Quantitative trait loci and genomewide association mapping in western Canadian spring wheat (*Triticum aestivum* L.)

by

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Abstract

Early maturity, grain yield and grain protein content are some of the important traits in western Canadian wheat breeding programs. A series of experiments were conducted to explore the genetic basis of days to heading, and maturity, plant height, grain protein, grain yield and related traits. In a spring wheat population of 187 recombinant inbred lines genotyped with 341 Diversity Array Technology (DArT) polymorphic markers, a total of 21 quantitative trait loci (QTLs) were identified for all phenotypic traits recorded, except plant height and grain protein content. Two earliness *per se* QTLs were mapped on chromosomes 1A (*QEps.dms-1A*) and 4A (*QEps.dms-4A*) in all three growing seasons, contributing 15-27% and 8-10%, respectively, to the total genetic variation in days to maturity. The two earliness QTLs and *Vrn-B1* exhibited additive interaction. In the same population, lines carrying the resistant allele of *Lr34/Yr18* were taller, matured earlier, yielded less grain with lower test weights than lines without *Lr34/Yr18.* Lines with *Lr34/Yr18* also exhibited lower leaf and stripe rust infection than lines with the susceptible allele. The failure to combine *Lr34/Yr18* with high yield, protein, and SDS sedimentation suggested single seed descent or doubled haploid populations for the combined selection of multiple quantitatively inherited traits, and simply one molecular marker, would require population sizes in excess of at least 500 to have any possibility of selection success. Genetic diversity analysis for earliness related and plant height reducing genes in 82 spring wheat cultivars registered in western Canada through eight diagnostic DNA markers suggested breeding efforts in western Canada have resulted in the incorporation of vernalization and photoperiod insensitive and height reducing genes in modern cultivars to promote early maturity, to make use of off-season nurseries in other parts of the world and to improve lodging tolerance. Using genome-wide association mapping (GWAS). we identified a total of 152 significant marker-trait associations; however, there were only 18 genomic regions that consisted of clusters of 3 to 20 significant single nucleotide

polymorphisms (SNPs) across 12 chromosomes, including two regions each for grain yield, test weight and protein content, six regions for plant height and six other coincident regions that were associated with two or three traits. The genomic region associated with plant height on chromosome 4B showed high linkage disequilibrium ($r^2 \geq 0.80$) with the semi-dwarfing gene *Rht-B1*. Results of these studies suggest that besides the widely used semi-dwarf and early maturity related genes, there is a wide spectrum of loci available that could be used for modulating plant height, days to maturity, grain yield and grain protein content in western Canadian wheat germplasm.

Preface

I joined the CDC Teal × CDC Go population study presented in Chapter 2 from 2010 to 2014. The population development was done before I joined the study. I was partially responsible for field experiment during 2011 and 2013, extracting and shipping the DNA samples to Diversity Array Technology (DArT) for high throughput genotyping and testing the polymorphism of *Vrn-B1* in the population. I was responsible for phenotypic data analysis and QTL mapping and kindly guided by Dr. Curtis Pozniak and Dr. Rong Cai Yang.

I collected agronomic trait data and scored diseases for leaf rust, tan spot and common bunt in diseases nursery from 2011 to 2013 at the Edmonton Research Center, University of Alberta. Score of stripe rust in Lethbridge and Creston were recorded by Drs. Harpinder Singh Randhawa and Dean Spaner, respectively. As for the selection study, all planting and data collection was done by the wheat breeding group of the University of Alberta. I was responsible for data analysis for Chapter 3 and guided by Dr. Dean Spaner.

Dr. Pierre Hucl developed the association mapping population used in Chapters 4 and 5. From 2011 to 2013, Neshat Pazooki Moakahr and I collected agronomic data of 82 Canadian western spring wheat in Edmonton Research Center. In Kernen Saskatoon, trials were planted by Curtis Pozniak's Durum wheat breeding group and data were recorded by Teketel Haile. In 2014, traits were recorded and the population grown curtesy of the wheat breeding group of the University of Alberta. I was responsible for all the phenotypic traits analysis and identified the polymorphisms on vernalization genes (*Vrn-A1*, *Vrn-B1* and *Vrn-D1*), photoperiod genes (*Ppd-A1*, *Ppd-B1* and *Ppd-D1*) and semi-dwarf genes (*Rht-B1* and *Rht-D1*) of these cultivars for Chapter 4, and was guided by Dr. Muhammad Iqbal. The SNP markers were filtered from Genome Studio by Dr. Curtis Pozniak's group at the University of Saskatchewan, I conducted the association studies for the 82 cultivars in Chapter 5 and was guided by Drs. Curtis Pozniak and Kassa Semagn.

I was responsible for data analysis and interpretation of all the studies of presented in this thesis. Drs. Dean Spaner and 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 Drs. Muhammad Iqbal and Dean Spaner.

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Chapter 1 Literature Review

1.1Wheat

Wheat is divided into three taxonomic groups based on its ploidy level: diploid wheat (2n=14), tetraploid wheat (2n=28) and hexaploid wheat (2n=42) (Feldman 2001). Through A-genome repeated nucleotide sequence variation from the diploid wheat species, *Triticum urartu* (2n=2x=14, AA) is the main A-genome donor of all polyploidy wheat species (Dvorak et al. 1988). Einkorn wheat (*T. monoccum,* A^mA^m) is the only cultivated diploid hulled wheat. The B genome experienced large changes at the DNA level when it formed polyploidy wheat species. *Aegilops. speltoides* is thought to be the maternal parent for both tetraploid and hexaploid wheat depending on cytoplasmic analysis (Wang et al. 1997). Wild emmer wheat (*T. dicoccoides*, 2n=4x=28, BBAA) is the progenitor of all modern cultivated tetraploid and hexaploid wheat species. Durum wheat (*T. turgidum*) is the most important cultivated tetraploid wheat for pasta and low-rising bread. Among all the wheat species, common wheat (*T. aestivum*, 2n=6x=42, BBAADD), which is derived from *T. dicoccoides* and *Ae. Tauschii*, is the most widely grown wheat globally. Wheat has a long cultivation and domestication history. The Fertile Crescent in southwest Asia is regarded as the center of origin and the current center of wheat distribution and diversity (DePauw and Hunt 2001). This area extends west to Mediterranean Sea, north to Turkey, east to Iran and south to the Syrian-Arabian desert and includes 4 wild wheat species and 17 species of *Aegilops* closely related to, wheat B and D genomes (Feldman 2001). Approximately 13,000 years ago, people started to collect grains of wild cereals and cultivate wild einkorn wheat and wild emmer wheat. Approximately 7000 years ago, domesticated tetraploid wheat with non-brittleness and free-threshing characters was formed. It hybridized with *Ae. Tauschii* to become common wheat. About 8000 years ago, wheat in southwest Asia migrated through Greece to Europe, Egypt to Africa and northern Iran to East Asia (Feldman 2001).

1.2 Wheat in Canada

Canada has a relative short wheat cultivation history. The first wheat planted in eastern Canada was in 1605 at a French settlement. In western Canada, the Selkirk settlers in the Red River settlement first planted wheat in 1812, with the first harvest in 1815 (Campbell and Shebeski 1986). Presently the three prairie provinces of Saskatchewan, Alberta and Manitoba account for

90% of Canadian wheat production (StatisticsCanada 2014). Canada is the seventh largest wheat producing country and the second largest wheat exporting country in the world (FAOSTAT 2015).

Hexaploid spring wheat is the predominant type produced in western Canada, and it is roughly grouped into six classes according to specific functional properties. Canada Western Red spring (CWRS) is the premium class in the three Prairie Provinces due to its wide adaptation, good flour milling characteristics, baking suitability and high price in world trade markets (McCallum and DePauw 2008). CWRS includes four milling grades and can be used for making high volume pan bread, noodle, steamed bread and common wheat pasta. A typical CWRS kernel is small to medium size with translucent red color quality (CGC 2015). CWRS has been planted in western Canada since 1812. The first famous cultivar in the class was Red Fife, brought into Canada around 1870 and it replaced previous cultivars with excellent end use quality (Campbell and Shebeski 1986). Because of adaptation problems, Red Fife was replaced by Marquis which matured earlier and was less susceptible to shattering than Red Fife (McCallum and DePauw 2008). Until 2014, CWRS included 79 registered cultivars. The genetic diversity and adaptation have been improved over the 100 years history of the class. About 12% of CWRS cultivars carried photoperiod insensitive alleles of *Ppd-1* genes before the mid-1980s. By 2008, 43% carried the photoperiod insensitive genes *Ppd-D1a* (Kamran et al. 2013). For disease resistance, the cultivar Thatcher (registered in 1935) was developed as a rust resistant cultivar and had the stem rust resistant genes *Sr5*, *Sr9g*, *Sr12* and *Sr16*. Thereafter, a series of CWRS cultivars were developed with different combinations of resistant genes and no major stem rust epidemic has occurred in Western Canada since 1961 (McCallum and DePauw 2008). The yield potential of CWRS has increased 6-9 kg per ha each year over the last 90 years (McCallum and DePauw 2008).

Canada Prairie Spring Wheat (CPS) was established in 1985 and can have either red or white kernel color (McCallum and DePauw 2008). Most registered CPS cultivars had more or less kinship with HY320, which was the first cultivar in this class (McCallum and DePauw 2008). A typical kernel is medium to large size, elliptical to conical shape with small to medium size embryo (DePauw and Hunt 2001).They are usually used to make hearth breads, flat breads, steamed breads and noodles (CGC 2015).

Canada Western Extra Strong (CWES) spring wheat with strong gluten properties is used in specialty products when high gluten strength is needed (CGC, 2014). There were 12 registered

varieties by 2014 (CGC, 2014). Cultivars Pictic62 and Glenlea were high yielding cultivars developed from CIMMYT and Brazil germplasm, respectively (Briggs 1975). From 2001 to 2007, the planting area of this class of wheat in western Canada has declined due to the decreasing international market demand and baking technique changes in North America (McCallum and DePauw 2008).

Canada Western Soft White Spring Wheat (CWSWS) is mostly planted in southern Alberta and under irrigation. The production area was increased in Saskatchewan, because of high yield and low protein content characters, which is good for producing biofuel. However, the total growing area has decreased, because of CWRS and Canada Western Amber Durum (CWAD) cultivars development. CWSWS has three milling grades. The kernel size is small to medium with ovate to oval shapes. It can be used for cookie, cake, pastry and flat bread (CGC 2015).

Canada Western Hard White Spring (CWHWS) class was established in 2001. It contained seven registered cultivars until 2014 (CGC 2015). CWHWS shared similar end use quality with CWRS, except for kernel color (McCallum and DePauw 2008). The first registered cultivar Snowbird was derived from CWRS cultivar AC Domain and a white seeded isoline of RL4137 (McCallum and DePauw 2008). Cultivars in this class showed superior milling quality producing flour with excellent color and could be used to make bread and Asian style noodle (CGC, 2015).

Canada Western General Purpose (CWGP) class was established in 2008 to develop high starch yield cultivars with protein content of 10% or less for ethanol production and livestock feeding (McCallum and DePauw 2008; Fowler 2012). There were a total of 19 registered cultivars in this class at the end of 2014 (CGC 2015). These cultivars varied in kernel size, color and hardness. For instance, AAC Proclaim is a soft red spring wheat (CGC 2015), Sunrise is soft red winter wheat (Fowler 2012) and Accipiter is a hard red winter wheat.

1.3 Genotyping platforms

Since 1980, when restriction fragment length polymorphism (RFLP) was first used in human genome mapping (Botstein et al. 1980), various types of molecular markers are available for genotyping but the most widely used types of markers in recent years includes microsatellite or simple sequence repeat (SSR), single nucleotide polymorphism (SNP), diversity arrays technology (DArT), and genotyping by sequencing (GBS). SSR markers are widely used by wheat

researchers because they are widely available, co-dominant, multiallelic, highly polymorphic, repeatable and uniformly distributed in the genome (Gupta et al. 2002). SSR fragment sizes can be separated using a wide range of methods, including agarose gels, polyacrylamide gels, gel-based sequencers and capillary sequencers. Differences in SSR allele size is often difficult to resolve on agarose and polyacrylamide gels and high resolutions can be achieved through the use of DNA sequencers. However, the establishment and running cost for an automatic DNA sequencer is expensive. One of the challenges of SSR markers is the difficulty 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's. Researchers often prefer to work with SSR markers containing tri-, tetra- or penta-nucleotide repeats arrays rather than di-nucleotide arrays because the former frequently give fewer "stutter bands" (multiple near-identical 'ladders' of PCR products which are one or two nucleotides shorter or longer than the full length product) (Diwan and Cregan 1997). Di-nucleotide repeat arrays occur much more frequently than the other arrays, but there is a compromise in the quality of the SSR data. The other limitations for SSR markers is the low throughput and high genotyping costs per data point, which have limited their use for some applications that requires high marker density, such as genomewide association studies (GWAS).

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). Because of their 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; Schlötterer 2004), SNPs have emerged as powerful tools for many genetic applications. SNP data can be obtained using one of the numerous uniplex or multiplex SNP genotyping platforms that combine a variety of chemistry, detection methods and reaction formats (Edwards et al. 2007). The availability of sequence information has paved the way for the identification and development of SNP markers for several crop species. SNPs have largely replaced SSRs in species that have been extensively sequenced and they are expected to replace other types of molecular markers in most species in the near future given the increased use of next generation sequencing technologies for genotyping. In wheat, high throughput SNP discovery and genotyping lag behind rice and maize

due to its larger genome size and the lack of reference genetic map. However, next generation sequencing technology used for the International Wheat Genome Sequencing Consortium (IWGSC) offers an alternative opportunity from conversional sequencing and amplification (www. wheatgenome.org). In wheat only 9,000 (9K) SNPs were incorporated in the bead-chip assay in 2013 (Cavanagh et al. 2013), which has increased to 90,000 gene-associated SNPs (90K) in 2014 using iSelect platform (Wang et al. 2014). The consensus genetic position of 43,999 of the SNPs was determined using eight mapping populations, which has provided tremendous opportunity for wheat researchers in conducting research that requires high marker density, such as GWAS.

DArT is a microarray hybridization-based technique that enables the simultaneous typing of several hundred polymorphic loci spread over the genome (Jaccoud et al. 2001; Wenzl et al. 2004; Semagn et al. 2006). However, the efficiency of DArT marker discovery depends on the intra species diversity. In wheat, the efficiency varied between 5% to 10% (Hong et al. 2009). The dominant inheritance (present vs absent variation) of DArT markers is one of the major drawbacks, as homozygous dominant and heterozygous individuals are not easily identified. To date, DArT technology has been updated to DArTseq platform, which deploys sequencing of the representations using the Next Generation Sequencing (NGS) platforms (www.diversityarrays.com). The DArTseq allows researchers to use DArT-based GBS markers that may change our current perception on the reliability of DArT markers in marker-assisted breeding. DArTseq GBS has been used for genetic characterization of more than 40,000 wheat germplasm accessions held by the International Wheat and Maize Improvement Center (CIMMYT) as part of its Seeds of Discovery initiative (Li et al. 2015).

The Cornell University-based GBS platform is an emerging method for SNP genotyping which is being increasingly adopted for discovery applications. This platform has been widely used for generating high density marker data for several species and it is cost effective in terms of wet chemistry, with genotyping and informatics cost ranging from US$ 20 to US$ 38 per sample, depending on sample size and multiplex level (http://igd.cornell.edu/). However, overall project costs can be expensive and time consuming due to a number of factors: (i) relatively slow data turnaround time for most marker-assisted selection projects; (ii) a large proportion of missing data is generated due to low sequence depth (Beissinger et al. 2013), although some of this can be

remedied using reliable imputation methods; (iii) the GBS methodology is still evolving, which requires re-analysis and re-examination of old data to ensure allele calls have not changed, as direct comparison of all data over different years may not be possible; (iv) GBS allele calls for heterogeneous and highly heterozygous samples is less accurate as compared with data derived from highly homozygous or doubled haploid (DH) lines (Semagn et al. 2014). GBS was first used in wheat for whole-genome predictions and genomic selection to a set of 254 elite breeding lines from CIMMYT (Poland et al. 2012).

1.4 Linkage-based QTL mapping

Most traits of interest in plant breeding show quantitative inheritance, which complicate the breeding process since phenotypic performances only partially reflects the genetic values of individuals. A quantitative trait is a measurable trait that depends on the cumulative action of many genes or quantitative trait loci (QTL) and their interaction with the environment that can vary among individuals over a given range to produce a continuous distribution of phenotypes (Sham et al. 2002). It is therefore necessary to simultaneously introgress several genes or QTLs into the same genetic background. Molecular markers can be used to speed up the development and deployment of improved germplasm in different ways, including marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS) and genomic selection (GS) (Bernardo 2008). The use of molecular markers in MABC involves finding a subset of markers that are significantly associated with genes or major effect QTLs that regulate the expression of traits of interest in breeding. Both linkage analysis and association mapping can be used for identifying significant marker-trait associations (MTAs), with each method having its own strength and weaknesses.

Linkage-based QTL analysis depends on well-defined populations, such as F_2 or their derived families, backcross (BC) populations, doubled haploids (DH) lines, recombinant inbred lines (RILs), and near isogenic lines (NILs). The choice of appropriate mapping population is very critical for the success of any QTL mapping project. Both F_2 and BC populations require only a short time to develop but have three major limitations. First, development of these populations allows for relatively few meiosis such that even markers that are far from the QTLs remain strongly associated with it. Second, F_2 and backcross populations are highly heterozygous and cannot be evaluated several times in different environmental conditions, years, locations, etc. Finally,

epistatic interactions could hardly be studied in both F_2 and backcross populations. RILs, NILs and DHs are permanent populations and they 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. 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). In wheat, RILs and DH lines are the most commonly used populations for QTL mapping studies.

There is usually a high cost associated with genotyping and phenotyping of large population size, particularly for traits requiring extensive field trials or complex analysis. Consequently, the size of the mapping population and the number of replications and sites (environments) for phenotyping is often limited. Most published experiments with replicated trials have used between 100 and 200 progenies but it should be noted that the reliability of the QTL results may be affected by such small population size in terms of the number of QTLs to be detected and their effect (Beavis 1998; Melchinger et al. 1998; Melchinger et al. 2004; Xu 2003). Having generated both the phenotype and genotype data for a given mapping population, researchers are often eager to test (i) the null hypothesis (H_0) that no QTL is present or a QTL is present but it is not linked to the marker(s) and (ii) the alternative hypothesis (H_A) that a QTL is present and it is linked to the marker(s). Various statistical methods exist for testing the two hypotheses, including single marker analysis (SMA), interval mapping (IM), composite interval mapping (CIM) (Xu 2010) and inclusive composite interval mapping (ICIM) (Wang 2009). The accuracy of QTL results and its effect can be influenced by the statistical methods and the specific parameters used for mapping.

1.5 Association mapping

The linkage disequilibrium based association mapping is a population-based survey that capitalizes on historical recombination to identify candidate genes affecting complex traits (Falconer et al. 1996). The terms linkage disequilibrium (LD) and association mapping have often been used interchangeably in the literature. According to Gupta et al. (2005), association mapping refers to significant association of a molecular marker with a phenotypic trait, while LD refers to non-random association between two markers (alleles at different loci), between two genes or

QTLs, between a gene/QTL and a marker locus. Thus, association mapping is one of the several uses of LD. The terms linkage and LD are also often confused. Linkage refers to the correlated inheritance of loci through the physical connection on a chromosome, whereas LD refers to the correlation between alleles in a population (Flint-Garcia and Thornsberry 2003) but not necessarily on the same chromosome. LD can be used in plant genomes for construction of LD maps, for studying marker-trait association (MTA) both independently and in combination with linkage analysis, and for the study of population genetics and evolution (Gupta et al. 2005).

Unlike linkage analysis, where familial relationships are used to predict correlations between phenotype and genotype, association mapping rely on previous, unrecorded sources of disequilibrium to create population-wide MTAs (Kruglyak 1999; Ewens and Spielman 2001; Jannink et al. 2001). Individuals in the association mapping are usually from various genetic backgrounds or pedigrees developed for a wide range of purposes and have large number of alleles and higher recombinant events. Such increase in recombination frequency leads to reshuffling of chromosome into smaller segments or gene blocks, which reduces LD levels in to a short distance (Zhu et al. 2008). Association mapping is divided into (i) candidate-gene association mapping, which relates polymorphisms in selected candidate genes that have purported roles in controlling phenotypic variation for specific traits; and (ii) genome-wide association mapping (GWAS), or genome scan, which surveys genetic variation in the whole genome to find signals of MTAs for traits of interest (Risch and Merikangas 1996). For candidate-gene association mapping, information regarding the location and function of genes involved in genetic, biochemical or physiological pathways that lead to trait variation is often required (Risch and Merikangas 1996; Rafalski 2002; Mackay 2001). On the other hand, GWAS requires high marker density to efficiently identify markers at a density that accurately reflects genome-wide LD structure and haplotype diversity.

In wheat, association studies have been used to map and characterize QTLs for a wide range of traits, including kernel size and milling quality (Breseghello and Sorrells 2006), α-amylase activity (Emebiri et al. 2010), agronomic traits (Yao et al. 2009), plant height (Zhang et al. 2011), grain weight (Mir et al. 2012), seed longevity and storage (Arif et al. 2012a), pre-harvest sprouting resistance (Arif et al. 2012b), and flowering variation (Rousset et al. 2011). These studies reported different numbers of significant MTAs that generally explained a significant proportion

of the phenotypic variance. However, most of these studies were conducted using spare number of markers. QTL results from association mapping may be affected by a number of factors, including marker density, population structure, population size, and breeding history (Gaut and Long 2003; Gupta et al. 2005). The number of markers required for GWAS depends on the genome size of the species and the expected LD decay (Yu and Buckler 2006; Myles et al. 2009).

1.6. Target breeding traits in Western Canada

1.6.1 Early maturity

Due to the short growing season (95-125 days) in western Canada, development of early maturing cultivars is an effective way to avoid frost damage, post-harvest sprouting and disease (Iqbal et al. 2007). In wheat, flowering time is determined by three gene groups: vernalization, photoperiod and earliness *per se* genes (Distelfeld et al. 2009). Vernalization genes are associated with temperature requirements in the seedling stage; photoperiod genes with day length sensitivity and earliness *per se* genes mediate basic development rate. The first two gene groups play a major role in determining flowering and maturity time than earliness *per se* genes.

Most vernalization genes are located on homoeologous group 5 chromosomes in wheat, except *VRN3* which is on the short arm of homoeologous group 7 (Yan et al. 2004; Yan et al. 2006; Yoshida et al. 2010). *VRN1, VRN2* and *VRN3* were widely identified from diploid wheat to polyploid wheat (Santra et al. 2009; Yan et al. 2004); while only one copy of *VRN4* has been reported on the D genome (Yoshida et al. 2010). A MADS-box transcription factor (Yan et al. 2003) in wheat is encoded by *VRN1* genes, orthologous with *Arabidopsis* meristem identity genes *APETALA1* (*AP1*), the dominant alleles of which are present in spring wheat alone or in combination. *VRN1* is essential for flowering. An attempt to delete *VRN1* genes resulted in wheat incapable of flowering, even with vernalization treatment and long days (Trevaskis et al. 2007; Fu et al. 2005). Both dominant and recessive alleles in diploid wheat have almost the same exon sequence (Yan et al. 2003), except one single nucleotide polymorphism (Diaz et al. 2012), and common variations occur in the promoter region and the first intron (Dubcovsky et al. 2006). The difference due to insertion-deletion or SNPs in the two areas results in various alterations of flowering time in spring wheat. For example, *Vrn-A1* has the strongest function to accelerate flowering, followed by *Vrn-D1* and *Vrn-B1* (Loukoianov et al. 2005). On chromosome 5D, *Vrn-D1a* accelerated flowering by 32 days compared to *Vrn-D1b*, with less leaves being produced

(Zhang et al. 2012). Despite the absence of close homologues in *Arabidopsis*, *VRN2* plays the same role as *FLOWERING LOCOUS C* (*FLC*) of *Arabidopsis*, which prevents flowering (Michaels and Amasino 1999), and can be down-regulated by vernalization or short day length (Yan et al. 2004). This difference suggests different evolutionary pathways for monocot and dicot plants (Distelfeld et al. 2009). Some research has demonstrated that even heterozygous alleles at the *VRN2* locus is sufficient to play a repression role to make wheat more cold-tolerant and delay flowering (Diallo et al. 2010). *VRN2* region includes two linked duplicated genes *ZCCT1* and *ZCCT2*, each of which includes a putative zinc finger and a CCT domain (Yan et al. 2004). Recessive alleles with deletion or substitution in the CCT domain lose the repression function (Yan et al. 2004; Dubcovsky et al. 2006). The *VRN3* gene, with three exons encoding a RAF kinase inhibitor-like protein (Yan et al. 2006), is similar to the Arabidopsis protein *FLOWERING LOCUS T* (*FT*). The three copies vary on the intron 1 length (390, 626 and 400 bp, respectively) and are more conserved on the intron 2 (Bonnin et al. 2008). The sequence difference between dominant and recessive alleles of *Vrn-B3* in hexaploid wheat occurs 591 bp upstream from the start codon, where a 5,295 bp repetitive element is inserted (Yan et al. 2006).

A model describing the interaction between vernalization genes in wheat has been modified by Chen and Dubcovsky (2012). Initiation of the flowering pathway is from leaf to apex. In the leaf, high expression levels of VRN2 represses the function of VRN3 before vernalization (which is considered as the integrator of photoperiod and vernalization), then during vernalization and long days, the up-regulated VRN1 in leaves down-regulates VRN2 to release VRN3. Protein production of VRN3 is transported to the plant apex through the phloem, interacting with FDL2 up-regulated VRN1 transcript in the apex, which triggers the switch from vegetative to the reproductive stage.

The photoperiod insensitive alleles (a) of photoperiod responsive (*Ppd*) genes (McIntosh et al. 2003) result in wheat that is insensitive to day length, and flower under short days. Cultivars carrying *Ppd-1a* exhibit slightly delayed flowering in short days, when compared to long days; hence they are referred to as semi-dominant genes. Cultivars carrying *Ppd-1b* (recessive alleles) may exhibit flowering delays of longer than 200 days under short days condition (Worland et al. 1994). Genes of *Ppd-1* are located on the homeologous group 2 chromosomes (Nishida et al. 2013; Jarillo et al. 2008). Genic polymorphisms between *Ppd-1a* and *Ppd-1b* alleles have been reported

in many studies (Beales et al. 2007). Comparison of the two allele sequences revealed differences in both introns and exons, the most important one was on the upstream of the coding regions (Beales et al. 2007; Wilhelm et al. 2009; Nishida et al. 2013). For instance, 160 day differences to heading between 'Ciano 67' (*Ppd-D1a*) and 'Chinese Spring' (*Ppd-D1b*) is resulted from a 2,089 bp deletion upstream from the start codon (Beales et al. 2007). Three other polymorphisms have also been detected in intron 1 and exon 7 of *Ppd-D1* (Beales et al. 2007). Two polymorphisms of *Ppd-A1a* were first found in tetraploid wheat (Wilhelm et al. 2009); *Ppd-A1a.2* and *Ppd-A1a.3* with 1,027 and 1,117 bp deletions upstream from the start codon, respectively, included an 886 bp common sequence. In hexaploid wheat, *Ppd-A1a.1* was detected in the Japanese cultivar Chihokukomugi (Nishida et al. 2013) with a 1,085bp deletion on the upstream, sharing the same region with *Ppd-D1a*. *Ppd-B1a* exhibited a 308bp insertion in the upstream area in Japanese winter wheat (Nishida et al. 2013).

Earliness *per se* (*Eps*) genes related to ear emergence in the field was first reported in 1965 (Yasuda and Shimoyama 1965). However, such flowering genes are not easily found, with only 2-7 days differential in the field; while greenhouse differentials can extend to 50 days (Yasuda and Shimoyama 1965). The chromosomes of *Eps* genes have been identified since 1980s in controlled environments, which are located on chromosomes 2B (Scarth and Law 1983), 2D (Worland 1996), 3A, 4D, 6B (Hoogendoorn 1985), 6D (Law 2013) and 7B (Flood and Halloran 1983). Earliness *per se* genes are considered to be a group with differential effects on different developmental phases of wheat. Earliness *per se* genes in hexaploid wheat are not studied in as much details as *Vrn* and *Ppd* due to the genome size and the complex interactions between genotype and environments (Worland 1996; Slafer 1996; Appendino and Slafer 2003). However, they are important for wheat adaptation under the continuous selection pressure. Diploid wheat (*Triticum. Monococcum* L.), with its relative small genome size, was widely used to investigate the location and function of *Eps* genes. *Eps-A^m1*, located on the distal region of chromosome 1A^mL (Bullrich et al. 2002), is associated with growth temperature under long-day photoperiods: *Eps-A^m1-early* flowered earlier than *Eps-A^m1-late* lines and flowering earlier at 23℃ than at 16℃ using single seed descendant (SSD) recombinant inbred lines from the cross DV92×G3116 (Appendino and Slafer 2003). The candidate gene *Mot1* for *Eps-A^m1* showed amino acid differences between DV92 and G3116, but the effect was still hard to predict (Faricelli et al. 2010). *Eps-3A^m* has been mapped

to chromosome $3A^m$ (Shindo et al. 2002; Hori et al. 2007; Gawronski and Schnurbusch 2012) using a recombinant inbred lines (RIL) population.

1.6.2 Plant height

Wheat breeders are primarily aiming on increasing grain yield by reducing plant height, to make the plants resistant to lodging in conditions of intensive agriculture. Many wheat breeders were successful in breeding semi-dwarf, high-yielding varieties that were well adapted to intensive agriculture (Borojevic and Borojevic 2005). The incorporation of Reduced height (*Rht*) genes has been a globally important wheat breeding strategy that launched the Green Revolution to increase grain yield through reduced lodging, improved harvest index, and greater grain in biomass assimilation (Evenson and Gollin 2003; Borojevic and Borojevic 2005). Dwarfing genes in wheat can be divided into two categories based on seedling response to exogenous gibberellic acid (GA) (Börner et al. 1987). *Rht-B1* on chromosome 4BS and *Rht-D1* on chromosome 4DS belong to the GA-insensitive group and both of them are derived from the Japanese wheat cultivar Norin10 (Gale and Youssefian 1985). Compared with wild type alleles (*Rht-B1a* and *Rht-D1a*), the two mutant alleles (*Rht-B1b* and *Rht-D1b*) have single base pair substitutions that cause a premature stop codon (Peng et al. 1999). Therefore, the mutations alter the function of the gene in height regulation, reducing plant height and increasing grain yield by 23 and 20% respectively, in isogeneic lines with different alleles of *Rht-B1* and *Rht-D1* (Gale and Youssefian 1985). *Rht-D1b* and/or *Rht-B1b* are present in 44% of the registered wheat cultivars in Germany (Knopf et al. 2008), and more than 90% of wheat cultivars in USA (Guedira et al. 2010).

GA sensitive dwarf genotypes contains *Rht-4*, *Rht-5*, *Rht-6*, *Rht-7*, *Rht-8* and *Rht-9*. The semi-dwarfing gene *Rht8* was mapped at a distance of 21.7 cM from *Ppd-D1* gene (Gasperini et al. 2012) and was reported to reduce height from 3.5% to 12.5% (Börner et al. 1993; Ellis et al. 2005) without reducing yield. As for the other four genes, they were mutated by irradiation or ethyl methyl sulphonate (EMS), but no commercial prospect is found in these genes due to yield penalties (Gale and Youssefian 1985).

1.6.3 Grain Yield

Grain yield of wheat is determined not only by the genes directly controlling yield and yield components, but also by the genes controlling plant development and maturity. Grain yield is a complex trait that is controlled by a number of QTLs and easily affected by the interaction

between genotype and environment. The heritability of grain yield varied from 0.2 to 0.9, depending on the type of population and environment (Cuthbert et al. 2007; Huang et al. 2004; Kumar et al. 2007; Bennett et al. 2012).

Several studies that aimed in mapping QTLs associated with grain yield and yield components have been conducted for many years. QTLs associated with yield have been found on all 21 chromosomes of bread wheat (Cuthbert et al. 2007; Huang et al. 2004; Kumar et al. 2007; Bennett et al. 2012). The number of QTLs reported for grain yield and the proportion of phenotypic variance explained by each QTL was high variable depending on the type of population, population size, the number of environments and the management conditions. For instance, only one QTL related to yield on chromosome 4AL was found in RIL population derived from cultivars 'Dharwar Dry' and 'Sitta' under drought conditions (Kirigwi et al. 2007); whereas in a DH population derived from two Canadian spring wheat cultivars, 16 QTLs were found significantly associated with grain yield (Huang et al. 2006).

Grain yield is a complex trait and it has high correlation with other traits such as thousand kernel weight and spike number, QTLs with pleiotropic effects were found in several studies. For example, the QTL on chromosome 4A in the RIL population derived from cultivars 'Dharwar Dry' and 'Sitta' had a confidence interval of 7.7 cM and was also significantly associated with grain fill rate, spike density, number of grains, biomass production, and drought susceptibility index (Kirigwi et al. 2007). In the DH population from 'AC Karma' and 'Glenlea', a QTL for grain yield on chromosome 7B was also significantly associated with plant height (Huang et al. 2006).

1.7 Conclusion

Bread wheat exhibits great adaptation in diverse environments in the world due to the exploitation of genes controlling different agronomic traits, genes resisting diseases and human selection in the local environment. Among these agronomic traits, early maturity is controlled by three gene categories: vernalization, photoperiod and *earliness per se* genes. Vernalization and photoperiod genes play main roles on early maturity and *earliness per se* genes are more important for fine regulation. Yield, kernel weight and test weight are more complex than early maturity, there are no main genes that have been found in these traits to explain the genetic controlling mechanism. The decreasing cost of genetic markers and high throughput genetic mapping protocols, genetic linkage maps are being developed to locate QTLs in shorter intervals in artificial

bi-parent population. Moreover, the new widely accepted method of association mapping incorporated more alleles in natural populations and has higher density so as to identify more QTLs; depending on linkage disequilibrium. In association mapping, the increased recombination frequency among several alleles leads to the reshuffling of chromosome segments into smaller pieces, which reduce LD levels over a short distance. Only physically close loci could be found significantly correlated. Through marker assisted selection, breeders may have greater efficiency to target phenotypes of interest without environment interference, and pyramid more genes into single plants.

1.8 Objectives

This thesis is based on four studies, each with two or more specific objectives as described below.

1. Map QTLs associated with flowering time and other phenotypic traits in a RIL population derived from two Canadian Western Hard Red Spring (CWRS) wheat cultivars carrying different alleles on *Vrn-B1 locus*, explore the function of *Vrn-B1* on agronomic traits and study the interactions between the stable QTLs associated with flowering and *Vrn-B1* gene.
2. Study the segregation of *Lr34/Yr18* gene complex, its effects on disease resistance and agronomic traits in a bi-parental Canadian western red spring wheat population; and investigate the efficiency of marker assistant selection in comparison with phenotypic selection.
3. Investigate genetic variation for vernalization (especially VRN1), photoperiod and semi-dwarfing (*Rht*) genes in a Canadian western spring wheat germplasm, quantify the effect of these genes on days to heading, maturity, plant height and grain yield in the field over three years.
4. Explore marker trait associations (MTAs) for days to heading, maturity, protein content, plant height, thousand kernels weight, test weight and grain yield in 82 Canadian historical and modern spring wheat cultivars and explore the linkage disequilibrium between known genes and SNP markers on their own chromosomes.

These objectives were tested under the following null hypotheses:

1. For the RIL population derived from the cross between CDC Teal × CDC Go:

a) There is no polymorphism identified on Vrn-B1 gene.
b) No genomic region affecting days to heading, flowering and maturity, plant height, protein content, sedimentation and yield and its related traits.
c) Disease resistant gene *Lr34/Yr18* has no effect on important agronomic traits as well as leaf rust, stripe rust, tan spot and common bunt.
d) Marker assisted selection combing *Lr34/Yr18* with high grain yield and high sedimentation will fail to select outstanding line in mapping population selection.

2. For the 82 historical and modern Canadian western spring wheat cultivars:
    a) They have same alleles of *Vrn* and *Ppd* and *Rht* genes and no difference will be found on days to heading and maturity, protein content, yield and its related traits.
    b) No marker-trait associations could be found in 82 historical and modern Canada western spring wheat cultivars on days to heading and maturity, protein content, yield and its related traits through SNP markers.
    c) No linkage disequilibrium could be found between candidate genes and SNP markers.

1.9 References

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Chapter 2 Earliness *per se* quantitative trait loci and their interaction with *Vrn-B1* locus in a spring wheat population [1]

2.1 Introduction

The wide adaptation of bread wheat (*Triticum aestivum* L) is partly due to the interaction between flowering and maturation timing, and local environmental conditions. In wheat, flowering time is determined by three gene groups: vernalization (*Vrn*), photoperiod (*Ppd*) and earliness *per se* (*Eps*) genes (Distelfeld et al. 2009). Vernalization genes are associated with temperature requirements in the seedling stage, photoperiod genes with sensitivity to day length and earliness *per se* genes mediate basic development rate. The first two gene groups play more important roles in determining flowering and maturity time than *Eps* genes.

Most vernalization genes are located on homoeologous group 5 chromosomes in wheat, except *VRN3* which is on the short arm of homoeologous group 7 (Yan et al. 2004; Yan et al. 2006; Yoshida et al. 2010). *VRN1, VRN2* and *VRN3* have been identified in diploid and polyploid wheat (Santra et al. 2009; Yan et al. 2004); while only one copy of *VRN4* has been reported on the D genome (Yoshida et al. 2010). A MADS-box transcription factor (Yan et al. 2003) in wheat is encoded by *VRN1* genes, orthologues with *Arabidopsis* meristem identity genes *APETALA1* (*AP1*), the dominant alleles of which are present in spring wheat, alone or in combination. Three recessive alleles in combination result in winter ecotypes in hexaploid wheat (Zhang et al. 2012; Preston and Kellogg 2008; Greenup et al. 2009). Such winter types require a low temperature treatment to reach the reproductive stage. *Vrn-A1* has the strongest effect on accelerating flowering, followed by *Vrn-D1* and *Vrn-B1* (Loukoianov et al. 2005). *Vrn-D1a* accelerated flowering by 32 days compared to *Vrn-D1b*, with fewer number of leaves produced (Zhang et al. 2012).

A model describing the interaction between vernalization genes in wheat has been modified by Chen and Dubcovsky (2012). They suggested the initiation of the flowering pathway is from leaf to apex. In leaves, high expression levels of *VRN2* represses the

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function of *VRN3* before vernalization (which is considered as the integrator of photoperiod and vernalization); during vernalization and long days, the up-regulated *VRN1* in leaves down-regulates *VRN2* to release *VRN3*. Protein production of *VRN3* is transported to the plant apex through the phloem, interacting with *FDL2* up-regulated *VRN1* transcript in the apex, which triggers the switch from vegetative to the reproductive stage.

Earliness *per se* genes related to ear emergence in the field were first reported in 1965 (Yasuda and Shimoyama 1965). Such genes are difficult to identify under field conditions due to their minor effect on flowering time (Yasuda and Shimoyama 1965). *Eps* genes have been identified since the 1980s in controlled environments on chromosomes 2B (Scarth and Law 1983), 2D (Worland 1996), 3A, 4D, 6B (Hoogendoorn 1985), 6D (Law 2013) and 7B (Flood and Halloran 1983). Earliness *per se* genes are considered to be a group with differential effects on different developmental phases of wheat. *Eps* genes in hexaploid wheat have not been studied in as great detail as *Vrn* and *Ppd* genes, in part due to the genome size and complex interaction between genotype and environment in hexaploid wheat (Worland 1996; Slafer 1996; Appendino and Slafer 2003). However, they are important for wheat adaptation under the continuous selection pressure. Diploid wheat (*Triticum monococcum*), with its relatively small genome size, has been widely used to investigate the location and function of *Eps* genes. *Eps-A^m1*, located on the distal region of chromosome 1A^mL (Bullrich et al. 2002), is associated with growth temperature under long-day photoperiods: *Eps-A^m1-early* flowered earlier than *Eps-A^m1-late* lines and flowered earlier at 23℃ than at 16℃ in a single seed descendant (SSD) homozygous lines from the cross DV92×G3116 (Appendino and Slafer 2003). The candidate gene *Mot1* for *Eps-A^m1* exhibited amino acid differences between DV92 and G3116, but the effect was difficult to predict (Faricelli et al. 2010). *Eps-3A^m* has been mapped to chromosome 3A^m (Shindo et al. 2002; Hori et al. 2007; Gawronski and Schnurbusch 2012) using a recombinant inbred line (RIL) population.

In western Canada, *Vrn-A1a* alone or in combination with *Vrn-B1*, *Vrn-D1* represents the major combinations in spring wheat germplasm (Iqbal et al. 2007). The present study was designed to 1) map QTLs associated with flowering time and other phenotypic traits in a population derived from two Canadian Western Hard Red Spring

wheat (CWRS) cultivars carrying different alleles at the *Vrn-B1* locus (Iqbal et al. 2007), 2) explore the function of *Vrn-B1* on agronomic traits, and 3) study the interactions between stable QTLs associated with flowering time and the *Vrn-B1* gene.

2.2 Materials and Methods

2.2.1 Population development

A population of 187 F6:7 recombinant inbred lines derived from two Canadian western red spring wheat cultivars (CDC Teal and CDC Go) was used in this study. CDC Teal is a tall, high yielding cultivar with good leaf and stem rust resistance and possesses the adult plant rust resistance gene *Lr34/Yr18* (Hughes and Hucl 1993) and the dominant allele *Vrn-A1a* at the *Vrn-A1* locus (Iqbal et al. 2007). CDC Go is a semi-dwarf, has high yield potential, strong straw (Hucl, 2004) and has the dominant alleles of the vernalization genes *Vrn-A1a* and *Vrn-B1* (Iqbal et al. 2007).

2.2.2 Field evaluation

The two parents, 187 RILs and seven check cultivars including AC Carberry (DePauw et al. 2011), AC Laura (DePauw et al. 1988), AC Lillian (DePauw et al. 2005), AC Splendor (Fox et al. 2007), CDC Kernen, CDC Osler and Katepwa (Campbell and Czarnecki 1987) were planted at the University of Alberta South Campus Crop Research facility in Edmonton, Canada (53°19'N, 113°35'W) on May 9th in 2011, May 7th in 2012 and May 11th in 2013, respectively. Each experiment was planted in a randomized incomplete block design with three (2012 and 2013) and two (2011) replications. Experimental plots were 4×1.08m, with five rows spaced 23 cm apart. Fertilizer (N-P_2O_5-K_2O:11-52-0) was applied at a rate of 36 kg ha^{-1} at sowing; and other standard agronomic practices were applied throughout the growing season to obtain even crop stands.

We recorded data for days to heading, flowering, maturity, plant height, leaf color, tillers m^{-2}, grain yield, grain protein content, SDS sedimentation volume, thousand kernel weight and test weight (TWt). Days to heading were recorded when 50% of the spikes in a plot had emerged out of the flag leaf, and flowering was recorded when 50% of the spikes in a plot exhibited protruded anthers. Physiological maturity was determined when 50% of the peduncles in a plot had completely lost green color. Leaf chlorophyll concentration was recorded as the mean value of ten flag leaves randomly selected in each plot using a SPAD-

502 meter (Konica-Minolta, Japan). Plant height was measured from the base of the plants to the tip of the heads excluding awns. The number of tillers was counted in a randomly chosen 50×50 cm quadrat consisting of two 50 cm rows in each plot and then this number was adjusted to be the number of tillers m^{-2}. Grain protein content was estimated by Near Infrared Reflectance (NIR) spectroscopy using a SpectraStar RTW apparatus (Unity Scientific SpectraStar^{TM} 2500, Unity Scientific Asia Pacific, Australia). Grain yield per plot was weighed and converted into Mg ha^{-1} on an air dry weight basis. SDS sedimentation volume was measured on whole grain flour samples gathered from an Udy cyclone mill (UDY Corporation, Fort Collins, USA) following approved standard SDS-lactic acid method 56-70 (AACC 2000). We measured grain weight following seed counts from each plot of 200 randomly selected cleaned grains using a digital seed counter (Agriculex Inc. Guelph, Canada). For TWt, we filled a one pint container with a clean grain sample from each plot using a hopper and stand (Seedburo Equipment Co. Des Plaines, USA) and then weighed the sample.

2.2.3 DNA extraction

Four seeds of each RIL and the parents were sown in trays containing commercial soil (Sunshine-LA4 Sun Grow Horticulture, Canada) in a green house. The growth conditions were maintained at 21℃ day and 19℃ night with 16-h daylight and 8-h darkness. Approximately 100-200 mg fresh leaf tissue from two-leaf-growth-stage plants was collected into 2-ml tubes, immediately frozen in liquid nitrogen, and stored in a -80℃ freezer for further processing. Genomic DNA was extracted following the protocol supplied by Diversity Array Technology (http://www.diversityarrays.com). DNA was quantified by NanoDrop® (ND-1000) and the concentration was adjusted to 50 and 100 ng μL^{-1}. 100 µl DNA of the two parents and RILs was sent to Diversity Arrays Technology Pty. Ltd., Yarralumla, Australia, for genotyping against 7000 cloned sequences using protocol similar to that described by Akbari et al. (2006).

2.2.4 *Vrn-B1* evaluation

PCR was performed in a Veriti® Thermal cycler (Applied Biosystems, Foster City, USA) in a total volume of 15 µL. The reaction mixture included 1×PCR buffer, 1.5 mM $MgCl_2$, 150 µM each of dNTPs, 10 pmol each of reverse and forward primers, 50 ng of

template DNA and 1 U of Taq DNA polymerase (QiaGen, Toronto, Canada). The sequences of primers for *Vrn-B1* were: Intr/B/F: 5'- CAA GTG GAA CGG TTA GGA CA -3'; Intr1/B/R3: 5'- CTC ATG CCA AAA ATT GAA GAT GA -3' (Fu et al. 2005). The PCR condition was: initial denaturation steps at 94℃for 10 min, followed by 38 cycles of denaturation at 94℃ for 45s, annealing at 63℃ for 45s, and extension at 72℃ for 43s with a final 5 minutes extension. The amplified PCR products were separated on 1.5% agarose gel stained with SYBR® Safe DNA Gel Stain (QiaGen, Toronto, Canada) and visualized with Typhoon Trio (GE Healthcare Life Science, Quebec, Canada).

2.2.5 Statistical analysis

Analysis of variance (ANOVA) for all traits was performed using PROC MIXED in SAS version 9.3.1 (SAS Institute Inc. Cary, USA). ANOVA was initially done on yearly data and subsequently combined over the 3 years. Each RIL line was considered as a fixed effect to calculate mean, while the effects of replication, incomplete blocks nested in replication were considered as random effects in each year. For the combined ANOVA, year was considered a random effect. The linear models for both ANOVAs were:

For each year:

$$Y_{ijlm} = \mu_i + G_{mi} + R_{ij} + B(R)_{ijl} + \varepsilon_{ijlm} \qquad (1)$$

For overall means:

$$Y_{ijklm} = \mu_i + G_{mi} + T_{ki} + R(T)_{ijk} + B(T*R)_{ijkl} + G_{mi}*T_{ki} + \varepsilon_{ijklm} \qquad (2)$$

In equation (1), μ_i is the mean effect on trait i, G_{mi} is the effect of genotype m on trait i, R_{ij} is the effect of replication j on trait i, $B(R)_{ijl}$ is the effect of block l within replication j on trait i. In equation (2), μ_i is the mean effect on trait i, G_{mi} is the effect of genotype m on trait i, T_{ki} is the effect of year k on trait i, $R(T)_{ijk}$ is the effect of replication j in year k, $B(T*R)_{ijkl}$ is the effect of block l within replication j in year k, $G_{mi}*T_{ki}$ is the interaction between genotype m and year k on trait i.

Broad-sense heritability of each trait on plot basis and phenotypic and genetic correlations among traits were computed by multivariate restricted maximum likelihood (REML) in PROC MIXED of SAS (Holland et al. 2003; Holland 2006).

2.2.6 QTL Analysis

Least square means of genotypes were used to detect QTLs associated with specific traits. Inclusive composite interval mapping was carried out using QTL IciMapping V3.3 (Wang et al. 2011) to detect QTLs influencing traits in individual years and combined over years. We chose ICIM for QTL mapping over other commonly used methods because it is computationally less intensive with a fast convergence rate. For mapping additive QTL effects, ICIM retains all advantages of composite interval mapping (CIM), one of the most commonly used methods for QTL mapping (Zeng 1994). In addition, it also avoids the possible increase in sampling variance and the complicated background marker selection process often encountered in CIM. We applied ICIM's two-step procedure for QTL mapping. First, a stepwise regression analysis was carried out to identify most significant markers in terms of p-values from each regression run by either adding or removing markers. Second, once the set of significant markers were confirmed in the first step, the phenotypic values were adjusted by all markers retained in the regression equation except the two adjacent markers flanking the QTL that were to be tested. Thus, the phenotypic value adjusted in this way contains the information on the position and additive effect of the tested QTL, and it also excludes the influence of any QTL located at other locations on the same or other chromosomes. The threshold LOD (logarithm of the odds) score of ICIM-additive module for declaring the significance of a QTL was determined with 1000 permutations and the type I error rate equal to 0.05. The sliding window width set for chromosome scan for QTLs was 1 cM per run for all traits. Epistatic QTLs were detected using a step of 5 cM in a genome-wide scan, probability of 0.0001 in stepwise regression, and a LOD threshold of 5.0 to claim the significant QTL using ICIM-EPI module. Graphical chromosome maps were generated with the aid of MapChart 2.1 software (Voorrips 2002).

2.3 Results

2.3.1 Phenotypic evaluation

Temperature was normal from May to September over the three trial years, while total precipitation varied considerably (data not given). On average, the earlier heading

parent CDC Go flowered 0.9 days earlier than CDC Teal. It was also 13.8 cm shorter than CDC Teal and yielded 0.3 Mg ha^{-1} more grain. CDC Teal had 1.3 % higher grain protein content and 7.9 mm greater SDS sedimentation volume than CDC Go (Table 2-1). Transgressive segregation was observed in the population for all traits. The highest heritability estimates were observed for SDS sedimentation volume, followed by kernel weight and days to flowering, with tillers m^{-2} exhibiting the lowest heritability value of 21 % (Table 2-2).

Days to heading, flowering and maturity had high to moderate genetic correlation with each other ($r_{hf} = 0.93$; $r_{hm} = 0.65$; $r_{fm} = 0.71$). Grain yield was positively correlated with leaf color (51 %), days to heading (49 %), flowering (40 %) and maturity (49 %); and plant height (24%). Grain yield was negatively correlated with grain protein content and SDS sedimentation. In addition, tillers m^{-2} was negatively correlated with plant height (r_g = -0.47), kernel weight (r_g = -0.68), and SDS sedimentation volume (r_g = -0.47).

2.3.2 *Vrn-B1* evaluation

The parents along with the RIL population were screened for *Vrn-B1* with functional marker (Fu et al. 2005). The expected 709 bp amplicon appeared in CDC Go, which has the spring type allele at the *Vrn-B1* locus, and not in CDC Teal, which has recessive allele *vrn-B1*. Of the 187 lines of the RIL population, 87 lines carried *Vrn-B1* allele and 100 lines *vrn-B1*, and thus there was no evidence of segregation distortion from a 1:1 ratio at this locus (χ^2=0.90<3.84= $\chi^2_{0.05}$). Lines carrying *Vrn-B1* headed 0.5 days ($P < 0.01$), flowered 0.3 days ($P < 0.05$) and matured 0.5 days ($P < 0.01$) earlier than lines with *vrn-B1*. Lines with *Vrn-B1* were taller and had lower test weights and SDS sedimentation volume than lines with *vrn-B1* (Table 2-3).

2.3.3 QTL mapping

Three hundred and forty one polymorphic DArT markers identified for 182 RIL lines derived from the cross of CDC Teal × CDC Go were used to construct the consensus map. The DArT markers and the consensus map were supplied by DArT Pty Ltd, Yarralumla, Australia. The map covered 17 chromosomes, excluding chromosomes 4D, 5A, 5D and 6D, and spanned a total distance of 1911.8 cM (A genome= 654.5 cM; B

genome= 853.1 cM; D genome = 404.2 cM) with an average distance of 9.6 cM between markers.

A total of 21 QTLs were mapped for nine of the 11 studied traits (two for days to heading, three for days to flowering, two for leaf color, four for days to maturity, four for tillers m^{-2}, two for SDS sedimentation, one for thousand kernel weight, two for TWt and one for grain yield). We found no QTL for plant height or grain protein content (Table 2-5, Figure 2-1). Nine of the 21 QTLs were located on A genome, 12 on B genome and no QTL were uncovered on the D genome. Consistent QTLs (those found in at least two years) were mapped for days to heading, flowering, and maturity, leaf color and grain yield. We mapped *QEps-dms.1A* (*QFld-dms.1A and QMat-dms.1A*) on chromosome 1A and *QEps-dms.4A* (*QHed-dms.4A, QFld-dms.4A and QMat-dms.4A.2*) on chromosome 4A for maturity-related traits over all three years (Table 2-4; Figure 2-1). Using ICIM-EPI module, we found one pair of markers with significant epistatic interaction for TKW, sedimentation volume, days to heading and flowering time (Table 2-6).

Lines with the dominant allele at *Vrn-B1*, *QEps-dms.1A* or *QEps-dms.4A* headed, flowered and matured 0.3, 0.8 and 0.5 days, respectively, earlier than those having a recessive allele at one of these loci. Lines carrying three dominant alleles in combination exhibited accelerated heading of 1.7 days, flowering 1.9 days and maturity 4 days (Table 2-5). Dominant alleles of *Vrn-B1* and *QEps-dms.1A* did not alter grain yield. However, lines with the recessive allele of *QEps-dms.4A* yielded 0.38t/ha less than lines with the dominant allele.

2.4 Discussion

We used the CDC Teal × CDC Go spring wheat population to map QTLs for earliness and other important agronomic traits, and to better understand the interaction between alleles of the flowering gene complex on important agronomic traits. We mapped two stable *Eps* QTLs on chromosomes 1A and 4A; and one stable QTL each for grain yield, leaf color, tillers m^{-2} and Twt. The vernalization-insensitive allele at *Vrn-B1* locus reduced days to heading, flowering and maturity, leaf color, plant height, TWt and SDS sedimentation volume, but did not alter tillers m^{-2}, grain yield, TKW or grain protein content. *Eps* QTLs identified in this study interacted in an additive fashion with the

vernalization insensitive gene *Vrn-B1*. We found positive genetic correlations among days to heading, flowering, maturity and grain yield, and negative correlations between flowering-related traits and TKW and grain protein content.

In western Canada, spring growth habit in wheat is mainly determined by *Vrn-A1a* allele (Iqbal et al. 2007; Kamran et al. 2013b). Both CDC Go and CDC Teal carry the insensitive allele *Vrn-A1a* (Iqbal et al. 2007), while CDC Go also possesses the insensitive allele *Vrn-B1*. Thus, this population was ideal to map QTL associated with earliness and agronomic traits and explore the effects of *Vrn-B1* on mapped QTL in the presence of common genetic backgrounds with dominant *Vrn-A1a* allele at *Vrn-A1*. Despite reportedly being the weakest gene for early maturity in the *VRN-1* group (Loukoianov et al. 2005), *Vrn-B1* still significantly altered early maturity related traits, leaf color, TWt, and SDS sedimentation but not grain yield or kernel weight in the present study. A study of Canadian soft white spring wheat cultivars reported that cultivars with *Vrn-A1a* and *Vrn-B1* matured earlier than cultivars with *Vrn-A1a* only (Kamran et al. 2014).

QTLs associated with days to heading, flowering and maturity were located on chromosomes 1A, 1B, 2B, 3B and 4A. Among these, *QEps.dms-1A* was identified for days to flowering (*QFld.dms-1A*) in 2012 and days to maturity (*QMat.dms-1A*) in all three years, and explained 15-27 % of the overall genetic variation and resulted in 1.3-1.9 days earlier maturity in field. According to the consensus map of DArT and SSR markers (Marone et al. 2012), the flanking marker *tpt-1419* of *QEps.dms-1A* was located on the distal end of chromosome 1A near the location of *Eps-A1*. *Eps-A1* has been mapped to 123 cM on this chromosome (http://wheat.pw.usda.gov/GG2/) and exhibited earlier heading in controlled and natural environments (Bullrich et al. 2002). *QEps.dms-4A* on chromosome 4A also resulted in a pleiotropic effect on days to heading (*QHed.dms-4A*), flowering (*QFld.dms-4A*) and maturity (*QMat.dms-4A.2*). It was 1.1 cM away from left flanking marker *wpt-8271* and 0.4 cM from right flanking marker *wpt-9183*. In the present study, this QTL resulted in 0.5-0.8 day earlier heading, 0.5-0.8 day earlier flowering and 0.6-0.7 day earlier maturity. Moreover, the genetic contribution of this QTL was 20 % for days to heading and maturity, and 15 % for days to flowering. This QTL may be the same as *QMat.crc-4A,* which was found linked with *Wx-B1* and maturity in the DH population of RL4452 ×'AC Domain' (McCartney et al. 2005), and *QEet.ocs-4A.1* that was found in single-chromosome

recombinant substitution lines from the cross between Chinese Spring and Kanto 107 4A (Araki et al. 1999), which accounted for about 40% variation in days to heading. On the short arm of chromosome 4A, a meta-QTL (Hanocq et al., 2007) was found controlling earlier heading from 13 different studies. The other four QTLs named *QHed.dms-1B*, *QMat.dms-2B*, *QFld.dms-3B* and *QMat.dms-4A.1* were only found in 2011 in the present study.

Having mapped two new stable QTLs associated with early maturity on chromosomes 1A and 4A in the present study, we were able to study the interactions of *Vrn-B1* with these loci on traits related to maturity and grain yield. We compared lines carrying dominant alleles at the three loci with those having recessive alleles at these loci. Lines with all dominant alleles headed, flowered and matured 1.7, 1.9 and 4 days earlier, respectively, than lines carrying all the recessive alleles. The combined effect of all three loci was almost equal to the sum of the individual additive effects of all three loci (Table 2-5). A similar additive effect of *Ppd-D1a* and population-specific QTLs was reported in the spring wheat RIL population Cutler × AC Barrie (Kamran et al. 2013a). We found positive genetic correlation among traits related to maturity and grain yield, which has also been reported in other studies (Baloch et al. 2003; Zhang et al. 2014). The three loci had an additive interaction on grain yield; the early allele of *QEps.dms-4A* decreased grain yield by 0.38t/ha, while *QEps.dms-1A* and *Vrn-B1* did not alter yield (Table 2-5). However, the three loci in combination resulted in a yield difference of up to 0.43Mg ha^{-1}.

Between marker *wpt-0266* and *wpt-744769* on chromosome 7B, there was a cluster of QTLs associated with yield, TWt, leaf color and SDS sedimentation. The leaf color QTL *QLc.dms-7B* was found in all the three growing seasons, while the yield QTL *QYld.dms-7B* was found in 2012 and 2013. This locus, derived from CDC Teal, increased SDS sedimentation volume, but decreased grain yield, test weight and leaf color. This negative relationship of SDS sedimentation volume and grain yield has been reported in previous studies (Tahir et al. 2006; Li et al. 2012). On chromosome 7B, *QYld.crc-7B* associated with grain yield was found in a DH population of two Canadian wheats 'AC Karma' and 87E03-S2B1 (Huang et al. 2006). In a backcross population, two QTLs controlling TKW were also found on this chromosome (Huang et al. 2004). A pleiotropic QTL located on the same

chromosome explained 15 and 17 % genetic variation on thousand kernel weight and tillers m^{-2}, respectively.

As for the epistatic interactions, the pair of QTLs with significant epistatic interaction for TKW on chromosomes 6A and 7B explained 12 and 38 % of phenotypic variation, respectively. Somewhat surprisingly, one of the two QTLs, *QTkw.dms-7B* identified with additive model was 20 cM away from the same QTL mapped with epistatic interaction model. In 2011 and in combined environments, loci on chromosomes 4A and 6B exhibited an epistatic interaction for sedimentation volume. These QTLs were not found using the additive model. Only two main effect QTLs were involved in epistatic effects. Similarly, the epistatic QTLs for plant height were only involved in 25% of main effect QTLs (Zhang et al. 2008). These observations indicate that individual loci involved in epistatic interactions may not have significant effects on a trait when considered alone, but they may affect the trait through epistatic interactions with other loci.

2.5 Conclusion

The RIL population CDC Teal × CDC Go was developed to investigate the genetics of maturity and yield-related traits. We found 21 QTLs for the nine studied traits in all three years. Lines carrying *Vrn-B1* flowered earlier than those carrying *vrn-B1*; *Vrn-B1* also had a pleiotropic effect on leaf color and SDS sedimentation volume. A group of QTLs was found on chromosome 7B that altered grain yield, kernel weight and TWt. Two stable *Eps* QTL associated with early flowering were mapped on chromosomes 1A and 4A. Lines carrying dominant alleles of the two *Eps* QTL and *Vrn-B1* headed, flowered and matured 1.7, 1.9 and 4 days earlier but yielded 0.43 Mg ha^{-1} less grain than lines with all recessive alleles. The three loci exhibited additive effects.

2.6 References

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2.7 Tables and Figures

Table 2-1 Mean and ranges of leaf color, number of tillers per square meter (tillers), days to heading, flowering and maturity, plant height, grain yield, thousand kernel weight (TKW), test weight (TW), protein content and sedimentation volume among parents, checks and RILs from CDC Teal x CDC Go population during 2011-13.

Traits	Environment	Parents		Parents	Checks	population(n=187)			Heritability
		CDC Teal	CDC Go	Diff.	n=7	Mean±SE	Range	F value	
Leaf Color	2011	40.5	45.6		44.9	43.4±5.4	22.5-53.8	7.28***	
	2012	45.5	48.5		46.9	46.3±3.7	30.7-57.2	7.96***	
	2013	47.6	45.1		44.5	46.6±3.4	32.4-55.6	5.57***	
	Overall	45.0	46.5	-1.5	45.5	45.7±4.3	22.5-57.2	7.44***	0.57
Tillers Number	2011	658	592		658	608±86	376-868	1.45*	
	2012	505	504		528	498±79	328-968	1.29*	
	2013	571	512		537	477±68	240-744	2.07**	
	Overall	568	529	39	564	517±94	240-968	3.08***	0.21
Heading	2011	55.5	55.0		55.8	55.4±1.8	52-60	7.06***	
(days)	2012	54.3	52.3		53.8	53.2±1.7	47-58	12.08***	
	2013	51.7	50.0		51.8	51.1±1.4	47-54	8.27***	
	Overall	53.6	52.1	1.5	53.6	53.0±2.3	47-60	11.65***	0.67
Flowering	2011	60.0	62.5		61.1	60±2	55-65	9.79***	
(days)	2012	60.7	59.0		59.9	58.8±1.9	54-63	15.84***	

Traits	Environment	Parents		Parents	Checks	population(n=187)			Heritability
		CDC Teal	CDC Go	Diff.	n=7	Mean±SE	Range	F value	
	2013	56.7	54.3		55.8	55±1.6	52-59	9.22***	
	Overall	59.0	58.1	0.9	58.7	57.7±2.8	52-65	11.35***	0.69
Maturity	2011	103.0	107.0		101.8	102.5±3	96-113	5.40***	
(days)	2012	93.3	96.7		92.8	92.9±2.9	84-102	6.86***	
	2013	99.7	102.3		98.7	97.8±2.8	91-106	4.68***	
	Overall	98.1	101.4	-3.3	97.3	97.2±4.8	84-113	7.16***	0.52
Height	2011	100.0	87.0		97.7	101.5±9.3	74-128	4.90***	
(cm)	2012	98.7	88.0		98.8	101.2±7.7	70-126	7.23***	
	2013	114.0	96.6		110.8	113.3±8.9	82-140	11.63***	
	Overall	104.8	91.0	13.8	103.0	105.8±10.4	70-140	12.39***	0.66
Yield(Mg ha^{-1})	2011	4.3	4.7		5.3	4.6±0.9	2.2-6.9	4.65***	
	2012	4.1	4.2		3.7	3.6±0.5	1.4-5	6.27***	
	2013	6.0	6.6		4.7	5.4±0.8	2.3-8.2	5.64***	
	Overall	4.9	5.2	-0.3	4.5	4.5±1.1	1.4-8.2	4.72***	0.44
TKW(g)	2011	36.7	37.3		37.1	39.5±4.6	26.3-52.2	24.55***	
	2012	34.9	37.2		33.9	35.4±3.4	25.6-44.4	14.45***	
	2013	41.2	43.0		39.6	42.2±3.8	31-54.5	21.30***	

Traits	Environment	Parents		Parents	Checks	population(n=187)			Heritability
		CDC Teal	CDC Go	Diff.	n=7	Mean±SE	Range	F value	
	Overall	37.7	39.4	-1.7	36.8	38.9±4.9	25.6-54.5	9.91***	0.69
TWT (kg hL^{-1})	2011	.	.	.	.	.	.	.	
	2012	75.9	76.5		76.4	74.6±1.7	69.1-79.1	11.86***	
	2013	79.3	79.6		79.3	78.5±1.5	73.2-82.8	5.46***	
	Overall	77.6	78.1	-0.5	77.8	75.3±2.5	69.1-82.8	6.13***	0.58
WPRO (%)	2011	13.3	12.6		13.3	13.2±0.6	11.38-15.48	4.75***	
	2012	13.1	12.7		13.1	13.1±0.7	11.1-15.3	11.26***	
	2013	14.8	12.1		13.6	13.7±0.9	11.3-19.2	2.67***	
	Overall	13.8	12.5	1.3	13.3	13.4±0.8	11.1-19.2	7.72***	0.5
Sedimentation	2011	.	.	.	.	.	.	.	
(mm)	2012	76.0	75.0		74.9	82.3±10.8	54-113	13.56***	
	2013	80.0	68.3		76.4	81.7±10.5	54-116	10.57***	
	Overall	78.0	70.1	7.9	74.3	80.2±11.5	54-116	18.40***	0.76

Table 2-2 Genotypic and Phenotypic correlation coefficients among leaf color (color), number of tillers per square meter (tillers), days to heading, flowering and maturity, plant height (height), grain yield (yield), thousand kernel weight(TKW), test weight (TW), protein content (WPRO) and sedimentation volume (sed) during 2011-13.

	Color	Tillers	Heading	Flowering	Maturity	Height	Yield	TKW	TW	WPRO	Sed
Color		-	-	-	-	0.24	0.51	0.49	-	0.38	-
Tillers	-0.10		-	0.25	0.18	-0.47	-	-0.68	0.30	-0.23	-0.47
Heading	-	-		0.93	0.65	0.31	0.41	-0.30	0.39	-0.22	-0.36
Flowering	-	0.11*	0.80		0.71	0.34	0.40	-0.29	0.52	-	-0.45
Maturity	-	-	0.46	0.48		-	0.49	-0.27	0.62	-0.37	-0.52
Height	0.14	-0.20	0.26	0.28	-		0.24	0.39	0.18	0.38	-
Yield	0.29	-	0.18	0.19	0.26	0.14		0.24	0.39	-0.19	-0.31
TKW	0.39	-0.32	-0.19	-0.18	-0.14	0.28	0.25		-	0.36	0.41
TW	0.07	-	0.22	0.31	0.37	-	0.25	-		-	-0.32
Protein	0.25	-0.11	-	-	-0.11	0.24	-0.14	0.28	-		0.37
Sed	-	-0.22	-0.24	-0.33	-0.30	-	-0.17	0.33	-0.26	0.27	

Values above and below the diagonal represents genotypic and phenotypic correlation coefficients, respectively
Only significant correlation coefficients given in this table.

Table 2-3 Effect of vernalization insensitive allele Vrn-B1 (early) and sensitive allele vrn-B1 (Late) on leaf color, number of tillers per square meter (tillers), days to heading, flowering, maturity, plant height, grain yield, thousand kernel weight(TKW), test weight(TW), protein content and SDS sedimentation.

Trait	*Vrn-B1*	*vrn-B1*	Difference	SE
Leaf Color	46.2	44.8	1.4**	0.2
Tillers Number	530.4	524.0	6.4	6.0
Heading (days)	53.0	53.4	-0.5**	0.1
Flowering (days)	57.8	58.1	-0.3*	0.1
Maturity (days)	97.5	98.0	-0.5**	0.1
Height (cm)	106.1	104.7	1.4**	0.5
Yield (t/ha)	4.48	4.54	-0.06	0.04
TKW (g)	39.1	38.9	0.2	0.2
TW (kg/hl)	76.4	76.6	-0.2*	0.5
Protein (%)	13.4	13.4	0	0.03
Sedimentation (mm)	76.7	78.1	-1.4*	0.6

*Significant at $P < 0.05$;
**Significant at $P < 0.01$

Table 2-4 Summary of quantitative trait loci (QTL) identified for 11 different traits in CDC Teal x CDC Go mapping population in Edmonton, Alberta, Canada during 2011-2013.

Trait Name	QTL name	Year	Chr.	Position	Left Marker	Right Marker	LOD	PVE(%)	Add
Heading	*QHed.dms-1B*	2011	1B	28.57	wPt-2019	wPt-741749	7.96	35.2	-1.13
	QHed.dms-4A	2012	4A	96.33	wPt-9183	wPt-1161	10.8	26.7	0.81
		2013	4A	95.33	wPt-8271	wPt-9183	6.9	15.4	0.47
		overall	4A	95.33	wPt-8271	wPt-9183	9.0	20.2	0.61
Flowering	*QFld.dms-1A*	2012	1A	120.83	wPt-4897	wPt-8016	6.8	24.3	-1
	QFld.dms-3B	2011	3B	136.11	wPt-0912	wPt-0280	3.9	7.98	0.97
	QFld.dms-4A	2012	4A	96.33	wPt-9183	wPt-1161	10.8	20.2	0.79
		2013	4A	95.33	wPt-8271	wPt-9183	6.5	13.0	0.53
		overall	4A	95.33	wPt-8271	wPt-9183	9.7	15.8	0.62
Maturity	*QMat.dms-1A*	2011	1A	124.83	wPt-4897	wPt-8016	12.8	26.6	-1.92
		2012	1A	125.83	wPt-8016	wPt-7339	7.0	15.0	-1.3
		2013	1A	120.83	wPt-4897	wPt-8016	6.2	27.3	-1.36
		overall	1A	124.83	wPt-4897	wPt-8016	9.5	20.2	-1.31
	QMat.dms-2B	2011	2B	29.21	wPt-1813	tPt-4627	7.6	13.6	1.43
	QMat.dms-4A.1	2011	4A	73.33	wPt-7280	wPt-4596	5.6	25.3	1.8
	QMat.dms-4A.2	2011	4A	95.33	wPt-8271	wPt-9183	3.7	6.1	0.69
		2012	4A	95.33	wPt-8271	wPt-9183	4.4	8.6	0.71
		overall	4A	95.33	wPt-8271	wPt-9183	4.97	8.9	0.66
Leaf color	*QLc.dms-6A*	2013	6A	23.93	wPt-666988	wPt-666224	3.8	31.6	2.43
	QLc.dms-7B	2011	7B	212.31	wPt-2356	wPt-744769	6.0	19.4	-2.29
		2012	7B	212.31	wPt-2356	wPt-744769	3.99	13.9	-1.22
		2013	7B	212.31	wPt-2356	wPt-744769	5.4	17.9	-1.15
		overall	7B	212.31	wPt-2356	wPt-744769	6.2	19.7	-1.44
sedimentation	*QSed.dms-1A*	2012	1A	14.83	wPt-734027	wPt-667558	9.4	14.5	-4.14

Trait Name	QTL name	Year	Chr.	Position	Left Marker	Right Marker	LOD	PVE(%)	Add
		overall	1A	18.83	wPt-734027	wPt-667558	7.9	23.9	-4.5
	QSed.dms-7B	2012	7B	212.31	wPt-2356	wPt-744769	4.1	9.3	3.12
		overall	7B	212.31	wPt-2356	wPt-744769	4.2	10.5	3.06
Tillers	*QTil.dms-1B*	2013	1B	28.57	wPt-3465	wPt-741749	6.2	23.0	-26.76
	QTil.dms-4A	overall	4A	90.33	wPt-6502	wPt-730913	3.6	6.7	-12.04
	QTil.dms-5B	2011	5B	118.97	wPt-1304	tPt-1253	8.5	33.7	48,32
		overall	5B	119.96	wPt-1304	tPt-1253	3.7	7.4	17.72
	QTil.dms-7B	2012	7B	167.31	wPt-1826	wPt-8233	4.1	15.7	-4.85
TKW	*QTkw.dms-7B*	2011	7B	167.31	wPt-1826	wPt-8233	5.1	17.8	2.11
TW	*QTw.dms-1B*	2012	1B	27.57	wPt-3465	wPt-741749	4.1	19.4	-4.51
		overall	1B	28.57	wPt-3465	wPt-741749	5.8	23.2	-4.12
	QTw.dms-7B	2013	7B	198.31	wPt-0266	wPt-2356	4.0	15.9	-2.88
Yield	*QYld.dms-7B*	2012	7B	212.31	wPt-2356	wPt-744769	4.4	15.1	-0.18
		2013	7B	212.31	wPt-2356	wPt-744769	5.1	17.4	-0.26
		overall	7B	212.31	wPt-2356	wPt-744769	5.2	17.8	-0.23

PVE= Phenotypic variation explained.
Add= Additive effect of QTL

Table 2-5 The comparison of the lines with *Vrn-B1a* and QTLs related early maturity to explain interaction between the insensitive alleles (early) and the sensitive alleles (late) for days to heading, days to flowering and days to maturity.

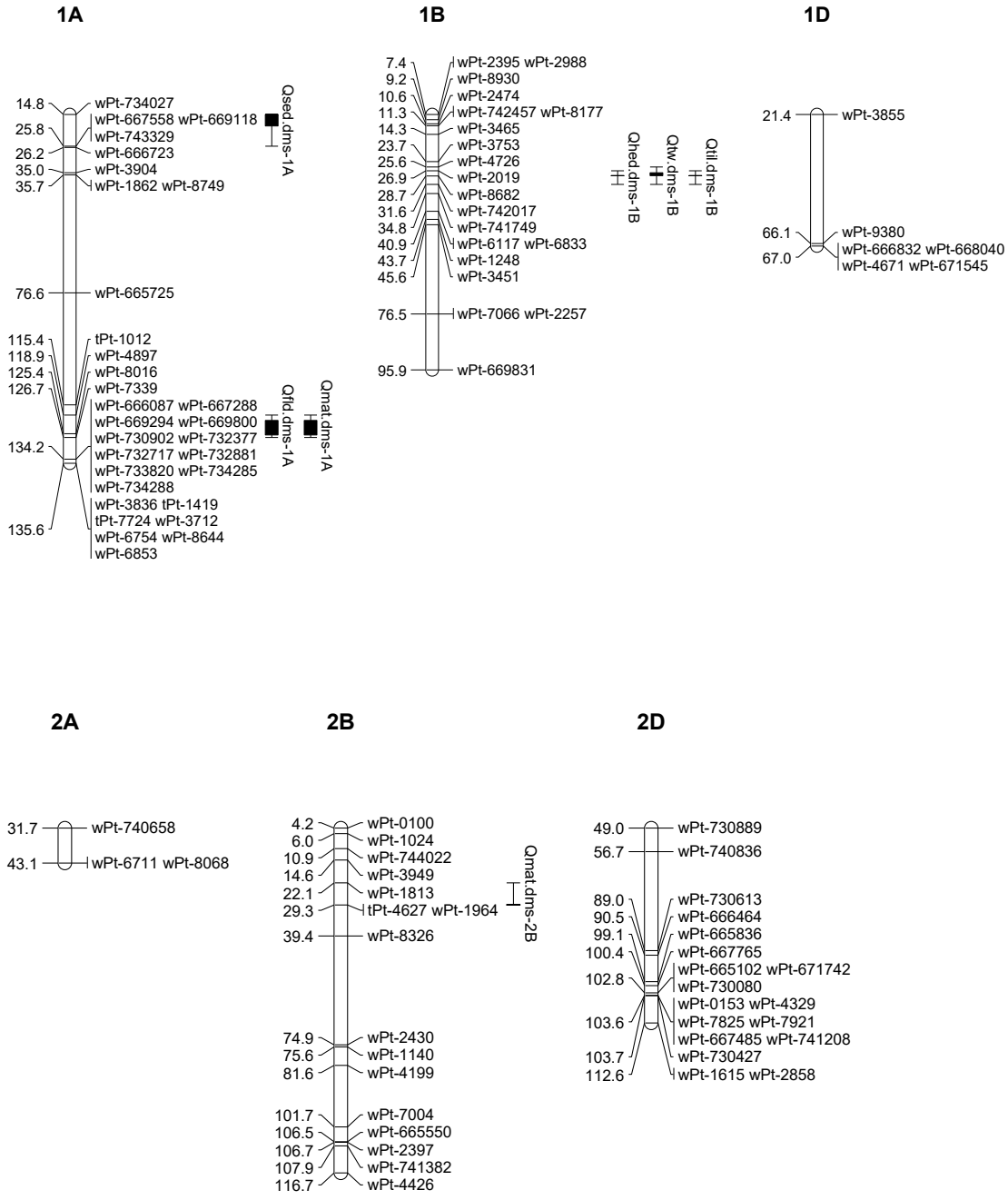
	Heading (Days)			Flowering (Days)			Maturity (Days)			Grain Yield (t/ha)		
Locus	Early	Late	Diff.	Early	Late	Diff.	Early	Late	Diff.	Early	Late	Diff.
QEps-1A[a]	53.2	53.5	-0.3	57.8	58.4	-0.6*	97.6	98.8	-1.2**	4.54	4.48	0.06
QEps-4A[b]	52.9	53.7	-0.8**	57.8	58.4	-0.7*	97.3	99.1	-1.8**	4.32	4.70	-0.38**
Vrn-B1	53.1	53.6	-0.5**	58.0	58.2	-0.2	97.9	98.5	-0.6*	4.47	4.55	-0.08
QEps-1A×*QEps-4A*	53.0	54.1	-1.1**	57.6	58.9	-1.3**	97.1	100	-2.9**	4.45	4.77	-0.32**
QEps-1A×*Vrn-B1*	53.0	53.7	-0.7**	57.7	58.5	-0.8**	97.5	99.3	-1.8**	4.53	4.55	-0.02
QEps-4A×*Vrn-B1*	52.9	54.2	-1.3**	58.1	59	-0.9**	97.2	99.7	-2.4**	4.23	4.70	-0.47**
QEps-1A×*QEps-4A*× *Vrn-B1*	53.0	54.7	-1.7**	57.8	59.7	-1.9**	97.0	101.0	-4.0**	4.38	4.81	-0.43**

a: *QEps-1A* means, *QFld.dms-1A* and *QMat.dms-1A*
b*: QEps-4A* means *Qhed.dms-4A*, *QFld.dms-4A* and *QMat.dms-4A*
*Significant at $P < 0.05$;
**Significant at $P < 0.0$

Table 2-6 Summary of epistatic interactions for thousand kernel weight (TKW), sedimentation (sed), days to heading and flowering in CDC Teal × CDC Go mapping population in Edmonton, Alberta, Canada during 2011-2013.

Trait	Chr.1	Site 1(cM)	Flanking markers	Chr.2	Site 2(cM)	Flanking markers	LOD	PVE(%)	Add by Add
TKW	6A	26.9	wPt-666988~wPt-666224	7B	187.3	wPt-8233~wpt-0266	5.5	38	2.2
TKW 13	6A	31.9	wPt-666988~wPt-666224	7B	187.3	wPt-8233~wpt-0266	5.7	12	1.3
Sed	4A	86.3	wPt-0538~wPt-6502	6B	13.2	wPt-3116~wpt-6594	5.8	9	4.8
Sed 12	4A	86.3	wPt-0538~wPt-6502	6B	13.2	wPt-3116~wpt-6594	5.4	9	5.2
Heading	2D	94.0	wPt-730613~wPt-665836	4A	101.3	wPt-9183~wpt1161	5.4	19	0.6
Flowering	2D	99.0	wPt-730613~wPt-665836	4A	101.3	wPt-9183~wpt1161	5.1	9	0.5

Figure 2-1 Diversity Arrays technology (DArT) marker linkage map of CDC Teal and CDC Go.



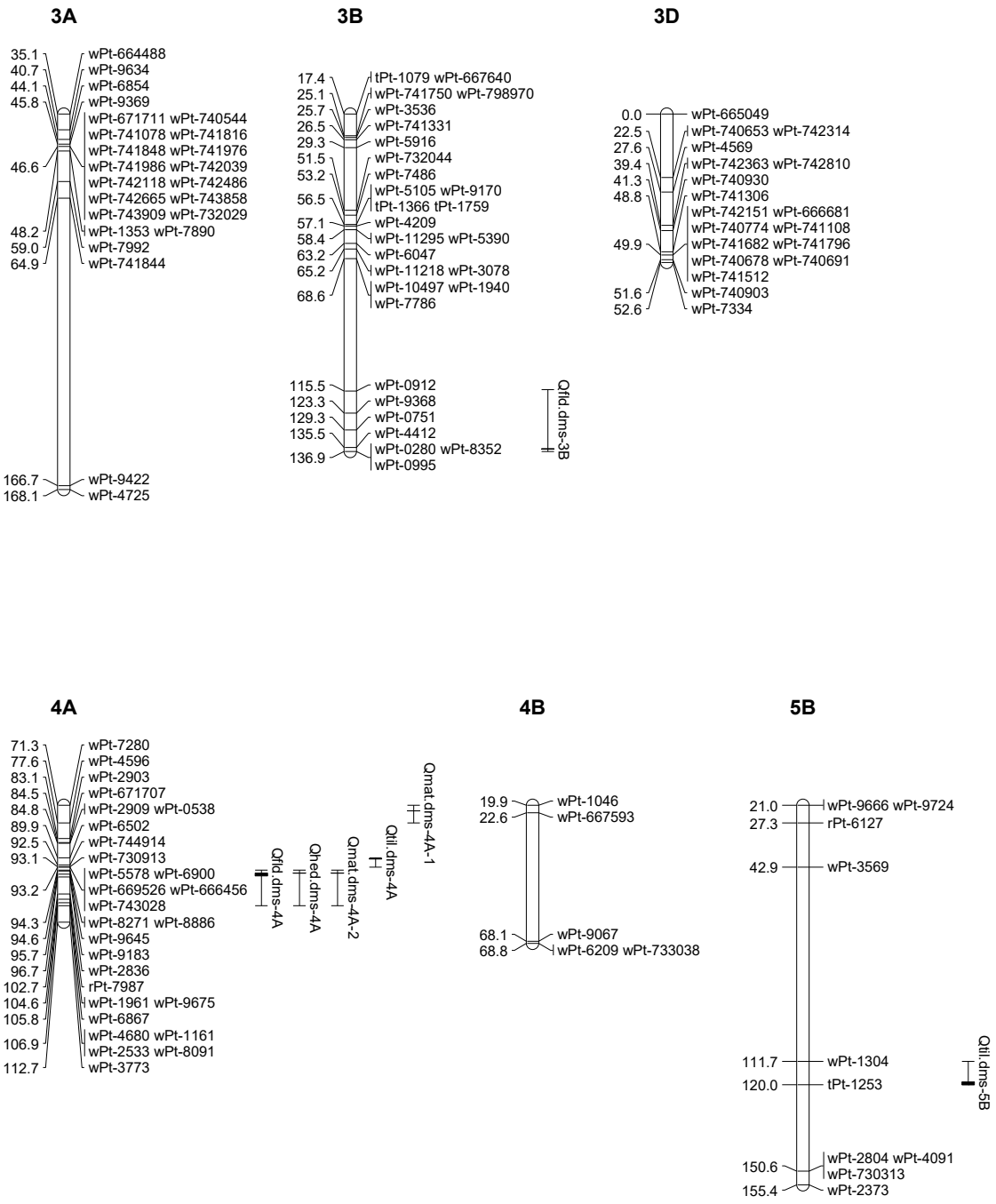


Figure 2-1 (Continued).

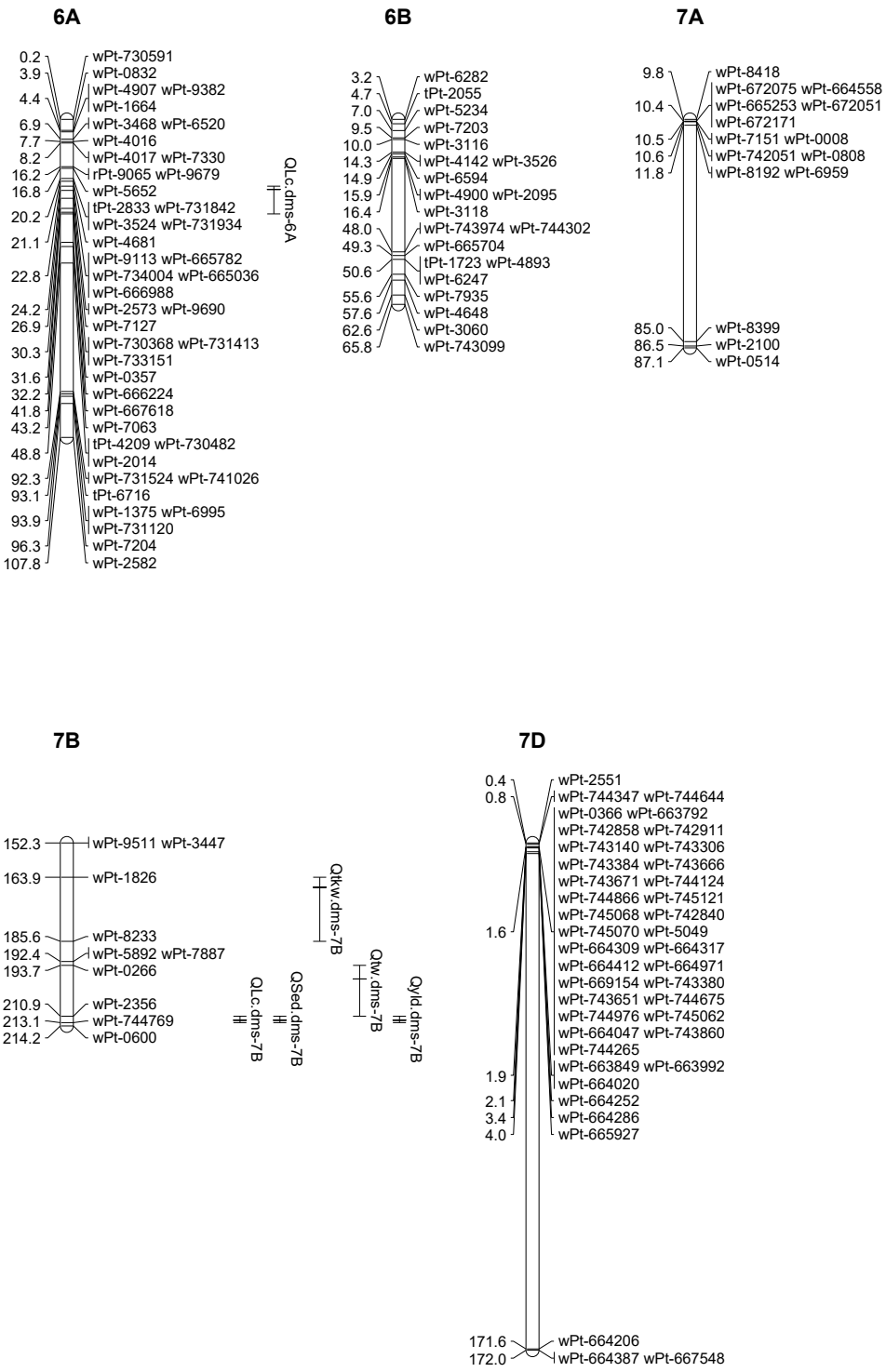


Figure 2-1 (Continued).

Chapter 3 the Effect of *Lr34/Yr18* on important traits in a spring wheat mapping population and marker assisted selection for this gene in a practical breeding program

3.1 Introduction

Plant breeding plays an important role in assembling desirable gene combinations into new cultivars, serving to improve adaptation to specific environments. It is important to take into account gene rich and pleiotropic regions of loci controlling economically important traits in wheat breeding. One such important locus in wheat is *Lr34/Yr18*, which plays an important role in conferring resistance to a number of fungal diseases and is thus an important component of global wheat breeding efforts (Marasas et al. 2003). This gene complex was first reported by Dyck (1987) on wheat chromosome 7D. Most rust resistance genes are race specific and can be overcome by pathogens through the evolution of new races. However, *Lr34/Yr18* is one of a few gene clusters that is non-race specific and therefore confers resistance against a variety of races of rust fungi. This locus carries multiple tightly linked genes including *Lr34* for leaf rust (caused by *Puccinia triticina*) resistance, *Yr18* for stripe rust (caused by *Puccinia striiformis*) resistance and *Pm38* for powdery mildew (caused by *Blumeria graminis*) resistance (Lagudah et al. 2009; Spielmeyer et al. 2005), as well as spot blotch and stem rust (caused by *Puccinia graminis*) (Lillemo et al. 2013; Dyck 1987) resistance genes. *Lr34/Yr18* is predominantly expressed at the adult plant stage and is also associated with leaf tip necrosis (*Ltn1*), a morphological marker. These genes (*Lr34/Yr18*) can provide adequate levels of resistance against leaf/stripe rust in most environments when combined with 3-4 minor genes (Singh 1992).

In response to pathogen infection, *Lr34/Yr18* exhibits increased latent period and reduced intercellular hyphal development as opposed to a hypersensitive response or papilla formation (Kolmer and Broers 1995). Although *Lr34/Yr18* is considered an adult plant resistance gene, it has also been reported to function as seedling stage resistance at 4 to 8℃ (Krattinger et al. 2009). Consistent sequence analysis and eight independent mutations demonstrated that *Lr34/Yr18* encodes an ATP-binding cassette (ABC) transporter (Krattinger et al. 2009), which is not similar to typical race specific resistant genes encoding proteins with a common nucleotide binding site (NBS) domain and an extended domain of leucine-rich repeat (LRR) (McDowell and Simon 2008). Sequence differences between cultivars with and without *Lr34/Yr18* were found in two exons and

one intron. In mRNA level between lines carrying *Lr34/Yr18* and those without *Lr34/Yr18*, both such lines exhibited higher RNA expression levels at the adult than at the seedling plant stage and there were no visible differences between the two (Krattinger et al. 2009). In hexaploid wheat, homoeologous genes of Lr34/Yr18 were also found on chromosome 7A and 4A (partly translated by chromosome 7BS) (Krattinger et al. 2011).

Molecular markers may aid in selecting superior plants carrying desirable alleles (Babu et al. 2004). Compared with phenotypic selection, marker assisted selection (MAS) theoretically shortens the breeding cycle and is independent of environmental variation (Collard and Mackill 2008). Marker assisted selection can be applied in early growth stages and, therefore, theoretically eliminates the requirement of growing plants until the adult plant stage or until maturity; two processes obviously required for phenotypic selection. Flanking SSR markers were originally used to map the position of *Lr34/Yr18* in earlier studies. Later, the co-dominant STS marker *csLV34*, 0.4 cM from *Lr34/Yr18*, was used to detect the presence/absence of this gene (Lagudah et al. 2006). Lagudah et al. (2009) developed five allele specific markers based on a 3 bp deletion in Exon 11 of the *Lr34/Yr18* gene. The combination of two dominant markers (*Xcssfr1* and *Xcssfr2*) serves as a co-dominant marker to determine the presence (571 bp) and absence (523 bp) of the *Lr34/Yr18* gene complex. Recently, SNP markers designed to explore the differences in exon and intron have been applied to identify the allelic variations and haplotypes in wheat germplasm based on the full length of the *Lr34* sequence. Kuchel et al. (2007) employed MAS for selecting lines with *Lr34/Yr18* and *Lr46/Yr29* gene complexes and reported a significant improvement in rust resistance of the selected lines. Similarly, Lillemo et al. (2008) also observed a large effect of these two gene complexes on resistance to stripe and leaf rust in bread wheat line 'Saar'. A number of studies have been conducted on the use of DNA markers for determining the presence/absence of genes controlling resistance to rusts, insects, wheat quality and important agronomic traits (Liu et al. 2014).

The objective of our present study was firstly to study the segregation of the *Lr34/Yr18* gene complex and its effects on agronomic traits and disease resistance in a bi-parental (CDC Teal × CDC Go) Canadian western red spring wheat RIL mapping population grown over three years. In addition, we used results from the first year phenotyping of the mapping population to examine a real plant breeding question. We selected superior lines from the population based on high yield,

protein, SDS sedimentation and the presence of the resistant allele of *Lr34/Yr18*. We thereafter grew the superior 10%, based on these selection criteria, in replicated, multi-site, multi-year yield trials. We were attempting to obtain the breeding result of combining *Lr34/Yr18* with high yield, protein, and SDS sedimentation appropriate to the Canadian Western Hard Red spring classification of wheat. Our breeding questions: would MAS for *Lr34/Yr18* eliminate superior genotypes in a practical breeding program and could we select a superior line with this strategy in a mapping population?

3.2 Materials and Methods

3.2.1 Plant materials

A population of 187 $F_{6:7}$ recombinant inbred lines derived from a cross between two Canadian western red spring wheat cultivars, CDC Teal and CDC Go, along with the two parents and three check cultivars, AC Splendor, CDC Osler and Katepwa, were used in this study. CDC Teal was registered in 1993; it is early maturing, has good yield potential and leaf rust and stem rust resistance and carries *Lr34/Yr18* (Hughes and Hucl 1993). CDC Go was registered in 2003; is semi-dwarf with strong straw features (Canadian Wheat Board, 2010). In Alberta, the area grown with CDC Teal decreased from 35,889 ha in 2009 to 11,465 ha in 2013 and its average yield was 2.71 t ha^{-1}(Statistics 2015). Conversely, the area under CDC Go increased from 88,329 ha to 222,845 ha during these five years and its average yield was 3.41 t ha^{-1} (Statistics 2015). AC Splendor was developed by Agriculture and Agri-Food Canada and licensed in 1996. It was reported as early maturing, having high protein content, good stem and leaf rust resistance and intermediate resistance to common bunt (Fox et al. 2007). CDC Osler was released by the Crop Development Centre, Saskatoon, SK (Hucl, 2004). It is an awnless, strong-strawed cultivar with high yield and early maturity, resistant to leaf and stem rust but susceptible to FHB. Katepwa (Campbell and Czarnecki 1987) was released in 1981. It has good milling and baking properties and intermediate resistance to sprouting and stem rust.

3.2.2 Mapping Population Study

The two parents, 187 RILs and three check cultivars including AC Splendor (Fox et al. 2007), CDC Osler and Katepwa (Campbell and Czarnecki 1987) were planted at the University of Alberta South Campus Crop Research facility in Edmonton, Canada, (53°19'N, 113°35'W) on May 9th in 2011, May 7th in 2012 and May 11th in 2013, respectively. Agronomic details, data collection protocols are described in Chen et al. (2015).

Disease Screening

The population of 187 RILs along with parents and three checks were planted in a randomized incomplete block design with two replications in leaf rust and tan spot screening nurseries in Edmonton during 2011-13. Reaction to stripe rust was tested at Lethbridge, AB (49.7° N, 112.83° W) during 2012-2013 and at Creston, BC (49.06° N, 116.31° W) during 2013. Ten seeds of each line were planted per hill with 25cm between hills or rows. Spreader rows of susceptible cultivars and check cultivars were planted every three rows. In the leaf rust nurseries, wheat cultivars AC Barrie (McCaig et al. 1996), Park (McCallum and DePauw 2008), Carberry (DePauw et al. 2011) and Peace (McCallum and DePauw 2008) were used as checks, while AC Barrie, Glenlea (Evans et al. 1972), Neepawa medium resistant to common bunt (Fu et al. 2005) and Unity (Fox et al. 2010) were used as checks in the leaf spot nurseries.

Leaf rust epidemics were established by inoculating the entire nursery with mixture of urediniospores of leaf rust races collected from spreader rows from the previous year in the nursery. Rust severity ratings and reaction types were recorded using a modified Cobb scale (Peterson et al. 1948), when the spreaders reached maximum severity. The host reaction type was on a scale from 0 (no pustules=resistant) to 9 (leaf area totally covered with pustules=highly susceptible) on each hill plot basis.

For leaf spot screening, plants were sprayed with a spore suspension of isolate AB7-2 (race 1) of *P.tritici-repentis* at heading. AB7-2 is a dominant race in Alberta, Canada. When wheat grains were at milk-stage, the infection scores were recorded on a scale of 0-10 to represent the percentage leaf area diseased. For bunt screening, seeds of each line were mixed with excess of bunt spores in an envelope (Sukhwinder et al. 2003). The bunt inoculum consisted of an even mixture of race T-19 of *Tilletia laevis* and race L-16 of *Tilletia tritici*. At dough stage, all heads of each line in a hill plot were examined for bunt infection. The rate of infection was recorded as the ratio of the number of grains infected to the total number of grains per hill plot. Stripe rust infection resulted from natural inoculum and the nurseries were evenly planted with susceptible spreader rows every 6^{th} row. Disease resistance score were based on the average percentage of leaf area covered with pustules at anthesis as described for leaf rust.

Marker Analysis for Lr34/Yr18

Genomic DNA extraction protocol has been described for this population in Chen et al. (2015). The primer sequences of the functional marker cssfr5 were: Lr34DINT9F: 5'- TTG ATG AAA CCA GTT TTT TTT CTA -3'; Lr34MINUSR: 5'-TAT GCC ATT TAA CAT AAT CAT GAA -3'; Lr34SPF: 5'- GGG AGC ATT ATT TTT TTC CAT CAT G -3'; Lr34DINT13R2: 5'- ACT TTC CTG AAA ATA ATA CAA GCA -3' (Lagudah et al. 2009). The PCR conditions were: 5 cycles each of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and elongation at 72°C for 2 min; followed by 30 cycles each of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and elongation at 72°C for 50 s and elongation at 72°C for 5 min. The amplified PCR products were separated on 1.5% agarose gel, stained with SYBR® Safe DNA Gel Stain (QiaGen, Toronto, Canada) and visualised with Typhoon Trio (GE Healthcare Life Science, Quebec, Cana da).

3.2.3 Breeding selection experiment

We selected 16 (≈10 %) superior lines from the population based on high yield, protein, SDS sedimentation and the presence of the resistant allele of *Lr34/Yr18* from the first year (2011 cycle) of the field phenotyping experiment of the CDC Teal × CDC Go mapping population. The 16 lines we selected for replicated, multi-site yield trials with checks are shown along bell curves of the resistant *Lr34/Yr18* members of the population for protein and yield in Figure 3-1. The 16 selected lines along with CDC Teal and the previously mentioned three check cultivars (chosen because these four cultivars were the check cultivars for the western Canadian variety registration trials at the time) were planted at Edmonton, AB, Ellerslie, AB (53°43'N, 113°45'W), Lethbridge, AB, Lamont, AB (53°46'N, 112°48'W) and Fort St. John (54°14'N, 120°44'W) BC in 2012. The trials were planted in randomized complete block designs with four replications. Agronomic treatment of trial sites followed standard protocols and data collection for yield trials is described in Chen et al., (2015). Following 2012 data collection and analyses, we chose the five superior lines based again on yield, protein, SDS sedimentation and the presence of *Lr34/Yr18*. These five lines along with CDC Teal, CDC Go and the previously mentioned three check cultivars were planted at Edmonton AB, Ellerslie AB, Neapolis, AB (51°65'N, 113°86'W) and Fort St. John BC in 2013.

Following completion of the 2013 replicated, multi-location yield trials, we compared the performance of our five selected lines with the three-year average performance of the best 20 lines (disregarding marker information for *Lr34/Yr18*) from the entire mapping population based on the

similar agronomic criteria of yield, protein, SDS sedimentation, as well as stripe and leaf rust resistance when grown in disease nurseries.

3.2.4 Statistical analyses

Statistical analyses for the mapping population have been described by Chen et al. (2015). For the breeding selection experiments, analysis of variance (ANOVA) for all agronomic traits was performed using PROC MIXED in SAS version 9.3.1 (SAS Institute Inc. Cary, USA). All data were analysed independently by the following linear model:

$$Y_{ijk} = \mu + E_j + R(E)_{kj} + G_i + G_i * E_j + \varepsilon_{ijk} ,$$

Where G, E and R were the genotypes, environments and replicates, respectively. All terms were analysed as fixed effects except G_i, which was considered a random effect.

The diseases reaction difference between lines with and without *Lr34/Yr18* were used in non-parametric analysis PROC NAPE1WAY, since data did not fit to normal distribution. For the two-sample data, it uses simple linear rank tests:

$$S = \sum_{i=1}^{n} c_i \, a(R_i)$$

where, R_i is the rank of the individual *i*; $a(R_i)$ is the score based on that rank; c_i is an indicator variable denoting the class to which the *i*th observation belongs based on the alleles of *Lr34/Yr18*, and n is the total number of the observations (SAS Institute Inc. Cary, N.C., USA). Disease data for each environment were separately analyzed due to possible variation in the pathogen races in different years.

3.3 Results

3.3.1 Mapping Population study

PCR amplification of genomic DNA with STS marker cssfr5 revealed a 523 bp amplicon in CDC Go, suggesting the absence of *Lr34/Yr18*. The 751 bp allele of cssfr5 was found in CDC Teal, indicating the presence of *Lr34/Yr18*. Among the 187 RILs, 102 lines carried *Lr34/Yr18;* 82 lines lacked this gene, while three lines were heterozygous at the *Lr34/Yr18* locus. These results followed a χ^2 distribution (χ^2=2.17<3.84= $\chi^2_{0.05}$). On average, the earlier heading parent CDC Go flowered 0.9 days earlier than CDC Teal. It was also 13.8 cm shorter than CDC Teal and yielded 0.3 Mg ha^{-1} more grain. CDC Teal had 1.3 % higher grain protein content and 7.9 mm greater SDS

sedimentation volume than CDC Go [see (Chen et al. 2015)]. Field trial results indicated that lines carrying *Lr34/Yr18* were 2cm taller ($P<0.01$), matured 0.6 day earlier ($P<0.01$), yielded 0.13 Mg ha^{-1} less grain weight with lower test weights ($P<0.01$) than lines without *Lr34/Yr18*. There were no differences ($P>0.05$) in grain protein content and sedimentation volume for lines with or without *Lr34/Yr18* (Table 3-1).

Both CDC Teal and CDC Go exhibited low leaf rust scores of 0 and 1, respectively, in an inoculated nursery in 2011 (Table 3-2). In 2012 and 2013, CDC Teal (*Lr34/Yr18* resistant allele) was intermediate to moderately resistant (3.5-5), while CDC Go (*Lr34/Yr18* susceptible allele) was moderately susceptible to susceptible (6-7.5). Average infection score of the population ranged from 1.4 in 2011 to 6.5 in 2013. Lines with *Lr34/Yr18* exhibited significantly ($P<0.0001$) lower leaf rust infection than lines without *Lr34/Yr18* over three years. Stripe rust resistance of the population was tested in Lethbridge, AB and Creston, BC over two years. Lines with *Lr34/Yr18* were generally resistant, whereas, lines without *Lr34/Yr18* were intermediate to weakly resistant in Lethbridge (Table 3-2). CDC Teal exhibited high to medium resistance to leaf spot disease with scores ranging from 2 to 5.5, while CDC Go was highly susceptible to leaf spot over the three years. No difference for reaction to leaf spot and common bunt was found between lines with and without the resistant allele of *Lr34/Yr18* (Table 3-2).

3.3.2 Breeding Selection Experiment

We selected 16 (≈10 %) superior lines from the 2011 planted trial of the CDC Teal × CDC Go mapping population. Our selections were based on high yield, grain protein content SDS sedimentation (Figure 3-1 (a) and (b)) and we chose only those with the presence of the resistant allele of *Lr34/Yr18*, as determined in the laboratory. Among the 16 selected lines, TG096 was the only line yielding numerically more than CDC Teal, the lower yielding parent, in multi-site trials in 2012. The yield difference, however, was not significantly ($P>0.05$) different (Table 3-3). All lines exhibited protein and SDS sedimentation attributes within the range of the checks and therefore suitable for CWRS designation in the Canadian system of rating hard red spring wheat. Only one line (TG041) was shorter than the taller parent (CDC Teal).

To enter a line into the official registration trials in western Canada, that line must have been tested in replicated, multi-environment studies over two years prior. We therefore chose the five top yielding lines from the 2012 multi-site replicated yield trials for testing in 2013 along with

check cultivars and both parents. None of the five selected lines yielded statistically (P>0.05) more grain than CDC Teal or CDC Go. The three highest yielding lines (TG096, TG142, and TG156) were all taller than the tall parent CDC Teal and lodged more than all checks (Table 3-4). All five lines could have been chosen for testing in official registration trials as they exhibited numerically higher grain yield than the parent CDC Teal, possessed *Lr34/Yr18*, and had quality attributes of the CWRS class. Nevertheless, they were all taller than the tall parent (some also had quite high lodging scores), and their yields were not statistically greater than the lowest yielding parent, which also has the resistant allele of *Lr34/Yr18*. We tested two of the highest yielding lines in the pre-registration "B" level yield trials in 2014 but both lodged heavily and were, therefore, culled (data not shown).

Following the process of selection and yield testing of lines from the original population, we are able to review the progress made from the selection decisions in 2011. Our original selection was based on high yield, grain protein, SDS sedimentation and the presence of resistant allele of *Lr34/Yr18.* Table 3-5 presents the three year means for rust disease reactions, and all agronomic traits recorded for the top 20 yielding lines of the CDC Teal × CDC Go mapping population along with three lines from the multi-site yield trials of 2013 (which did not rank in the top twenty), and the parents and check cultivars. Using our selection criteria we eliminated three lines (TG033, TG071, and TG114) not possessing the resistant allele of *Lr34/Yr18* but with yield potential greater than CDC Teal. Only TG033, however, had average leaf and stripe rust scores acceptable for registration in Canada.

Out of the population of 187 lines, we did not find a single line in this study yielding greater than the highest parent and having acceptable protein and SDS sedimentation levels of the CWRS class of wheat. High yielding lines with the resistant allele of *Lr34/Yr18* did not yield as much grain as the high yielding parent CDC Go and were taller with weak straw. High yielding lines with the susceptible allele of *Lr34/Yr18* were generally susceptible to leaf and stripe rust. One line with the susceptible allele of *Lr34/Yr18* (TG033) had acceptable yield and quality attributes but was not selected because it lacked the resistant allele of *Lr34/Yr18*.

3.4 Discussion and Conclusions

3.4.1 Mapping Population study: the effect of *Lr34/Yr18*

In this study, we investigated the effects of *Lr34/Yr18* on agronomic traits and four diseases (leaf and stripe rust, leaf spot and common bunt) in the Canadian spring wheat mapping population CDC Teal × CDC Go. On average, lines carrying the resistant allele of *Lr34/Yr18* were taller, matured earlier, yielded less grain and had lower test weights than lines without *Lr34/Yr18*. Lines with or without the resistant allele of *Lr34/Yr18* did not differ for grain protein content and or sedimentation volume. Lines with the resistant allele of *Lr34/Yr18* exhibited lower leaf and stripe rust infection than lines with the susceptible allele. Lines with and without the resistant allele of *Lr34/Yr18* did not differ for resistance to leaf spot or common bunt.

Previous studies have reported that wheat cultivars with *Lr34/Yr18* alone or in combination with other resistance genes, had lower yield reduction than cultivars without *Lr34/Yr18* under high leaf rust infection (McCallum et al. 2007; Sayre et al. 1998; Drijepondt et al. 1990). For example, 'Thatcher' (susceptible) had 10 times higher severity than 'RL6058' (resistant). This resulted in a 25 % reduction in grain yield and a 16 % in grain weight in 'Thatcher' but no or little reduction in 'RL6058' (Drijepondt et al. 1990). Our results are in agreement with other studies conducted in rust free environments in which cultivars with the susceptible allele of *Lr34/Yr18* yielded more grain than those with the resistant allele. Lines with *Lr34/Yr18* yielded 3 % lower and had 1 % lower test weight than lines without *Lr34/Yr18* in our study. In a study of 22 near isogenic lines, 11 lines with *Lr34/Yr18* had 5.9% lower mean grain yield as well as lower biomass, kernels per spike and kernels m^{-2} than lines without *Lr34/Yr18* (Singh and Huerta-Espino 1997). A yield penalty associated with disease resistance genes has been reported in wheat and in other cereals. In wheat, the eyespot resistance gene *Pch1* (Worland et al. 1990) and leaf rust resistance gene *Lr9* (Ortelli et al. 1996) also hampered yield. In barley, the mildew resistance gene *mlo* was reported to be associated with grain yield reductions; the resistant lines had 4.2% lower mean yield and 5.4% lower grain weight than the susceptible lines in a doubled haploid population (Brown 2002). There are two possible explanations for the association of disease resistance genes and yield reduction. Firstly, disease resistance genes may be tightly linked with genes negatively affecting yield. Thus, breaking this negative linkage is a good way to solve this problem. Secondly, resistance genes may have pleiotropic effects on yield. This may be the case in our study. Wheat cultivars with *Lr34/Yr18* almost always shows leaf tip necrosis on the flag leaf, which decreases

the green leaf area and possibly photosynthesis efficiency, ultimately resulting in grain yield reduction (Parry et al. 2010).

Lines with the resistant allele of *Lr34/Yr18* were, on average, more resistant to leaf and stripe rust in all environments than those without this allele. Lines with and without the resistant allele of *Lr34/Yr18* did not differ for resistance to tan spot and common bunt. In a doubled haploid population of a cross between Japanese wheat 'Fukuho-komugi' and Israeli wheat 'Oligouculm', *Lr34/Yr18* had the largest effect on both leaf and stripe rust severities (Suenaga et al. 2003). The importance of incorporating resistant genes in wheat cultivars has been long recognized in wheat breeding. Absence of rust resistance genes and/or the presence of only race-specific genes have led to rust epidemics in many parts of the world, including stem rust epidemics in Canada (McCallum et al. 2007), and stripe rust epidemics in China (Wan et al. 2007) and the USA (Chen 2007).

3.4.2 Breeding Selection Experiment

We used the first year phenotyping results of the mapping population to examine a real life plant breeding question. We selected superior lines from the population based on high yield, protein, SDS sedimentation and the presence of the resistant allele of *Lr34/Yr18*. We thereafter grew the superior 16 lines (10% of the population), based on these selection criteria, in replicated, multi-site, multi-year yield trials. We were attempting to combine the resistant allele of *Lr34/Yr18* with high yield, grain protein, and SDS sedimentation suitable for the Canadian Western Hard Red spring class of wheat. This study was conducted using a standard mapping population size of $F_{6:7}$ 187 recombinant inbred lines derived from a cross between two Canadian western red spring wheat cultivars, CDC Teal and CDC Go. In wheat, population size of as low as 79 RILs from a cross between the Canadian hard red spring wheat cultivar AC Barrie and the CIMMYT derived cultivar Attila has been used by Reid et al. (2011) for grain yield selection in conventional and organically managed system. In lupine breeding, an F_8 population of 187 RILs was used to select resistant lines for stem blight disease (Yang et al. 2013). Six RIL populations in the F_6 generation were used to identify lines carrying rice blast resistance genes *Pi25* and high yield in rice (Zhou et al. 2008). Given that our original selection criteria included the presence of the resistant allele of *Lr34/Yr18*, we eliminated 82 lines at the outset and chose high yielding, high protein, elevated dough strength

lines out of the remaining 102 lines. From this subset, we were able to combine the resistant allele of *Lr34/Yr18* with reasonable yield potential and adequate quality attributes of the CWRS wheat class. Unfortunately all of these lines were tall with weak straw. We also missed one line of good agronomic potential with reasonable rust reaction, but with the susceptible allele of *Lr34/Yr18*, through our selection strategy.

Our results from our three year population study indicated that there were transgressive segregants for all traits in this population. Thus our population size was simply not sufficient to select one marker (*Lr34/Yr18*) and obtain high yielding lines that were as short or strong-strawed as the parent CDC Go, combined with dough strength and protein levels of the CWRS class of wheat. In artificial selection, keeping population size as large as possible is a common recommendation for selection programs. Large population size reduce the effect of inbreeding on the fixation of undesirable genes and reduces the loss of genetic variation (Hill and Robertson 1966). To obtain rice restorer line resistant to blast and bacterial blight in rice breeding, two parents and 883 RILs showed polymorphism among 25 crosses between five blast and five bacterial blight resistant germplasm accessions and only one line named R8012 was selected from this population. This one line out of 883 RILs exhibited dual resistance to bacterial blight and blast, good grain quality and high yield potential (Zhan et al. 2012). In soybean breeding, two cycles of marker assisted selection were used to develop two lines with transgressive seed protein content. A new RIL population of 262 F_2 plants was further developed from the two selected lines. After the second cycle of MAS based on three loci associated with seed protein content, five F_2-derived superior families were selected which were, on average, higher than the high parent value (Zhang et al. 2015).

Our original question from the introduction of this paper was: would MAS for the resistant allele of *Lr34/Yr18* eliminate superior genotypes in a practical breeding program and could we select a superior line with this strategy in a mapping population? Our results suggest that selecting for only one marker reduces the effective population size in half. Thereafter, selection for yield, protein, elevated dough strength and disease resistance requires much larger populations than a standard mapping population study. We suggest that single seed descent or doubled haploid populations to select for simply one marker and multiple quantitatively inherited traits would require population sizes in excess of 500 individuals to have any possibility of selection success.

Much greater population sizes would obviously increase the potential of pooling genes into a superior genotype. Failing to obtain such a population structure implies that bulk or modified bulk selection strategies may be more appropriate.

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3.6 Table and figures

Table 3-1 Effect of the resistant allele of Lr34/Yr18 (present) and the susceptible allele of Lr34/Yr18 (absent) on leaf color, tillers number, days to heading, flowering and maturity, plant height, grain yield, thousand kernel weight (TKW), test weight, protein content and SDS sedimentation in the CDC Teal × CDC Go mapping population grown 2011 to 2013.

Trait	*Lr34/Yr18*			
	Present	Absent	Difference	SE Diff.
Leaf color	45.0	46.1	-1.1*	0.21
Tillers number	132	132	0.0	0.98
Heading (days)	53.1	53.4	-0.3**	0.09
Flowering(days)	57.7	58.1	-0.4**	0.10
Maturity(days)	97.5	98.1	-0.6**	0.15
Plant Height(cm)	106	104	2**	0.45
Grain yield(t/ha)	4.46	4.59	-0.13**	0.04
TKW(g)	38.9	39.1	-0.2	0.24
Test weight(kg/hl)	76.3	76.7	-0.4**	0.10
Sedimentation(mm)	77.3	77.8	-0.5	0.59
Protein content (%)	13.3	13.3	0.0	0.04

*: significant at the 0.05 probability level

**: significant at the 0.01 probability level

***: significant at the 0.001 probability level

Table 3-2 Mean and significance of the difference† for the resistant allele of Lr34/Yr18 (present) and susceptible allele of Lr34/Yr18 (absent) on leaf rust, leaf spot, common bunt and stripe rust the CDC Teal × CDC Go mapping population grown in disease nurseries 2011 to 2013.

Year	*Lr34/Yr18*	Leaf Rust[1]		Leaf Spot[1]		Common Bunt[1]		Stripe Rust[2]		Stripe Rust[3]	
		Mean	P value†	Mean	P value	Mean	P value	Mean	P value	Mean	P value
2011	CDC Teal	0.0		5.0		8.5		-		-	
	CDC Go	1.0		7.0		0.6		-		-	
	Present	1.3	0.008	6.1	0.32	10.7	0.32	-	-	-	-
	Absent	2.4		5.9		10.1		-		-	
2012	CDC Teal	5.5		2.0		8.1		1.0		-	
	CDC Go	8.0		5.0		3.8		3.0		-	
	Present	4.4	0.02	5.5	0.98	19.6	0.78	2.1	<0.0001	-	-
	Absent	5.1		5.5		18.9		4.0		-	
2013	CDC Teal	3.5		6.0		12.6		1.5		1.0	
	CDC Go	6.5		8.0		21.5		4.3		3.0	
	Present	5.8	0.02	7.2	0.15	27.3	0.91	3.5	<0.0001	2.5	0.15
	Absent	7.1		7.8		27.6		5.2		2.6	

1: Leaf rust, leaf spot and common bunt nurseries were grown in Edmonton AB
2. Stripe rust nurseries were grown in Lethbridge AB
3. Stripe rust nursery grown in Creston BC.

Table 3-3 Least square means and statistical analyses of plant height, days to maturity, test weight, grain protein content (WPRO), sedimentation (Sed.) and yield with rank by yield for sixteen selected lines from the CDC Teal× CDC Go mapping population grown replicated yield trials in 2012 over five locations (Edmonton, Ellerslie, Fort St. John, Lamont and Lethbridge) in BC and AB, Canada.

Entry	Lodging (1-9)	Height (cm)	Maturity (days)	Test weight (Kg/hl)	WPRO (%)	Sed. (mm)	Yield (Mg/ha)	Rank by Yield	Yield of Teal
AC Splendor	2.0	95	95	74.7	13.8	97.4	3.28	8	96
CDC Osler	1.8	90	94	75.4	13.8	83.0	3.52	2	103
CDC Teal	1.9	93	94	74.9	13.5	89.6	3.42	3	100
Katepwa	1.9	97	94	74.9	13.2	80.8	3.36	5	98
TG035	1.8	99	97	74.0	13.4	93.6	3.24	10	95
TG041	1.9	91	94	72.8	13.9	94.2	2.97	19	87
TG042	2.3	95	94	74.3	13.8	89.8	3.41	4	100
TG064	2.3	99	99	74.8	14.5	81.8	3.06	16	89
TG068	2.3	101	95	73.5	13.5	97.6	3.30	7	96
TG069	1.9	91	94	74.7	13.2	93.8	3.19	13	93
TG076	2.2	98	92	73.9	13.7	84.6	3.19	12	93
TG096	3.2	99	94	73.2	13.9	84.2	3.70	1	108
TG107	2.0	102	92	72.5	13.8	93.2	2.95	20	86
TG142	2.3	104	95	73.7	13.6	85.2	3.35	6	98
TG147	3.3	99	93	73.3	13.6	89.0	3.21	11	94
TG156	2.3	101	94	73.5	13.4	84.4	3.25	9	95
TG158	1.9	93	92	72.8	13.8	91.4	3.02	18	88
TG162	2.2	98	92	75.8	13.8	86.0	3.16	14	92
TG171	1.5	99	92	73.0	14.6	92.4	3.08	15	90
TG181	1.7	95	94	74.7	14.5	100.4	3.02	17	88
F test	2.63***	7.79***	3.32**	5.59***	5.80***	10.65***	7.31***		
SE of diff.	0.39	1.96	1.32	0.55	0.23	2.47	0.24		

***: significant at the 0.001 probability level; ns: not significant

Table 3-4 Least square means and statistical analyses of plant height, days to maturity, test weight, grain protein content (WPRO), sedimentation(Sed.) and yield with rank by yield for five selected lines from the CDC Teal× CDC Go mapping population in 2013 over four locations (Edmonton, Ellerslie, Fort St. John and Neapolis) in BC and AB, Canada.

Entry	Lodging (1-9)	Height (cm)	Maturity (days)	Test weight (Kg/hl)	WPRO (%)	Sed. (mm)	Yield (Mg/ha)	Rank by Yield	Yield of Teal
AC Splendor	2.5	111	103	78.3	12.7	81.8	5.53	7	103
CDC Go	1.3	94	106	79.5	12.2	71.0	6.21	1	116
CDC Osler	2.3	107	104	79.4	13.1	70.0	5.73	3	107
CDC Teal	1.8	109	106	79.0	12.8	73.0	5.35	9	100
Katepwa	2.2	116	105	78.5	12.4	81.5	5.13	10	96
TG042	2.3	112	105	78.3	12.5	70.5	5.59	6	104
TG068	2.5	118	105	77.4	12.5	82.3	5.50	8	103
TG096	3.0	115	104	77.9	12.5	74.8	5.68	5	106
TG142	2.9	119	106	78.1	12.8	74.8	5.69	4	106
TG156	2.9	120	105	78.8	12.9	83.0	5.77	2	108
F test	4.43***	71.67***	4.36***	4.11***	2.48*	8.13***	3.87***		
SE of diff	0.43	1.61	0.82	0.59	0.33	4.30	0.29		

*: significant at the 0.05 probability level

***: significant at the 0.001 probability level;

Table 3-5 Means for leaf rust, stripe rust, plant height, days to maturity, test weight, protein content (WPRO), sedimentation (Sed.) and grain yield with rank by yield for twenty selected lines from the CDC Teal × CDC Go mapping population grown 2011 to 2013 Edmonton AB Canada.

Entry	*Lr34/Yr18*	Leaf rust (1-9)	Stripe rust (1-9)	Height (cm)	Maturity (days)	Test Weight (Kg/hl)	WPRO (%)	Sed. (mm)	Grain Yield (Mg/ha)	Rank by yield	Yield % Teal
CDC Teal	P††	2.7	1.5	103	99	75.1	13.6	78.0	4.79	60	100
CDC Go	A	4.5	2.7	91	102	75.6	12.6	70.3	5.18	16	108
AC Splendor	A	4.2	2.3	107	94	74.1	13.6	78.9	4.69	82	98
CDC Osler	P	3.8	2.5	105	95	76.0	13.4	65.4	5.08	26	106
Katepwa	A	5.7	4.4	108	94	74.6	12.9	66.9	4.72	75	98
TG012	P	4.7	4.0	115	99	74.5	14.0	77.8	5.39	7	112
TG020	A	5.8	5.7	117	102	77.0	13.0	76.3	5.22	13	109
TG033	A	3.5	2.1	108	102	75.5	12.7	65.8	5.76	2	120*
TG042	P	3.7	2.0	103	98	73.9	13.4	70.4	4.92	40	103
TG068	P	5.5	2.2	112	99	73.4	13.7	84.3	4.98	34	104
TG071	A	5.2	2.8	108	102	75.4	12.9	70.3	5.70	3	119*
TG073	A	7.3	6.8	118	102	76.2	13.4	75.6	5.30	11	111
TG074	A	4.0	2.5	96	98	73.8	12.9	70.5	5.18	15	108
TG078	A	4.5	2.7	111	95	73.5	13.4	72.4	5.45	4	114
TG084	P	5.7	3.5	111	101	75.1	12.9	79.8	5.30	12	111
TG096	P	3.5	1.7	110	99	74.5	13.7	67.1	4.94	36	103
TG104	P	5.2	2.3	108	102	75.3	13.1	61.7	5.34	10	112
TG107	P	4.7	1.8	109	94	72.2	13.5	84.1	5.16	17	108
TG114	A	5.5	3.2	103	103	73.7	12.2	62.1	5.95	1	124**
TG119	A	6.8	3.2	119	103	77.0	13.3	64.5	5.40	5	113
TG142	P	3.5	4.2	116	99	74.3	13.7	72.6	5.13	20	107
TG156	P	4.3	1.8	117	98	73.3	13.7	72.1	4.97	35	104
TG166	A	4.5	2.8	111	97	74.4	13.7	99.9	5.15	18	107
TG176	P	3.8	1.8	103	100	75.3	12.9	77.7	5.37	8	112
TG178	A	6.3	5.2	111	99	76.3	12.9	66.2	5.36	9	112

Entry	*Lr34/Yr18*	Leaf rust (1-9)	Stripe rust (1-9)	Height (cm)	Maturity (days)	Test Weight (Kg/hl)	WPRO (%)	Sed. (mm)	Grain Yield (Mg/ha)	Rank by yield	Yield % Teal
TG181	P	2.2	2.5	117	102	75.3	13.6	50.6	5.21	14	109
TG185	P	3.8	2.2	100	99	75.2	13.4	77.7	5.14	19	107
TG200	P	5.2	2.7	117	98	73.8	13.1	78.2	5.40	6	113
F Value				12.39***	7.16***	4.75***	13.56***	11.61***	4.72***		
SE of diff.		0.19	0.53	2.91	1.14	0.75	0.28	3.15	0.34		

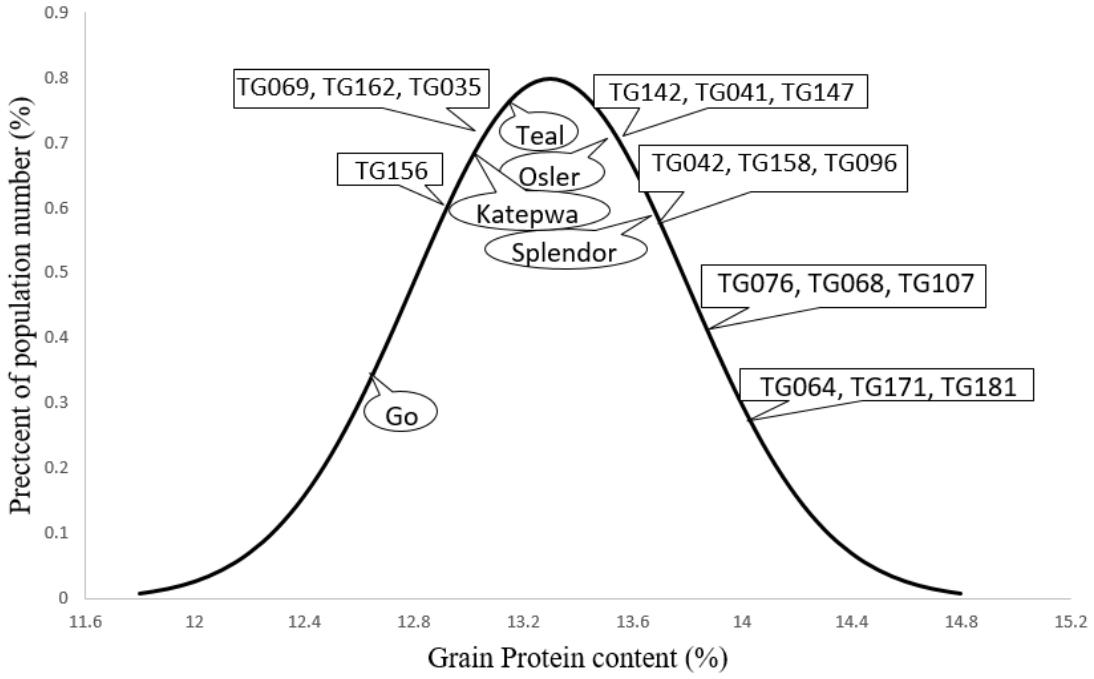
† Significance level of the genotype effect F-test;

†† Presence (P) or absence (A) of the resistant allele of *Lr34 / Yr18*

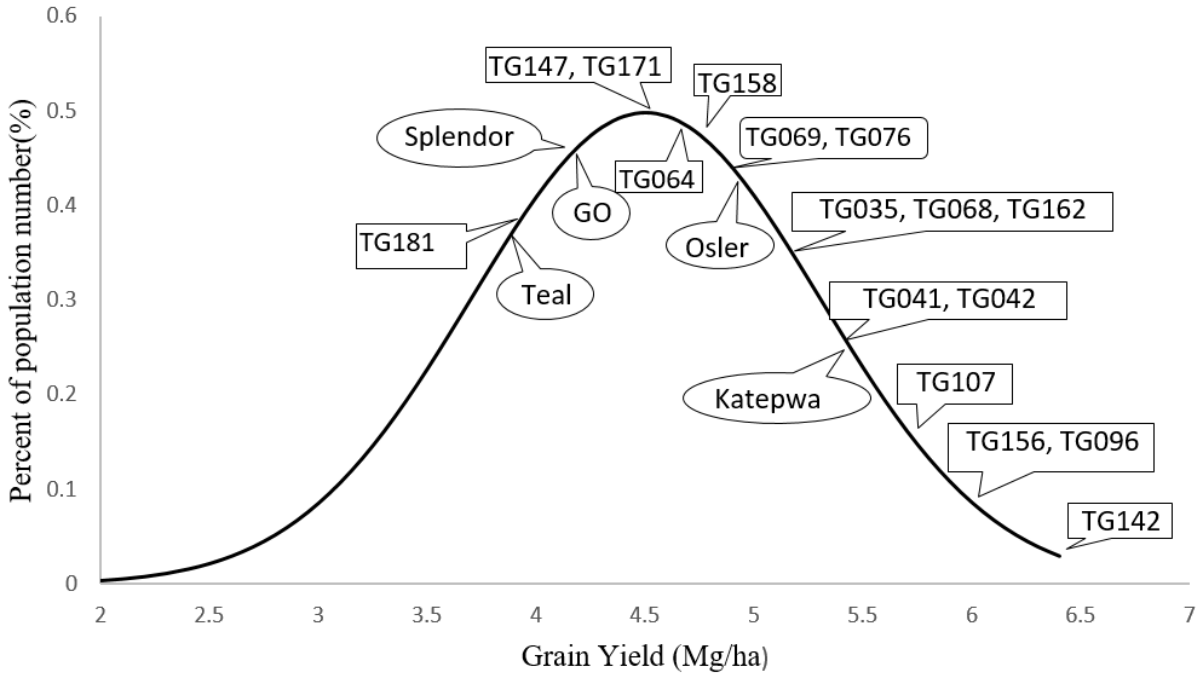
*: significant different at the 0.05 probability with CDC Teal;

**: significant different at the 0.01 probability with CDC Teal;

***: significant at the 0.001 probability level by F test;


Prectcent of population number (%)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
11.6
12
12.4
12.8
13.2
13.6
14
14.4
14.8
15.2
Grain Protein content (%)
TG069, TG162, TG035
TG142, TG041, TG147
Teal
Osler
TG156
TG042, TG158, TG096
Katepwa
Splendor
TG076, TG068, TG107
TG064, TG171, TG181
Go

(a)



1

(b)

Figure 3-1 Distribution based on protein content (a) and grain yield (b) of RILs derived from the CDC Teal × CDC Go population carrying the resistant allele of Lr34/Yr18 in 2011. The relative position of the lines selected in 2011 from the population are shown. Square indicate the lines selected and oval indicate check cultivars CDC Osler (Osler), Katepwa, AC Splendor (Splendor) and the two parents [CDC Teal (Teal) and CDC Go (Go)].

Chapter 4 Genetic Variation for Flowering Time and Height Reducing Genes and Important Traits in Western Canadian Spring Wheat [2]

4.1 Introduction

Wheat is one of the most important cereals globally, and has been the most important crop in terms of production and export over the last 10 years in Canada (FAOSTAT 2015). Genetic variation is a prerequisite for improving economically important traits in crop plants. Plant breeders exploit genetic variation present in two or more cultivars of a crop species for combining desirable traits into a single genotype or creating additional genetic variation through genetic recombination of different alleles present in the parent cultivars. Afterwards, selection is practised within the recombinants to obtain lines with desirable traits that become commercial cultivars after going through the regional trials and cultivar evaluation process.

Due to the short growing season in western Canada, development of early maturing cultivars is an effective way to avoid some biotic and abiotic stresses (Iqbal et al. 2007a). In wheat, flowering time is controlled by vernalization genes (*Vrn*), photoperiod genes (*Ppd*) and earliness *per se* genes (*Eps*) (Distelfeld et al. 2009). Three *VRN* genes *VRN1, VRN2* and *VRN3* have been identified in diploid and polyploid wheat (Santra et al. 2009; Yan et al. 2004). *VRN1* genes, which encode a MADS-box transcription factors, have been identified on homeologous group 5 chromosomes and are orthologous to *Arabidopsis* meristem identity genes *APETALA1* (*AP1*) (Yan et al. 2003). Despite the absence of close homologues in *Arabidopsis*, *VRN2* plays the same role as *FLOWERING LOCUS C* (*FLC*) of *Arabidopsis* and can be down-regulated by vernalization or short day length (Yan et al. 2004). The *VRN3* gene, with three exons encoding a RAF kinase inhibitor-like protein (Yan et al. 2006), is similar to the Arabidopsis protein *FLOWERING LOCUS T* (*FT*). A model describing the interaction between vernalization genes in wheat has been modified by Chen and Dubcovsky (2012) according to which initiation of the flowering pathway is from leaf to apex. In leaves, high expression levels of *VRN2* represses the function of *VRN3* before vernalization (which is considered as the integrator of photoperiod and vernalization); during vernalization and long days, the up-regulated *VRN1* in leaves down-regulates *VRN2* to release *VRN3*. Protein production of *VRN3* is transported to the plant apex through the phloem, interacting

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with FDL2 up-regulated *VRN1* transcript in the apex, which triggers the switch from vegetative to the reproductive stage. Dominant alleles at one of the three *Vrn1* loci (Chen and Dubcovsky 2012), results in a spring habit, which does not require low temperature vernalization for transition from vegetative to reproductive growth. Global wheat germplasm possess different combinations of alleles at *VRN1* loci, conferring adaptability under variable climatic conditions. In China, the dominant allele of *Vrn-D1* predominates in spring and facultative wheat (Zhang et al. 2012), *Vrn-B1* in Turkey (Andeden et al. 2011) and *Vrn-A1a* in the pacific northwest region of USA and Canada (Iqbal et al. 2007b; Santra et al. 2009).

The photoperiod insensitive alleles (a) of photoperiod response (*Ppd*) genes (McIntosh et al. 2003) result in wheat that is insensitive to day length, thus flowering under short days. Cultivars carrying *Ppd-1a* exhibit slightly delayed flowering in short days, when compared to long days; hence they are referred to as semi-dominant genes. Cultivars carrying *Ppd-1b* (recessive alleles) may exhibit flowering delays of longer than 200 days under short days (Worland et al. 1994). *Ppd-1* genes are located on the homeologous group 2 chromosomes (Nishida et al. 2013; Jarillo et al. 2008).*Ppd-D1* showed the most effective photoperiod insensitivity, followed by *Ppd-B1* and *Ppd-A1*(Beales et al. 2007). In Europe, *Ppd-D1* was the major factor affecting flowering time in 410 winter wheat varieties (Langer et al. 2014). It was also the major gene in Canadian spring wheat germplasm (Kamran et al. 2013b).

The incorporation of height reducing (*Rht*) genes has been a globally important wheat breeding objective that 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). Dwarfing genes in wheat can be divided into two categories based on seedling response to exogenous gibberellic acid (GA) (Börner et al. 1987). *Rht-B1* on chromosome 4BS and *Rht-D1* on chromosome 4DS belong to the GA-insensitive group and both of them derive from the Japanese wheat cultivar Norin10 (Gale and Youssefian 1985). These genes are now present in more than 70% of globally registered wheat cultivars (Evans 1998). Compared with wild type alleles (*Rht-B1a* and *Rht-D1a*), the two mutant alleles (*Rht-B1b* and *Rht-D1b*) have single base pair substitutions that code for a premature stop codon (Peng et al. 1999). Therefore, they alter the function of height regulation, reducing plant height and increasing grain yield by 15 and 24 %, respectively (Gale and Youssefian 1985). Based on PCR-based markers, *Rht-D1b* and *Rht-B1b*

were present in 44 % of the registered wheat cultivars in Germany (Knopf et al. 2008). Another study showed that 47 and 25 % of 220 Chinese winter wheat cultivars carried *Rht-D1b* and *Rht-B1b*, respectively (Zhang et al. 2006). More than 90% of modern wheat cultivars carried either *Rht-B1b* or *Rht-D1b* in eastern and central USA (Guedira et al. 2010).

Due to harsh winter in western Canada, wheat is planted under short growing season with long day length. Early maturity is the third most important traits for wheat in these areas following high yield and protein content. The objectives of the present study were to, 1) investigate genetic variation for vernalization (especially *VRN1*), photoperiod and semi-dwarfing (*Rht*) genes 2) explore the difference among cultivar groups including different alleles combinations on days to heading, maturity, plant height and grain yield in the field over three years, and 3) explore associations among different traits in spring wheat cultivars registered in western Canada.

4.2 Material and Methods

4.2.1 Plant material

A set of 82 historical and modern Canadian spring wheat cultivars registered from 1885 to 2011 (Table 4-1), including 8 Canada Prairie Spring (CPS) cultivars, 6 Canada Western Extra Strong (CWES) cultivars, 3 Canada Western General Purpose (CWGP) cultivars, 2 Canada Western Hard White Spring (CWHWS) cultivars, 60 Canada Western Red Spring (CWRS) cultivars and 3 Canada Western Soft White Spring (CWSWS) cultivars was used in this study. CWRS is a hard red spring wheat class with superior milling and baking quality and is the predominant class grown in western Canada; CPS class has medium hard kernels with medium strong dough; CWES is a hard red spring wheat class with extra strong gluten; CWSWS is a soft white spring wheat class with low protein and is used for manufacturing cookies, cakes, pastry and flat breads; CWHWS is a hard white spring wheat class with superior milling quality and excellent flour color; CWGP is a wheat class that is not required to meet the strict quality requirements of the other milling classes (Canadian Grain Commission; https://www.grainscanada.gc.ca/wheat-ble/classes/classes-eng.htm).

4.2.2 Field evaluation

The 82 wheat cultivars were planted at the University of Alberta South Campus Crop Research facility in Edmonton, Canada, (53°19'N, 113°35'W) on May 9th in 2011, May 7th in 2012 and May 11th 2013, respectively. In 2011 and 2012, it was also seeded at the University of

Saskatchewan Kernen Crop Research center (52°10'N, 106°43'W) in Saskatoon, Canada. In 2013, it was planted at the University of Alberta Research Centre, St. Albert, Canada, which is 15 km north of Edmonton. Each of the six field experiments was grown in randomized incomplete block designs with three replications at Edmonton (2012 and 2013) and St. Albert (2013) and with two replications in Edmonton (2011) and Saskatoon (2011 and 2012). Experimental plots were 4 × 1.08 m, with five rows spaced 23 cm apart. The target planting density was 300 seeds m^{-2}. Fertilizer (N-P_2O_5-K_2O:11-52-0) was applied at a rate of 36 kg/ha at sowing; and other standard agronomic practices were carried throughout the growing season to obtain even crop stands.

Data were recorded on days to heading, maturity, plant height, grain yield, grain protein content, thousand kernel weight and test weight. Days to heading was recorded when 50% of the spikes had emerged out of the flag leaf. Physiological maturity was determined when 50% of the peduncles in a plot had completely lost green color. Plant height was measured from the base of the plants to the tip of heads excluding awns. Grain protein content was estimated by Near Infrared Reflectance (NIR) spectroscopy using a SperctraStar RTW apparatus (Unity Scientific SpectraStar^{TM} 2500, Unity Scientific Asia Pacific, Australia) that was calibrated with reference samples analyzed using LECO combustion analysis. Grain yield per plot was weighed and converted to yield per hectare based on the area harvested. We measured grain weight following seed counts of 200 randomly selected cleaned grains from each plot using a digital seed counter (Agriculex Inc. Guelph, Canada). Test weight (kg hL-1) was assessed using AACC approved Method 55-10 (AACC 2000)(AACC International, 2000).

4.2.3 DNA extraction and molecular markers

Genomic DNA of the 82 wheat cultivars and two check cultivars Pitic 62 (Fisher 1973) and McNeal (Sherman et al. 2014) for *Rht-D1b* were extracted by Wizard® Genomic DNA Purification Kit (Promega, USA). For vernalization, photoperiod and semi-dwarfing genes, primers information is provided in Table 4-2. Except for *Ppd-B1*, PCR reactions comprised a 10 μL reaction mix of 1× Green GoTaq Reaction Buffer (Promega), 0.2 mM each of the four dNTPs, 0.5 μM each of reverse and forward primers, 0.5 U Taq DNA Polymerase and 50 ng of DNA template. The PCR profile consisted of an initial denaturation at 94 ℃ for 5 min, followed by 38 cycles of denaturation at 94 ℃ for 30 s, annealing at the indicated temperature for 30 s and extension at 72 ℃ for 1min kb^{-1}, followed by final extension for 10 min at 72 ℃ (Table 4-2). A

touchdown PCR was used for *Ppd-B1* following Nishida et al. (2013). Amplified PCR products were separated on a 1.5% agarose gel stained with ethidium bromide in 1× TBE buffer and visualized under UV light.

4.2.4 Statistical analysis for phenotypic Data

Data for all traits were analyzed using PROC MIXED in SAS version 9.3.1 (SAS Institute Inc. Cary, USA). Analysis was initially performed by individual environment and subsequently combined over the three years. Each cultivar was considered as a fixed effect, while the effect of replication and incomplete blocks nested in replication were considered as random effects in each year. For the combined ANOVA, location and year were considered as random effects. The linear models for both ANOVAs were:

For each year each location:

$$Y_{ijlm} = \mu_i + G_{mi} + R_{ij} + B(R)_{ijl} + \varepsilon_{ijlm} \qquad (1)$$

For each year and combined locations:

$$Y_{ijklm} = \mu_i + G_{mi} + L_{ki} + R(L)_{ijk} + B(L*R)_{ijkl} + G_{mi} * L_{ki} + \varepsilon_{ijklm} \quad (2)$$

For three years overall means:

$$Y_{ijklm} = \mu_i + G_{mi} + T_n + L\,(T)_{kni} + R(T*L)_{ijnk} + B(T*R*L)_{ijkln} + G_{mi} * T_{ni} + G_{mi} * (L)T_{kni} + \varepsilon_{ijklm} \qquad (3)$$

In Eq (1), μ_i was the mean effect on trait i, G_{mi} is the effect of genotype m on trait i, R_j was the effect of replication j on trait i, $B(R)_{ijl}$ is the effect of block l within replication j on trait i. In Eq (2), μ_i is the mean effect on trait i, G_{mi} is the effect of genotype m on trait i, L_{ki} is the effect of location k on trait i, $R(L)_{ijk}$ is the effect of replication j in location k, $B(L*R)_{ijkl}$ is the effect of block l within replication j in location k, $G_{mi} * L_{ki}$ was the interaction between genotype m and location k on trait i. In Eq (3), μ_i is the mean effect on trait i, G_{mi} is the effect of genotype m on trait i, T_n is the effect of year n on trait i, $L\,(T)_{kni}$ is the effect of location k on trait i in year n, $R(L*T)_{ijkn}$ is the effect of replication j in location k and year n, $B(L*R*T)_{ijkln}$ is the effect of block l within replication j in location k and year n, $G_{mi} * (L)T_{kni}$ is the

interaction between genotype m and location k on trait i and $G_{mi} * T_{ni}$ is the interaction between year n and genotype m.

Broad-sense heritability of each trait on plot basis and phenotypic and genetic correlations among traits were computed by multivariate restricted maximum likelihood (REML) in PROC MIXED of SAS (Holland et al. 2003; Holland 2006).

4.3 Results

The cultivars used in this study were registered for commercial production in western Canada over the last 100 years. These cultivars represent cultivars released during this period and, therefore, form a representative set to study the genetic progress of agronomic traits of Canadian spring wheat as a result of breeding efforts in local environments. Sixty of the 82 cultivars were from Canada Western Red Spring Class. The oldest spring bread wheat cultivar in western Canada is 'Red Fife' (1885). In this study, 57 (95%) cultivars from CWRS exhibited both higher grain yield and grain protein content than 'Red Fife'. The latest cultivar CDC Plentiful (registered in 2011) yielded 23 % more grain and produced 15 % more protein content than 'Red Fife'. The other two cultivars 'Marquis' and 'Garnet' registered in early 1900s showed superiority over 'Red Fife' only for protein content. All the modern wheat cultivars headed and matured earlier than 'Red Fife'. The earliest cultivar 'AC Splendor' in this study was 7 days early heading and 13 days earlier maturing than 'Red Fife'. Moreover, plant height in CWRS class decreased consistently over time as a result of incorporation of semi-dwarfing genes/quantitative trait loci. The shortest cultivar 'Muchmore' (registered in 2009) was 35cm shorter than 'Red Fife'.

Significant difference ($P<0.05$) was observed among genotypes for all the traits in the combined environments (Table 4-3). The 82 cultivars from six classes exhibited a wide variation of all the traits, Days to heading ranged from 49 to 73 days, plant height from 68 to 142 cm, days to maturity from 86 to 128 days, grain yield from 1.4 to 9.6 t ha^{-1}, test weight from 68.5 to 83.4 kg hL^{-1}, kernel weight from 23.4 to 60.5 g and protein content from 7.9 to 18.8 %. Among the seven traits, protein content exhibited the highest heritability (73 %), followed by plant height (69 %) and kernel weight (64 %); the lowest heritability was found for days to maturity (27 %). The heritability of both test weight and days to heading was 39%, which was 1% higher than that of grain yield. As for the genetic and phenotypic correlations between traits (Table 4-4), days to heading and maturity showed high genetic correlation ($r_{g\,hm}$ =0.65; $r_{p\,hm}$ =0.44). The two traits

were positively correlated with grain yield and kernel weight and negatively correlated with test weight and protein content. Plant height was positively correlated with protein content ($r_{g\,hp}$ =0.53; $r_{p\,hp}$ =0.42), but negatively correlated with grain yield ($r_{g\,hg} = -0.47$; $r_{p\,hg} = -0.14$) and no significant correlation was found with days to heading. The negative genetic correlation coefficient between yield and protein was -0.57.

We explored allelic diversity for genes associated with early maturity (vernalization and photoperiod) and plant height (*Rht-B1* and *Rht-D1*) in the 82 western Canadian spring wheat cultivars. All cultivars were monomorphic on *Ppd-A1* and *Ppd-B1*. Our results indicated that *Vrn-A1a* and *Vrn-B1* have existed in Canadian Spring wheat germplasm for more than 100 years; however, *Ppd-D1a* was incorporated around 1986. The two semi-dwarfing genes *Rht-B1* and *Rht-D1* were only found in cultivars registered after 1990. For *Vrn-A1* gene, the winter type allele was only found in five cultivars belonging to CPS class and 'AC Andrew' from CWSWS class. Forty cultivars carried the spring type allele of *Vrn-B1*, whereas 42 had the recessive allele. All cultivars in CWHWS and CWSWS classes carried the spring type allele of *Vrn-B1*; whereas, all three cultivars in CWGP class had winter type allele of *Vrn-B1*. From the amplification results of *Vrn-D1*, 'CDC Stanley' and 'Roblin' from CWRS class carried the spring habit allele, whereas, 79 cultivars had the winter habit allele. The genotype of 'AC Andrew' at *Vrn-D1* locus remains unknown as both dominant and recessive allele specific markers for this locus did not amplify the associated PCR products. For *Ppd-D1* locus, 26 cultivars had photoperiod insensitive allele *Ppd-D1a* and 56 cultivars had the sensitive allele *Ppd-D1b*. Cultivars in CWSWS and CWGP classes had *Ppd-D1a*, while cultivars in CWES and CWHWS classes possessed *Ppd-D1b*. Only eight cultivars, three from CWSWS and five from CWRS, had the semi-dwarfing allele *Rht-B1b*, whereas 13 cultivars (eight from CPS, one from CWES, three from CWGP and one from CWRS) had the semi-dwarfing allele *Rht-D1b*.

Since only five cultivars carried winter type allele at *Vrn-A1* locus and two had spring type allele of *Vrn-D1* among the 82 cultivars, we were not able to assess the differences of the two genes on phenotypic traits in the two groups. Moreover, only 21 cultivars had the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* and no cultivar carried both alleles, therefore, cultivars carrying semi-dwarfing genes were considered as one group to analyse their effects on the seven phenotypic traits (Table 4-5). Cultivars with and without vernalization gene *Vrn-B1* significantly differed for plant

height, grain yield and grain protein content (Table 4-5). Similarly, cultivars with *Ppd-D1a* allele differed for days to heading, plant height, test weight and protein content from cultivars with *Ppd-A1b* allele (Table 4-5). The two groups of cultivars carrying and lacking semi-dwarfing genes differed for all the seven traits. Cultivars with combination of *Vrn-B1*, *Ppd-D1a* and *Rht-1b* alleles had 16 cm shorter plants and 0.92 t ha^{-1} higher grain yield than cultivars with *vrn-B1*, *Ppd-D1b* and *Rht-1a* alleles (Table 4-5).

4.4 Discussions

We investigated genetic variation for vernalization response, photoperiod sensitivity and height reducing genes and agronomic traits in 82 western Canadian spring wheat cultivars in six environments and found that: a) genetic diversity for earliness related (*Vrn* and *Ppd*) genes was relatively higher than that for height reducing genes in the studied cultivars; b) all the alleles associated with earlier flowering decreased plant height and grain protein content but increased grain yield; c) *Vrn-B1*, *Ppd-D1* and *Rht-1* loci showed additive interaction effects on seven phenotypic traits; and d) breeding efforts over the last 100 years resulted in earlier heading and maturing cultivars with higher grain yield and protein content and shorter plants.

In the present study, there was no polymorphism on *Ppd-A1* and *Ppd-B1* loci, which is probably due to the copy number variation rather than the sequence changes. According to Diaz et al. (2012), alleles with an increased copy number of *Ppd-B1* confer an early flowering. In a sample of 410 winter wheat cultivars, copy number variation explained 3.2 % genotypic variance. Different alleles at *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Ppd-D1*, *Rht-B1* and *Rht-D1* loci were observed in the studied cultivars. The highest frequency (94%) was observed for the dominant allele of *Vrn-A1a*, which is consistent with the results of Iqbal et al. (2007b). In their study, 35 of the 42 Canadian spring wheat cultivars/lines carried *Vrn-A1a* allele. Our marker screening results of seven cultivars for *Vrn-B1* and three cultivars for *Vrn-D1* differed from previous studies (Iqbal et al. 2007b; Kamran et al. 2014) possibly due to the use of different seed source. Cultivars 'Bhishaj', '5702PR', 'Sadash' and 'Marquis' were found having spring habit allele of *Vrn-B1* in our study, whereas these cultivars were previously reported carrying winter habit allele (Kamran et al. 2014) We found that in the presence of both spring type *Vrn-B1* and *Ppd-D1a*, cultivars with *Vrn-A1a* allele headed 1.8 days and matured 1.6 days earlier and yielded 1.2 t ha^{-1} more than cultivars with *vrn-A1* (Table 4-6). Cultivars with *Ppd-D1a* were 0.6 days and 0.4 days earlier on days to heading

and maturity and yielded 0.18 t ha^{-1} more than cultivars with *Ppd-D1b* in the presence of only *Vrn-A1a* allele. Our result are consistent with those of (Kamran et al. 2013a) who studied a mapping population developed from two spring wheat cultivars carrying similar *Vrn-A1a* allele but different *Ppd-D1* alleles. *Ppd-D1a* induced 0.7days and 1.3 days earlier flowering and maturity in that population. However, the cultivars with *Ppd-D1a*, were still 4.8 and 6.5 days later heading and maturity than cultivars without *Ppd-D1b* when they carried spring type *Vrn-B1* and winter type *Vrn-A1*. Cultivars with *Ppd-D1a* also headed and matured later than cultivars with *Ppd-D1b* in the presence of the spring type *Vrn-A1a* and *Vrn-B1* alleles. This may be due to *Vrn-A1* having the strongest effect on decreasing flowering time among the three *VRN-1* genes (Trevaskis et al. 2003) and its epistatic nature over the other dominant alleles (Leonova et al. 2003). In the present study, only one cultivar carried *vrn-A1* in combination with spring habit *Vrn-B1* and photoperiod sensitive Ppd-D1b alleles, so we were not able to explore the difference of *Vrn-A1* loci on days to heading and maturity. Among the four polymorphic genes, *Vrn-A1a* alone or in combination with *Vrn-B1* and *Ppd-D1b* was frequently present in Canadian wheat cultivars, which is consistent with the results of Kamran et al. (2013b). Cultivars with *Vrn-A1a* in combination with *Vrn-B1* or *Ppd-D1a* headed 2 - 3 days earlier than those having *Vrn-A1a*, *Vrn-B1* and *Ppd-D1a* in combination. Different combinations of vernalization and photoperiod response genes are widely used in wheat breeding programs around the world to improve wheat's adaptation. Due to the differences in local environment and germplasm origin, allele frequencies and effects of early maturity related genes on days to heading varied in different studies. In 254 European wheat accessions, the earliest combination included *Vrn-A1a*, *Vrn-B1c* and *Ppd-D1b* (Shcherban et al. 2015); in Southern Europe and Asia, the photoperiod insensitive allele of *Ppd-D1* and *Ppd-B1*, *Vrn-D1* and *vrn-A1* or *vrn-B1* headed earlier than other combinations (Kiss et al. 2014).

The presence of *Ppd-D1a* resulted in 4.4 cm shorter plants on average in current study. Similar results have been reported in 'Reeder' × 'Conan' spring wheat population (Blake et al. 2009). *Ppd-D1a* derives from the Japanese cultivar 'Akakomugi' (Worland et al. 1988), from which the semi-dwarfing gene *Rht8* was mapped at a distance of 21.7 cM from *Ppd-D1* gene (Gasperini et al. 2012). Unlike *Rht-1* gene, *Rht8* is a GA sensitive gene and widely spread in southern European wheat cultivars (Korzun et al. 1998; Worland et al. 1998). *Rht8* has been reported to reduce height from 3.5 to 12.5 % (Börner et al. 1993; Ellis et al. 2005).

The CPS cultivar 'AC Taber' (registered in 1991) and the CWSWS cultivar 'AC Andrew' (registered in 2000) were the first registered cultivars carrying *Rht-D1b* and *Rht-B1b*, respectively. No Canadian spring wheat cultivar carried the two semi-dwarfing alleles of *Rht-B1* and *Rht-D1* in combination. This may be due to the fact that the additive effects of the two alleles might result in very short plants with a negative impact on harvest index and grain yield. In 368 global bread wheat accessions, only nine cultivars carried *Rht-B1b* and *Rht-D1b* in combination; cultivars carrying *Rht-B1b*, *Rht-D1b* and the two alleles in combination had plant heights of 101cm, 108cm and 71.6cm, respectively, while the average height of lines carrying *Rht-B1a* and *Rht-D1a* alleles was 138.6cm (Wilhelm et al., 2013a). Similar plant height reduction due to *Rht-1* genes were previously reported by Allan (1989) who studied five winter wheat populations and found that lines with both semi-dwarfing alleles had lower grain yield, grain number per spike, grain weight and grain volume weight than lines with only *Rht-B1b* or *Rht-D1b*. Height reducing genes contributed greatly towards increased grain yield in wheat and rice during 'Green Revolution' (Hedden 2003). We also found a negative correlation between plant height and grain yield; cultivars with *Rht-1b* alleles were 8 cm shorter and yielded 0.59 t ha^{-1} more grains than cultivars with *Rht-1a* alleles. Although the cultivars we tested were not isogenic lines and had different genetic background, we divided them into groups to investigate the effects of different *Vrn*, *Ppd* and *Rht* genes. Cultivars with *Vrn-B1*, *Rht-1b* and *Ppd-D1a* in combination yielded the highest and were shortest than all other combination of these genes; whereas, cultivars with *vrn-B1*, *Rht-1a* and *Ppd-D1b* in combination yielded the lowest grain and were 16 cm taller than those having *Vrn-B1*, *Rht-1b* and *Ppd-D1a* in combination (Table 4-5).

The 60 CWRS cultivars presented considerable genetic variation and improvement over time as a result of breeding efforts. The oldest cultivar only had *Vrn-A1a*, later on Canadian wheat breeders incorporated *Vrn-B1*, *Vrn-D1*, *Ppd-D1a*, *Rht-B1b* and *Rht-D1b.* The 37 cultivars registered after 2000 headed, on average, 0.2 days earlier, matured 0.8 days later and were 6.9cm shorter than cultivars registered before 2000. The longer grain filling period and relatively shorter plants are likely the reason of the increased grain yield without considerable delay in maturity time. Even modern cultivars without *Rht-B1b* and *Rht-D1b* alleles were shorter and higher yielding than the oldest cultivar 'Red Fife'. This indicated that more genes/QTLs associated with higher grain yield and shorter stature heights were pooled into CWRS class by Canadian wheat breeders over the period of time.

4.5 Conclusions

High yield and high protein content are major goals in global wheat breeding programs. In addition, early maturity is also important in Canadian wheat breeding programs due to the short growing seasons. Among the wheat cultivars released over the last more than 100 years in Canada, 39 % of all the cultivars carried both *Vrn-A1a* and *Vrn-B1*, followed by 28 % cultivars carried only carried *Vrn-A1a*. Only 21 cultivars carried semi-dwarf genes *Rht-B1b* or *Rht-D1b*. As a result of breeding efforts in western Canada, grain yield and grain protein content have been increased in addition to the incorporation of diverse genes/QTLs associated with earliness and plant height in modern wheat cultivars. Since early maturity is one of the most important traits in western Canada, copy number variation of *Ppd-A1* and *Ppd-B1* needs to be explored in future. Also, since *Vrn-A1* was the most common gene in Canada cultivars, it was important to explore its function in common genetic background. Besides the known *Rht-1b* alleles, the studied cultivars showed genetic variation for plant height and grain yield suggesting that the genetic basis of such variation need to be further explored to find genes or QTLs associated with these traits.

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4.7 Tables

Table 4-1 Genotype at the *VRN-1*, *Ppd-1* and *Rht-1* loci of 82 Canadian spring wheat cultivars from Canada Prairies Spring (CPS), Canadian western extra strong (CWES), Canada Western General Purpose (CWGP), Canada Western Hard white Spring (CWHWS) cultivars, Canada Western Red Spring cultivars (CWRS) and Canada Western Soft White Spring (CWSWS) classes.

Variety	Year	Class	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	*Ppd-D1*	*Rht-D1*	*Rht-B1*
AC Taber	1991	CPS	*vrn-A1*	*Vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1b*	*Rht-B1a*
AC Foremost	1995	CPS	*vrn-A1*	*Vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1b*	*Rht-B1a*
AC Crystal	1996	CPS	*vrn-A1*	*Vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1b*	*Rht-B1a*
AC Vista	1996	CPS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1b*	*Rht-B1a*
5700 PR	2000	CPS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1b*	*Rht-B1a*
5701 PR	2001	CPS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1b*	*Rht-B1a*
5702 PR	2007	CPS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1b*	*Rht-B1a*
Conquer	2009	CPS	*vrn-A1*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1b*	*Rht-B1a*
Glenlea	1972	CWES	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
CDC Rama	2001	CWES	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
CDC Walrus	2003	CWES	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Burnside	2004	CWES	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Glencross	2007	CWES	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
CDC Bison	2008	CWES	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1b*	*Rht-B1a*
Minnedosa	2008	CWGP	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1b*	*Rht-B1a*
NRG 003	2009	CWGP	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1b*	*Rht-B1a*
NRG 010	2009	CWGP	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1b*	*Rht-B1a*
Snowbird	2000	CWHWS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Snowstar	2006	CWHWS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Red Fife	1885	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Marquis	1910	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Garnet	1925	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Park	1963	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Neepawa	1969	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Columbus	1980	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Katepwa	1981	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Kane	2006	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Laura	1986	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*
Roblin	1986	CWRS	*Vrn-A1a*	*vrn-B1*	*Vrn-D1a*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*
CDC Teal	1991	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*
CDC Merlin	1992	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
AC Eatonia	1993	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*
AC Domain	1993	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
AC Barrie	1994	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
AC Elsa	1996	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*

Variety	Year	Class	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	*Ppd-D1*	*Rht-D1*	*Rht-B1*
AC Cadillac	1996	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
AC Intrepid	1997	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*
AC Splendor	1997	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*
McKenzie	1997	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
AC Abbey	1998	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*
Prodigy	1998	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
5600HR	1999	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
CDC Bounty	1999	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
5500HR	2000	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
5601HR	2001	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
AC Superb	2001	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1b*
Journey	2002	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Lovitt	2002	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
CDC Imagine	2002	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
CDC Osler	2003	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
PT 559	2003	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
CDC Go	2003	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1b*
CDC Alsask	2004	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*
5602 HR	2004	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Infinity	2004	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Lillian	2004	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Harvest	2004	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1b*	*Rht-B1a*
Somerset	2005	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Alvena	2006	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*
Helios	2006	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
CDC Abound	2006	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1b*
Fieldstar	2007	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Goodeve	2007	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*
Unity	2007	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Waskada	2007	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
5603 HR	2008	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
859 CL	2008	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Stettler	2008	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
CDC Kernen	2009	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
CDC Stanley	2009	CWRS	*Vrn-A1a*	*vrn-B1*	*Vrn-D1a*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*
CDC Utmost	2009	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*
BW878	2009	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Glenn	2009	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Thrive	2009	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Shaw	2009	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
Carberry	2009	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1b*
MuchMore	2009	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1b*

Variety	Year	Class	*Vrn-A1*	*Vrn-B1*	*Vrn-D1*	*Ppd-D1*	*Rht-D1*	*Rht-B1*
Vesper	2010	CWRS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	*Rht-D1a*	*Rht-B1a*
CDC Plentiful	2011	CWRS	*Vrn-A1a*	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1a*
AC Andrew	2000	CWSWS	*vrn-A1*	*Vrn-B1*	-	*Ppd-D1a*	*Rht-D1a*	*Rht-B1b*
Bhishaj	2003	CWSWS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1b*
Sadash	2007	CWSWS	*Vrn-A1a*	*Vrn-B1*	*vrn-D1*	*Ppd-D1a*	*Rht-D1a*	*Rht-B1b*

Table 4-2 PCR markers for the different *VRN-1* alleles, *Ppd-1* alleles and *Rht-1* alleles in wheat.

Trait	Locus	Primers	Sequence (5' → 3')	Expected size (bp)	Annealing Tm	Reference
Photoperiod	*Ppd-A1*	TaPpd-A1prodelF1	CGTACTCCCTCCGTTTCTTT		57	(Nishida et al. 2013)
		TaPpd-A1prodelR2	GTTGGGGTCGTTTGGTGGTG	338		
		TaPpd-A1prodelR3	AATTTACGGGGACCAAATACC	299		
	Ppd-B1	TaPpd-B1proinF1	CAGCTCCTCCGTTTGCTTCC	620	a	(Nishida et al. 2013)
		TaPpd-B1proinR1	CAGAGGAGTAGTCCGCGTGT	312		
	Ppd-D1	Ppd-D1_F	ACGCCTCCCACTACACTG		54	(Beales et al. 2007)
		Ppd-D1_R1	GTTGGTTCAAACAGAGAGC	414		
		Ppd-D1_R2	CACTGGTGGTAGCTGAGATT	288		
Vernalization	*Vrn-A1*	Vrn1AF	GAAAGGAAAAATTCTGCTCG	650 and 750	55	(Yan et al. 2003)
		Vrn1R	TGCACCTTCCCCCGCCCCAT	500		
	Vrn-B1	Intr1/B/F	CAAGTGGAACGGTTAGGACA			(Fu et al. 2005)
		Intr1/B/R3	CTCATGCCAAAAATTGAAGATGA	709	58	
		Intr1/B/R4	CAAATGAAAAGGAATGAGAGCA	1149	56	
	Vrn-D1	Intr1/D/F	GTTGTCTGCCTCATCAAATCC			(Fu et al. 2005)
		Intr1/D/R3	GGTCACTGGTGGTCTGTGC	1671	55	
		Intr1/D/R4	AAATGAAAAGGAACGAGAGCG	997	55	
Semi-dwarf		Rht-B-F1	AGGCAAGCAAAAGCTTGAGA			
	Rht-B1a	Rht-B1a-R2	CCATGGCCATCTCCAGATG	265	60	
	Rht-B1b	Rht-B1b-R2	CCCATGGCCATCTCCAGATA			
		Rht-D-F5	GCTCGTTCTCCTCCCAGTTC	385		(Wilhelm et al. 2013)
	Rht-D1a	Rht-D1a-R2	ATGGCCATCTCGAGCTGTTC		60	
	Rht-D1b	Rht-D1b-R2	CATGGCCATCTCGAGCTGTTA			

a: primers for *Ppd-B1* used touchdown PCR

Table 4-3 Summary of mean, ranges, F test (for genotype effect) and heritability estimates for 7 traits of 82 Canadian spring wheat cultivars.

Trait	Mean	SE	Min	Max	F value	Heritability
Heading (days)	57.5	0.1	49	73	7.75**	0.39**
Height (cm)	99	0.3	68	142	21.43**	0.69**
Maturity (days)	99.2	0.2	86	128	4.17**	0.27**
Yield(t ha^{-1})	4.90	0.04	1.40	9.6	7.27**	0.38**
Test Weight (kg hL^{-1})	78.3	0.06	68.5	83.4	3.31*	0.39**
Thousand kernel wt (g)	37.7	0.15	23.4	60.5	15.06**	0.64**
Protein (%)	13.1	0.05	7.9	18.8	29.53**	0.73**

SE standard error

*: significant at $P = 0.05$; **: significant at $P = 0.01$

Table 4-4 Genotypic (G) and Phenotypic (P) correlation coefficients among 7 traits over six environments in western Canada.

		Height	Maturity	Yield	TWT	TKW	Protein
Heading	G	0.1	0.65*	0.36*	-0.24*	0.35*	-0.35*
	P	0.01	0.44*	0.12*	-0.05	0.2*	-0.15
Height	G		-0.37*	-0.47*	0.28*	0.05	0.53*
	P		-0.22*	-0.14*	0.1	0.02	0.42*
Maturity	G			0.55	-0.16	0.36*	-0.64*
	P			0.23*	0.02	0.21*	-0.2*
Yield	G				-0.17	0.54*	-0.57*
	P				0.11*	0.46*	-0.27*
TWT	G					-0.03	0.52*
	P					0.21*	0.28*
TKW	G						-0.07
	P						-0.0016

*: significant at $P = 0.05$

Table 4-5 Allele effects of *Vrn-B1*, *Ppd-D1* and *Rht-1*(*Rht-B1 and Rht-D1*) and their interactions on seven traits over 6 environments.

Genotype	Allele	Number	Heading (days)	Height (cm)	Maturity (days)	Yield (t ha^{-1})	TWT (kg hL^{-1})	KW (g)	Protein (%)
Vrn-B1	*Vrn-B1*	40	58.2	93	100.8	5.09	78.0	37.2	12.6
	vrn-B1	42	57.9	97	99.6	4.90	78.1	38.0	13.2
	Difference		0.3	-4**	1.2	0.19*	-0.1	-0.8	-0.6**
	SE of diff		0.3	1	0.6	0.09	0.2	0.5	0.1
Ppd-D1	*Ppd-D1a*	26	58.5	93	100.3	5.07	77.6	37.7	12.5
	Ppd-D1b	56	57.7	97	100.1	4.92	78.6	37.5	13.3
	Difference		0.8**	-4**	0.2	0.15	-1.0**	0.2	-0.8**
	SE of diff		0.3	1	0.6	0.09	0.2	0.4	0.1
Rht-1	*Rht-1b*	21	58.5	91	101.9	5.29	77.7	38.8	12.1
	Rht-1a	61	57.7	99	98.5	4.70	78.5	36.4	13.6
	Difference		0.8**	-8**	3.4**	0.59**	-0.8**	2.4**	-1.5**
	SE of diff		0.3	1	0.6	0.09	0.2	0.5	0.1
*Rht-1*Vrn-B1*	*Vrn-B1*Rht-1b*	13	58.8	89	102.9	5.35	77.4	37.5	11.6
	*vrn-B1*Rht-1a*	34	57.7	102	98.3	4.56	78.4	35.8	13.7
	Difference		1.1**	-13**	4.6**	0.79**	-1.0**	1.7**	-2.1**
	SE of diff		0.3	1	0.8	0.10	0.3	0.4	0.1
*Rht-1*Ppd-D1*	*Ppd-D1a*Rht-1b*	12	59.3	89	102.5	5.35	76.9	38.7	11.2
	*Ppd-D1b*Rht-1a*	47	57.7	102	98.8	4.60	78.7	35.9	13.5
	Difference		1.6**	-13**	3.7**	0.75**	-1.8**	2.8**	-2.3**
	SE of diff		0.3	1	0.8	0.10	0.3	0.4	0.1
*Vrn-B1*Ppd-D1*	*Vrn-B1*Ppd-D1a*	7	59.2	91	101.0	5.21	77.5	37.4	12.0
	*vrn-B1*Ppd-D1b*	23	58.1	99	99.6	4.87	78.6	37.9	13.4
	Difference		1.1*	-8**	1.4	0.34*	-1.1**	-0.5	-1.4**
	SE of diff		0.4	1	1.0	0.15	0.4	0.8	0.2
Mixed	*Vrn-B1*Rht-1b*Ppd-D1a*	6	60.4	88	103.3	5.44	76.4	37.1	10.5
	*vrn-B1*Rht-1a*Ppd-D1b*	21	58.2	104	99.0	4.52	78.7	35.7	13.6
	Difference		2.2**	-16**	4.3**	0.92**	-2.3**	1.4	-3.1**
	SE of diff		0.4	1	1.1	0.14	0.4	1.3	0.1

TWT test weight, *TKW* thousand kernel weight

*: significant at $P = 0.05$; **: significant at $P = 0.01$

Table 4-6 Haplotype diversity at *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Ppd-D1* loci and the average values on days to heading, days to maturity and grain yield in each group over six environments.

Haplotype				# of cultivars	Heading (days)	Maturity (days)	Yield (t/ha)
vrn-A1	*Vrn-B1*	-	*Ppd-D1a*	1	60.8	102.7	6.42
vrn-A1	*Vrn-B1*	*vrn-D1*	*Ppd-D1a*	3	60.8	103.3	4.58
vrn-A1	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	1	56.0	97.8	4.69
Vrn-A1a	*vrn-B1*	*vrn-D1*	*Ppd-D1a*	17	57.8	99.0	4.82
Vrn-A1a	*vrn-B1*	*vrn-D1*	*Ppd-D1b*	23	58.4	99.4	4.64
Vrn-A1a	*vrn-B1*	*Vrn-D1*	*Ppd-D1a*	2	56.0	97.6	4.77
Vrn-A1a	*Vrn-B1*	*vrn-D1*	*Ppd-D1a*	3	59.0	101.7	5.78
Vrn-A1a	*Vrn-B1*	*vrn-D1*	*Ppd-D1b*	32	57.1	99.2	4.77

Chapter 5 Genetic diversity and association mapping of QTL associated with phenotypic traits in historical and modern Canadian Western Spring wheat

5.1 Introduction

Conventional breeding methods have a proven track record of developing improved germplasm combining a wide range of abiotic and biotic stress resistance. Progress with conventional methods is generally slow, mainly due to the polygenic nature of most traits associated with stress, requiring the simultaneous introgression of several genes or quantitative trait loci (QTL) into one genotype. Molecular markers can be used to hasten the development and deployment of improved germplasm in different ways, including marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS) and genomic selection (GS) (Bernardo 2008). The use of molecular markers in MABC involves finding a subset of markers that are significantly associated with genes or major effect QTLs that regulate the expression of traits of interest in breeding. Both linkage analysis and association mapping have been used to identify significant marker-trait associations (MTAs) in various species, including wheat (Arif et al. 2012b; Oraguzie et al. 2007; Gupta et al. 2005).

Linkage-based QTL analysis depends on well-defined populations, such as F_x derived families, backcross populations, doubled haploid (DH) lines, recombinant inbred lines (RILs), and near isogenic lines (NILs). These populations are developed by crossing two inbred parents with clear contrasting difference in phenotypic trait(s) of interest. Such mapping populations take a long time to develop. The parents used for the population development may be obsolete after the five or more years that is normally required to complete genotypic and phenotypic data necessary for mapping (Semagn et al. 2010). In addition, most populations for linkage-based QTL mapping have limited number of recombination events that only allows researchers to identify chromosomal regions, not individual genes (Holland 2007). The linkage disequilibrium (LD) based 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, association mapping rely on previous, unrecorded sources of disequilibrium to create population-wide MTAs (Kruglyak 1999; Ewens and Spielman 2001; Jannink et al. 2001). Individuals in the association mapping are usually from various genetic backgrounds or pedigrees developed for a wide range of

purposes and have large number of alleles and higher recombinant events. Such increase in recombination frequency leads to reshuffling of chromosomes into smaller segments or gene blocks, which reduces LD levels to short distances (Zhu et al. 2008).

Association mapping is divided into (i) candidate-gene association mapping, which relates polymorphisms in selected candidate genes that have purported roles in controlling phenotypic variation for specific traits; and (ii) genome-wide association mapping (GWAS), or genome scan, which surveys genetic variation in the whole genome to find signals of MTAs for traits of interest (Risch and Merikangas 1996). For candidate-gene association mapping, information regarding the location and function of genes involved in genetic, biochemical or physiological pathways that lead to trait variation is often required (Risch and Merikangas 1996; Rafalski 2002; Mackay 2001). On the other hand, GWAS requires high marker density to efficiently identify markers at a density that accurately reflects genome-wide LD structure and haplotype diversity. The availability of low cost and high density single nucleotide polymorphic (SNP) markers made GWAS highly appealing for QTL discovery in various species. SNP data can be obtained using one of the numerous uniplex or multiplex SNP genotyping platforms that combine a variety of chemistry, detection methods and reaction formats (Syvanen 2001; Gut 2001; Chen and Sullivan 2003; Sobrino et al. 2005), including the iSelect platform from Illumina (http://www.illumina.com) and genotyping by-sequencing (Elshire et al. 2011; Poland et al. 2012). The recent availability of the 90,000 (90K) Illumina iSelect genotyping platform for wheat, of which 52% of the SNPs have been genetically mapped using a combination of eight mapping populations (Wang et al. 2014), provides a tremendous opportunity for conducting GWAS in this species.

In wheat, association studies have been used to map and characterize QTLs for a wide range of traits, including kernel size and milling quality (Breseghello and Sorrells 2006), α-amylase activity (Emebiri et al. 2010), agronomic traits (Yao et al. 2009), plant height (Zhang et al. 2011), grain weight (Mir et al. 2012), seed longevity and storage (Arif et al. 2012a), pre-harvest sprouting resistance (Arif et al. 2012b), and flowering variation (Rousset et al. 2011). These studies reported different numbers of significant MTAs that generally explained a significant proportion of the phenotypic variance. However, most of these studies were conducted using limited numbers of markers. QTL results from association mapping may be affected by a number of factors, including marker density, population structure, population size, and breeding history (Gaut and

Long 2003; Gupta et al. 2005). The number of markers required for GWAS depends on the genome size of the species and the expected LD decay (Yu et al. 2006; Myles et al. 2009).

A wide range of spring wheat cultivars have been released in western Canada over the last one hundred years (Chen et al.). Very little is known about these cultivars in terms of the extent of genetic differences at the molecular level and whether there are genomic regions associated with traits of agronomic importance. The objectives of the present study were therefore to (i) understand the extent of genetic differences and pattern of relationship among 82 historical and modern Canadian spring wheat cultivars released over a period of 100 years; and (ii) identify genomic regions and SNPs associated with seven traits (heading date, maturity, protein content, plant height, kernel weight, test weight and grain yield) using genomewide association mapping.

5.2 Materials and Methods

5.2.1 Plant material and phenotyping

We used an association mapping panel (population) consisting of a total of 82 historical and modern Canadian spring wheat cultivars released over a period of 100 years (Appendix 1). The population consisted of 9 Canada Prairies Spring (CPS), 6 Canada Western Extra Strong (CWES), 3 Canada Western General Purpose (CWGP), 2 Canada Western Hard White Spring (CWHWS), 58 Canada Western Red Spring (CWRS) and 4 Canada Western Soft White Spring (CWSWS) cultivars. The population was phenotyped at 6 environments over a period of 3 years as follows: (i) the University of Alberta research stations at South Campus Crop Research facility (53°19'N, 113°35'W), Edmonton, Canada in 2011, 2012 and 2013; (ii) the University of Alberta research stations at Saint Alberta (53°19'N, 113°35'W), Canada in 2013; and (iii) the University of Saskatchewan Kernen Crop Research center, Saskatoon, Canada (52°10'N, 106°43'W) in 2011 and 2012. Each experiment was conducted in randomized incomplete block designs with two to three replications depending on seed availability. Each plot was 4.0 m long and 1.08 m wide, with five rows spaced 23 cm apart. Fertilizer (N-P_2O_5-K_2O:11-52-0) was applied at a rate of 36 kg ha^{-1} at sowing. The experiments were conducted under rainfed condition using standard agronomic and cultural practices.

Each genotype was evaluated for the following seven traits. Heading date was recorded when 50% of the spikes emerged from the flag leaf. Physiological maturity was determined when 50% of the peduncles in a plot lost their greenness. Plant height was measured from the base of

the plants to the tip of heads, excluding awns, at maturity. Grain yield per plot was weighed after air drying the grains and used to estimate yield per hectare. Kernel weight and test weight were used as measure of the kernel mass and density, respectively. Kernel weight was estimated by weighing 200 randomly selected clean grains using a digital seed counter (Agriculex Inc. Guelph, Canada), while test weight was obtained by weighing one pint container filled with clean grains from each plot using a hopper and stand (Seedburo Equipment Co. Des Plaines, USA). Grain protein content was estimated by Near Infrared Reflectance (NIR) spectroscopy using a SperctraStar RTW apparatus (Unity Scientific SpectraStar^{TM} 2500, Unity Scientific Asia Pacific, Australia).

5.2.2 DNA extraction and genotyping

Genomic DNA was extracted by bulking equal leaf tissue from three plants per cultivar using Sigma DNA extraction kit (http://www.sigmaaldrich.com) as described in the user's manual. The quality of the isolated DNA was checked by running aliquots of DNA samples on a 1 % agrose gel that contained 0.3 g/mL with SYBR Safe DNA Gel Stain (Bio-Rad, Canada). DNA concentration was measured using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, USA), and normalized to about 100 ng/µL. One hundred uL of the normalized genomic DNA per sample was forwarded to the University of Saskatchewan, Saskatoon, Canada for genotyping. DNA samples were genotyped with the 90K using Illumina iSelect SNP array as described by 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 AA, AB, and BB genotypes for a bi-allelic SNP, and those that had very weak signal (weak amplification) were excluded from scoring.

5.2.3 Data analyses

SNP data cleaning and analyses

We received allele calls for 32,147 SNPs (35.7 % of the initial 90K) from the University of Saskatchewan. We further excluded SNPs with minor allele frequencies (MAF) <0.05 using TASSEL v3.0 (Bradbury et al. 2007) and also excluded all SNPs that do not have linkage map positions using the information recently reported for hexaploid wheat (Wang et al. 2014). We finally retained a subset of 15,987 SNPs (17.8 % of the initial 90K) for statistical analyses. Shared allele-frequency based genetic distance matrix was calculated between each pair of cultivars using

PowerMarker v3.25 (Liu and Muse 2005). A dendrogram was constructed from the genetic distance matrix using the neighbor-joining algorithm with PowerMarker and the resulting trees were visualized using MEGA version 6.0 (Tamura et al. 2007). Principal component analysis was performed using the Discriminant Analysis of Principal Components (DAPC) implemented in R for windows-based *adegenet* software (Jombart et al. 2010).The first two principal components were plotted for visual examination of the clustering pattern of cultivars.

The extent of LD among the SNPs was evaluated by computing the r^2 values between pairs of SNP markers in a sliding window of 50 makers using TASSEL version 3.0 (Bradbury et al. 2007). LD decay was estimated at the point where a second degree LOESS curve intersects the threshold of the critical LD. Critical LD was evaluated following Breseghello and Sorrells (2006). The r^2 values for all available marker pairs on different chromosomes were square root transformed and the 95th percentile was chosen as the threshold value to declare linkage. To prevent bias associated with very low minor allele frequencies, only markers with a minor allele frequency greater than 0.10 were used in the LD analyses. An admixture model-based clustering method implemented in the software package STRUCTURE, version 2.3.3 (Pritchard et al. 2000) was used to infer population structure among lines. STRUCTURE was run by varying the number of clusters (K) from 1 to 10; each K was run 3 times with a burn-in period of 100,000 and 100,000 MCMC (Markov Chain Monte Carlo) replications after burn-in. The best K value was determined as described by Evanno et al (2005) using the online program CLUMPP (Jakobsson and Rosenberg 2007).

Phenotypic data analyses and association mapping

Analysis of variance (ANOVA) for all traits was performed using PROC MIXED in SAS version 9.3.1 (SAS Institute Inc. Cary, USA). The analyses were conducted on each environment separately and also combined across all the six environments. Each cultivar was considered as a fixed effect, while replications, blocks and environments were considered as random effect. Broad-sense heritability across the combined data of all environments, and repeatability within each environment, was computed using PROC MIXED of SAS which employs multivariate restricted maximum likelihood (REML) algorithms (Holland et al. 2003; Holland 2006).

GWAS was performed using the mixed linear model (MLM) implemented in the software package TASSEL v3.0 (Bradbury et al. 2007) using the following datasets: (a) the SNP data; (b) the kinship matrix (K) computed from the SNP data using TASSEL v3.0; (c) the Q and P matrices from STRUCTRE and DAPC, respectively, as covariates to account for population structure; and d) the phenotype data of the seven traits for each environment and combined across all environments. The best model was chosen on a trait-by-trait basis by comparing the likelihoods of each mixed linear model using Bayesian information criterion (BIC) value. The model with the smallest BIC was selected as the best model. The threshold of Bonferroni correction for multiple testing was calculated by dividing all P values lower than 0.05 with the number of SNP markers used in the analysis (Cheverud 2001). The chromosomal position of genomic regions significantly associated with one or more traits were plotted using MapChart v2.3 software (Voorrips 2002).

5.3 Results

5.3.1 Markers and germplasm

Among the 90K SNPs that were used for genotyping the 82 diverse spring wheat cultivars, approximately 82% of the markers were discarded either during scoring or prior to data analyses for several reasons. Approximately 24% of the markers were discarded due to redundancy (100% similarity among pairs of markers and/or the same map positions), 21 % were discarded due to lack of linkage map positions, and 5% were discarded due to their representation in more than one chromosome. The remaining 32% were discarded due to their inability to clearly discriminate the three expected genotypes (clusters), very weak amplification, lack of polymorphism and/or minor allele frequency <0.05. Thus, only 15,897 SNPs (17.7% of the 90K) that were unambiguously called with MAF >0.05 and had known genetic positions in the consensus wheat map were used for all statistical analyses. The number of SNPs retained for analyses varied from 93 on chromosome 4D to 1497 on chromosome 2B and the overall average per chromosome was 757 (Table 5-1). As shown in Table 5-1, the proportion of SNPs belonging to the A, B and D genome was 38.9%, 50.8% and 10.3%, respectively.

LD between markers was measured using r^2, which varied from 0.0 to 1.0 with a median of 0.11. The 95th percentile of the distribution of r^2 was 0.14, which we used as threshold value to declare linkage; markers that are on the same chromosome with r^2 values higher than 0.14 were considered to be probably due to genetic linkage. Approximately 24% of the marker pairs were in

LD. Based on the threshold r^2 value of 0.14, LD decayed at about 6.9 cM for the A genome, 9.5 cM for the B genome and 12.9 cM for the D genome. LD for the whole genome decayed at approximately 9.0 cM (Figure 5-1).

The genetic distance between pairwise comparisons of the 82 cultivars ranged from 0.002 to 0.457, and the overall average was 0.328. Most of the cultivars exhibited genetic differences, with 71% of the distance matrix ranging between 0.3 and 0.457 (Figure 5-2). Only eleven pairs of cultivars had very low genetic differences. For example, Fieldstar is nearly identical with Waskada, with only 0.2% of the SNP markers differing (31 out of 15,215 SNPs excluding those that were missing in one or both cultivars).

Between AC Vista and GP003, only 109 out of the 15,487 SNPs (0.7% of the markers) were different. The other nine pairs of cultivars that showed a genetic distance <0.05 were the following: McKenzie vs Unity, AC Superb vs CDC Abound, Katepwa vs Neepawa, Burnside vs Glenlea, AC Crystal vs AC Taber, AC Vista vs Minnedosa, Columbus vs Neepawa, Columbus vs Katepwa and Minnedosa vs GP003 (Figure 5-3). We then examined the genetic relationship and population structure among the 82 cultivars using cluster analysis (Figure 5-3), principal component analysis (Figure 5-4) and population STRUCTURE. All these three multivariate methods described three distinct groups (subpopulations).The first two principal components (PCs) from principal component analysis explained 32% of the total SNP variations among samples (Figure 5-4). The grouping of the 82 cultivars into three clusters (subpopulations) exhibited some level of pattern based on Western Canadian wheat classes but it was not distinct. Group 1 was the largest cluster that consisted of 37 cultivars that all belong to the Canada Western Red Spring wheat class. The second cluster consisted of 22 cultivars that belongs to CPS (8 cultivars), CWES (6 cultivars), CWGP and CWSW (3 cultivars for each) and CWRS (2 cultivars). The third cluster had 23 cultivars that belong to the Canada Western Red Spring wheat class (21 cultivars) and Canadian Western Hard white spring wheat (2 cultivars).

5.3.2 Phenotypic traits

On average, over six site-years, cultivars varied in height from 68 to 142 cm, and headed and matured from 49 to 73 and from 86 to 128 days, respectively (Table 5-2). The average grain yield per cultivar varied from 1.4 to 9.6 t ha^{-1} and the overall mean across the six environments was 4.9 t ha^{-1}. Protein content ranged from 7.9 to 18.5%, with an average of 13.1%. Kernel and

test weight varied from 23.4 to 60.5 mg and from 68.5 to 83.4 g hL^{-1}, respectively. The average kernel and test weight was 37.7 mg and 78.2 g hL^{-1}, respectively. Broad sense heritability combined over the six environments varied from 0.29 for days to maturity to 0.76 for grain protein. Days to heading, test weight and grain yield had heritability estimates ranging between 0.40 and 050, while kernel weight and plant height had high heritability estimates (0.68 and 0.72, respectively). The repeatability within each environment was variable, with some traits (e.g., days to maturity and protein content) having consistently higher repeatability, while others (e.g., kernel and test weight) exhibiting inconsistent repeatability. Repeatability of grain yield in five environments varied from 0.62 to 0.86 but was only 0.21 for Edmonton 2011. Genotypes differed ($p < 0.01$) in individual and when combined over six environments for all traits except heading date in Edmonton 2013 (Table 5-2).

5.3.3 Association mapping

We first compared BIC values of the three different models (K model, K+Q model and K+P model) to select the best model that should be used for association analyses. We chose the K+P model, which included both the kinship matrix from TASSEL and the four principal components from DAPC method, as the most appropriate model with the lowest BIC values for all traits (Table 5-3 and Appendix 2). The K+P model was then used with the mixed linear model (MLM) to identify SNP markers that were significantly associated with the seven phenotypic traits evaluated across six environments. At a threshold of $-\log_{10}$ P value >3.0, we found a total of 152 significant marker-trait associations that involved 150 SNPs, with two of the SNPs detected twice for both grain yield and protein content. The number of significant marker trait associations were 2 for days to heading, 4 for kernel weight, 14 for test weight, 24 for days to maturity, 31 for grain yield, 37 for protein content and 40 for plant height (Appendix 3). The threshold value for Bonferroni correction for multiple testing in our data was 4.1. Only 29 out of the 150 significant SNPs had higher than the 4.1 threshold value (Table 5-4), which included one SNP for grain yield, six SNPs for plant height, eight SNPs for days to maturity, and 14 SNPs for protein content (Appendix 3). The 6 SNPs significantly associated with plant height after Bonferroni correction mapped at 124-125 cM on 3A and at 68-69 cM on 4B, and all of them were detected on four of the six environments plus combined data of all environments. Among the eight SNPs for days to maturity that passed the Bonferroni correction threshold value, five mapped at 26-27 cM on 2B and were detected in three six environments and the combined analysis environments. Of the

fourteen SNPs associated with protein content after Bonferroni correction, six SNPs mapped between 18.6 and 20.1 cM on 2A and all of them were detected in four of the individual environments plus the combine data of all six environments. One of the SNPs for protein content that mapped at 87.4 cM on 3A (wsnp_Ku_c8334_14181247) was detected in five individual environments and also combined data of all six environments.

In order to better understand the significant marker trait associations, we examined genomic regions consisting of clusters of three or more SNPs within a reasonably short genetic interval. Employing these criteria, we identified 18 genomic regions across 12 chromosomes consisting of clusters of 3 to 20 SNPs that were significantly associated with one or more traits. This included two regions for each of test weight, protein content and grain yield, six regions for plant height plus six other coincident regions that were significantly associated with two or three traits (Table 5-5, Figure 5-5 and Appendix 4). No genomic region with clusters of at least three significant SNPs was found for days to heading. For test weight, the two genomic regions with clusters of three to four significant SNPs mapped at 88 - 89 cM on 2B and at 71 - 72 cM on 6B, which on average explained 15.7% (2B) and 15.0% (6B) of the phenotypic variance for test weight. The genomic regions on 5A (82-83 cM) and 7A (33.4-42.5 cM) consisted of clusters of three to four SNPs and explained on average between 14.6% and 15.5% of the phenotypic variance for protein content. Haplotypes with favorable allele combinations across the clusters of SNPs on both 5A and 7A had about 1% higher protein content than those with unfavorable alleles The genomic regions associated with grain yield mapped on 1D (at 85.6 - 92.9 cM) and 3B (at 34 - 35 cM), and explained on average 13.7% and 14.3% of the phenotypic variance for grain yield over six environments, respectively. Haplotypes that consisted of combinations of favorable alleles across the clusters of SNPs significantly associated with grain yield on 1D on average produced 745 kg ha^{-1} higher grain yield than those that had the unfavorable alleles. For plant height, the six genomic regions were located on 2B (157.2 - 161.4 cM), 3A (124 - 125 cM), 4B (56.0 - 68.5 cM), 5B (45.4 - 51.2 cM), 6A (74 - 75 cM) and 6D (82.1 - 83.4 cM). Altogether the six genomic regions accounted for 96.2% of the phenotypic variance for plant height over six environments, with each region explaining between 14.1 and 19.8%. Each haplotype with the favorable allele combinations on 3A, 4B, 2B and 6D were 14.3 cm, 12.0 cm, 6.8, and 5.9 cm shorter, respectively.

Among the genomic regions associated with two or more traits, three regions on chromosomes 2A, 3A and 6B were significantly associated with three traits. The interval between 18.6 and 25.9 cM on 2A consisted of clusters of 13 SNPs that were significantly associated with protein content, days to maturity and TKW; this region on average explained 18% of the phenotypic variance for each trait. The genomic region between 85.1 and 90.6 cM on 3A consisted of clusters of nine SNPs that were significantly associated with protein content, days to maturity and grain yield, and explained on average 17.6% of the phenotypic variance of each trait across six environments. On chromosome 6B, the region between 57.1 and 63.5 cM consisted of clusters of six SNPs that were significantly associated with protein content, days to maturity and test weight, and explained on average 16.1% of the phenotypic variance of each trait over six environments. Although the coincident genomic regions on both 2A and 3A had complicated haplotypes, haplotypes with favorable allele's combinations at 57.1 - 63.5 cM interval on 6B increased protein content by 1.2% and decreased days to maturity by 3.6 days but did not alter test weight. The three other coincident genomic regions were significantly associated with two traits; located between 23.9 - 28.5 cM on 2B for both days to maturity and grain yield; and between 135.6 - 144.6 cM and 176.6 - 182.2 cM on 5B for protein content and plant height, and protein content and test weight, respectively. On average, the coincident regions on 2B and 5B consisted of clusters of four to fourteen significant SNPs and explained on average between 14.6 and 18.6% of the phenotypic variance of each trait over six environments. When haplotypes with combinations of favorable alleles were considered, (i) the coincident region from 23.9 to 28.5 cM on 2B increased grain yield by 204 kg ha^{-1} but did not alter days to maturity; (ii) the region from 135.6 to 144.6 cM on 5B increased both protein content and plant height by 0.8% and 4.4 cm, respectively; and (iii) the interval between 176.6 and 182.2 cM on 5B increased both protein content and test weight by 1.3% and 1.6 kg hL^{-1}, respectively.

5.4 Discussion

Results from the present study showed two pairs of nearly identical cultivars that may be due to errors in labeling or due to release of genetically the same germplasm by different names. The two pairs of cultivars that are nearly identical are Fieldstar vs Waskada and AC Vista vs GP003. Other nine pairs of cultivars were very similar (Figure 5-3) but at least one percent of the SNP markers were different between any of the pairs. According to the pedigree information, some of the cultivars are conversions of other highly popular cultivars by only introgressing a single or

few genes from donor parents, but we expected at least one percent of the markers to show differences between any two pairs of cultivars. Our results also showed the presence of clear population structure, with three distinct clusters or subpopulations (Figure 5-4). A substantial number of cultivars shared one or more common parents. The two groups (group 1 and 3 in Figure 5-4) consisted of cultivars that belong to the Canadian Western Red Spring wheat class, with most cultivars having Red Fife (registered in 1885) as one of their parents (McCallum and DePauw 2008). However, we were not able to explain as to why the same class of wheat was divided into two groups. The third group consisted of mixture of cultivars from most spring wheat classes. In order to minimize false discovery rate due to such clear population subdivision, we tested three different models to account for population structure and selected the best model for genomewide association analyses.

The power of QTL detection depends on the sample size, the genetic architecture of the trait, and trait heritability (Yu et al. 2008). The number of cultivars in our association mapping panel is relatively smaller than some other studies, which is the major limitation in the present study. However, most of the cultivars used in the panel were highly diverse and showed highly significant genotypic differences for all the seven traits evaluated across six environments. The quality of our phenotype data across the six years was generally very good, with high heritability (0.68 to 0.76) for plant height, TKW and protein content, and moderate heritability (0.29 and 0.43) for days to maturity, grain yield, test weight and days to heading. Knowledge on the extent of LD is important for determining the number of markers required for reasonably accurate marker-trait association analyses (Gaut and Long 2003). In our study, LD decayed at about 6.9 cM for A genome, 9.5 cM for B genome, and 12.9 cM for D genome, which is generally in agreement with several other studies my(Edae et al. 2014; Chao et al. 2010; Lopes et al. 2014), suggesting the need for higher marker density to identify genes in the D genome than both the A and B genomes. GWAS identified a total of 152 significant marker-trait associations, which includes two for days to heading, four for TKW, 14 for test weight, 24 for days to maturity, 31 for grain yield, 37 for protein content and 40 for plant height. However, we think that the most reliable genomic regions are only 18 that are distributed across 12 chromosomes, with each region consisting of clusters of 3 to 20 significant SNPs. One of the most important regions for plant height mapped between 56.0 and 68.5 cM on 4B that on average explained 15.6% of the phenotypic variance for plant height across six environments. Cultivars consisting of favorable alleles across the set of 6 significant

SNPs were on average 12 cm shorter than those cultivars with unfavorable alleles. The significant markers in this region were on high LD ($r^2 \geq 0.80$) with the known semi-dwarf *Rht-B1* gene (Peng et al. 1999) (Figure 5-6), suggesting the reliability of this genomic region in significantly affecting plant height. Regions on chromosomes 2B, 3A and 5B have also been previously reported to be significantly associated with plant height in winter wheat association mapping study (Zanke et al. 2014). According to the shared SNP markers information, the significant region on 5B is orthologous of gibberellin acid (GA) receptor in rice (Zanke et al. 2014). Three regions on chromosomes 2B, 3A and 6B were significantly associated with days to maturity in the present study. The photoperiod gene *Ppd-B1* is one of the major genes controlling flowering and located on 2B (Nishida et al. 2013) but we are not able to compare the exact position of this gene with the significant SNP markers due to differences on linkage map position. Some QTLs of earliness *per se* have also been reported on chromosomes 3A and 6B (Shah et al. 1999; Worland 1996).

The genomic region between 85.1 and 90.6 cM on 3A has been found to have significant effect on protein content, days to maturity and grain yield (Table 5-5). One of the significant SNP markers, 'wsnp_Ku_c8334_14181247' was strongly associated with protein content on four of the 6 environments and also combined data of all six environments. Cultivars that consisted of the favorable allele at this SNP marker showed 2% higher protein content than those with the unfavorable allele. Although it is difficult to locate the exact position of previous QTL positions with our data due to difference in marker type, other studies have reported QTLs for protein content on the same chromosome at about approximately the same position (Bogard et al. 2011; Groos et al. 2003; Li et al. 2009). One of the studies reported stable QTLs on both chromosome 3A and 4D that were associated with protein content in a biparental mapping population of 194 F_7 recombinant inbred lines derived from 'Renan' and 'Recital' and evaluated over six environments. That QTL on 3A explained 8.3% for phenotypic variation for protein content (Groos et al. 2003). Six of the eighteen genomic regions identified by GWAS on chromosomes 2A, 2B, 3A, 5B and 6B consisting of clusters of four to fourteen SNPs that were significantly associated with two or three of the seven traits. Each region explained on average between 14.6 and 18.6% of the phenotypic variance of each trait over six environments. Identification of genomic regions that are significantly associated with two traits can result from four alternative scenarios (Tuberosa et al. 2002): (i) two tightly linked genes or QTLs modulating the expression of separate traits, but not separated by the statistical tests adopted; (ii) a single gene or QTL leads to a sequence of causally related events;

(iii) a single gene or QTL with an effect on two or more traits independent of each other; or (iv) two tightly linked genes or QTLs with effects on two or more traits. The first scenario is due to linkage, the fourth is due to both linkage and pleiotropy, while the second and third scenarios are pleiotropy, which refers to a situation where a single gene or genomic region influences two or more seemingly unrelated phenotypic traits. In the present study, it is unclear whether such coincidental regions were due to tight linkage or pleiotropy. Tight linkage between genes or QTLs can be determined by screening large numbers of recombinants to break up the linkage (Kolb et al. 2001), which is easily done in biparental populations but likely to be complicated in GWAS studies where a diverse set of germplasm is used for mapping. Furthermore, we have also observed a genomic region between 135.6 and 144.6 cM on 5B which increased both protein content and plant height by 0.8% and 4.4 cm, respectively. Since breeders in western Canada are primarily interested in developing short cultivars with high protein content, the ideal situation is identifying major genes or genomic regions that increased protein content and decreased plant height, but not increase both traits, as has been the case in our study, which would not be useful in breeding.

5.5 Conclusions

Our study showed high genetic diversity among most of the historical and modern Canadian spring wheat cultivars released over a period of 100 years and also the presence of clear population subdivision. GWAS identified 18 genomic regions from 12 chromosomes that are associated with grain yield, test weight and protein content (two regions for each trait), six regions for plant height, and six other coincident regions that were associated with two or three traits. The coincidental regions were located on 2A for protein content, days to maturity and TKW; 3A for protein content, days to maturity and grain yield; 6B for protein content, days to maturity and test weight; 2B for days to maturity and grain yield; 5B at two regions for protein content and plant height plus protein content and test weight, respectively. Such coincident regions may be due to tight linkage or pleiotropy, which was unclear from our data. Each of the 18 QTLs explained on average between 13.7 and 19.8% of the phenotypic variance. The genomic region associated with plant height on chromosome 4B showed high linkage disequilibrium ($r^2 \geq 0.80$) with the semi-dwarf gene *Rht-B1*. The results presented in this study would provide highly useful information to wheat breeders.

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5.7 Tables and figures

Table 5-1 Summary of the SNP markers used in the present study.

Chromosome	Length (cM)	Number of SNPs	No. of SNPs per cM
1A	142.57	924	6.5
1B	173.62	1319	7.6
1D	200.37	336	1.7
2A	185.46	889	4.8
2B	181.91	1497	8.2
2D	152.83	421	2.8
3A	197.19	764	3.9
3B	156.05	1201	7.7
3D	156.06	255	1.6
4A	164.12	828	5.0
4B	119.45	628	5.3
4D	170.43	93	0.5
5A	148.49	883	5.9
5B	217.64	1337	6.1
5D	214.42	191	0.9
6A	170.56	824	4.8
6B	122.92	1113	9.1
6D	160.58	148	0.9
7A	244.16	1069	4.4
7B	181.93	984	5.4
7D	235.56	193	0.8
A genome	1252.55	6181	4.9
B genome	1153.52	8079	7.0
D genome	1290.25	1637	1.3

Table 5-2 Summary of the seven phenotypic traits evaluated in 82 Canadian spring wheat cultivars across 6 environments. F tests for genotypes were based on analysis of variance.

Trait	Year	Location	Mean	Stdev	Min	Max	F-test $_{genotype}$	Repeatability /Heritability	SE
Heading	2011	Edmonton	62	0.3	54	73	3.22**	0.52	0.08
(days)	2011	Kernen	57	0.1	53	61	11.64**	0.84	0.03
	2012	Edmonton	57	0.2	50	62	23.45**	0.89	0.02
	2012	Kernen	59	0.1	55	64	28.49**	0.93	0.02
	2013	Edmonton	58	0.2	53	64	0.83	-	-
	2013	St. Albert	54	0.2	49	60	12.28**	0.77	0.04
	Overall		58	0.1	49	73	7.75**	0.40	0.04
Height	2011	Edmonton	101	0.9	72	124	11.97**	0.73	0.05
(cm)	2011	Kernen	96	0.7	74	120	15.88**	0.88	0.02
	2012	Edmonton	97	0.6	68	118	22.57**	0.88	0.02
	2012	Kernen	89	0.6	70	106	2.61**	0.45	0.09
	2013	Edmonton	109	0.5	88	142	11.11**	0.77	0.04
	2013	St. Albert	99	0.6	78	136	10.1**	0.72	0.04
	Overall		99	0.3	68	142	21.43**	0.72	0.03
Maturity	2011	Edmonton	109	0.7	91	128	3.82**	0.60	0.07
(days)	2011	Kernen	96	0.2	90	102	9.3**	0.76	0.05
	2012	Edmonton	94	0.3	86	105	13.45**	0.81	0.03
	2012	Kernen	98	0.1	94	102	8.22**	0.73	0.05
	2013	Edmonton	98	0.2	91	105	8.36**	0.69	0.05
	2013	St. Albert	102	0.2	93	107	2.5**	0.35	0.07
	Overall		99	0.2	86	128	4.17**	0.29	0.04
Yield	2011	Edmonton	5549	95.4	2257	9632	1.7*	0.21	0.10
(kg ha^{-1})	2011	Kernen	4772	45.5	3252	6314	18.33**	0.78	0.04
	2012	Edmonton	4229	39.2	2395	5695	10.26**	0.72	0.04
	2012	Kernen	3100	43.0	1422	5279	14.9**	0.86	0.03
	2013	Edmonton	6081	63.3	2929	9100	8.78**	0.69	0.05
	2013	St. Albert	4996	61.7	1936	7741	7.95**	0.62	0.05

Trait	Year	Location	Mean	Stdev	Min	Max	F-test $_{genotype}$	Repeatability /Heritability	SE
	Overall		4853	36.2	1422	9632	7.27**	0.43	0.04
Test	2011	Edmonton	78.0	0.2	72.0	82.7	4.16**	0.62	0.07
weight	2011	Kernen	79.8	0.1	77.3	83.1	26.12**	0.92	0.02
(kg hL^{-1})	2012	Edmonton	75.6	0.1	69.2	79.6	5.43**	0.59	0.06
	2012	Kernen	78.0	0.1	74.2	82.1	16.07**	0.88	0.02
	2013	Edmonton	79.5	0.1	74.1	82.8	7.3**	0.68	0.05
	2013	St. Albert	78.8	0.1	68.5	83.4	1.48*	0.87	0.02
	Overall		78.2	0.1	68.5	83.4	3.31*	0.40	0.05
Kernel weight	2011	Edmonton	41.3	0.3	31.5	54.2	9.79**	0.81	0.04
(mg)	2011	Kernen	38.6	0.3	30.3	48.1	22.05**	0.91	0.02
	2012	Edmonton	31.9	0.3	23.4	44.5	8.25**	0.62	0.05
	2012	Kernen	34.6	0.3	25.4	46.0	19.43**	0.90	0.02
	2013	Edmonton	42.3	0.2	35.0	60.5	10.1**	0.72	0.04
	2013	St. Albert	37.7	0.3	25.0	52.0	16.75**	0.84	0.03
	Overall		37.7	0.2	23.4	60.5	15.06**	0.68	0.04
Protein	2011	Edmonton	12.2	0.1	7.9	15.0	4.66**	0.63	0.06
(%)	2011	Kernen	12.7	0.1	9.1	15.4	6.54**	0.68	0.06
	2012	Edmonton	12.6	0.1	8.4	15.1	19.43**	0.86	0.02
	2012	Kernen	15.7	0.1	11.4	18.8	23.05**	0.89	0.02
	2013	Edmonton	13.4	0.1	9.2	16.1	34.52**	0.91	0.02
	2013	St. Albert	12.4	0.1	8.2	14.8	20.88**	0.87	0.02
	Overall		13.1	0.1	7.9	18.8	29.53**	0.76	0.03

*** Significant at $p \leq 0.001$
** Significant at $p \leq 0.01$

Table 5-3 Comparisons of three mixed linear models for correcting population structure using kinship (K) model, K matrix and population structure from STRUCTURE software (K +Q model), and K matrix and DAPC method (K+P model).

Trait	K model		K+Q model		K+P model	
	-2Ln(L)	BIC	-2Ln(L)	BIC	-2Ln(L)	BIC
Days to heading	297	306	277	**290**	277	**290**
Plant height	511	520	488	501	479	**492**
Days to maturity	351	360	332	345	330	**343**
Protein content	163	172	151	164	148	**161**
Kernel weight	375	384	358	371	341	**354**
Test weight	218	227	210	223	202	**215**
Grain yield	1202	1211	1146	1159	1137	**1150**

*BIC value: Bayesian information criterion. The K+P model with the smaller BIC values sown in was used for marker-trait association analyses.

Table 5-4 Summary of the 29 SNP markers that were significantly associated with the protein content, days to maturity, grain yield and plant height in wheat after Bonferroni correction.

Trait	Marker	Chr	Position (cM)	Environments						Overall	R^2 (%)
				E11	E12	E13	K11	K12	St13		
Protein	RFL_Contig4030_493	2A	18.6		4.2	4.3	3.6	3.2		4.3	13-19
Protein	wsnp_Ex_c11950_19164041	2A	18.6		4.1	4.2	3.3	3.3		4.4	14-19
Protein	wsnp_Ex_c11950_19164191	2A	18.6		4.1	4.3	3.4	3.5		4.5	14-20
Protein	Kukri_c22152_730	2A	18.6		4.1	3.3	3.4	3.5		4.5	15-18
Protein	CAP8_c2783_336	2A	18.6		4.4	4.4	3.2	3.5		4.6	15-20
Protein	Excalibur_c386_894	2A	20.1		3.5	5.1				4.9	16-21
Maturity	BobWhite_c26803_89	2A	26.0	4.3	3.7	6.2				4.7	18-21
Maturity	Kukri_c22513_1780	2B	26.5	4.3	3.7	6.2				4.7	16-27
Maturity	wsnp_Ex_c25445_34710489	2B	26.5	4.1	3.8	6.4				4.7	17-28
Maturity	Excalibur_c52842_317	2B	26.5	4.2	4.0	6.6				5.1	18-23
Maturity	BobWhite_c45060_188	2B	26.5	4.2	4.0	6.7				5.1	18-23
Maturity	Excalibur_c25445_1061	2B	26.5	4.2	4.0	6.7				5.1	18-23
Maturity	Kukri_c77179_54	2D	4.7	4.8		5.3				4.4	22-25
Yield	D_F5XZDLF02GZM9K_79	3A	85.4						3.6	4.3	15-19
Protein	wsnp_Ku_c8334_14181247	3A	87.4		6.7	6.2	8.0	4.5	5.9	7.3	20-31
Protein	Ra_c11263_2353	3A	90.6			3.2				4.1	14-17
Protein	Tdurum_contig68855_91	3A	90.6			3.2				4.1	14-17
Height	BobWhite_c5337_225	3A	124.3	4.5	4.3	3.7			4.3	4.5	16-20
Height	BS00035364_51	3A	124.3	4.5	4.3	3.7			4.3	4.5	16-20
Height	BS00088755_51	3A	124.3	4.5	4.3	3.7			4.3	4.5	16-20
Height	BobWhite_c26330_284	3A	125.0	4.5	4.3	3.5			4.1	4.4	15-19
Protein	RAC875_c27536_611	4B	54.6			6.0	3.5		4.9	5.1	16-22
Height	RAC875_c6865_349	4B	68.5	3.8	3.2	3.7	4.9			4.1	13-21
Height	TA003248-0911	4B	68.5	4.5	3.7	3.6	5.1			4.4	15-22
Protein	RFL_Contig5341_816	4B	86.6	3.8					3.7	4.2	16-18
Protein	RAC875_c52775_296	5B	135.6			3.3				4.1	15-17
Maturity	Excalibur_c47748_83	6B	63.5		5.0	3.4	4.7			4.1	15-23
Protein	Ku_c586_811	6D	58.8		4.7	4.4	5.2			5.1	19-22
Protein	Tdurum_contig17365_336	7A	42.1	3.7		3.4				4.1	14-18

Table 5-5 Summary of the 18 genomic regions from 12 chromosomes of wheat that were significantly associated with one or more traits evaluated across six environments.

Chromosome	Position (cM)	No. of SNPs	-log P value (range)	R^2 range (%)	Mean R^2	Trait(s)*
1D	85.6-92.9	20	3.1-3.5	13.4-154	13.7	GY
2A	18.6-25.9	13	3.2-4.9	13.4-21.8	18.0	PC, PM, TKW
2B	23.9-28.5	14	3.3-5.1	14.8-23.5	18.6	PM, GY
2B	88.9-89.3	4	3.3-3.9	14.4-17.1	15.7	TW
2B	157.2-161.4	5	3.4-3.5	14.5-15.5	15.1	PH
3A	85.1-90.6	9	3.1-7.3	13.9-31.3	17.6	PC, PM, GY
3A	124.0-125.0	3	4.5-4.5	19.6-20.0	19.8	PH
3B	34.0-35.0	3	3.3-3.3	14.2-14.4	14.3	GY
4B	56.0-68.5	6	3.2-4.4	13.3-19.7	15.6	PH
5A	82.0-83.0	4	3.2-3.5	13.6-15.2	14.6	PC
5B	45.4-51.2	5	3.1-3.7	13.6-16.3	15.3	PH
5B	135.6-144.6	7	3.1-3.4	13.4-18.0	15.4	PC, PH
5B	176.6-182.2	4	3.2-3.6	13.8-16.2	14.6	PC, TW
6A	74.0-75.0	3	3.7-3.7	16.3-16.4	16.3	PH
6B	57.1-63.5	6	3.1-4.1	13.1-19.0	16.1	PC, PM, TW
6B	71.0-72.0	3	3.1-3.6	13.9-15.9	15.0	TW
6D	82.1-83.4	6	3.1-3.4	13.4-14.9	14.1	PH
7A	33.5-42.5	3	3.1-4.1	13.6-18.4	15.5	PC

*GY: grain yield; PM: days to maturity; PH: plant height; PC: protein content; TKW: thousand kernel weight; TW: test weight

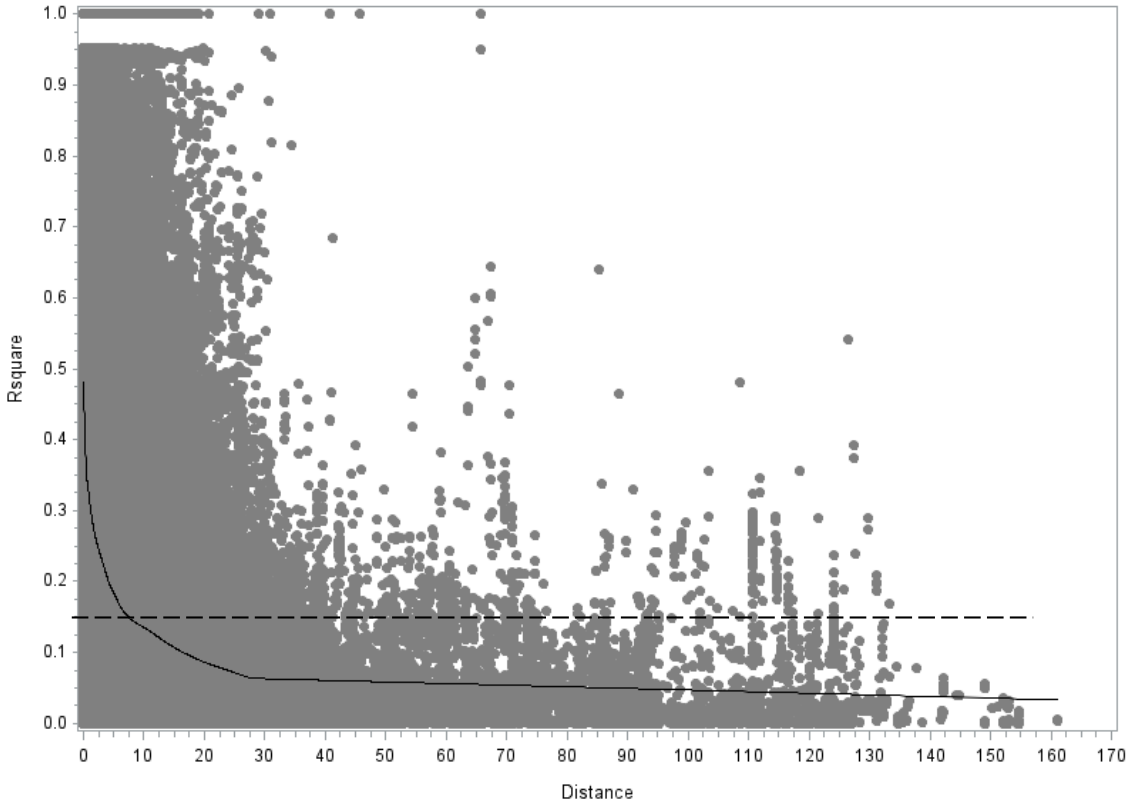

Figure 5-1 Relationship between linkage disequilibrium (LD) using the squared allele-frequency correlation (r^2) against marker distance (cM) for the whole wheat genome. The horizontal dotted line indicates the 95th percentiles of the distribution that is used as a threshold r^2 value to declare for linkage.

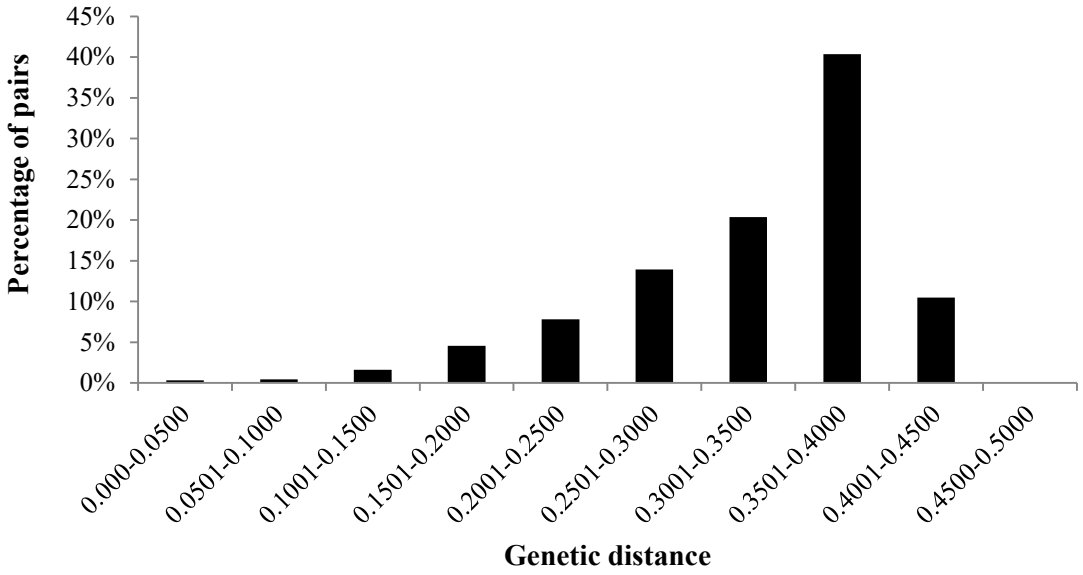

Figure 5-2 Distribution of pairwise genetic distances among 82 wheat cultivars using 15,897 SNP markers.

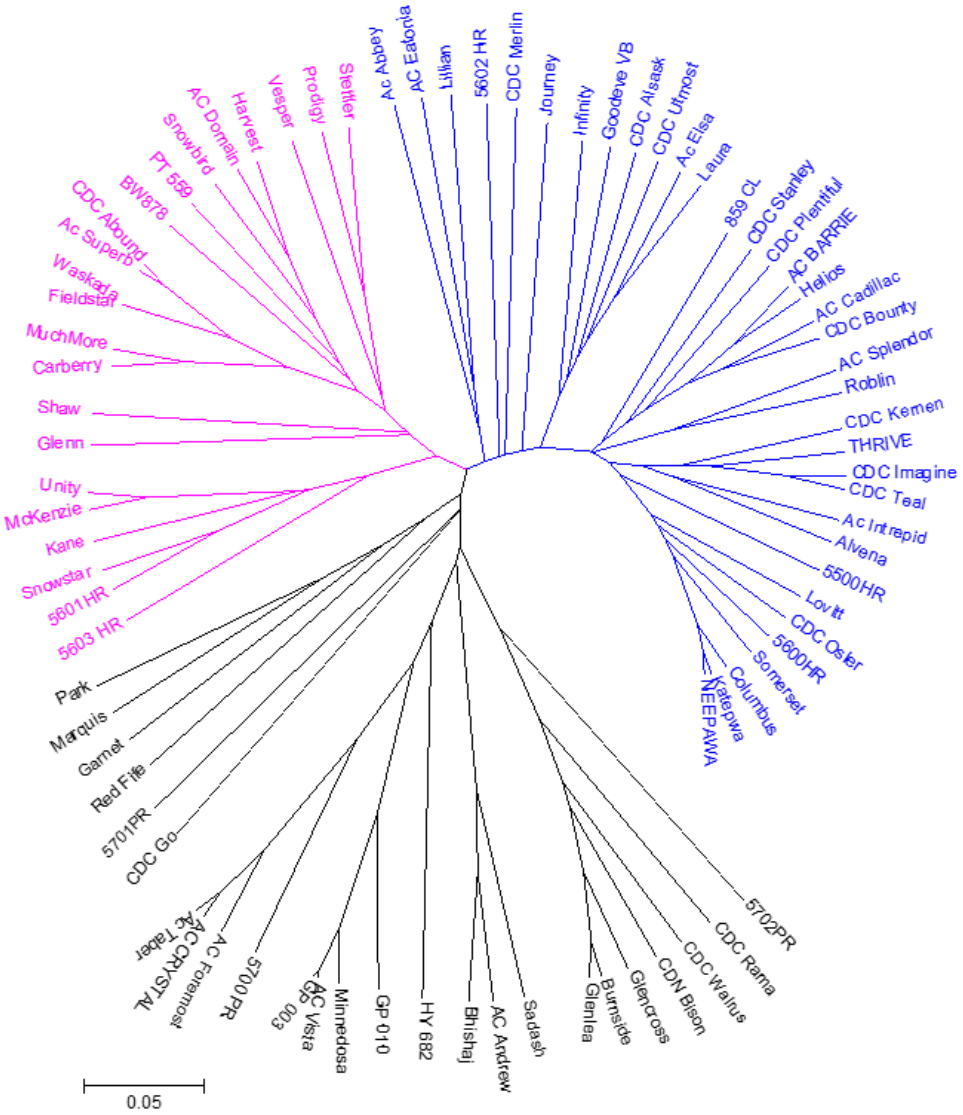

Figure 5-3 Neighbor-joining tree for 82 wheat cultivars based on genetic distance calculated from 15,897 SNP markers. The groups are indicated with different color.

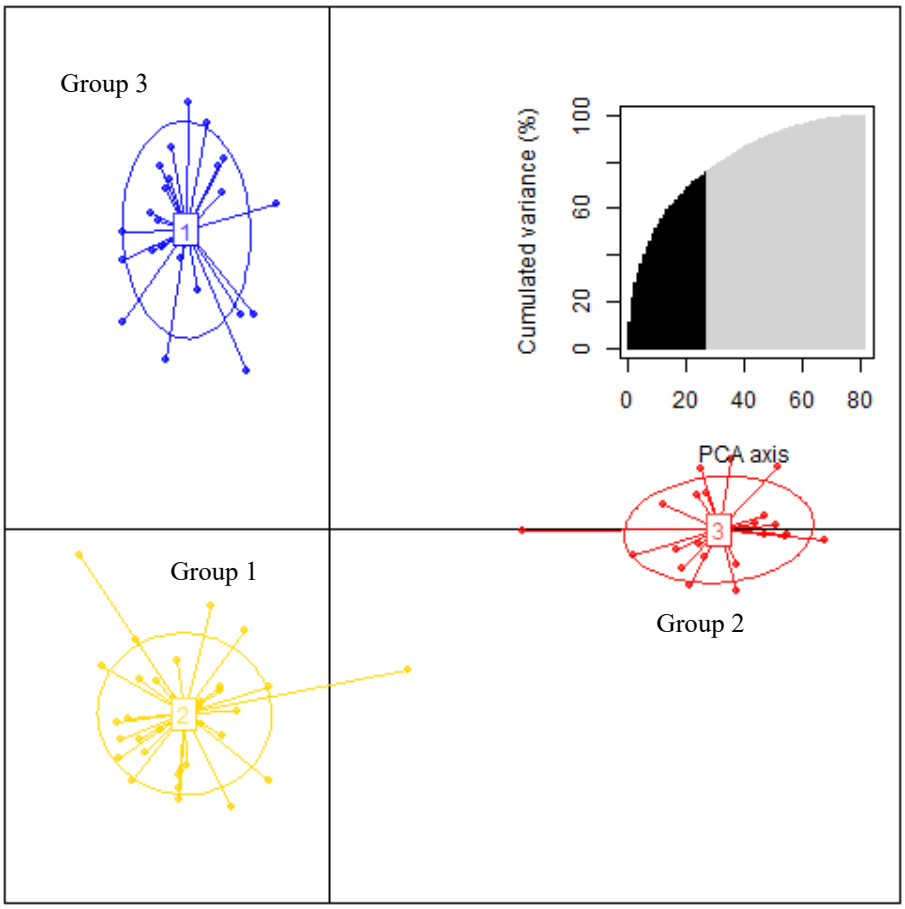

Figure 5-4 Population structure of 82 cultivars based on the first two principal components (PC) from Discriminant Analysis of Principal Components (DAPC) using 15,897 SNPs.

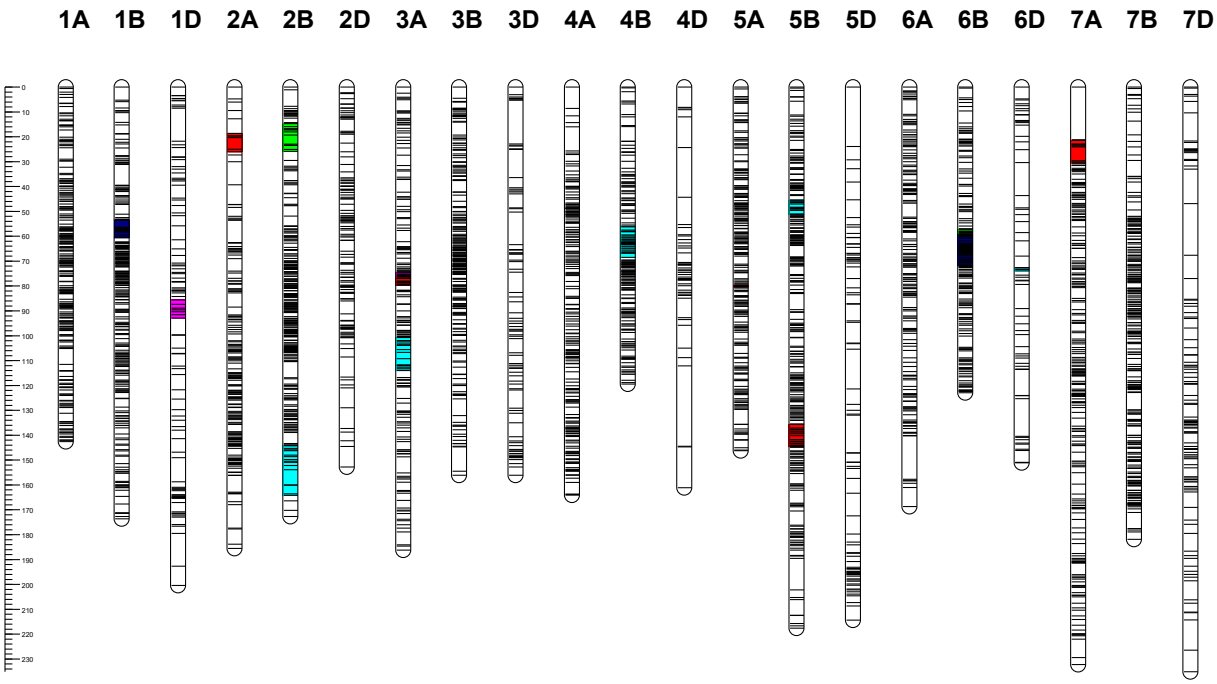

Figure 5-5 Overview of the position of the 18 genomic regions of wheat that were significantly associated with one or more of the traits evaluated across six environments. The significant regions are shaded in different colors, with pink for grain yield, red for protein content, green for days to maturity, light blue for plant height, dark blue for test weight.

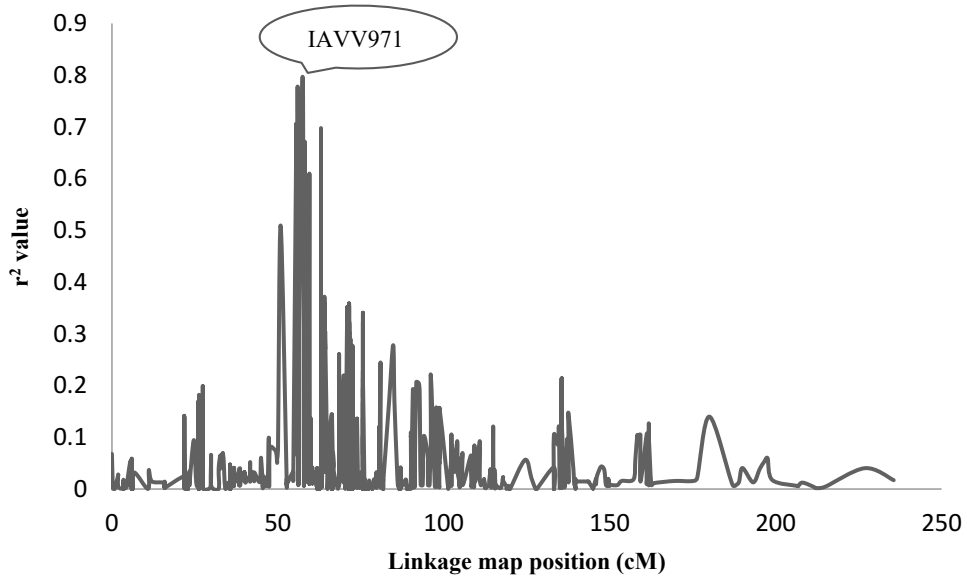

Figure 5-6 Linkage disequilibrium (LD) between the semi-dwarf *Rht-B1* gene and SNP markers on chromosome 4B of wheat. IAVV971 showed the highest LD with *Rht-B1* gene.

Chapter 6 General Discussion and Conclusions

6.1 Introduction

Wheat is one of the most important staple crops in the world. Two species of wheat (common bread wheat, *Triticum. asetivum*, 2n = 6x = 42 and durum *T. turgidum*, 2n = 4x = 28) are among the most important food crops globally, supplying protein, starch and other nutrients for humans. Global wheat production was 716 million ton in 2013 of which Canada contributed 37.5 million ton and was ranked the 6th largest wheat producer in the world (FAOSTAT 2015). More wheat is produced in Canada than any other crop, followed by canola (*Braccica napus*) (17.9 million ton) and maize (*Zea mays*) (14.1 million ton) (FAOSTAT 2015). The Canadian prairie provinces of Alberta, Manitoba and Saskatchewan produced 10.5 million ton, 5.2 million ton and 18.3 million ton in 2013, respectively (Statistics Canada, 2015).

Due to the short growing season in western Canada, development of early maturing cultivars is an effective way to avoid some biotic and abiotic stresses (Iqbal et al. 2007). In wheat, flowering time is determined by three gene groups: vernalization, photoperiod and earliness *per se* genes (Distelfeld et al. 2009). Vernalization genes are associated with temperature requirements in the seedling stage; photoperiod genes with day length sensitivity and earliness *per se* genes mediate basic development rate. The first two gene groups play a greater role in determining flowering and maturity time than earliness *per se* genes.

The incorporation of reduced height (*Rht*) genes has been a globally important wheat breeding strategy. The Green Revolution increased grain yield through height reduction which reduced lodging, improved harvest index, and led to greater grain biomass assimilation potential (Evenson and Gollin 2003; Borojevic and Borojevic 2005). Dwarfing genes in wheat can be divided into two categories based on seedling response to exogenous gibberellin acid (GA) (Börner et al. 1987). *Rht-B1* on chromosome 4BS and *Rht-D1* on chromosome 4DS belong to the GA-insensitive group and both derive from the Japanese wheat cultivar Norin10 (Gale and Youssefian 1985). These genes are now present in more than 70% of globally registered wheat cultivars (Evans 1998).

Since 1980, when restriction fragment length polymorphism (RFLP) was first used in human genome mapping (Botstein et al. 1980), various molecular markers have been developed,

invented and applied to genetic research, such as Diversity arrays technology (DArT) (Jaccoud et al. 2001) and single nucleotide polymorphism (SNP) (Edwards et al. 2007). Based on these molecular markers, many studies have been conducted to map quantitative trait loci (QTL) of importance for crop adaptation processes. In wheat, recombinant inbred lines (RILs) and doubled haploid (DH) populations are commonly employed for mapping studies, deriving from crosses between two parents which differ for traits of interest. Such parents only represent a small variation within the species. QTLs in different populations often exhibit specificity. Compared to linkage mapping, association mapping uses natural populations which lessens the population construction time. Individuals in an association mapping study are usually from various genetic backgrounds, such as different germplasm, growing areas and/or pedigrees. Populations generally include broader allele coverage and higher recombinant events during historical evolution. The increased recombination frequency among several alleles leads to reshuffling of chromosome segments into smaller pieces, which reduces LD levels in short distances. Only physically close loci can be found significantly correlated thereby allowing high resolution (Zhu et al. 2008).

The goal of this thesis was to explore and understand the genetic control of various phenotypic traits in Canadian spring wheat. The specific objectives were: 1) to map and investigate the QTLs of phenotypic traits in a population of recombinant inbred lines (RILs) from two Canadian hard red western spring wheat cultivars; 2) to investigate the advantages and disadvantages of marker assisted selection of a mapping population in an actual breeding program; 3) to identify specific vernalizaiton, photoperiod and semi-dwarf gene combinations that may offer advantages in western Canadian growing conditions; and 4) to identify marker traits association in Canadian western spring wheat germplasm.

6.2 Contribution to Knowledge

The first experimental study of this thesis project was to explore the effect of the three flowering and maturity gene complexes on the expression of 11 agronomic traits in the CDC Teal × CDC Go Canadian western red spring wheat mapping population. The population of 187 recombinant inbred lines was genotyped with 341 DArT polymorphic markers and a functional *Vrn-B1* marker; and phenotyped over three years in replicated trials. The dominant allele of *Vrn-B1* reduced the number of days to heading, flowering and maturity, and increased leaf color concentration and plant height, but did not affect grain yield in the presence of common genetic

backgrounds with dominant *Vrn-A1a* alleles at *Vrn-A1*. A total of 21 QTLs were identified for all phenotypic traits recorded, except plant height and grain protein concentration. Two earliness *per se* QTLs were mapped on chromosomes 1A (*QEps.dms-1A*) and 4A (*QEps.dms-4A*) in all three growing seasons, contributing 15-27% and 8-10%, respectively, to the total genetic variation in days to maturity. The two earliness QTLs and *Vrn-B1* exhibited additive interaction. Lines carrying dominant alleles at these three loci headed, flowered and matured 1.7, 1.9 and 4 days earlier, respectively, but yielded 0.43t ha^{-1} less than lines with recessive alleles.

I also investigated the differences in disease response and agronomic traits of a CDC Teal × CDC Go spring wheat population of 187 RILs in relation to the presence/absence of the rust resistance gene complex *Lr34/Yr18.* Lines carrying the resistant allele of *Lr34/Yr18* were taller, matured earlier, yielded less grain with lower test weights than lines without *Lr34/Yr18*. Lines with or without the resistant allele of *Lr34/Yr18* did not differ for grain protein content or SDS sedimentation volume. Lines with the resistant allele of *Lr34/Yr18* exhibited lower leaf and stripe rust infection than lines with the susceptible allele. Lines with and without the resistant allele of *Lr34/Yr18* did not differ for resistance to leaf spotting or common bunt. We used results from the first year phenotyping of the mapping population to examine a real plant breeding question. We selected superior lines from the population based on high yield, protein, SDS sedimentation and the presence of the resistant allele of *Lr34/Yr18* and grew them with continued selection in replicated yield trials over nine site years. We were attempting to obtain the breeding result of combining *Lr34/Yr18* with high yield, protein, and SDS sedimentation appropriate to the Canadian Western Hard Red spring classification of wheat. Selecting for only one marker reduces the effective population size in half. Thereafter, selection for yield, protein, strong dough and disease resistance requires much larger populations than a standard mapping population study. We suggest that single seed descent or doubled haploid populations for the combined selection of multiple quantitatively inherited traits, and simply one molecular marker, would require population sizes in excess of at least 500 to have any possibility of selection success. Failing to obtain such a population structure implies that bulk or modified bulk selection strategies may be more appropriate for desirable field-scale breeding results.

Genetic variation is a prerequisite in global wheat improvement programs. High grain yield and protein content and early maturity are some of the major goals in global as well as Canadian

wheat breeding programs. In current study, we investigated genetic diversity for earliness related and plant height reducing genes in 82 spring wheat cultivars registered in western Canada through eight diagnostic DNA markers. Allelic variation was observed at the *Vrn-A1*, *Vrn-B1*, *Vrn-D1* and *Ppd-D1* loci but not for *Ppd-A1* and *Ppd-B1* loci in the studied wheat genotypes. Spring type allele of *Vrn-A1* was present in 94% cultivars, whereas only two cultivars carried spring allele of *Vrn-D1*. Among the four earliness related genes, the most frequent combination was *Vrn-A1a*, *Vrn-B1*, *vrn-D1* and *Ppd-D1b,* which was found in 32 Canadian spring wheat cultivars. As for the *Rht* genes, eight cultivars had *Rht-B1b* and 13 cultivars had *Rht-D1b*. All cultivars carrying spring type allele of *Vrn-B1*, photoperiod-insensitive allele of *Ppd-D1* and height reducing allele of *Rht-1* had shorter plants and higher grain yield but lower grain protein content. Days to heading and maturity showed positive genetic (r_g=0.65) and phenotypic correlation (r_p= 0.44), and were also positively correlated with grain yield and kernel weight but negatively correlated with test weight and protein content. Plant height was positively correlated with grain protein content (r_g= 0.53; r_p= 0.42), but negatively correlated with grain yield (r_g = -0.47; r_p= -0.14). Grain yield and protein content showed negative genetic correlation (r_g=0.57). Among the sixty cultivars from Canada Western Red Spring Class released over 100 years, the newest cultivar yielded 23% more grain and had 15% higher grain protein than the oldest cultivar Red Fife. Breeding efforts in western Canada have resulted in the incorporation of vernalization and photoperiod insensitive and height reducing genes in modern cultivars to promote early maturity, to make use of off-season nurseries in other parts of the world and to improve lodging tolerance.

Days to heading, plant height, plant maturity, test weight, kernel weight, grain yield, protein content and disease resistance are important traits for wheat breeders in Canada. The objectives of the present study were to (i) understand the extent of genetic differences and pattern of relationship among 82 historical and modern Canadian spring wheat cultivars released over a 100 year period; and (ii) identify genomic regions and single nucleotide polymorphic (SNP) markers associated with the above seven traits using genome wide association (GWAS) mapping. The 82 cultivars were phenotyped in six environments and genotyped with 15,897 high quality SNP markers. The mapping population showed the presence of clear population structure, primarily based on Western Canadian wheat classes. The quality of the phenotype data across the six years was generally very good, with high heritability (0.68 to 0.76) for plant height, TKW and protein content, and moderate heritability (0.29 and 0.43) for days to heading and maturity, test

weight and grain yield. Using GWAS we identified a total of 152 significant marker-trait associations, which include two for days to heading, 4 for kernel weight, 14 for test weight, 24 for days to maturity, 31 for grain yield, 37 for protein content and 40 for plant height. However, there were only 18 genomic regions that consisted of clusters of 3 to 20 significant SNPs across 12 chromosomes, with each region explaining on average between 13.7 and 19.8% of the phenotypic variance. This includes two regions for each of grain yield, test weight and protein content, six regions for plant height and six other coincident regions that were associated with two or three traits. The coincident regions, which could be due to tight linkage or pleiotropy were located on 2A for protein content, days to maturity and TKW; on 3A for protein content, days to maturity and grain yield on 6B for protein content, days to maturity and test weight; 2B for days to maturity and grain yield; two regions on 5B for protein content and plant height plus protein content and test weight, respectively. The genomic region associated with plant height on chromosome 4B showed high linkage disequilibrium ($r^2 \geq 0.80$) with the semi-dwarf gene *Rht-B1*.

6.3 General discussion

Most phenotypic traits are complex and the genetic basis of them remains poorly understood in wheat (Bennett et al. 2012; Blake et al. 2009; Groos et al. 2003). I explored the underlying genetics of some important breeding traits in western Canada through two different mapping populations: a recombinant inbred line population and a natural population including historical and modern western Canadian spring wheat cultivars. I mapped a total of 21 QTLs in the 187 RIL population and found 152 significant marker trait associations (MTAs) in the association mapping study of a natural population. My results suggest that association mapping may identify more potential QTL or genes for more simply inherited phenotypic traits than QTL mapping due to broader genetic background of the population structure. Both of these methods found significant QTLs or MTAs for day to heading on chromosome 4A, for test weight on chromosome 1B and for days to maturity on chromosomes 1A, 2B and 4A respectively. Cultivars CDC Teal and CDC Go differed on chromosome 2B for days to maturity in association mapping. Different types of molecular markers were applied in the mapping populations, it was difficult to confirm the marker trait association on chromosome 2B in the bi-parental population in my thesis. However, this strategy has been applied in soybean association analysis (Sonah et al. 2015; Butenhoff 2015). Genome wide association studies are not yet well established in wheat because high throughput genotyping platforms have only recently been developed and the genome size of

wheat is very large and complex (Wang et al. 2014; Eversole et al. 2014). The physical map of the whole genome is still a work in process and genetic regions are not well annotated in wheat. For complex traits, such as grain yield, it was difficult to assess the degree of success achieved by accuracy of marker trait associations identified. Thus, QTL mapping could play an important role to validate the loci found from GWAS.

In genetic mapping, the density of markers has an impact upon QTLs identification. According to my molecular characterization of early maturity and semi-dwarf genes in Western Canadian spring wheat, cultivars CDC Teal and CDC Go carried different alleles for vernalizaiton gene *Vrn-B1*, photoperiod gene *Ppd-D1* and semi-dwarf gene *Rht-B1* loci by Diagnostic markers from theses loci. However, in the bi-parents population, no QTL identified from the population related to these three genes might due to the relatively small number of DArT markers on chromosome 5B, 2D and 4B, respectively. Through the simulation for interval mapping, the power of QTL detection and the genetic effect were affected by marker density less than 10 cM in a backcross population (Piepho 2000). For association mapping, only 2% of total 90K SNP markers was applied for marker trait association. On chromosome 4D, my failure to find high linkage disequilibrium markers for *Rht-D1* may be due to the reality that there were only 93 SNPs on the chromosome.

According to the diagnostic and SNP markers, the 82 historical and modern cultivars had polymorphism for vernalization, photoperiod and reduced height loci. Every cultivar carried at least one spring type allele of three vernalization genes, but only a few cultivars carried the semi-dwarf genes. The genetic distances among Canadian western spring wheat cultivars applied in our study was relative small. Some cultivars shared the same progenitor. For instance, Neepwa as a recurrent parents was employed to cross with other breeding lines and produced Katepwa (Campbell and Czarnecki 1987) and Columbus (Campbell and Czarnecki 1981).

For candidate genes association analysis, all the genes of Vernalization (*Vrn-A1*, *Vrn-B1* and *Vrn-D1*), photoperiod (*Ppd-D1*) and plant height reduced (*Rht-B1* and *Rht-D1*) exhibited no strong significant effects ($P< 0.001$) on the phenotypic traits they controlled, but *Rht-B1* and *Ppd-D1* exhibited moderate significant effect ($P<0.01$) on plant height and grain yield respectively. They also showed high linkage disequilibrium with SNP markers on their own chromosomes. All the cultivars applied in association mapping were spring wheat. The frequency of spring type

alleles were 93%, 48% and 2% for *Vrn-A1*, *Vrn-B1* and *Vrn-D1*, respectively. The spring type allele of *Vrn-A1* was not only insensitive to varnalizaiton but also epistatic to *Vrn-B1* and *Vrn-D1*(Shindo and Sasakuma 2002). In our study, most of the cultivars carried the spring type allele of *Vrn-A1*, which might be the reason that non-significant effect of *Vrn-B1* were found on days to heading and maturity.

6.4 Future Research

To better understand the genetic background of Canadian western spring wheat, therefore:

1) The molecular marker number is important in genetic linkage mapping, so the 90K SNPs marker chip is a wise choice in the future genetic study. This would allow comparison of results from mapping populations;
2) In western Canada, the frequency of *Rht-B1b* and *Rht-D1b* are relatively small in the current germplasm, we need to introduce *Rht-B1b* or *Rht-D1b* into more cultivars, because these two alleles decrease plant height and increase grain yield. We also need explore polymorphism of other plant height reducing genes, such as *Rht-4*, *Rht-5* and *Rht-8* in Canada western spring wheat cultivars.
3) For future association mapping analysis, preselection of cultivars will be necessary to expand the genetic background. The population size for association mapping could be increased as well.
4) Early maturity is important in western Canada, so it is essential to create specific population to explore the genetic interaction among vernalizaiton genes and photoperiod genes and within their own categories. Also, we found a region on chromosome 2B was associated with days to maturity. It should be confirmed whether it is the same as *Ppd-B1* by other methods or primers.
5) According to linkage mapping and association mapping, a QTL/region located on chromosome 4A are associated with days to maturity. This location should be confirmed in the future genetic study.

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Appendices

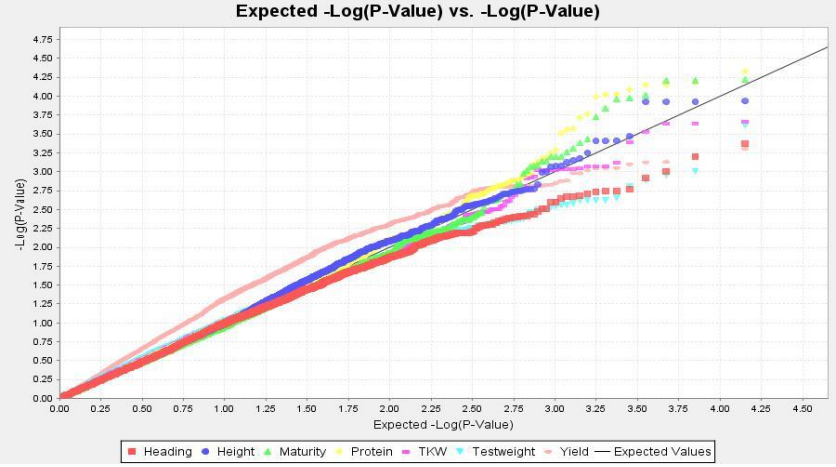
Appendix 1 Summary of the 82 spring wheat cultivars used in the present study.

Variety	Year of release	Class	Group membership from DAPC	Group membership from cluster analysis
5500HR	2000	CWRS	1	2
5600HR	1999	CWRS	1	2
5601HR	2001	CWRS	3	3
5602 HR	2004	CWRS	1	2
5603 HR	2008	CWRS	3	3
5700 PR	2000	CPS	2	1
5701 PR	2001	CPS	2	3
5702 PR	2007	CPS	2	1
859 CL	2008	CWRS	1	2
AC Abbey	1998	CWRS	1	2
AC Andrew	2000	CWSWS	2	1
AC Barrie	1994	CWRS	1	2
AC Cadillac	1996	CWRS	1	2
AC Crystal	1996	CPS	2	1
AC Domain	1993	CWRS	3	3
AC Eatonia	1993	CWRS	1	2
AC Elsa	1996	CWRS	1	2
AC Foremost	1995	CPS	2	1
AC Intrepid	1997	CWRS	1	2
AC Splendor	1997	CWRS	1	2
AC Superb	2001	CWRS	3	3
AC Taber	1991	CPS	2	1
AC Vista	1996	CPS	2	1
Alvena	2006	CWRS	1	2
Bhishaj	2003	CWSWS	2	1
Burnside	2004	CWES	2	1
BW878	2009	CWRS	3	3
Carberry	2009	CWRS	3	3
CDC Abound	2006	CWRS	3	3
CDC Alsask	2004	CWRS	1	2
CDC Bison	2008	CWES	2	1
CDC Bounty	1999	CWRS	1	2
CDC Go	2003	CWRS	3	3
CDC Imagine	2002	CWRS	1	2
CDC Kernen	2009	CWRS	1	2
CDC Merlin	1992	CWRS	1	2

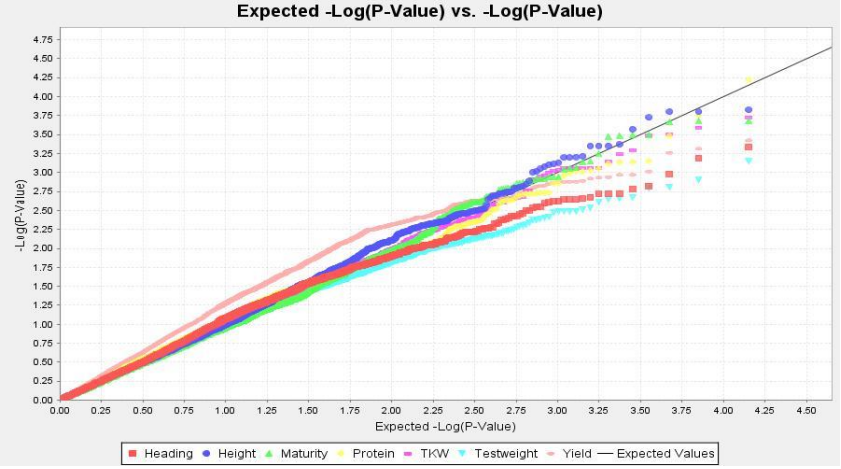
Variety	Year of release	Class	Group membership from DAPC	Group membership from cluster analysis
CDC Osler	2003	CWRS	1	2
CDC Plentiful	2011	CWRS	1	2
CDC Rama	2001	CWES	2	1
CDC Stanley	2009	CWRS	1	2
CDC Teal	1991	CWRS	1	2
CDC Utmost	2009	CWRS	1	2
CDC Walrus	2003	CWES	2	1
Columbus	1980	CWRS	1	2
Conquer	2009	CPS	2	1
Fieldstar	2007	CWRS	3	3
Garnet	1925	CWRS	2	3
Glencross	2007	CWES	2	1
Glenlea	1972	CWES	2	1
Glenn	2009	CWRS	3	3
Goodeve VB	2007	CWRS	1	2
Harvest	2004	CWRS	3	3
Helios	2006	CWRS	1	2
Infinity	2004	CWRS	1	2
Journey	2002	CWRS	1	2
Kane	2006	CWRS	3	3
Katepwa	1981	CWRS	1	2
Laura	1986	CWRS	1	2
Lillian	2004	CWRS	1	2
Lovitt	2002	CWRS	1	2
Marquis	1910	CWRS	1	3
McKenzie	1997	CWRS	3	3
Minnedosa	2008	CWGP	2	1
MuchMore	2009	CWRS	3	3
Neepawa	1969	CWRS	1	2
GP 003	2009	CWGP	2	1
GP 010	2009	CWGP	2	1
Park	1963	CWRS	1	3
Prodigy	1998	CWRS	3	3
PT 559	2003	CWRS	3	3
Red Fife	1885	CWRS	2	3
Roblin	1986	CWRS	1	2
Sadash	2007	CWSWS	2	1
Shaw	2009	CWRS	3	3
Snowbird	2000	CWHWS	3	3
Snowstar	2006	CWHWS	3	3
Somerset	2005	CWRS	1	2

Variety	Year of release	Class	Group membership from DAPC	Group membership from cluster analysis
Stettler	2008	CWRS	3	3
Thrive	2009	CWRS	1	2
Unity	2007	CWRS	3	3
Vesper	2010	CWRS	3	3
Waskada	2007	CWRS	3	3

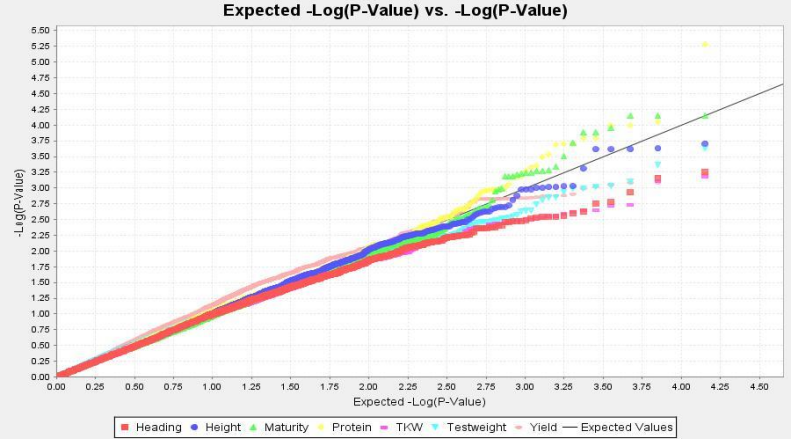
Appendix 2 Comparison of QQ plots for three different mixed linear models for seven phenotypic traits: (A) mixed model with kinship matrix; (B) mixed model with kinship matrix and population structure from STRUCTURE software; and (C) mixed model with kinship matrix and population structure from DAPC method from R package.



(A)



(B)



(C)

Appendix 3 The 152 signficant marker-trait assocaition uncovered for the 7 traits evalauted across six environmnets in Canada. A SNP was declared significant at –log10P value of >3.0. The SNPs that passed the 4.1 -$\log_{10}$(P-value) threshold value for Bonferroni correcion are indicated in boldface.

Trait	Marker	Chro[1].	Position (cM)	P value	-$\log_{10}$P value	Marker R^2
Maturity	RFL_Contig3203_1971	1A	77.8	8.88E-04	3.1	13.5%
Test weight	IAAV7541	1B	53.4	5.01E-04	3.3	15.7%
Test weight	BS00065896_51	1B	60.6	5.76E-05	4.2	19.2%
Height	RAC875_c92464_53	1B	70.1	7.77E-04	3.1	13.6%
1000 kernel weight	wsnp_RFL_Contig4298_4993464	1B	143.6	2.29E-04	3.6	12.4%
Yield	D_GBF1XID01DNWRS_378	1D	85.6	6.47E-04	3.2	13.5%
Yield	BS00004282_51	1D	87.4	6.82E-04	3.2	13.6%
Yield	BS00079095_51	1D	87.4	6.47E-04	3.2	13.5%
Yield	Kukri_c3253_83	1D	87.4	8.23E-04	3.1	13.4%
Yield	RAC875_c54260_127	1D	87.4	6.82E-04	3.2	13.6%
Yield	wsnp_Ex_c13164_20793506	1D	87.4	7.44E-04	3.1	13.6%
Yield	wsnp_Ex_c6920_11929922	1D	87.4	6.79E-04	3.2	13.6%
Yield	Excalibur_c48609_101	1D	88.9	6.54E-04	3.2	13.8%
Yield	IAAV868	1D	89.6	6.75E-04	3.2	13.8%
Yield	BobWhite_c10977_834	1D	90.3	5.46E-04	3.3	13.9%
Yield	BS00075001_51	1D	90.3	7.34E-04	3.1	13.6%
Yield	RAC875_c51177_261	1D	90.3	7.81E-04	3.1	13.7%
Yield	RFL_Contig5534_1826	1D	90.3	7.25E-04	3.1	13.7%
Yield	wsnp_Ex_c1609_3069103	1D	90.3	7.92E-04	3.1	13.7%
Yield	wsnp_Ex_c57601_59245965	1D	90.3	6.87E-04	3.2	13.6%
Yield	wsnp_Ku_c5560_9853214	1D	90.3	7.25E-04	3.1	13.7%
Yield	BS00018250_51	1D	92.9	7.08E-04	3.2	13.7%
Yield	CAP11_c3464_68	1D	92.9	5.65E-04	3.2	14.0%
Yield	IAAV5841	1D	92.9	8.23E-04	3.1	13.4%
Yield	wsnp_Ku_rep_c83972_79348562	1D	92.9	3.25E-04	3.5	15.4%
Kernel weight	wsnp_Ex_c19516_28480622	2A	6.0	1.77E-04	3.8	14.1%
Protein content	CAP8_c2783_336	2A	18.6	2.28E-05	4.6	20.6%
Protein content	Kukri_c22152_730	2A	18.6	3.16E-05	4.5	19.7%
Protein content	RFL_Contig4030_493	2A	18.6	5.01E-05	4.3	19.0%
Protein content	wsnp_Ex_c11950_19164041	2A	18.6	4.21E-05	4.4	19.5%
Protein content	wsnp_Ex_c11950_19164191	2A	18.6	3.54E-05	4.5	19.7%
Protein content	Excalibur_c386_894	2A	20.1	1.17E-05	4.9	21.8%
Protein content	Kukri_c33374_1048	2A	20.1	1.36E-04	3.9	16.8%
Protein content	wsnp_Ex_c997_1906900	2A	20.3	1.76E-04	3.8	16.7%
Protein content	wsnp_Ku_c33374_42877546	2A	20.3	1.82E-04	3.7	16.4%
Kernel weight	Tdurum_contig21761_267	2A	25.0	2.23E-04	3.7	13.4%
Maturity	BobWhite_c26803_89	2A	26.0	2.02E-05	4.7	21.2%

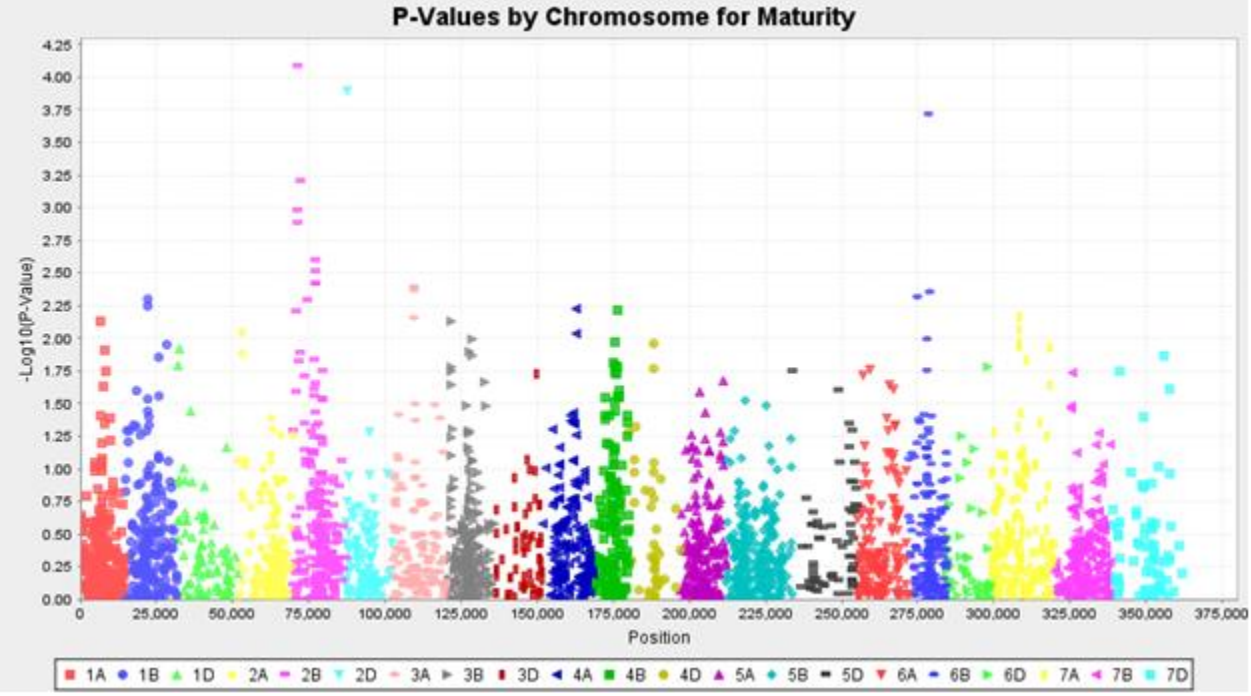
Trait	Marker	Chro[1].	Position (cM)	P value	-log$_{10}$P value	Marker R^2
Protein content	Excalibur_c7682_652	2A	26.0	5.78E-04	3.2	14.1%
Protein content	TA002095-0637	2A	26.0	2.75E-04	3.6	15.4%
Yield	wsnp_Ra_c1501_2991585	2B	19.2	3.73E-04	3.4	14.8%
Maturity	Kukri_c98858_299	2B	23.9	2.31E-04	3.6	16.3%
Maturity	BobWhite_c2949_1083	2B	25.1	2.28E-04	3.6	17.0%
Maturity	BobWhite_c5668_115	2B	25.1	2.31E-04	3.6	16.3%
Maturity	BobWhite_c7145_355	2B	25.1	1.49E-04	3.8	17.5%
Maturity	BobWhite_rep_c64787_359	2B	25.1	2.50E-04	3.6	16.4%
Maturity	BS00043055_51	2B	26.1	4.82E-04	3.3	15.0%
Maturity	Tdurum_contig54649_798	2B	26.1	4.95E-04	3.3	14.8%
Maturity	BobWhite_c45060_188	2B	26.5	7.39E-06	5.1	23.5%
Maturity	Excalibur_c25445_1061	2B	26.5	7.39E-06	5.1	23.5%
Maturity	Excalibur_c52842_317	2B	26.5	8.20E-06	5.1	23.5%
Maturity	Kukri_c22513_1780	2B	26.5	2.17E-05	4.7	21.4%
Maturity	RAC875_c6538_123	2B	26.5	8.99E-05	4.0	18.7%
Maturity	wsnp_Ex_c25445_34710489	2B	26.5	1.85E-05	4.7	21.7%
Yield	Kukri_rep_c69177_180	2B	28.5	3.56E-04	3.4	15.1%
Maturity	RAC875_c38003_164	2B	35.1	1.94E-04	3.7	16.9%
Test weight	BS00070001_51	2B	88.9	3.74E-04	3.4	15.6%
Test weight	IAAV29	2B	88.9	3.22E-04	3.5	15.9%
Test weight	RAC875_rep_c70402_527	2B	88.9	1.26E-04	3.9	17.1%
Test weight	wsnp_Ex_c12671_20140295	2B	89.3	5.00E-04	3.3	14.4%
Height	wsnp_Ex_c5193_9204522	2B	153.2	8.64E-04	3.1	13.1%
Height	Excalibur_c11727_837	2B	157.2	4.40E-04	3.4	14.5%
Height	BS00032381_51	2B	161.4	3.09E-04	3.5	15.5%
Height	Excalibur_c48404_59	2B	161.4	3.48E-04	3.5	15.1%
Height	Kukri_c38413_121	2B	161.4	3.58E-04	3.4	15.2%
Height	wsnp_Ex_c15646_23969140	2B	161.4	3.48E-04	3.5	15.1%
Height	RAC875_c16993_444	2B	173.4	6.61E-04	3.2	13.8%
Height	RAC875_c23769_347	2B	173.4	1.47E-04	3.8	16.7%
Maturity	Kukri_c77179_54	2D	4.7	4.37E-05	4.4	21.2%
Maturity	BS00022148_51	3A	85.1	7.23E-04	3.1	14.1%
Yield	D_F5XZDLF02GZM9K_79	3A	85.4	5.21E-05	4.3	19.5%
Protein content	wsnp_Ku_c8334_14181247	3A	87.4	4.67E-08	7.3	31.3%
Yield	Ra_c88203_1055	3A	89.5	6.50E-04	3.2	13.9%
Yield	Ra_c88203_455	3A	89.5	5.99E-04	3.2	13.9%
Protein content	Ra_c11263_2353	3A	90.6	7.90E-05	4.1	17.9%
Protein content	Tdurum_contig68855_91	3A	90.6	7.90E-05	4.1	17.9%
Yield	Ra_c11263_2353	3A	90.6	2.96E-04	3.5	15.1%
Yield	Tdurum_contig68855_91	3A	90.6	2.96E-04	3.5	15.1%
Height	Excalibur_c39508_88	3A	111.6	1.95E-04	3.7	16.1%

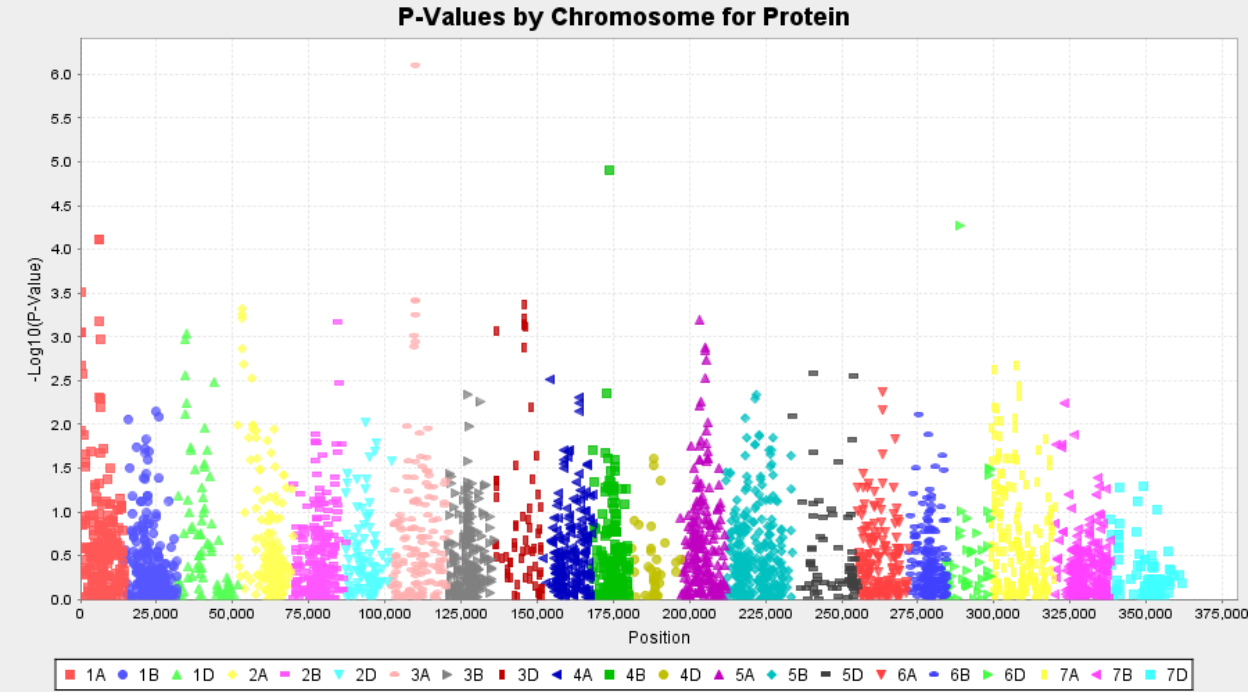
Trait	Marker	Chro[1].	Position (cM)	P value	-log$_{10}$P value	Marker R^2
Height	BobWhite_c5337_225	3A	124.3	3.30E-05	4.5	19.6%
Height	BS00035364_51	3A	124.3	3.30E-05	4.5	19.6%
Height	BS00088755_51	3A	124.3	2.88E-05	4.5	20.0%
Height	BobWhite_c26330_284	3A	125.0	3.66E-05	4.4	19.7%
Yield	BS00046375_51	3B	34.2	5.39E-04	3.3	14.2%
Yield	RAC875_c7724_1312	3B	34.2	5.18E-04	3.3	14.4%
Yield	Tdurum_contig13898_648	3B	34.2	4.63E-04	3.3	14.3%
Protein content	D_GBB4FNX02JKG8H_167	3D	97.4	6.64E-04	3.2	14.3%
Protein content	CAP8_c2305_193	3D	97.7	7.81E-04	3.1	13.7%
Height	wsnp_BE444579D_Ta_2_3	3D	143.0	6.87E-04	3.2	13.7%
Heading date	Ex_c7227_53	4A	37.1	5.57E-04	3.3	18.2%
Maturity	Tdurum_contig20987_1271	4A	61.9	1.85E-04	3.7	16.8%
Protein content	RAC875_c27536_611	4B	54.6	8.66E-06	5.1	22.4%
Height	wsnp_Ku_c28756_38667953	4B	56.0	5.89E-04	3.2	14.1%
Height	IAAV971	4B	57.5	5.14E-04	3.3	14.2%
Height	Kukri_c11415_1074	4B	68.5	7.80E-04	3.1	13.3%
Height	Kukri_c94033_432	4B	68.5	5.79E-04	3.2	14.3%
Height	RAC875_c6865_349	4B	68.5	8.33E-05	4.1	17.8%
Height	TA003248-0911	4B	68.5	3.63E-05	4.4	19.7%
Protein content	RFL_Contig5341_816	4B	86.6	6.94E-05	4.2	18.1%
Height	Kukri_s112067_110	5A	64.2	8.60E-04	3.1	13.3%
Protein content	BobWhite_c6782_180	5A	82.0	6.69E-04	3.2	13.6%
Protein content	BS00035256_51	5A	82.7	4.03E-04	3.4	14.7%
Protein content	Kukri_c54152_242	5A	82.7	4.24E-04	3.4	14.8%
Protein content	wsnp_Ku_c21275_31007309	5A	82.7	3.32E-04	3.5	15.2%
Height	CAP7_c5481_96	5B	29.1	3.74E-04	3.4	14.8%
Height	BS00077423_51	5B	45.4	8.10E-04	3.1	13.6%
Height	Kukri_rep_c69113_985	5B	45.4	6.65E-04	3.2	14.1%
Height	Ex_c66350_301	5B	51.2	1.89E-04	3.7	16.2%
Height	Ex_c66350_302	5B	51.2	2.01E-04	3.7	16.3%
Height	Ex_c66350_322	5B	51.2	1.89E-04	3.7	16.2%
Protein content	RAC875_c52775_296	5B	135.6	7.25E-05	4.1	18.0%
Height	Tdurum_contig32091_224	5B	137.1	8.25E-04	3.1	13.4%
Protein content	IACX3775	5B	138.3	3.96E-04	3.4	14.7%
Protein content	Ex_c8305_422	5B	139.4	3.96E-04	3.4	14.7%
Protein content	IAAV1779	5B	142.6	3.00E-04	3.5	16.1%
Protein content	BobWhite_c36154_81	5B	143.6	2.71E-04	3.6	15.7%
Protein content	RFL_Contig372_339	5B	144.6	5.26E-04	3.3	15.0%
Protein content	BobWhite_c10921_1183	5B	176.6	6.05E-04	3.2	13.8%
Protein content	BobWhite_c16916_658	5B	176.6	6.05E-04	3.2	13.8%
Protein content	GENE-2399_666	5B	176.6	6.88E-04	3.2	14.4%

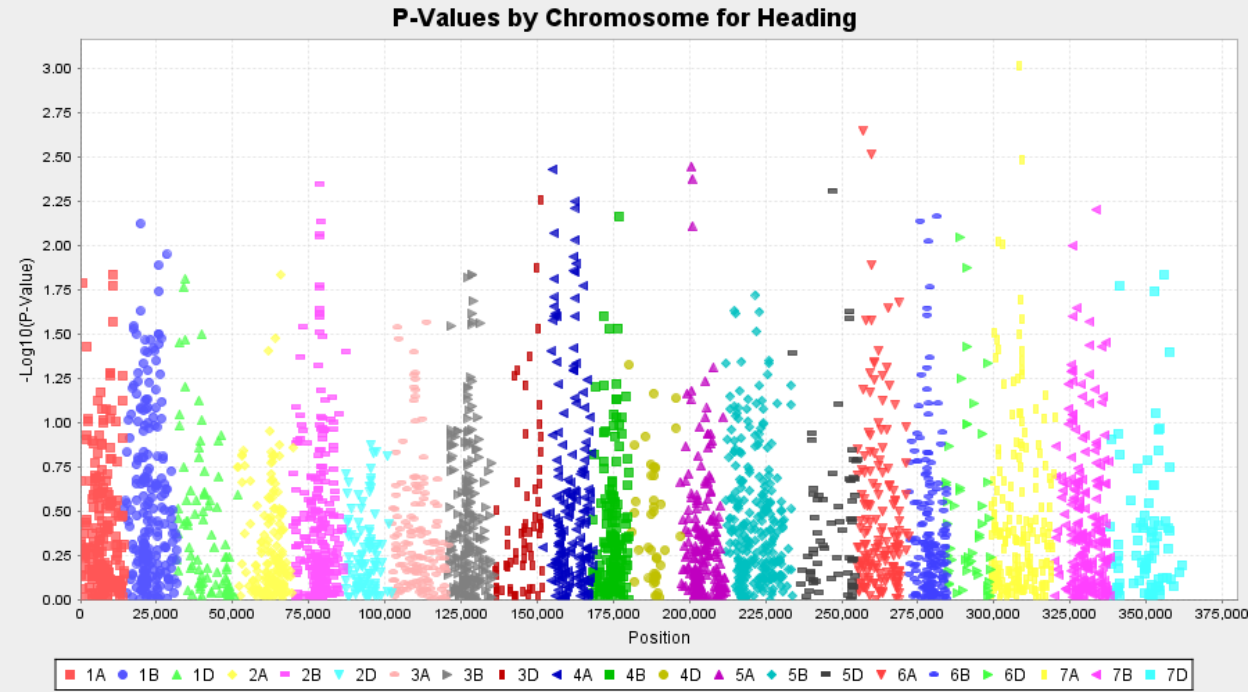
Trait	Marker	Chro[1].	Position (cM)	P value	-log$_{10}$P value	Marker R^2
Test weight	RFL_Contig3285_1009	5B	182.2	2.32E-04	3.6	16.2%
Test weight	Kukri_c7546_3035	5B	206.1	1.95E-04	3.7	16.3%
Test weight	Ra_c3287_1461	5B	206.1	1.95E-04	3.7	16.3%
Protein content	RAC875_c47278_818	6A	37.0	2.19E-04	3.7	15.9%
Height	BS00004377_51	6A	74.2	2.20E-04	3.7	16.4%
Height	IACX6453	6A	74.2	2.00E-04	3.7	16.4%
Height	wsnp_Ex_rep_c102845_87922204	6A	74.2	1.88E-04	3.7	16.3%
Test weight	RAC875_rep_c107929_341	6B	39.2	2.75E-04	3.6	16.6%
Protein content	BS00074041_51	6B	57.1	8.87E-04	3.1	13.1%
Maturity	RAC875_rep_c107892_142	6B	57.4	1.74E-04	3.8	16.9%
Maturity	GENE-4074_1167	6B	58.2	2.25E-04	3.6	16.6%
Maturity	Kukri_c38398_164	6B	58.2	1.74E-04	3.8	16.9%
Test weight	Kukri_c38732_225	6B	59.2	6.62E-04	3.2	14.2%
Maturity	Excalibur_c47748_83	6B	63.5	8.72E-05	4.1	19.0%
Test weight	Tdurum_contig44173_792	6B	71.8	7.3E-04	3.1	13.9%
Test weight	Tdurum_contig7981_70	6B	71.8	2.8E-04	3.6	15.8%
Test weight	wsnp_CAP11_c166_172556	6B	71.8	3.2E-04	3.5	15.3%
Yield	BS00034554_51	6B	93.5	8.7E-04	3.1	13.3%
Height	Tdurum_contig54917_597	6D	39.9	3.5E-04	3.5	15.1%
Protein content	Ku_c586_811	6D	58.8	8.1E-06	5.1	22.4%
Height	Kukri_c35951_337	6D	82.1	5.3E-04	3.3	14.3%
Height	RAC875_c18002_58	6D	82.1	5.8E-04	3.2	14.0%
Height	wsnp_Ex_c37749_45436366	6D	82.1	4.2E-04	3.4	14.9%
Height	wsnp_BE445201D_Ta_1_1	6D	82.2	6.2E-04	3.2	14.0%
Height	Excalibur_rep_c70026_113	6D	83.4	7.2E-04	3.1	13.8%
Height	wsnp_BF428701D_Ta_1_1	6D	83.4	8.5E-04	3.1	13.4%
Protein content	Excalibur_c34115_226	7A	33.5	8.3E-04	3.1	13.6%
Protein content	Tdurum_contig17365_336	7A	42.1	7.3E-05	4.1	18.4%
Protein content	Tdurum_contig17756_518	7A	42.5	3.9E-04	3.4	14.7%
Heading date	BS00038787_51	7A	125.8	6.8E-04	3.2	16.1%
Maturity	Excalibur_rep_c108991_181	7A	125.8	3.7E-04	3.4	15.8%
Kernel weight	BobWhite_c13431_1372	7A	212.7	7.0E-04	3.2	11.4%
Height	wsnp_Ex_c4637_8299644	7D	114.1	8.3E-04	3.1	13.4%

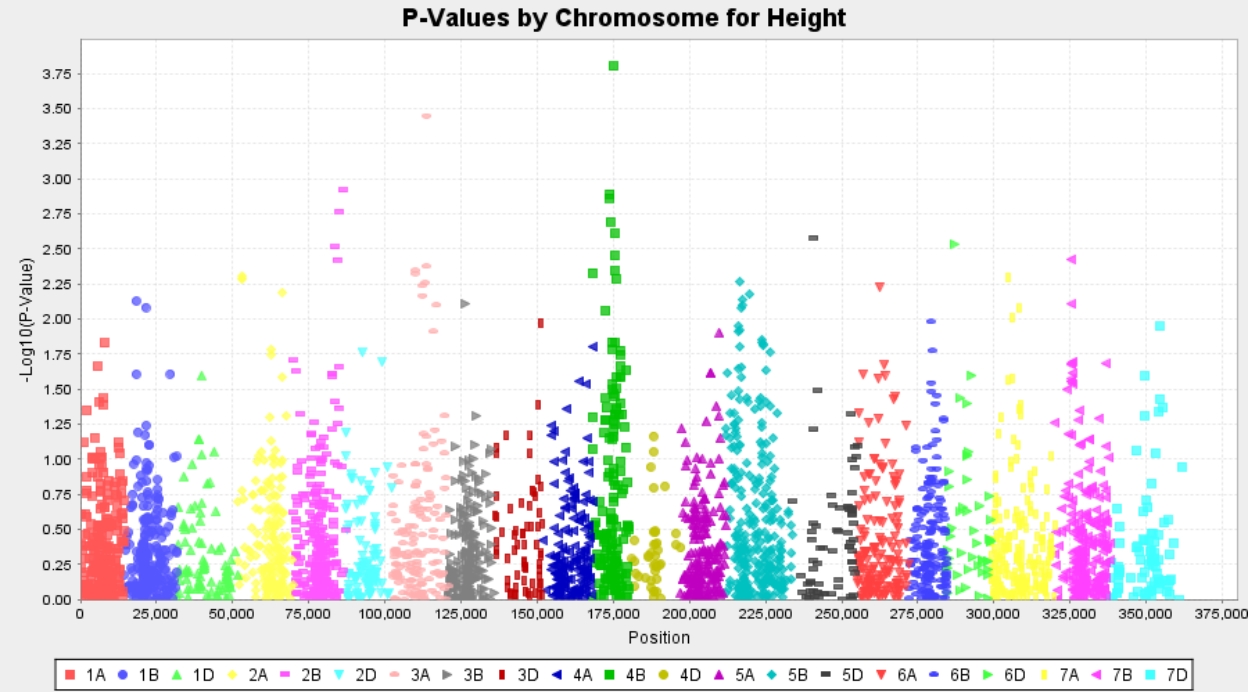
1. Chro: chromosome.

Appendix 4 Manhattan plots of seven phenotypic traits based on the combined environments least square mean. The dotted horizontal line indicates the threshold $-\log_{10}$P value of 3.






P-Values by Chromosome for Heading
-Log10(P-Value)
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
25,000
50,000
75,000
100,000
125,000
150,000
175,000
200,000
225,000
250,000
275,000
300,000
325,000
350,000
375,000
Position
1A 1B 1D 2A 2B 2D 3A 3B 3D 4A 4B 4D 5A 5B 5D 6A 6B 6D 7A 7B 7D


P-Values by Chromosome for Height
-Log10(P-Value)
3.75
3.50
3.25
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
25,000
50,000
75,000
100,000
125,000
150,000
175,000
200,000
225,000
250,000
275,000
300,000
325,000
350,000
375,000
Position
1A 1B 1D 2A 2B 2D 3A 3B 3D 4A 4B 4D 5A 5B 5D 6A 6B 6D 7A 7B 7D