.4158	0.4252	0.3436	0.4234	0.4168	0.4002
26	CDCAbound	CDC_Abound	2006	CWRS	1	1	0.3754	0.3281	0.3933	0.3897	0.4009	0.2572	0.4785
27	CDCAlsask	CDC_Alsask	2004	CWRS	3	3	0.2225	0.2089	0.2709	0.3435	0.3526	0.2930	0.4387
28	CDCBison	CDN_Bison	2008	CWES	2	2	0.4185	0.4386	0.4387	0.3627	0.4115	0.4190	0.3499
29	CDCBounty	CDC_Bounty	1999	CWRS	3	3	0.2061	0.1732	0.2607	0.3012	0.3550	0.3054	0.4305
30	CDCGo	CDC_Go	2003	CWRS	1	1	0.4098	0.3991	0.4465	0.3958	0.4341	0.2774	0.4433
31	CDCImagine	CDC_Imagine	2002	CWRS	3	3	0.2582	0.2199	0.2592	0.2984	0.3691	0.3126	0.4008
32	CDCKernen	CDC_Kernen	2009	CWRS	3	3	0.2385	0.1915	0.2583	0.3192	0.3555	0.3180	0.4304
33	CDCMerlin	CDC_Merlin	1992	CWRS	3	3	0.2992	0.2554	0.3237	0.3682	0.3741	0.3320	0.4791
34	CDCOsler	CDC_Osler	2003	CWRS	3	3	0.2184	0.1577	0.2245	0.3160	0.3200	0.2784	0.4239
35	CDCPlentiful	CDC_Plentiful	2011	CWRS	3	3	0.2663	0.2634	0.2912	0.3005	0.3675	0.3310	0.4083
36	CDCRama	CDC_Rama	2001	CWES	2	2	0.4096	0.4105	0.4043	0.3616	0.4038	0.4051	0.4215
37	CDCTeal	CDC_Teal	1991	CWRS	3	3	0.2375	0.2051	0.2527	0.3187	0.3407	0.3227	0.4288
38	CDCThrive	CDC_Thrive	2009	CWRS	3	3	0.2404	0.1950	0.2573	0.2954	0.3554	0.2977	0.4197
39	CDCUtmost	CDC_Utmost	2009	CWRS	3	3	0.3069	0.2848	0.3291	0.3546	0.3644	0.3325	0.4261
40	CDCVRMorris	CDC_VR_Morris	2010	CWRS	3	3	0.2572	0.2268	0.2750	0.3380	0.3572	0.3696	0.4161
41	CDCWalrus	CDC_Walrus	2003	CWES	2	2	0.3916	0.4056	0.4218	0.3798	0.4140	0.4455	0.4385
42	CONQUER	CONQUER=HY_682	2009	CPSR	2	2	0.4010	0.3957	0.4146	0.4007	0.3967	0.4154	0.3208
43	Carberry	Carberry	2009	CWRS	1	1	0.3691	0.3454	0.4005	0.3844	0.3918	0.2793	0.4468
44	Columbus	Columbus	1980	CWRS	3	3	0.2042	0.1138	0.1215	0.3419	0.3034	0.2763	0.4285
45	Glencross	Glencross	2007	CWES	2	2	0.3852	0.4102	0.4188	0.3509	0.3986	0.4200	0.3909
46	Glenlea	Glenlea	1972	CWES	2	2	0.4054	0.4337	0.4377	0.3424	0.4196	0.4295	0.3993
47	Glenn	Glenn	2009	CWRS	1	1	0.3953	0.3764	0.4060	0.3890	0.3826	0.3131	0.4248
48	GoodeveVB	Goodeve_VB	2007	CWRS	3	3	0.3062	0.2774	0.3233	0.3407	0.3409	0.3416	0.4262
49	Harvest	Harvest	2004	CWRS	1	1	0.2954	0.2925	0.3271	0.3757	0.3611	0.2227	0.4195
50	Helios	Helios	2006	CWRS	3	3	0.2018	0.1817	0.2324	0.2448	0.3823	0.2883	0.4188
51	Infinity	Infinity	2004	CWRS	3	3	0.2858	0.3003	0.3097	0.3580	0.3882	0.3535	0.3808
52	Journey	Journey	2002	CWRS	3	3	0.3267	0.2709	0.3115	0.3401	0.3315	0.2934	0.4212
53	Kane	Kane	2006	CWRS	1	1	0.3192	0.2734	0.2937	0.3252	0.3153	0.2289	0.4093
54	Katepwa	Katepwa	1981	CWRS	3	3	0.1587	0.0958	0.1889	0.3368	0.3360	0.2836	0.4527
55	Laura	Laura	1986	CWRS	3	3	0.3745	0.3413	0.3761	0.3671	0.3961	0.3909	0.4224
56	Lillian	Lillian	2004	CWRS	3	3	0.3106	0.2822	0.3064	0.3784	0.3385	0.3513	0.4257
57	Lovitt	Lovitt	2002	CWRS	3	3	0.1857	0.1503	0.2369	0.3217	0.3331	0.2806	0.4281
58	McKenzie	McKenzie	1997	CWRS	1	1	0.3087	0.2649	0.2934	0.3679	0.1997	0.2615	0.4157
59	Minnedosa	Minnedosa	2008	CWGP	2	2	0.3501	0.3859	0.3631	0.3590	0.3937	0.3930	0.3366
60	Muchmore	Muchmore	2009	CWRS	1	1	0.3738	0.3621	0.3983	0.3942	0.3901	0.2981	0.4497
61	NRG010	NRG010=GP_010	2009	CWGP	2	2	0.3796	0.3914	0.3715	0.3710	0.3937	0.3846	0.3313
62	Neepawa	Neepawa	1969	CWRS	3	3	0.1513	0.1033	0.1897	0.3354	0.3345	0.2851	0.4531
63	PT559	PT_559	2003	CWRS	1	1	0.3762	0.3400	0.3821	0.3804	0.3939	0.2495	0.4411
64	Park	Park	1963	CWRS	3	3	0.2709	0.2474	0.2998	0.3503	0.3393	0.3086	0.4520
65	Prodigy	Prodigy	1998	CWRS	1	1	0.3085	0.2515	0.3204	0.3412	0.3351	0.2768	0.4212
66	RedFife	Red_Fife	1885	CWRS	2	2	0.3473	0.3837	0.3790	0.4032	0.3721	0.3885	0.4213
67	Roblin	Roblin	1986	CWRS	3	3	0.2998	0.2117	0.2698	0.3169	0.3645	0.3369	0.3833
68	SWS285	SWS285=Bhishaj	2003	CWSWS	2	2	0.4357	0.4409	0.4602	0.4102	0.4393	0.4326	0.4042
69	SY985	SY985	2010	CPSR	2	2	0.3724	0.3977	0.3889	0.3579	0.3704	0.4088	0.3071
70	Sadash	Sadash	2007	CWSWS	2	2	0.4071	0.4142	0.4379	0.4244	0.4254	0.4437	0.4224
71	Snowbird	Snowbird	2000	CWHWS	1	1	0.3324	0.2626	0.2780	0.3459	0.3466	0.2253	0.4348
72	Snowstar	Snowstar	2006	CWHWS	1	1	0.3445	0.2908	0.2925	0.3647	0.2687	0.2941	0.4418
73	Somerset	Somerset	2005	CWRS	3	3	0.2152	0.1692	0.2486	0.3503	0.3572	0.2897	0.4286
74	Stanley	CDC_Stanley	2009	CWRS	3	3	0.2543	0.1672	0.2479	0.2891	0.3813	0.2925	0.4508
75	Stettler	Stettler	2008	CWRS	1	1	0.3434	0.2835	0.3549	0.3798	0.3910	0.2918	0.4443
76	Superb	Superb	2001	CWRS	1	1	0.3868	0.3380	0.4004	0.3944	0.3991	0.2653	0.4744
77	Thatcher	Thatcher	1935	CWRS	3	3	0.2351	0.1802	0.2603	0.3070	0.3166	0.2795	0.4554
78	Unity	Unity	2007	CWRS	1	1	0.2922	0.2656	0.2884	0.3656	0.2270	0.2929	0.4030
79	Vesper	Vesper	2010	CWRS	1	1	0.2727	0.2473	0.3006	0.3348	0.3702	0.2655	0.4349
80	WR859CL	WR859CL	2008	CWRS	3	3	0.2314	0.2247	0.2508	0.3350	0.3557	0.2763	0.4019
81	Waskada	Waskada	2007	CWRS	1	1	0.3734	0.3433	0.3936	0.3921	0.3901	0.2637	0.4552
Minimum							0.1513	0.0958	0.1215	0.2448	0.1997	0.1913	0.1746
Maximum							0.4497	0.4710	0.4660	0.4321	0.4658	0.4648	0.4791
Overall average							0.3539						

Appendix 5 (cont.) Pairwise genetic distance matrix and other categorical variables for 81 spring wheat cultivars based on 19,930 markers

5701PR	5702PR	ACAbbey	ACAndrew	ACBarrie	ACCadillac	ACCrystal	ACDomain	ACEatonia	ACElsa	ACForemost	ACIntrepid	ACReed	ACSplendor	ACTaber	ACVista
0.3413															
0.4089	0.4224														
0.4482	0.4044	0.4120													
0.3696	0.3996	0.3543	0.4636												
0.3597	0.3989	0.3443	0.4385	0.1510											
0.4005	0.3905	0.3860	0.4044	0.3860	0.4012										
0.3871	0.4229	0.4327	0.4427	0.3457	0.3874	0.4454									
0.3868	0.4431	0.2874	0.4331	0.3528	0.3410	0.4064	0.3752								
0.3835	0.3840	0.3793	0.4729	0.2876	0.2296	0.3998	0.4312	0.3604							
0.4096	0.4088	0.3822	0.4083	0.4055	0.4153	0.0745	0.4504	0.4069	0.4202						
0.4056	0.3942	0.3072	0.4327	0.2820	0.2708	0.3711	0.4037	0.3454	0.2748	0.4049					
0.4468	0.3904	0.4155	0.1110	0.4692	0.4351	0.3935	0.4495	0.4184	0.4797	0.4023	0.4432				
0.3510	0.3698	0.3683	0.4779	0.2227	0.2208	0.4290	0.3677	0.3287	0.2597	0.4568	0.2282	0.4865			
0.4082	0.3839	0.3659	0.3924	0.3669	0.3893	0.0290	0.4340	0.4051	0.3886	0.0910	0.3596	0.3840	0.4177		
0.3994	0.3629	0.3861	0.3733	0.3968	0.3919	0.2662	0.4042	0.3986	0.4224	0.2586	0.4155	0.3735	0.4071	0.2668	
0.3678	0.3710	0.3420	0.4349	0.1998	0.1910	0.3861	0.3962	0.3603	0.2965	0.4193	0.1575	0.4345	0.2184	0.3722	0.4040
0.4076	0.2912	0.4380	0.4280	0.3748	0.3847	0.4070	0.4432	0.4421	0.3889	0.4252	0.4287	0.4085	0.3942	0.4031	0.4009
0.4020	0.4454	0.4220	0.4447	0.3617	0.3865	0.4801	0.1860	0.4047	0.4533	0.4976	0.4062	0.4464	0.3954	0.4692	0.4418
0.3753	0.3974	0.3257	0.4461	0.2393	0.2022	0.4358	0.3804	0.3152	0.1628	0.4674	0.2111	0.4473	0.2099	0.4203	0.4009
0.3775	0.2615	0.4311	0.4427	0.4055	0.3914	0.3644	0.4250	0.4368	0.3707	0.3827	0.4122	0.4281	0.4048	0.3612	0.3822
0.3570	0.3829	0.3256	0.4328	0.1810	0.0930	0.4278	0.3698	0.3110	0.2442	0.4400	0.2451	0.4278	0.2060	0.4090	0.4014
0.3851	0.4459	0.4140	0.4596	0.4304	0.4145	0.4626	0.2760	0.4341	0.4351	0.4809	0.4036	0.4635	0.4089	0.4507	0.4627
0.3682	0.3865	0.3150	0.4059	0.2582	0.2562	0.3706	0.3781	0.3342	0.3619	0.3946	0.1584	0.4174	0.2606	0.3615	0.3709
0.3766	0.3974	0.3182	0.4285	0.2365	0.1807	0.4200	0.3838	0.3199	0.3044	0.4403	0.2086	0.4363	0.2255	0.4127	0.4125
0.3972	0.4137	0.3157	0.4685	0.3352	0.3186	0.4531	0.3549	0.3264	0.3774	0.4921	0.2916	0.4583	0.3148	0.4394	0.4228
0.3633	0.3686	0.3333	0.4523	0.2482	0.2166	0.4422	0.3545	0.3103	0.3060	0.4740	0.2270	0.4421	0.1746	0.4261	0.4020
0.3739	0.3720	0.3900	0.4743	0.1568	0.1751	0.4101	0.4037	0.3834	0.1337	0.4270	0.2986	0.4795	0.2383	0.3976	0.4099
0.4093	0.3674	0.4360	0.4285	0.4078	0.3826	0.4247	0.4261	0.4365	0.3913	0.4316	0.4097	0.4126	0.4012	0.4185	0.4102
0.3847	0.3956	0.3146	0.4171	0.2537	0.2356	0.3907	0.3911	0.3330	0.3402	0.4265	0.1322	0.4331	0.2477	0.3830	0.3925
0.3582	0.3784	0.3325	0.4070	0.2379	0.2056	0.4074	0.3471	0.3250	0.2997	0.4241	0.2117	0.4180	0.2269	0.3908	0.3807
0.4086	0.4106	0.3513	0.4590	0.3159	0.2556	0.4278	0.4038	0.3593	0.1441	0.4635	0.2382	0.4563	0.2613	0.4140	0.4196
0.3416	0.3889	0.3514	0.4383	0.2515	0.1705	0.4457	0.3996	0.3494	0.2958	0.4415	0.2828	0.4345	0.2603	0.4409	0.4196
0.4384	0.3159	0.4286	0.3905	0.3903	0.4019	0.3982	0.4320	0.3991	0.4209	0.4298	0.4027	0.3703	0.4145	0.3961	0.3927
0.4098	0.3750	0.3525	0.4355	0.3928	0.4071	0.2888	0.4345	0.3956	0.3987	0.2825	0.3578	0.4427	0.4094	0.2896	0.3534
0.3621	0.4129	0.4140	0.4543	0.3739	0.3751	0.4842	0.2586	0.4116	0.4375	0.4932	0.4035	0.4555	0.3774	0.4710	0.4448
0.4052	0.4264	0.3321	0.4711	0.1902	0.2741	0.4476	0.3002	0.3050	0.3586	0.4720	0.2812	0.4584	0.2109	0.4324	0.3906
0.4112	0.3084	0.4201	0.4337	0.3862	0.3871	0.3994	0.4288	0.4159	0.3914	0.4135	0.4117	0.4320	0.3849	0.3960	0.3851
0.4036	0.2806	0.4452	0.4236	0.3881	0.3870	0.4035	0.4421	0.4451	0.3853	0.4191	0.4308	0.4059	0.4042	0.3996	0.3939
0.3873	0.4004	0.4275	0.4642	0.3963	0.4008	0.4654	0.3107	0.4156	0.4268	0.4637	0.4267	0.4514	0.3963	0.4615	0.4397
0.3904	0.3694	0.3647	0.4369	0.2950	0.2635	0.4313	0.3317	0.3563	0.1645	0.4439	0.2731	0.4536	0.2659	0.4229	0.3999
0.4036	0.4111	0.3764	0.4457	0.3200	0.3381	0.4330	0.1093	0.3347	0.4110	0.4354	0.3544	0.4415	0.3471	0.4193	0.3933
0.3705	0.4091	0.3339	0.4495	0.0713	0.1204	0.4061	0.3326	0.3433	0.2799	0.4147	0.2860	0.4541	0.2304	0.3919	0.3996
0.3867	0.4121	0.3607	0.4748	0.2592	0.2518	0.3787	0.3868	0.3644	0.1801	0.4004	0.2816	0.4631	0.2944	0.3707	0.3894
0.3668	0.4018	0.3707	0.4447	0.3221	0.3105	0.4470	0.3314	0.3897	0.3867	0.4630	0.2879	0.4633	0.2910	0.4389	0.4235
0.3745	0.3962	0.3725	0.4333	0.3017	0.3543	0.4255	0.1569	0.3727	0.4281	0.4337	0.3664	0.4275	0.3464	0.4118	0.3939
0.3809	0.3909	0.2954	0.4584	0.2066	0.2183	0.4335	0.3445	0.2612	0.3193	0.4648	0.1836	0.4470	0.1659	0.4149	0.4017
0.4080	0.3817	0.3958	0.4635	0.3657	0.3435	0.4002	0.4309	0.3893	0.1319	0.4172	0.2574	0.4741	0.2753	0.3952	0.4169
0.3972	0.4053	0.3190	0.4699	0.2980	0.3465	0.4411	0.3931	0.2636	0.3296	0.4570	0.3473	0.4674	0.2739	0.4386	0.4099
0.3788	0.4066	0.3303	0.4547	0.1660	0.1489	0.4161	0.3540	0.3014	0.2829	0.4443	0.2458	0.4422	0.2092	0.3986	0.3975
0.3778	0.3797	0.3488	0.4386	0.3468	0.3686	0.4515	0.3529	0.3473	0.3968	0.4714	0.3363	0.4244	0.3052	0.4333	0.4073
0.3894	0.3672	0.3546	0.3833	0.3731	0.3688	0.2884	0.3939	0.3792	0.4102	0.2740	0.3891	0.3854	0.3814	0.2862	0.0479
0.3752	0.4276	0.4053	0.4642	0.3916	0.3995	0.4852	0.2719	0.4111	0.4557	0.4916	0.4225	0.4654	0.3974	0.4727	0.4501
0.3487	0.3701	0.3734	0.3954	0.3921	0.3957	0.3105	0.3747	0.3773	0.4345	0.3023	0.4010	0.3946	0.3890	0.3119	0.1335
0.3858	0.3953	0.2921	0.4608	0.2088	0.2192	0.4332	0.3433	0.2595	0.3171	0.4662	0.1802	0.4455	0.1691	0.4145	0.4009
0.3800	0.4238	0.4095	0.4406	0.3760	0.4072	0.4617	0.1379	0.3902	0.4551	0.4665	0.3810	0.4485	0.3844	0.4519	0.4200
0.3987	0.3909	0.3046	0.4481	0.3232	0.2911	0.4708	0.3887	0.3298	0.3208	0.4960	0.3076	0.4347	0.2574	0.4530	0.4079
0.3937	0.3877	0.3983	0.4640	0.3108	0.3160	0.4578	0.2922	0.3572	0.3705	0.4726	0.3144	0.4538	0.2921	0.4426	0.4187
0.3926	0.4025	0.3502	0.3530	0.3957	0.3714	0.3963	0.3921	0.3798	0.4325	0.3976	0.3795	0.3758	0.4102	0.3885	0.3519
0.3429	0.3755	0.3510	0.4644	0.2364	0.2241	0.3987	0.4110	0.3831	0.2747	0.4161	0.2542	0.4697	0.1630	0.3813	0.4127
0.4461	0.3956	0.4085	0.0957	0.4612	0.4348	0.3918	0.4446	0.4274	0.4691	0.3997	0.4299	0.1497	0.4736	0.3835	0.3549
0.2108	0.2617	0.4365	0.4327	0.3870	0.3894	0.3573	0.4100	0.4143	0.3819	0.3856	0.4108	0.4230	0.3586	0.3604	0.3685
0.4683	0.4200	0.4006	0.1805	0.4454	0.4207	0.3973	0.4427	0.4027	0.4683	0.4061	0.4259	0.1772	0.4506	0.3905	0.3639
0.3918	0.4072	0.4016	0.4411	0.3067	0.3509	0.4387	0.1323	0.3452	0.3875	0.4481	0.3649	0.4464	0.3094	0.4244	0.3575
0.4403	0.4426	0.3896	0.4406	0.3488	0.4024	0.4313	0.2739	0.3607	0.4095	0.4634	0.3659	0.4396	0.3528	0.4165	0.4053
0.3906	0.4054	0.3022	0.4497	0.2529	0.2498	0.4400	0.3472	0.2821	0.3224	0.4565	0.2223	0.4399	0.2179	0.4231	0.4115
0.4162	0.4066	0.3456	0.4233	0.1496	0.2059	0.4342	0.3538	0.3494	0.3432	0.4463	0.2740	0.4309	0.2629	0.4205	0.4186
0.4188	0.4132	0.4067	0.4434	0.3242	0.3516	0.4574	0.2196	0.3890	0.4283	0.4650	0.3707	0.4325	0.3662	0.4467	0.4121
0.4068	0.4470	0.4364	0.4484	0.3744	0.3971	0.4834	0.1644	0.4097	0.4585	0.4917	0.4074	0.4535	0.4027	0.4725	0.4394
0.3646	0.3976	0.3006	0.4631	0.2592	0.2589	0.4726	0.3505	0.2706	0.2598	0.5037	0.2514	0.4541	0.1860	0.4547	0.4181
0.3781	0.3805	0.3391	0.4317	0.3355	0.3747	0.4348	0.3358	0.3557	0.3968	0.4557	0.3395	0.4174	0.3131	0.4165	0.3938
0.3918	0.4161	0.3927	0.4465	0.2055	0.2577	0.4422	0.2874	0.3887	0.3764	0.4642	0.3219	0.4466	0.3037	0.4280	0.4050
0.3697	0.4106	0.3577	0.4521	0.2134	0.2125	0.4106	0.3320	0.3381	0.3133	0.4329	0.2864	0.4446	0.2386	0.4014	0.3876
0.3935	0.4382	0.4342	0.4533	0.3760	0.4027	0.4700	0.1244	0.3941	0.4597	0.4762	0.4140	0.4492	0.4044	0.4597	0.4289
0.2108	0.2615	0.2874	0.0957	0.0713	0.0930	0.0290	0.1093	0.2595	0.1319	0.0910	0.1322	0.1497	0.1630	0.2668	0.0479
0.4683	0.4470	0.4452	0.4779	0.4692	0.4351	0.4852	0.4504	0.4451	0.4797	0.5037	0.4432	0.4865	0.4736	0.4727	0.4627

Appendix 5 (cont.) Pairwise genetic distance matrix and other categorical variables for 81 spring wheat cultivars based on 19,930 markers

Alvena	Burnside	CDCAbound	CDCAlsask	CDCBison	CDCBounty	CDCGo	CDCImagine	CDCKernen	CDCMerlin	CDCOsler	CDCPlentiful	CDCRama	CDCTeal	CDCThrive	CDCUtmost
0.3773															
0.3970	0.4810														
0.2289	0.4023	0.3752													
0.3863	0.1549	0.4582	0.4143												
0.1469	0.3974	0.3731	0.1910	0.4003											
0.4129	0.4538	0.1728	0.4012	0.4153	0.4013										
0.1812	0.3943	0.3996	0.2408	0.3978	0.2125	0.4014									
0.1782	0.3920	0.3632	0.2036	0.4056	0.1284	0.3739	0.1500								
0.3029	0.4453	0.3165	0.2982	0.4529	0.2975	0.3230	0.2964	0.2928							
0.2262	0.4143	0.3750	0.1950	0.4231	0.1651	0.3786	0.2243	0.2043	0.2792						
0.2540	0.3640	0.4365	0.2081	0.3758	0.2027	0.4495	0.3105	0.2751	0.3650	0.2679					
0.4041	0.1999	0.4704	0.3892	0.2658	0.3873	0.4396	0.3685	0.3818	0.4013	0.4028	0.3669				
0.1429	0.4187	0.3804	0.2183	0.4259	0.1931	0.4059	0.0515	0.1328	0.2767	0.2039	0.3058	0.3990			
0.1882	0.3925	0.3889	0.2187	0.3878	0.1161	0.3937	0.1011	0.1341	0.2963	0.1861	0.2579	0.3565	0.1384		
0.2852	0.4118	0.4048	0.1801	0.3888	0.2669	0.3872	0.2936	0.2694	0.3356	0.2599	0.2257	0.3912	0.2741	0.2770	
0.2143	0.4296	0.3886	0.2519	0.4274	0.1288	0.4271	0.2345	0.1690	0.3347	0.2313	0.2631	0.4239	0.2236	0.1823	0.3205
0.3743	0.1520	0.4816	0.4078	0.2219	0.4017	0.4734	0.3717	0.3945	0.4178	0.4079	0.3944	0.2609	0.3926	0.3688	0.4441
0.3791	0.4134	0.4454	0.4221	0.3801	0.3967	0.4521	0.3692	0.3881	0.4159	0.4014	0.4088	0.4472	0.3714	0.3788	0.4169
0.3784	0.4516	0.1466	0.3658	0.4166	0.3689	0.2076	0.3891	0.3610	0.3218	0.3412	0.4025	0.4365	0.3817	0.3943	0.3859
0.2664	0.4290	0.3434	0.2476	0.4553	0.2470	0.4180	0.2617	0.2600	0.2727	0.2070	0.2872	0.4247	0.2515	0.2486	0.3219
0.3797	0.0986	0.4693	0.3877	0.1982	0.3911	0.4610	0.3911	0.3803	0.4453	0.3891	0.3780	0.2483	0.3964	0.3922	0.4070
0.3877	0.0318	0.4866	0.4079	0.1290	0.4060	0.4613	0.3970	0.4030	0.4511	0.4202	0.3680	0.1800	0.4250	0.3912	0.4062
0.4065	0.4304	0.2641	0.3997	0.4198	0.3844	0.2652	0.4189	0.3906	0.3533	0.3692	0.3844	0.3958	0.4231	0.4059	0.4018
0.2679	0.3829	0.3325	0.2185	0.3880	0.2439	0.3755	0.3086	0.2739	0.3052	0.2835	0.2103	0.3866	0.2895	0.2683	0.2333
0.3373	0.4359	0.2495	0.3484	0.4326	0.3193	0.3221	0.3375	0.3421	0.2964	0.3252	0.3731	0.4265	0.3292	0.3201	0.3912
0.2138	0.3783	0.3523	0.2263	0.4030	0.1522	0.4152	0.2536	0.2172	0.3334	0.2412	0.1762	0.3926	0.2541	0.2214	0.3086
0.2851	0.3977	0.4239	0.2408	0.3942	0.2619	0.4408	0.3337	0.3132	0.3519	0.3096	0.2136	0.4421	0.3263	0.3112	0.2421
0.2819	0.4406	0.3009	0.3006	0.4143	0.2739	0.3191	0.2097	0.2347	0.3045	0.2578	0.3440	0.4155	0.1865	0.2472	0.3298
0.3434	0.4018	0.2447	0.3562	0.3897	0.3254	0.3349	0.3237	0.3457	0.3532	0.3102	0.3705	0.4174	0.3388	0.3174	0.3912
0.1960	0.4366	0.3602	0.1636	0.4537	0.1524	0.3937	0.1877	0.1754	0.2365	0.1013	0.2808	0.4205	0.1694	0.1677	0.2645
0.3265	0.4240	0.4508	0.2496	0.3828	0.3296	0.4256	0.3885	0.3512	0.4021	0.3294	0.2274	0.3977	0.3673	0.3511	0.2042
0.3051	0.3901	0.4373	0.2934	0.4128	0.3190	0.4337	0.3417	0.3269	0.3387	0.2931	0.3159	0.3935	0.3306	0.3241	0.3448
0.1774	0.4104	0.3651	0.1827	0.4402	0.1397	0.4152	0.2437	0.2055	0.2527	0.1789	0.2399	0.4086	0.2173	0.2033	0.2697
0.3457	0.4137	0.3654	0.3290	0.4055	0.3382	0.3898	0.3486	0.3531	0.3334	0.2778	0.3530	0.4209	0.3245	0.3534	0.3633
0.3906	0.4194	0.4270	0.3733	0.3923	0.3813	0.4480	0.3502	0.3911	0.3927	0.3713	0.3929	0.4393	0.3717	0.3618	0.4035
0.3937	0.4573	0.1632	0.3853	0.4336	0.3883	0.2036	0.4119	0.3795	0.2980	0.3561	0.4193	0.4312	0.4005	0.4124	0.4046
0.3963	0.3929	0.3973	0.4008	0.3800	0.4008	0.4194	0.3528	0.3860	0.3879	0.3852	0.4185	0.4066	0.3750	0.3707	0.4278
0.1894	0.4339	0.3634	0.1663	0.4560	0.1550	0.3928	0.1880	0.1777	0.2358	0.1092	0.2812	0.4148	0.1667	0.1690	0.2619
0.3898	0.4597	0.2115	0.3930	0.4355	0.3898	0.1877	0.3667	0.3694	0.3291	0.3599	0.4330	0.4390	0.3648	0.3640	0.4015
0.3117	0.4384	0.3969	0.2636	0.4443	0.2723	0.3835	0.2980	0.2818	0.2935	0.1940	0.3117	0.3959	0.2782	0.2836	0.2767
0.3063	0.4242	0.3179	0.3194	0.4242	0.2887	0.3091	0.3237	0.3116	0.3114	0.2509	0.3209	0.4102	0.3040	0.3136	0.3394
0.3655	0.3997	0.3961	0.3868	0.4029	0.3592	0.4260	0.3331	0.3554	0.3785	0.3781	0.4250	0.4167	0.3333	0.3470	0.4058
0.2444	0.3989	0.4206	0.2645	0.3641	0.2255	0.3970	0.2625	0.2441	0.3352	0.2244	0.2577	0.3960	0.2504	0.2441	0.2732
0.4332	0.4222	0.4454	0.4386	0.4277	0.4289	0.4631	0.3989	0.4179	0.4637	0.4562	0.4732	0.4265	0.4140	0.4013	0.4459
0.3646	0.3111	0.4468	0.3976	0.2329	0.3863	0.4511	0.3829	0.4030	0.4403	0.3864	0.3651	0.3516	0.3889	0.3801	0.3941
0.4247	0.4287	0.4372	0.4409	0.4528	0.4200	0.4738	0.4149	0.4070	0.4612	0.4358	0.4651	0.4376	0.4088	0.4244	0.4434
0.3654	0.4464	0.2602	0.3334	0.4360	0.3256	0.3391	0.3380	0.3528	0.3235	0.3131	0.3540	0.4164	0.3419	0.3033	0.3781
0.3978	0.4585	0.3261	0.3543	0.4413	0.3899	0.3914	0.3712	0.3959	0.3543	0.3379	0.3826	0.4599	0.3586	0.3676	0.3664
0.2492	0.4171	0.3548	0.1983	0.4403	0.2044	0.3651	0.2202	0.2052	0.2495	0.1984	0.3219	0.4151	0.2118	0.2118	0.2731
0.2340	0.3994	0.3586	0.2582	0.4427	0.1964	0.4102	0.2178	0.2157	0.3236	0.2273	0.2547	0.3951	0.2241	0.2221	0.3162
0.3467	0.4509	0.1658	0.3644	0.4359	0.3331	0.2699	0.3685	0.3515	0.3507	0.3327	0.3879	0.4448	0.3492	0.3669	0.3966
0.3994	0.4849	0.0233	0.3875	0.4562	0.3857	0.1878	0.4055	0.3752	0.3241	0.3833	0.4430	0.4676	0.3860	0.4026	0.4166
0.2579	0.4275	0.3676	0.1823	0.4403	0.2116	0.3612	0.2649	0.2268	0.2485	0.1571	0.2660	0.3797	0.2392	0.2295	0.2328
0.3304	0.4009	0.3687	0.3333	0.3989	0.3385	0.3967	0.3496	0.3525	0.3476	0.2826	0.3560	0.4182	0.3294	0.3511	0.3612
0.2717	0.4181	0.2376	0.2842	0.4403	0.2640	0.3274	0.3190	0.2937	0.3299	0.2787	0.3131	0.4432	0.2896	0.3217	0.3536
0.2539	0.4080	0.3492	0.2243	0.4350	0.2299	0.3778	0.2833	0.2513	0.2884	0.2255	0.2559	0.4047	0.2641	0.2717	0.2975
0.4044	0.4709	0.1168	0.4046	0.4531	0.3826	0.2370	0.3956	0.3771	0.3335	0.3702	0.4386	0.4425	0.3862	0.3801	0.4258
0.1429	0.0318	0.0233	0.1636	0.1290	0.1161	0.1877	0.0515	0.1328	0.2358	0.1013	0.1762	0.1800	0.1384	0.1677	0.2042
0.4332	0.4849	0.4866	0.4409	0.4562	0.4289	0.4738	0.4189	0.4179	0.4637	0.4562	0.4732	0.4676	0.4250	0.4244	0.4459

Appendix 5 (cont.) Pairwise genetic distance matrix and other categorical variables for 81 spring wheat cultivars based on 19,930 markers

CDCVRMoris	CDCWalrus	CONQUER	Carberry	Columbus	Glencross	Glenlea	Glenn	GoodeveVB	Harvest	Helios	Infinity	Journey	Kane	Katepwa	Laura
0.4362															
0.3979	0.4271														
0.3798	0.4711	0.4476													
0.2766	0.4164	0.4139	0.3661												
0.4184	0.1970	0.3650	0.4448	0.4269											
0.4320	0.1285	0.4195	0.4529	0.4429	0.0908										
0.3986	0.4532	0.4314	0.2147	0.3827	0.4365	0.4344									
0.2822	0.3956	0.4017	0.3339	0.2981	0.3775	0.3881	0.3823								
0.3592	0.4089	0.4214	0.2740	0.2703	0.4192	0.4395	0.3120	0.3182							
0.2318	0.3907	0.4055	0.3620	0.1926	0.3905	0.3896	0.3895	0.2699	0.2966						
0.3137	0.4087	0.3782	0.4086	0.3061	0.3973	0.4075	0.4180	0.2508	0.3362	0.2423					
0.2896	0.4414	0.4231	0.2961	0.2888	0.4273	0.4429	0.3298	0.3145	0.3133	0.3044	0.3952				
0.3711	0.3963	0.4079	0.2873	0.2445	0.4040	0.4047	0.3179	0.3377	0.2185	0.2947	0.3636	0.3176			
0.2203	0.4132	0.3972	0.3485	0.1554	0.4123	0.4499	0.3822	0.2975	0.2910	0.2274	0.2647	0.2842	0.3015		
0.3640	0.4333	0.4145	0.4404	0.3872	0.4231	0.4184	0.4281	0.2470	0.4245	0.3695	0.2532	0.4092	0.4463	0.3311	
0.3750	0.4013	0.4017	0.3974	0.2622	0.4037	0.4116	0.3834	0.3126	0.3461	0.3060	0.3569	0.3660	0.3635	0.2733	0.3394
0.2201	0.4037	0.3997	0.3666	0.1730	0.4021	0.4211	0.3945	0.2979	0.2957	0.1664	0.2125	0.3155	0.3071	0.1141	0.3509
0.3762	0.4204	0.4078	0.3287	0.2501	0.4041	0.4254	0.3217	0.3584	0.3270	0.3494	0.3616	0.3165	0.1994	0.2656	0.4103
0.4015	0.4063	0.3557	0.4312	0.3579	0.4045	0.4118	0.4421	0.3794	0.3802	0.3730	0.3720	0.4045	0.3699	0.3717	0.4134
0.3969	0.4734	0.4519	0.0630	0.3624	0.4481	0.4572	0.1989	0.3466	0.2782	0.3857	0.4364	0.2943	0.3011	0.3648	0.4463
0.3933	0.3888	0.3528	0.3748	0.3766	0.3916	0.3938	0.4010	0.3915	0.3629	0.3889	0.3944	0.4056	0.3589	0.3890	0.4285
0.2221	0.4114	0.4010	0.3561	0.1526	0.4098	0.4476	0.3846	0.2933	0.2830	0.2296	0.2684	0.2856	0.3003	0.0124	0.3286
0.4084	0.4511	0.4249	0.2667	0.3415	0.4464	0.4581	0.3088	0.3562	0.2078	0.3704	0.4330	0.3065	0.2359	0.3615	0.4466
0.3359	0.4383	0.4336	0.3869	0.2669	0.4323	0.4433	0.3913	0.3212	0.3656	0.3139	0.3604	0.3365	0.3520	0.2189	0.3314
0.3442	0.4283	0.4430	0.2966	0.2617	0.4249	0.4269	0.3081	0.3306	0.2538	0.3080	0.3617	0.3088	0.2935	0.2533	0.3746
0.3790	0.3955	0.3837	0.4022	0.3859	0.3805	0.4005	0.4244	0.3879	0.3802	0.3797	0.4106	0.3874	0.3611	0.3705	0.4553
0.2580	0.4166	0.3817	0.3845	0.2876	0.4037	0.4061	0.3997	0.3376	0.3714	0.2406	0.2859	0.2739	0.3699	0.2430	0.3083
0.4314	0.3879	0.4272	0.4582	0.4671	0.4287	0.4182	0.4558	0.4447	0.4462	0.4457	0.4704	0.4351	0.4296	0.4503	0.4582
0.3987	0.3521	0.3709	0.4242	0.4277	0.3198	0.2954	0.4246	0.3889	0.4291	0.4035	0.4052	0.4006	0.3951	0.4086	0.3953
0.4169	0.4055	0.4270	0.4548	0.4429	0.4142	0.4334	0.4360	0.4400	0.4284	0.4360	0.4690	0.4313	0.4328	0.4265	0.4502
0.3665	0.4253	0.4228	0.3336	0.2135	0.4425	0.4496	0.3416	0.3259	0.1871	0.2959	0.3718	0.3354	0.2193	0.2951	0.3925
0.3981	0.4396	0.4052	0.3662	0.2363	0.4376	0.4639	0.3643	0.3714	0.3109	0.3548	0.3853	0.3489	0.2396	0.3221	0.4148
0.2671	0.4142	0.3864	0.3524	0.2068	0.3862	0.4426	0.3891	0.3076	0.2886	0.2520	0.2839	0.3113	0.3320	0.1064	0.3425
0.2635	0.4146	0.4232	0.3755	0.2116	0.4062	0.4148	0.3874	0.3262	0.3347	0.1653	0.3289	0.2977	0.3140	0.1928	0.3990
0.3671	0.4356	0.4390	0.2218	0.3013	0.4383	0.4521	0.3150	0.3430	0.2135	0.3065	0.3895	0.3344	0.2416	0.3174	0.4166
0.3953	0.4827	0.4423	0.1380	0.3540	0.4633	0.4900	0.2711	0.3257	0.2280	0.3632	0.4233	0.3077	0.2457	0.3701	0.4486
0.2771	0.4340	0.4228	0.3551	0.2104	0.4191	0.4337	0.3812	0.2630	0.3335	0.2695	0.3109	0.3094	0.3356	0.1303	0.2686
0.3794	0.4091	0.3931	0.3393	0.2470	0.3878	0.4124	0.3365	0.3386	0.3200	0.3356	0.3484	0.3426	0.1971	0.2672	0.4113
0.3178	0.4148	0.4217	0.2824	0.2563	0.4137	0.4326	0.3481	0.3148	0.2832	0.2161	0.3280	0.3403	0.2610	0.2543	0.4110
0.2831	0.4184	0.4208	0.3434	0.2301	0.3864	0.4231	0.3788	0.3098	0.2765	0.2187	0.2688	0.3197	0.3232	0.2047	0.3488
0.4014	0.4704	0.4391	0.1846	0.3450	0.4513	0.4738	0.2774	0.3485	0.1966	0.3625	0.4227	0.3321	0.2240	0.3628	0.4554
0.2201	0.1285	0.3528	0.0630	0.1526	0.0908	0.2954	0.1989	0.2470	0.1871	0.1653	0.2125	0.2739	0.1971	0.0124	0.2686
0.4362	0.4827	0.4519	0.4582	0.4671	0.4633	0.4900	0.4558	0.4447	0.4462	0.4457	0.4704	0.4351	0.4463	0.4503	0.4582

Appendix 5 (cont.) Pairwise genetic distance matrix and other categorical variables for 81 spring wheat cultivars based on 19,930 markers

Lillian	Lovitt	McKenzie	Minnedosa	Muchmore	NRG010	Neepawa	PT559	Park	Prodigy	RedFife	Roblin	SWS285	SY985	Sadash	Snowbird
0.3156															
0.3118	0.2910														
0.3867	0.3754	0.4001													
0.3951	0.3797	0.3330	0.4359												
0.4053	0.3908	0.3953	0.1434	0.3869											
0.2716	0.1143	0.2635	0.3713	0.3687	0.3904										
0.4116	0.3840	0.3536	0.4068	0.2600	0.3798	0.3564									
0.2928	0.2734	0.3301	0.3730	0.3829	0.4113	0.2087	0.3725								
0.3314	0.2789	0.2936	0.3978	0.3007	0.3815	0.2551	0.3027	0.2943							
0.3977	0.3735	0.3743	0.3438	0.4104	0.3644	0.3705	0.4031	0.3660	0.4169						
0.3148	0.2368	0.3380	0.3978	0.3997	0.4037	0.2488	0.4228	0.3119	0.3180	0.4218					
0.4731	0.4459	0.4320	0.3618	0.4684	0.3792	0.4527	0.4394	0.4470	0.4634	0.3486	0.4616				
0.3931	0.4196	0.3697	0.3892	0.4395	0.3761	0.4115	0.4122	0.3945	0.4186	0.3934	0.3542	0.4259			
0.4473	0.4318	0.4173	0.3744	0.4587	0.3900	0.4299	0.4381	0.4379	0.4486	0.3532	0.4532	0.1554	0.4451		
0.3257	0.3038	0.3224	0.3395	0.3436	0.3452	0.2942	0.2402	0.3369	0.2725	0.3811	0.3645	0.4356	0.4098	0.4300	
0.3675	0.3371	0.1919	0.3961	0.3776	0.4013	0.3227	0.3149	0.3639	0.3369	0.4048	0.3928	0.4403	0.4279	0.4234	0.2545
0.2988	0.1722	0.3116	0.3834	0.3690	0.3941	0.1022	0.3564	0.2685	0.2803	0.3724	0.2711	0.4451	0.4256	0.4368	0.2984
0.3513	0.2072	0.3458	0.4010	0.3891	0.4167	0.1948	0.3703	0.2940	0.3039	0.3818	0.2885	0.4175	0.4269	0.3973	0.3256
0.3934	0.3325	0.3591	0.3992	0.2473	0.3686	0.3209	0.2511	0.3642	0.1638	0.4081	0.3959	0.4463	0.4389	0.4250	0.2648
0.4360	0.3747	0.3702	0.4281	0.1551	0.3950	0.3732	0.1899	0.4102	0.3217	0.3994	0.4272	0.4523	0.4494	0.4382	0.2533
0.2506	0.1957	0.3006	0.3782	0.3526	0.3972	0.1205	0.3607	0.1384	0.2611	0.3943	0.2644	0.4575	0.4033	0.4486	0.2918
0.2937	0.2935	0.0486	0.3880	0.3403	0.3868	0.2655	0.3592	0.3241	0.3087	0.3653	0.3500	0.4242	0.3648	0.4099	0.3106
0.3597	0.2309	0.3184	0.3905	0.3076	0.3796	0.2528	0.3117	0.3342	0.2609	0.3901	0.3327	0.4531	0.4229	0.4336	0.2979
0.3185	0.1896	0.3112	0.3751	0.3588	0.3771	0.1987	0.3546	0.3122	0.2798	0.4043	0.2719	0.4450	0.4107	0.4332	0.2982
0.4029	0.3778	0.3641	0.4202	0.2007	0.3891	0.3646	0.1760	0.3994	0.2917	0.3887	0.4306	0.4545	0.4232	0.4441	0.2228
0.2506	0.1143	0.0486	0.1434	0.1551	0.3452	0.1022	0.1760	0.1384	0.1638	0.3486	0.2644	0.1554	0.3648	0.3973	0.2228
0.4731	0.4459	0.4320	0.4359	0.4684	0.4167	0.4527	0.4394	0.4470	0.4634	0.4218	0.4616	0.4575	0.4494	0.4486	0.3256

Appendix 5 (cont.) Pairwise genetic distance matrix and other categorical variables for 81 spring wheat cultivars based on 19,930 markers

Snowstar	Somerset	Stanley	Stettler	Superb	Thatcher	Unity	Vesper	WR859CL
0.3466								
0.3500	0.2456							
0.3389	0.3269	0.3325						
0.3296	0.3658	0.3740	0.1618					
0.3298	0.1876	0.2485	0.3518	0.3774				
0.2228	0.3117	0.3539	0.3559	0.3736	0.3021			
0.3430	0.2775	0.2521	0.2249	0.2475	0.2960	0.3093		
0.3635	0.2261	0.2626	0.3272	0.3555	0.2629	0.3171	0.2514	
0.3117	0.3608	0.3764	0.1906	0.0938	0.3810	0.3682	0.2542	0.3553
0.2228	0.1876	0.2485	0.1618	0.0938	0.2629	0.3093	0.2514	0.3553
0.3635	0.3658	0.3764	0.3559	0.3774	0.3810	0.3682	0.2542	0.3553

Appendix 6. Summary of the marker and phenotype data

Gene				Vrn-A1	Vrn-B1	Ppd-D1	Rht-D1	Rht-D1	Rht-B1	Rht-B1	Rht8	Rht8	Rht8	Rht8	Rht8	Rht8	Rht8
Chrom												2D	2D	2D	2D	2D	2D
Position												12	21	22	22	22	22
number	Name	Year of Release	Wheat class	Vrn-A1	Vrn-B1	Ppd-D1	Rht-D1	wMAS000002-RhtD1	Rht-B1	wMAS000001-RhtB1	Rht8	wsnp_CAP12_c455_248396	BS00090678_51	JD_c63957_1176	Kukri_c51992_290	Kukri_rep_c106786_230	Kukri_rep_c113120_104
64	Park	1963	CNHR	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
62	Neepawa	1969	CNHR	CC	AA	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
46	Glenlea	1972	CWES	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
44	Columbus	1980	CNHR	CC	AA	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
54	Katepwa	1981	CNHR	CC	AA	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
55	Laura	1986	CWRS	CC	AA	AA	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
67	Roblin	1986	CWRS	CC	AA	AA	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
20	AC_Reed	1991	SWSW	NN	NN	NN	NN	CC	NN	AA	AA	CC	AA	AA	CC	CC	CC
22	AC_Taber	1991	CNHR	AA	CC	AA	CC	AA	AA	GG	AA	CC	AA	AA	CC	CC	CC
37	CDC_Teal	1991	CWRS	CC	AA	AA	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
33	CDC_Merlin	1992	CWRS	CC	AA	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
15	AC_Domain	1993	CWRS	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
16	AC_Eatonia	1993	CNHR	CC	AA	AA	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
12	AC_Barrie	1994	CWRS	CC	AA	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
18	AC_Foremost	1995	CNHR	AA	CC	AA	CC	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
13	AC_Cadillac	1996	CWRS	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
14	AC_Crystal	1996	CNHR	AA	CC	AA	CC	AA	AA	GG	AA	CC	AA	AA	CC	CC	CC
17	AC_Elsa	1996	CWRS	CC	AA	AA	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
23	AC_Vista	1996	CPSR	CC	AA	AA	CC	AA	AA	GG	CC	NN	CC	CC	AA	AA	AA
19	AC_Intrepid	1997	CWRS	CC	AA	AA	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
21	AC_Splendor	1997	CWRS	CC	AA	AA	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
58	McKenzie	1997	CNHR	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
10	AC_Abbey	1998	CNHR	CC	CC	AA	AA	NN	AA	NN	AA	CC	AA	AA	CC	CC	CC
65	Prodigy	1998	CWRS	CC	AA	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
29	CDC_Bounty	1999	CWRS	CC	AA	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
7	5700_PR	2000	CPSR	CC	AA	AA	CC	AA	AA	GG	AA	CC	AA	AA	CC	CC	CC
11	AC_Andrew	2000	CWSWS	AA	CC	AA	AA	CC	CC	AA	CC	AA	CC	CC	AA	AA	AA
71	Snowbird	2000	CWHWS	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
8	5701PR	2001	CPSR	CC	AA	AA	CC	AA	AA	GG	AA	CC	AA	AA	CC	CC	CC
36	CDC_Rama	2001	CWES	CC	AA	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
76	Superb	2001	CWRS	CC	CC	CC	AA	CC	CC	AA	AA	CC	AA	AA	CC	CC	CC
52	Journey	2002	CWRS	CC	AA	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
57	Lovitt	2002	CWRS	CC	CC	CC	AA	CC	AA	GG	NN	CC	AA	AA	CC	CC	CC
30	CDC_Go	2003	CWRS	CC	CC	CC	AA	CC	CC	AA	NN	CC	AA	AA	CC	CC	CC
34	CDC_Osler	2003	CNHR	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
41	CDC_Walrus	2003	CWES	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
68	Bhishaj	2003	CWSWS	CC	CC	AA	AA	CC	CC	AA	CC	AA	CC	CC	AA	AA	AA
4	5602_HR	2004	CWRS	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
25	Burnside	2004	CWES	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
27	CDC_Alsask	2004	CWRS	CC	AA	AA	AA	??	AA	??	AA	CC	AA	AA	CC	CC	CC
49	Harvest	2004	CNHR	CC	AA	AA	CC	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
51	Infinity	2004	CWRS	CC	AA	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
56	Lillian	2004	CNHR	CC	CC	CC	AA	CC	AA	GG	NN	CC	AA	AA	CC	CC	CC
73	Somerset	2005	CWRS	CC	AA	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
24	Alvena	2006	CNHR	CC	AA	AA	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
26	CDC_Abound	2006	CWRS	CC	CC	CC	AA	CC	CC	GG	AA	CC	AA	AA	CC	CC	CC
50	Helios	2006	CWRS	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
53	Kane	2006	CNHR	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
72	Snowstar	2006	CWHWS	CC	CC	CC	AA	CC	AA	GG	NN	CC	AA	AA	CC	CC	CC
9	5702PR	2007	CPSR	CC	CC	CC	CC	AA	AA	GG	AA	CC	AA	AA	CC	CC	CC
45	Glencross	2007	CWES	CC	CC	CC	AA	CC	AA	GG	NN	CC	AA	AA	CC	CC	CC
48	Goodeve_VB	2007	CWRS	CC	AA	AA	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
70	Sadash	2007	CWSWS	CC	CC	AA	AA	??	CC	AA	CC	AA	CC	CC	AA	AA	AA
81	Waskada	2007	CWRS	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
5	5603_HR	2008	CNHR	CC	AA	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
59	Minnedosa	2008	CWSP	CC	AA	AA	CC	AA	AA	GG	CC	NN	CC	CC	AA	AA	AA
75	Stettler	2008	CWRS	CC	AA	CC	AA	CC	AA	AA	AA	CC	AA	AA	CC	CC	CC
80	WR859CL	2008	CWRS	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
6	5604HRCL	2009	CWRS	CC	CC	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
32	CDC_Kernen	2009	CWRS	CC	AA	CC	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
38	CDC_Thrive	2009	CWRS	CC	AA	CC	AA	CC	AA	GG	AA	CC	CC	AA	AA	AA	AA
39	CDC_Utmost	2009	CWRS	CC	AA	AA	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
42	CONQUER	2009	CNHR	AA	CC	CC	CC	??	AA	GG	CC	CC	CC	CC	AA	AA	AA
43	Carberry	2009	CWRS	CC	CC	CC	AA	CC	CC	AA	AA	CC	AA	AA	CC	CC	CC
60	Muchmore	2009	CWRS	CC	CC	CC	AA	CC	CC	AA	AA	CC	AA	AA	CC	CC	CC
74	CDC_Stanley	2009	CWRS	CC	AA	AA	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC
40	CDC_VR_Morris	2010	CWRS	NN	NN	NN	NN	CC	NN	GG	AA	CC	AA	AA	CC	CC	CC
69	SY985	2010	CPSR	NN	NN	NN	NN	AA	NN	GG	AA	CC	AA	AA	CC	CC	CC
79	Vesper	2010	CWRS	CC	CC	CC	AA	CC	AA	GG	CC	AA	CC	CC	AA	AA	AA
35	CDC_Plentiful	2011	CWRS	CC	AA	AA	AA	CC	AA	GG	AA	CC	AA	AA	CC	CC	CC

Rht8	Rht8	Rht8	Rht8	Rht8	Rht8	Sr2	Sr26	Lr34	Lr34	Lr34	Lr46/Yr29	Lr68	Yr5	Yr26	Yr36	Yr10	Yr10	Yr10	Yr10	Yr10	Yr10	Yr10
2D	2D	2D	2D	2D	2D												1B	1B	1B	1B	1B	1B
22	22	22	23	23	77												6	6	6	6	6	8
wsnp_Ex_c14779_22892053	wsnp_JD_rep_c63957_407	wsnp_JD_rep_c63957_4079812	RAC875_c74_204	tplb0053n05_793	Ku_c1918_1569	Sr2	Sr26	wMAS000003-Lr34	wMAS000004-Lr34	Lr34	Lr46_Yr29	Lr68	Yr5	Yr26	Yr36	Yr10	Kukri_c37738_417	RAC875_c30138_595	Ra_c78638_309	TA001594-0748	Tdurum_contig28703_293	Excalibur_c30569_384
AA	AA	CC	AA	AA	AA	AA	AA	AA	TT	AA	AA	AA	AA	CC	AA	AA	AA	AA	CC	CC	AA	CC
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AA	AA	CC	AA	AA	AA	CC	AA	AA	TT	AA	CC	CC	AA	CC	CC	CC	CC	AA	AA	AA	AA	AA
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Yr10	Yr10	Yr10	Yr10	Yr10	Tsn1	Tsn1	Tsn1	Tsn1	Tsn1	Tsn1	Tsn1	Tsn1							
1B	1B	1B	1B	1B			5B	5B	5B	5B	5B	5B							
8	9	9	9	9			73	74	73	74	73	73							
Kukri_c8390_1515	BS00066271_51	Excalibur_c43567_633	Kukri_c8390_547	RAC875_c8282_644	Xfcp1_Tsn1	Xfcp394_Tsn1	Kukri_c54078_114	Tdurum_contig25513_123	Tdurum_contig57027_347	wsnp_Ku_c17396_26488733	wsnp_Ku_c3102_5810751	wsnp_Ku_c3102_5811860	Mat	Pht	Yield	Yr	Lr	Ls	Ptr ToxA
AA	AA	CC	CC	CC	CC	CC	AA	CC	AA	AA	CC	CC	97.5	102.1	4211.2	5.8	7.4	7.0	1
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	97.9	104.3	4075.5	4.5	7.2	6.0	1
AA	CC	AA	AA	AA	AA	AA	AA	AA	AA	AA	CC	CC	101.1	113.9	5390.9	3.2	4.0	3.8	1
AA	CC	AA	AA	AA	AA	AA	AA	CC	AA	AA	CC	CC	99.9	111.2	4376.9	4.3	5.6	4.5	1
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	96.4	102.9	4257.7	4.4	4.4	6.0	1
CC	AA	CC	CC	CC	CC	CC	CC	CC	CC	CC	AA	AA	100.0	103.6	4180.4	3.0	2.6	6.0	0
CC	AA	CC	CC	CC	AA	CC	AA	CC	AA	AA	CC	CC	95.8	98.0	4296.3	3.6	5.6	6.5	0
AA	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	100.0	83.2	5434.9	4.9	6.2	7.3	-
AA	CC	AA	AA	AA	AA	AA	AA	CC	AA	AA	CC	CC	104.4	88.8	4494.8	5.7	6.2	6.0	0
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	98.2	101.1	4948.3	3.5	4.6	4.8	1
AA	AA	NN	CC	CC	NN	NN	CC	CC	NN	CC	AA	AA	100.9	106.9	4562.7	3.7	5.8	6.5	0
CC	AA	CC	CC	CC	AA	NN	AA	AA	AA	AA	CC	CC	98.5	94.4	4096.1	2.5	4.4	5.8	1
CC	AA	CC	CC	CC	CC	CC	CC	CC	CC	CC	AA	AA	99.9	103.3	4088.1	2.4	6.0	5.0	0
CC	AA	CC	CC	CC	AA	CC	AA	CC	AA	AA	CC	CC	98.5	99.1	4576.1	6.1	5.2	5.5	0
AA	CC	AA	AA	AA	AA	CC	AA	CC	AA	AA	CC	CC	102.0	80.2	5045.9	6.1	6.0	4.8	0
CC	AA	CC	CC	CC	AA	CC	AA	CC	AA	AA	CC	CC	97.8	106.5	4770.7	2.9	4.0	4.5	0
AA	CC	AA	AA	AA	AA	NN	AA	CC	AA	AA	CC	CC	103.4	88.9	4177.4	6.1	6.2	5.3	0
CC	AA	CC	CC	CC	CC	NN	CC	CC	CC	CC	AA	AA	98.8	99.1	4546.7	2.0	3.6	4.5	0
AA	CC	AA	AA	AA	AA	CC	AA	CC	AA	AA	CC	CC	100.5	90.4	5667.7	4.1	5.8	4.5	1
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	95.3	101.6	4823.5	2.0	2.4	4.8	1
AA	CC	AA	AA	AA	AA	CC	AA	CC	AA	AA	CC	CC	93.2	103.4	4234.7	4.3	4.2	6.0	0
AA	CC	AA	AA	AA	AA	AA	AA	AA	AA	AA	CC	CC	97.1	99.3	4623.3	4.6	3.8	6.5	1
CC	AA	CC	CC	CC	AA	CC	AA	CC	AA	AA	CC	CC	98.6	93.0	4936.7	2.9	5.2	5.8	0
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	98.1	106.2	4489.3	4.1	4.6	5.0	1
CC	AA	CC	CC	CC	NN	NN	AA	AA	AA	AA	CC	CC	98.4	104.2	4460.3	3.7	5.8	5.0	1
CC	AA	CC	CC	CC	AA	CC	AA	CC	AA	AA	CC	CC	103.0	83.2	4879.3	6.7	4.2	4.8	0
AA	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	102.7	90.9	6423.4	2.6	5.4	3.8	1
AA	CC	AA	AA	AA	AA	AA	AA	AA	AA	AA	CC	CC	100.3	102.0	4665.3	2.6	3.4	5.5	1
CC	AA	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	101.3	86.4	5319.8	2.2	2.4	4.8	1
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	103.1	109.4	5255.4	1.6	4.8	5.0	1
CC	AA	CC	CC	CC	NN	NN	AA	AA	AA	AA	CC	CC	101.0	92.8	5055.4	5.4	5.0	5.0	1
CC	AA	CC	CC	CC	AA	CC	AA	AA	AA	AA	CC	CC	98.5	96.6	4368.4	4.4	4.0	5.0	1
AA	CC	AA	AA	AA	AA	AA	AA	AA	AA	AA	CC	CC	96.8	101.6	3994.4	5.6	4.4	5.5	1
CC	AA	CC	CC	CC	NN	NN	AA	AA	AA	AA	CC	CC	100.9	87.6	5340.5	3.8	3.8	6.3	1
CC	AA	CC	CC	CC	AA	NN	AA	CC	AA	AA	CC	CC	96.5	98.4	4545.3	3.7	3.0	7.0	1
AA	CC	AA	AA	AA	AA	AA	AA	AA	AA	AA	CC	CC	100.7	105.3	5306.8	2.6	4.0	3.3	1
AA	AA	CC	CC	CC	NN	NN	AA	AA	AA	AA	CC	CC	101.7	90.5	6115.6	2.0	5.2	6.0	1
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	99.1	97.5	4929.3	2.8	1.8	4.0	1
AA	CC	AA	AA	AA	AA	AA	AA	AA	AA	AA	CC	CC	98.1	108.6	5308.3	1.6	2.8	4.0	1
CC	AA	CC	CC	CC	CC	CC	CC	CC	NN	CC	AA	AA	98.1	103.7	4909.0	2.6	1.8	4.3	0
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	96.9	94.1	4754.3	3.8	4.2	6.5	1
AA	CC	AA	AA	AA	AA	CC	CC	CC	CC	CC	AA	AA	96.2	99.2	4408.0	4.4	5.0	5.5	0
CC	AA	CC	CC	CC	AA	CC	AA	CC	AA	AA	CC	CC	98.0	100.1	4634.5	2.0	4.4	4.5	0
AA	CC	AA	AA	AA	AA	AA	AA	AA	AA	AA	CC	CC	97.8	108.3	4676.1	3.0	2.5	5.0	1
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	97.0	99.5	4839.6	3.0	4.2	4.0	1
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	101.4	89.9	5076.5	5.1	4.0	6.0	1
CC	AA	CC	CC	CC	AA	CC	AA	CC	AA	AA	CC	CC	97.8	99.0	4689.7	5.3	4.8	5.5	0
AA	CC	AA	AA	AA	AA	AA	AA	AA	AA	AA	CC	CC	99.2	93.7	4976.9	3.4	2.8	5.8	1
AA	CC	AA	AA	AA	AA	CC	AA	AA	AA	AA	CC	CC	96.6	92.2	4086.0	5.8	4.6	6.3	1
AA	CC	AA	AA	AA	AA	AA	AA	AA	AA	AA	CC	CC	102.4	91.9	5616.7	3.7	4.4	4.8	1
AA	CC	AA	AA	AA	AA	NN	AA	AA	AA	AA	CC	CC	96.7	110.1	5448.8	2.6	4.2	5.3	1
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	99.2	98.4	4261.3	3.6	2.7	5.8	1
AA	CC	NN	NN	NN	AA	AA	AA	AA	AA	AA	CC	CC	104.7	89.7	6292.0	2.1	4.6	5.3	1
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	98.9	102.0	4762.2	3.3	2.8	4.5	1
AA	CC	AA	AA	AA	CC	NN	NN	CC	NN	CC	AA	AA	98.9	101.1	4928.5	5.2	3.6	4.8	0
AA	CC	AA	AA	AA	AA	CC	AA	CC	AA	NN	CC	CC	99.4	100.2	4503.8	2.4	3.4	5.3	1
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	101.1	94.3	4859.0	2.0	3.0	5.0	1
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	101.2	91.9	4902.2	3.2	2.2	5.3	1
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	97.6	94.9	4487.0	5.3	3.4	6.8	-
CC	AA	CC	CC	CC	AA	NN	AA	AA	AA	AA	CC	CC	101.3	101.7	5158.0	4.1	4.0	6.0	1
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	97.0	101.4	4728.8	4.2	6.0	5.8	1
CC	AA	CC	CC	CC	CC	AA	CC	CC	CC	CC	AA	AA	96.5	94.8	4760.7	3.3	6.4	4.8	0
AA	CC	AA	AA	AA	CC	CC	CC	CC	CC	CC	AA	AA	104.5	98.9	5905.8	1.8	6.8	2.8	0
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	103.8	85.1	4680.0	1.7	2.2	4.3	1
CC	AA	CC	CC	CC	NN	AA	AA	AA	AA	AA	CC	CC	103.1	83.2	5097.8	2.5	2.6	5.0	1
AA	AA	CC	CC	CC	AA	AA	AA	CC	CC	CC	CC	CC	99.3	96.4	5251.0	3.2	3.2	3.3	0
CC	AA	CC	CC	CC	CC	AA	CC	CC	CC	CC	CC	CC	97.9	98.1	5175.5	2.3	4.0	4.8	-
AA	CC	AA	AA	AA	CC	CC	CC	CC	CC	CC	AA	AA	102.5	90.3	5410.9	3.9	2.8	4.3	-
CC	AA	CC	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	99.4	97.0	4689.9	6.3	3.6	5.3	1
CC	AA	CC	CC	CC	CC	CC	CC	CC	NN	CC	AA	AA	97.1	96.0	4881.8	1.9	2.0	3.5	-

Appendix 7. Summary of markers identified (selected) by the different methods.

Primer name	Gene	Trait	Method
wMAS000003	*Lr34/Yr18*	Yellow rust	PLSR
wMAS000004	*Lr34/Yr18*	Yellow rust	PLSR
cssfr5	*Lr34/Yr18*	Yellow rust	PLSR
Xfcp1	*Tsn1*	Yellow rust	PLSR
Kukri_c54078_114	*Tsn1*	Yellow rust	PLSR
Tdurum_contig57027_347	*Tsn1*	Yellow rust	PLSR
wsnp_Ku_c17396_26488733	*Tsn1*	Yellow rust	PLSR
wsnp_Ku_c3102_5810751	*Tsn1*	Yellow rust	PLSR
Vrn-A1	*Vrn-A1*	Leaf rust	PLSR
wMAS000003	*Lr34/Yr18*	Leaf rust	PLSR
wMAS000004	*Lr34/Yr18*	Leaf rust	PLSR
cssfr5	*Lr34/Yr18*	Leaf rust	PLSR
csGS	*Lr68*	Leaf rust	PLSR
Xbarc181	*Yr26*	Leaf rust	PLSR
wMAS000003	*Lr34/Yr18*	Tan spot	PLSR
wMAS000004	*Lr34/Yr18*	Tan spot	PLSR
cssfr5	*Lr34/Yr18*	Tan spot	PLSR
Xfcp1	*Tsn1*	Tan spot	PLSR
Kukri_c54078_114	*Tsn1*	Tan spot	PLSR
Tdurum_contig25513_123	*Tsn1*	Tan spot	PLSR
Tdurum_contig57027_347	*Tsn1*	Tan spot	PLSR
wsnp_Ku_c17396_26488733	*Tsn1*	Tan spot	PLSR
wsnp_Ku_c3102_5810751	*Tsn1*	Tan spot	PLSR
Xfcp1	*Tsn1*	Ptr ToxA	PLSR
Xfcp394	*Tsn1*	Ptr ToxA	PLSR
Kukri_c54078_114	*Tsn1*	Ptr ToxA	PLSR
Tdurum_contig25513_123	*Tsn1*	Ptr ToxA	PLSR
Tdurum_contig57027_347	*Tsn1*	Ptr ToxA	PLSR
wsnp_Ku_c17396_26488733	*Tsn1*	Ptr ToxA	PLSR
wsnp_Ku_c3102_5810751	*Tsn1*	Ptr ToxA	PLSR
wMAS000003	*Lr34/Yr18*	Yellow rust	Correlation
wMAS000004	*Lr34/Yr18*	Yellow rust	Correlation
cssfr5	*Lr34/Yr18*	Yellow rust	Correlation
Kukri_c54078_114	*Tsn1*	Yellow rust	Correlation
Tdurum_contig57027_347	*Tsn1*	Yellow rust	Correlation
wsnp_Ku_c17396_26488733	*Tsn1*	Yellow rust	Correlation
Vrn-A1	*Vrn-A1*	Leaf rust	Correlation

wMAS000003	*Lr34/Yr18*	Tan spot	Correlation
wMAS000004	*Lr34/Yr18*	Tan spot	Correlation
cssfr5	*Lr34/Yr18*	Tan spot	Correlation
csGS	*Lr68*	Tan spot	Correlation
Tdurum_contig57027_347	*Tsn1*	Tan spot	Correlation
wsnp_Ku_c17396_26488733	*Tsn1*	Tan spot	Correlation
Vrn-A1	*Vrn-A1*	Ptr ToxA	Correlation
Ppd-D1	*Ppd-D1*	Ptr ToxA	Correlation
S23M41	*Yr5*	Ptr ToxA	Correlation
Xfcp1	*Tsn1*	Ptr ToxA	Correlation
Xfcp394	*Tsn1*	Ptr ToxA	Correlation
Kukri_c54078_114	*Tsn1*	Ptr ToxA	Correlation
Tdurum_contig25513_123	*Tsn1*	Ptr ToxA	Correlation
Tdurum_contig57027_347	*Tsn1*	Ptr ToxA	Correlation
wsnp_Ku_c17396_26488733	*Tsn1*	Ptr ToxA	Correlation
wsnp_Ku_c3102_5810751	*Tsn1*	Ptr ToxA	Correlation
wsnp_Ku_c3102_5811860	*Tsn1*	Ptr ToxA	Correlation
wMAS000003	*Lr34/Yr18*	Yellow rust	Stepdisc
Vrn-B1	*Vrn-B1*	Yellow rust	Stepdisc
Xfcp394	*Tsn1*	Yellow rust	Stepdisc
JD_c63957_1176	*Rht8*	Yellow rust	Stepdisc
Xwmc44	*Lr46/Yr29*	Yellow rust	Stepdisc
cssfr5	*Lr34/Yr18*	Yellow rust	Stepdisc
Vrn-A1	*Vrn-A1*	Leaf rust	Stepdisc
Tdurum_contig25513_123	*Tsn1*	Leaf rust	Stepdisc
Kukri_c54078_114	*Tsn1*	Leaf rust	Stepdisc
BS00090678_51	*Rht8*	Leaf rust	Stepdisc
cssfr5	*Lr34/Yr18*	Tan spot	Stepdisc
S23M41	*Yr5*	Tan spot	Stepdisc
csGS	*Lr68*	Tan spot	Stepdisc
Vrn-B1	*Vrn-B1*	Tan spot	Stepdisc
wMAS000001	*Rht-B1*	Tan spot	Stepdisc
Tdurum_contig25513_123	*Tsn1*	Ptr ToxA	Stepdisc
wsnp_Ku_c3102_5810751	*Tsn1*	Ptr ToxA	Stepdisc
Ku_c19185_1569	*Rht8*	Ptr ToxA	Stepdisc
Xfcp1	*Xfcp1*	Ptr ToxA	Stepdisc
Vrn-A1	*Vrn-A1*	Ptr ToxA	Stepdisc
Excalibur_c43567_633	*Yr10*	Ptr ToxA	Stepdisc