QTL mapping and genetic studies in the Attila x CDC Go spring wheat (*Triticum aestivum*

L.) mapping population

by

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Abstract

Bread wheat (*Triticum aestivum L.*) is one of the most important staple crops in the world. Wheat breeders in Canada primarily aim to develop cultivars with favored agronomic traits such as short stature, early maturity, high yielding, preferred end-use quality such as high protein content, and at least moderate resistance to priority diseases, such as leaf rust, stem rust, yellow rusts, fusarium head blight and common bunt. Almost all the traits mentioned above are quantitatively inherited, and therefore controlled by many genes of small effect. We used a mapping population of 167 recombinant inbred lines (*RILs*) developed from a cross between two spring wheat cultivars, 'Attila' and 'CDC Go' in our study, and evaluated the RIL population for agronomic traits and grain protein content at organic (2008 to 2011) and conventionally managed environments (2008 to 2015), and resistances to diseases in the field from 2012 to 2014. Then we genotyped this population with the Wheat 90K single nucleotide polymorphic (SNP) array. Inclusive composite interval mapping was conducted using average phenotypic data and a subset of 1200 informative SNPs out of the Wheat 90K SNP array. In addition, we compared our results for agronomic traits and grain protein content with the previous study conducted by our group with DArT markers and with phenotypic data from 2008 to 2011. In the present study with phenotypic data from 2008 to 2011, five moderate- and eleven minor-effect QTLs were detected across all three organic environments, including 13 QTLs that were not previously detected. Up to five QTLs were detected for each trait, except grain protein content, which individually accounted for 5.5 to 18.8% of phenotypic variance. For each trait, the total phenotypic and genetic variance explained by all detected QTLs varied from 9.3 to 39.4 and from 24.6 to 96.8%, respectively, which was much greater than our previous study. The results indicated that compared with 579 DArT markers that were used in our previous studies, the high-density SNP

markers were useful in identifying three-fold more number of QTLs. Although direct comparison of the QTL results between the three and seven environments was not simple, we think that the increase in the number of testing environments neither improved new QTL detection nor their effect. For the combined phenotypic data across seven environments, we found a total of 6 minor- and 8 moderate-effect QTLs which individually explained 6.1-18.4% of the phenotypic variance. For wheat disease resistance, in the combined phenotypic data across all the environments, we found a total of 10 QTLs associated with resistances to four diseases, which included three QTL for each of leaf rust, stripe rust, and tan spot; one QTLs for resistance to common bunt.

Preface

Wheat breeders in Canada primarily aim to develop cultivars with favored agronomic traits such as short stature, early maturing, high yielding, preferred end-use quality such as high protein content, and at least moderately resistant to priority diseases, such as leaf rust, stem rust, yellow rusts, fusarium head blight and common bunt. Therefore, it is important to better understand the underlying genetics for those traits in western Canadian wheat breeding programs.

The mapping population development for all my studies was done before I joined the programme. A version of Chapter 2 has been accepted for publication by the journal Crop Science as J. Zou, K. Semagn, M. Iqbal, A. N'Diaye, H. Chen, M. Asif, A. Navabi, E. Perez-Lara, C. Pozniak, R.C. Yang, H. Randhawa, and D. Spaner. Mapping QTLs controlling agronomic traits in the Attila x CDC Go spring wheat population under organic management using 90K SNP array doi: 10.2135/cropsci2016.06.0459; Date posted: September 12, 2016. The phenotypic data from 2008 to 2011 were collected by our research group. I conducted experiments and collected the phenotypic data from 2012 to 2015 and screened the population for all the genetic markers. Dr. Kassa Semagn, Dr. Muhammad Iqbal, and Dr. Chen Hua provided assistance in statistical analyses. I mapped the population with IciMapping software. I wrote manuscript. Dr. Dean Spaner and Dr. Kassa Semagn assisted with manuscript editing and interpretation of the results.

For the second study in Chapter 3, a version has been submitted to PLOS ONE and is under review as Jun Zou, Kassa Semagn, Muhammad Iqbal, Hua Chen, Mohammad Asif, Amidou N'Diay, Alireza Navabi, Enid Perez-Lara, Curtis Pozniak, Rong-Cai Yang, Harpinder Randhawa, and Dean Spaner. Effect of marker density and number of testing environments in

mapping QTLs for agronomic traits in spring wheat. I conducted experiments and collected the phenotypic data from 2012 to 2015 and screened the population for all the genetic markers. I analyzed the data and mapped the population with IciMapping software. I wrote the manuscript. The roles of Dr. Dean Spaner and Dr. Kassa Semagn and Dr. Muhammad Iqbal were similar to those explained above for Chapter 2.

For the third study in chapter 4 (QTL mapping of disease resistance in a RIL population derived from a cross of wheat cultivars Attila and CDC Go), I collected the phenotypic data for disease resistances to leaf rust, tan spot and common bunt from 2012 to 2014 at the Edmonton south campus research center, University of Alberta. Stripe rust in Lethbridge and Creston were recorded by Drs. Harpinder Singh Randhawa and Dean Spaner. I analyzed the data and mapped the population with IciMapping software. I wrote the manuscript. The role of Dr. Dean Spaner and Dr. Kassa Semagn were similar to those explained above for Chapter 2.

Acknowledgments

Finally, I would like to thank my parents for their endless love and support in all aspects of my life, and all my friends who have always been there for me by sharing the laughter and comforting tears in my life.

Contents

List of Tables

List of Figures

Appendices

Chapter 1 Literature review

1.1 Wheat

Bread wheat (*Triticum aestivum L.*) is one of the most important staple crops, feeding around 35% of the world population. It is originally from the Levant region of the Near East and the Ethiopian Highlands but is now cultivated worldwide. Globally, wheat has a higher protein content than maize or rice, which makes it one of the major sources of vegetable protein in human food. (Huang and Röder, 2004).

Hexaploid or bread wheat (2n=6x=42, AABBDD) has a very large genome size and carries three genetically related subgenomes A, B, and D (MuKai et al., 1993). Bread wheat originated from the hybridization of the cultivated tetraploid wheat (*Triticum turgidurn L.,* 2n =4x= 28, AABB) and diploid *Aegilops tauschii Coss.* (2n=2x=14, DD) (Jia et al., 2013; Dvorak et al., 1998). In bread wheat, AA is thought to come from Triticum urartu and DD from *Ae. Tauschii.* BB was from a species that is unknown but which may be of the section *Sitopsis* (to which *Aegilops speltoides* belongs) (Brenchley et al., 2012). The large size, high ploidy level, and the high content of repetitive DNA sequences (80%; Moore, 1995) contribute to the complexity and difficulty of fully understanding the wheat genome (Yahiaoui et al., 2004).

1.2 Wheat in the Canadian prairies

Average wheat productivity in Canada has increased from 2.7 t ha^{-1} in 2005 to 3.1 t ha^{-1} in 2014 (http://faostat3.fao.org), which makes Canada the sixth largest wheat producing and the second largest wheat exporting country (http://apps.fas.usda.gov/psdonline/circulars/production.pdf). Wheat has had a major role in the financial and cultural development of western Canada since the 1920s (Rawlinson and Granatstein 1997). Nearly all of Canada's wheat is produced in the prairie provinces of Manitoba,

Saskatchewan, and Alberta with a relatively small area in British Columbia and eastern Canada (Statistics Canada; McCallum et al., 2008). There are three main types of wheat produced in western Canada, spring hexaploid wheat (*Triticum aestivum L.*), winter hexaploid wheat (*Triticum aestivum L.)* and durum wheat [*Triticum turgidum L. ssp. durum (Desf.) Husn.].* Of these, spring hexaploid wheat has been the predominant type produced (McCallum et al., 2008).

Wheat breeders in Canada primarily aim to develop cultivars with favored agronomic traits such as short stature, early maturing, high yielding, preferred end-use quality such as high protein content, and at least moderate resistance to priority diseases, such as leaf rust, stem rust, yellow rusts, fusarium head blight and common bunt (http://www.pgdc.ca). Almost all the traits mentioned above are quantitatively inherited , and therefore controlled by many genes of small effect (Sham et al. 2002, Koinange et al. 1996, White and Doebley 1998). Due to their complexity, relatively less is known about the genetic control of them compared with the traits that follow Mendelian segregation patterns (McCallum et al., 2008; McCartney et al., 2005).

1.3 Genetic markers

The expression of a quantitative trait depends on the cumulative action of many genes or quantitative trait loci (QTL) and their interaction with the environment. This 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. Wheat breeders employ molecular markers in their breeding programs for different purposes, including parental selection, quality control analysis of advanced lines (cultivars) to examine the level of genetic purity and identity, and for marker-assisted selection (MAS) (Randhawa *et al.*, 2013). Currently, Diversity Arrays Technology (DArT), simple

sequence repeats (SSRs) and single nucleotide polymorphism (SNP) markers have been commonly used for QTL mapping in wheat.

1.3.1 Diversity arrays technology (DArT) markers

DArT, as a microarray hybridization-based technique, enables the simultaneous typing of several hundred polymorphic loci spread over the genome (Jaccoud *et al.*, 2001, Semagn *et al.*, 2006, Wenzl *et al.*, 2004), but the dominant inheritance (present vs absent variation) of DArT markers is one of the major drawbacks, as homozygous dominant and heterozygous individuals cannot be easily identified. In the absence of human error (e.g., contamination, labeling error) that compromises genetic purity in highly homozygous and uniform mapping populations such as RIL, however, the dominant inheritance of DArT markers may not be a major limitation for QTL mapping.

1.3.2 Simple sequence repeat (SSR) markers

SSR markers are widely used by wheat researchers, but one of the challenges of SSR markers is the difficulty in comparing data produced by different laboratories or the same lab at different times due to inconsistencies in allele size calling. Such inconsistencies are mainly due to differences in SSR marker repeat length, and the large variety of automatic sequencing machines used for fragment analyses, each providing different migration, fluorescent dyes, and allele calling software (Vignal *et al.*, 2002). In addition, SSR markers have low genome coverage, and they are not amenable for high throughput and low cost genotyping.

1.3.3 Single nucleotide polymorphism (SNP) markers

Single nucleotide polymorphisms (SNP) have emerged as powerful tools for many genetic applications due to their low assay cost, high genomic abundance, locus-specificity, co-dominant inheritance, simple documentation, high throughput analysis and relatively low

genotyping error rates (Rafalski, 2002, Schlotterer, 2004). Currently, a total of 90,000 (90K) gene-associated SNPs are available for wheat researchers through the iSelect platform (Wang *et al.*, 2014), which has provided a tremendous opportunity for wheat researchers conducting research requiring high marker density. As reviewed elsewhere (Semagn et al. 2014), there are numerous SNP genotyping platforms (Gut 2001; Syvanen 2001; Chen and Sullivan 2003; Sobrino et al. 2005) that combine a variety of chemistry, allele discrimination techniques, detection methods, and reaction formats. In wheat, a total of 81,587 gene-associated SNPs (90K) is available through the Illumina iSelect SNP array platform (Wang et al. 2014) of which at least 5 to 13% could be polymorphic in a given biparental mapping population (Babiker et al. 2015; Liu et al. 2016; Perez-Lara et al. 2016; Zou et al. 2016). Although the final number of SNPs retained from the 90K SNP array for QTL mapping could even be less than 2000, due to cosegregation (mapping at exactly the same population), they provide greater opportunity to identify new QTLs not identified using other genotyping platforms, such as DArTs (Perez-Lara et al. 2016; Zou et al. 2016). The Cornell University-based genotyping by sequencing (GBS) (Elshire et al. 2011; Poland and Rifeb 2012) has become a very popular SNP genotyping method that could generate high density genotype data (up to a million in crops such as maize) at lower cost than most of the chip-based genotyping platforms. However the current GBS technology has some limitations which includes the following: (i) it often generates a large proportion of missing data that requires reliable imputation methods (Beissinger et al. 2013; Nazzicari et al. 2016); (ii) GBS methodology is still evolving and may require re-analysis and re-examination of old data using improved computational tools to ensure allele calls have not changed; and (iii) GBS allele calls are still less accurate for heterogeneous and highly heterozygous germplasm as compared with highly homozygous lines (Semagn et al. 2015). The DArTseq (Sansaloni et al.

2011)-based GBS platform is an alternative method that generates a lower density of markers (50,000 to 350,000 SNPs) but with better coverage and lower levels of missing data than the Cornell-base GBS method (Chen et al. 2016).

1.4 QTL mapping

Traditional MAS involves a discovery phase (QTL mapping) to identify a subset of markers that are significantly associated with genes or major effect QTLs regulating the expression of traits of interest in breeding (Collard *et al.*, 2005, Semagn *et al.*, 2010). QTL mapping can be done using linkage-based QTL analysis, association mapping or both. Linkage-based QTL mapping depends on well-defined populations, such as F_2 or their derived families, backcross (BC) populations, doubled haploid (DH) lines, near isogenic lines (NILs) and recombinant inbred lines (RILs). In wheat, both DH and RILs are frequently used for mapping studies because (i) they are homozygous or 'true-breeding' lines that can be multiplied and used for multi-location phenotyping; and (ii) seeds can be exchanged between different collaborators to facilitate phenotyping and genotyping activities (Collard *et al.*, 2005)

1.5 Important traits of wheat in western Canada

1.5.1 Plant height

Plant height is an important trait for wheat breeding that is related to lodging resistance, planting density and grain yield. Appropriate plant height enable wheat to achieve an adequate lodging resistance while at the same time, maintain a good yield level (Zhang et al., 2008; Cadalen et al., 1998). It is a complex trait controlled by Mendelian genes as well as quantitative genes. Genes and QTLs affecting plant height can be found on almost all wheat 21 chromosomes (Snape et al. 1977; Law et al. 1973). According to their response to exogenously applied gibberellins (GAs), dwarf mutants can be classified into two categories, one is GA sensitive

mutants (Synthesis mutants) and the other is GA insensitive mutants. As for GA sensitive mutants, the absence of endogenous gibberellins results in dwarf plants, and normal growth can be recovered by GA application (Reid, 1986). While for GA insensitive mutants, they have a reduced response or complete insensitivity to applied GA (Reid, 1986). Among the GA insensitive mutants, dwarfing genes *Rht1 (Rht-B1b)* on 4BS and *Rht2* (*Rht-D1b*) on 4DS have been the mostly widely used ones in wheat breeding programs globally to develop high-yielding cultivars with reduced plant height (Cadalen et al., 1998). GA sensitive dwarfing genes are more difficult to identify and study compared with the GA insensitive genes. As a result, the locations for most of the GA sensitive genes still remain unknown (A. Borner et al., 1996). According to the studies reported by far, Rht4 was found to locate on 2BL, *Rht5* on chromosome 3BS, *Rht8* on chromosome 2DS, *Rht9* on chromosome 5AL, Rht12 on chromosome 5AL, and Rht13 on 7BS (Ellis et al., 2005; A. Borner et al., 1996). In addition to the above mentioned dwarfing genes identified already, many other QTLs have been detected in studies on wheat plat height. By using a doubled-haploid (DH) population derived from the cross between the cultivars 'Courtot' and 'Chinese Spring', p. Sourdille (2003) found four QTLs significantly associated with plant height on chromosome 4BS, 4DS, 7AL and 7BL, each explained 19.1, 16.7, 11.9, 12.5% of the phenotypic variance respectively. Cuthbert, J. L. (2008) used a F1 derived doubled haploid (DH) population of 402 lines adapted from the spring wheat cross Superb/BW278 and identified five QTLs on chromosome 1A, 3A, 3B, 5A and 5B, each of which explained 5.0-12.1% of the phenotypic variance.

1.5.2 Flowering and maturity time

Spring wheat cultivars with early maturity are preferred by wheat researchers, especially in western Canada where there is a short growing season (95–125 days), long days (>14 h), and

low temperatures early and late in the growing season (Iqbal et al., 2007). The short growing season in western Canada makes early maturity of wheat important to avoid frost damage which can reduce production and lower quality (Iqbal et al., 2007). Additionally, in years with cold and wet harvest conditions, wheat cultivars with early maturity may show less pre-harvest sprouting and less down-grading (Hucl and Matus-Cadiz 2002).

Flowering and maturity time of wheat is controlled mainly by three gene groups; photoperiod response genes (*Ppd* genes), vernalization response genes (*Vrn* genes), and earliness *per se* (*Eps* genes) (Snape et al. 2001). Vernalization and photoperiod response genes accelerate or delay flowering in response to environmental stimuli, so that they can help to ensure that floral initiation occurs at optimum temperatures (A. Kamran et al., 2014; Law and Worland 1997). Among them, the vernalization response genes accounts for about 70-75% of the genetic variability in the heading time of wheat, while the number for photoperiod response genes and earliness per se genes are 20-25% and 5% (Stelmakh, 1998).

1.5.2.1 Vernalization response

Vernalization is the ''acquisition or acceleration of the ability to flower by a chilling treatment'' (Chouard 1960). Winter type wheat requires vernalization to promote flowering while spring type wheat does not. However, when exposed to cold temperatures, some spring wheat cultivars flower early (Jedel et al. 1986; Iqbal et al. 2006). Vernalization response is associated with the intensity of temperature and duration of exposure (A. Kamran et al., 2014; Wang et al. 1995a, b; Rawson et al. 1998). Vernalization genes (*Vrn* genes) are associated with sensitivity to vernalization and determine the control of winter and spring type difference (Snape et al., 2001). According to substitution line studies, four loci controlling vernalization response have been identified, they are *Vrn-A1* on chromosome 5A (*Vrn1*), Vrn-D1 on chromosome 5D

(Vrn3), *Vrn- B1* on chromosome 5B (*Vrn2*) and Vrn- B4 on chromosome 7B (Law et al., 1976; Worland et al., 1987; Snape et al., 1985; Snape et al, 2001; A. Kamran et al., 2014). Spring wheats are controlled by the presence of one or more dominant alleles at *Vrn-1, Vrn-3* or *Vrn-4* loci, which confer partial or no sensitivity to cold treatment. While winter wheats have dominant allele (s) at *Vrn-2* locus and recessive alleles at the other three loci. As a result, they require exposure to cold temperatures before the onset of flowering (A. Kamran et al., 2014). Among the above mentioned vernalization response genes, *VrnA1* has the highest level of the ability to inhibit the vernalization requirement, followed by *Vrn-D1, Vrn-D5* and *Vrn-B1*(Goncharov 2004). In addition, *Vrn-A1* is also epistatic to the dominant *Vrn-B1, Vrn-D1,* and *Vrn-D5* genes (Pugsley 1971, 1972; A. Kamran et al., 2014).

1.5.2.2 Photoperiod response

photoperiodism is a kind of phenomenon that plant sense and respond to altering day and/or night length by receiving signals in the form of cryptochrome or phytochrome to induce flowering (Fosket 1994). Although vernalization is the most important factor affecting winter and spring growth habit, but flowering time of autumn sown spring or winter wheats are not largely affected by Vrn genes, as their vernalization requirement is generally satisfied (Worland and Snape 2001). Under such conditions, flowering time is determined mainly by sensitivity/insensitivity to photoperiod. Photoperiod response is mainly determined by *Ppd-D1* (*Ppd1), Ppd-B1 (Ppd2)* and *Ppd-A1 (Ppd3)* on chromosomes, 2D, 2B and 2A, respectively (Scarth and Law, 1983, Law et al., 1978). Photoperiod-insensitivity is conferred by the dominant alleles. Generally speaking, *Ppd-D1* confers a higher level of insensitivity (measured as days to flowering) than *Ppd-B1* (Worland, 1996; Stelmakh, 1998; Worland et al., 1998). Photoperiod insensitive alleles are designated by the suffix 'a' while sensitive alleles are with the suffix 'b'.

Therefore, *Ppd-D1a, Ppd-B1a* and *Ppd-A1a* indicate insensitivity to photoperiod, whereas *Ppd-D1b, Ppd-B1b* and *Ppd-A1b* stand for sensitive alleles at the three loci (McIntosh et al. 2007; A. Kamran et al., 2014).

1.5.2.3 Earliness *per se*

Earliness per se is the difference in flowering times of varieties whose requirements of vernalization and photoperiod have been satisfied (Kato et al. 2001). It is an inherent ability of wheat to flower earlier without interaction with the environment (Miura and Worland, 1994). Compared with vernalization and photoperiod response genes, *Earliness per se* genes have smaller effect on flowering and maturity time. According to the studies by far, it is generally controlled by minor QTLs rather than major genes (Snape et al, 2001). Since earliness per se is highly heritable, it can be used in breeding programs to shorten wheat's life cycle without the infection of other environmental factors that can affect flowering time (Kato and Wada 1999). With a DH mapping population developed by the cross of 'Courtot' and 'Chinese Spring', P. Sourdille et al. (2000) detected a QTL on chromosome 7BS for earliness per se. By using a doubled haploid population developed from a cross between Kukri and RAC876, Bennett et al. (2012) detected earliness per se QTLs on chromosomes 1A, 4A, 4B, 5A, 5B, 7A and 7B. With a mapping population of 177 recombinant inbred lines developed by crossing two spring wheat cultivars, Cutler and AC Barrie, A. Kamran et al., (2013) identified three QTLs of earliness per se affecting days to maturity and flowering on chromosomes 1B (*QEps.dms-1B1 and QEps.dms-1B2*) and 5B (*QEps.dms-5B1*). By using a mapping population of 187 recombinant inbred lines, Chen et al., (2015) found Two earliness per se QTLs on chromosomes 1A (*QEps.dms-1A)* and 4A (*QEps.dms-4A*) across all three growing seasons, accounting for 15–27 and 8–10 % to the total genetic variation for days to maturity.

1.5.3 Grain protein content

Wheat grain protein content (GPC) is very important for nutritional enhancement and improved processing performance. Due to its quantitative nature and strong interaction with environmental it is very difficult to select this trait effectively (Kulwal et al., 2005). In addition, it has been found that breeding efforts aimed at genetic improvement of grain yield often resulted with a lower grain yield, which means wheat grain protein content and wheat grain yield are negatively related to each other (Blanco et al., 2012). Joppa et al (1997) found a major QTL accounting for 66% of the phenotypic variance for grain protein content in wild tetraploid wheat in 1997. Latter, this major QTL was transferred in to hexaploid what cultivars such as Glupro, Yecora Rojo (Mesfin et al. 1999; Khan et al. 2000). C.Groos et al used a mapping population of 194 F7 recombinant inbred lines and identified stable QTLs on chromosomes 2A, 3A, 4D and 7D, each explaining about 10% of the phenotypic variation for grain protein content in 2002. Blanco et al. (2001), they identified seven QTLs for GPC, located on the chromosome arms 4BS, 5AL, 6AS, 6BS, 7AS and 7BS. In 2013, Heo H. et al., published their study on identification of QTL for grain protein content. They analyzed the recombinant inbred lines (RILS) derived from a cross between spring wheat and spring version of winter wheat, and detected two QTLs on chromosome 3B and 5B. Based on a RIL mapping population, M. Prasad et al. (2002) found five QTLs (*QGpc.ccsu-2B.1; QGpc.ccsu- 2D.1; QGpc.ccsu-3D.1 and QGpc.ccsu-7A.1*) (*QGpc.ccsu-3D.2)* associated with grain protein content. Blanco et al. (2012), identified Ten independent genomic regions involved in the expression of GPC with a recombinant inbred line population derived from two elite durum wheat cultivars. By using a randomly derived recombinant inbred line (RIL) population (n = 163) from a cross between CIMMYT spring wheat 'Attila' and the Canadian 'CDC Go', Asif et al. (2015) reported

one QTL on chromosome 6A for GPC across all the environments, which explained 29.6% of the phenotypic variance.

1.5.4 Grain yield

Grain yield is one of the most important yet complex traits of wheat. Several yield components can be studied to analyze grain yield, such as thousand kernel weight, test weight, number of spikes per unit land area (tillering numbers), product of number of plants per land area and number of grains per spike, number of spikes per plant (Moragues et al., 2006). Among those, tillering occurs in the early growth and mainly affected by the sowing density and the availability of water and nitrogen (Simane et al., 1993; Garcı́a del Moral et al., 1991). Wheat grain yield and its components are strongly affected by the environment. QTLs associated with yield and other agronomic traits have been reported on all 21 chromosomes of bread wheat (Bennett *et al.*, 2012, Cuthbert *et al.*, 2007, Huang *et al.*, 2004, Kumar *et al.*, 2007). The number of QTLs and the proportion of phenotypic variance explained by each QTL was highly variable, depending on the type of population, population size, the number of environments and management conditions. By using a randomly derived recombinant inbred line (RIL) population (n = 163) from a cross between CIMMYT spring wheat 'Attila' and the Canadian 'CDC Go', Asif et al. (2015) reported one QTL on chromosome 6A for grain yield across all the conventional environments. This QTL explained 17% of the phenotypic variance. With A doubled haploid (DH) population derived from a cross between the Japanese cultivar 'Fukuho-kumogi' and the Israeli wheat line 'Oligoculm, Bahram et al. (2011) identified QTLs on 6A2 and 6D controlling wheat grain yield. Sanyukta et al. (2014) used mapping population of 206 recombinant inbred lines derived from WL711/C306 to detect genomic regions. They repoted a

novel genomic region for GY under WDS, qGYWD.3B.1 was detected on chromosome 3BS of wheat.

1.5.5 Resistance to leaf rust

Leaf rust which is caused by *Puccinia triticina Eriks.*, is the most common rust disease of wheat globally (McCallum et al., 2007). Genetic studies of leaf rust resistance in wheat have been conducted by wheat researchers worldwide. Among all the control strategies, the most economical and efficient method is the genetic resistance. As of 2010, there were 71 reported leaf rust resistance genes in wheat which have been mapped to specific chromosome location and given gene designations (McIntosh et al., 2010). Race-specific resistance, which is mostly conferred by single or a few major genes such as *Lr1, Lr10* (Lu et al., 2009), is effective in both the seedling plants and in the adult plant stage. However, wheat cultivars with this type of resistance can be easily rendered susceptible as a result of the mutation and selection of the pathogen population (Carter et al., 2009). In contrast, non-race-specific resistance or adult-plant resistance (APR), which is quantitatively inherited, is better expressed in adult plants. This type of resistance can be less effective but more durable (Li et al., 2010). For instance, one of the most important adult disease resistance genes in wheat is *Lr34* that was firstly described by Dyck et al. (1966). Wheat cultivars with *Lr* genes combined with *Lr34* usually have higher level of resistance than lines that only have Lr34 or the other resistant genes (Liu and Kolmer, 1997). The locus of *Lr34* also conditions resistance to stem rust (Dyck et al., 1985; Hiebert et al., 2010), stripe rust (*Yr18,* Singh, 1992), powdery mildew (Pm38, Spielmeyer et al., 2005), as well as barley yellow dwarf virus (Bdv1, Singh, 1993). In addition, *Lr34* is able to condition slow rusting resistance and is fully linked with leaf tip necrosis (Singh, 1992). In 2012, Sybil et al.,

identified a new gene *Lr68 located on chromosome 7BL* conferring adult plant resistace to leaf rust in wheat.

1.5.6 Resistance to stripe rust

Stripe rust of wheat, caused by *Puccinia striiformis f.sp. tritici*, is one of the dominant factors affecting yield potential of wheat. Breeding for resistant cultivars is the best way to control stripe rust. Over 40 stripe rust resistant loci have been found. Similar to leaf rust, race-specific *Yr* genes are not durable. To obtain prolonged resistance, efforts have been made to develop multiple gene combinations. Many wheat breeding programs in the US are combining stripe resistant genes such as *Yr5* and *Yr15* into wheat cultivars for higher level resistance (Carver, 2009). Currently, wheat cultivars that show resistance to stripe rust in Canada may have *Lr34/ Yr18* genes in combination with other genes conferring partial resistance. The leaf rust resistant gene *Lr37* is found to be closely linked to *Yr17* and *Sr38* in 'CDC Stanley'. Likewise, *Lr 46* is linked with *Yr 29, Yr47* is linked with *Lr52,* and *Yr46* is with *Lr67*. All of them are of great value to breeders to manipulate disease resistance in common wheat (Randhawa. H. S. etal., 2013).

Unlike race-specific resistance, high-temperature adult-plant resistance (HTAP) cannot be detected in the seedling stage. However, with HTAP, the resistance is more apparent at higher temperatures in older plants (Carver, 2009). To avoid potential epidemics of stripe rust in Canada, understanding stripe rust resistance is important to assist field breeders (. Ramburan et al. (2004), using a population of 150 doubled haploid lines generated from a cross between Kariega and the susceptible cultivar Avocet S, mapped two major QTLs located on chromosomes 7D (*QYr.sgi-7D*) and 2B (*QYr.sgi-2B.1*), contributing 29% and 30% to the phenotypic variance, respectively. Using a mapping population of 188 recombinant inbred lines (RIL) from a Louise (resistant) by

'Penawawa' (susceptible) cross, one major QTL, designated *QYrlo.wpg-2BS*, associated with HTAP resistance in Louise, was detected on chromosome 2BS (LOD scores ranging from 5.5 to 62.3 across locations and years) within a 16.9 cM region flanked by Xwmc474 and Xgwm148 (Arron Hyrum Carter et al., 2009).

1.5.7. Resistance to stem rust

In the early 20th century, many epidemics of wheat stem rust (black rust), caused by *Puccinia graminis f. sp. tritici Erikss. &Henning*, lead to large yield losses for common wheat (Zurn et al., 2015). Although a popular wheat variety ''Enkoy'' suffered major losses during 1993 and 1994, which generated the last major stem rust epidemics occurred in Ethiopia (Shank, 1994), but wheats in rest of the world have not been affected by stem rust for over three decades (Singh et al., 2008). Since worldwide epidemics of leaf (or brown) rust caused by *P. triticina* and stripe (or yellow) rust caused by *P. striiformis*, were more severe recently, less attention has been focused on stem rust research and breeding, so that in some countries breeding for stem rust resistance have been suspended (Singh et al., 2008). In 1998, severe stem rust infections were observed on wheat in Uganda, and a new race U*g99* with virulence on *Sr31,* was detected (Pretorius et al., 2000). Latter, *Ug99* was found in n Kenya and Ethiopia in 2005 (Wanyera et al., 2006), and in Sudan and Yemen in 2006 (Singh et al., 2008). A variant of *Ug99* with added virulence to *Sr38* and *Sr24* was detected (Afzal et al., 2016). As a result, wheat breeders worldwide now begin to respond positively to the alarm raised by the *Ug99*. By far, around 50 stem rust resistance genes have been catalogued and only a few are valuable against *Ug99* (Singh et al.,2006,2008). Three pleiotropic adult plant resistance genes have been identified to confer non-race specific durable resistance. They are *Sr55 (=Lr67/Yr46/Pm46), Sr57 (=Lr34/ Yr18/*

*Pm38/ Sb1/ Bdv*1), and *Sr58 (=Lr46/Yr29/Pm39).* In addition, another adult plant resistance gene *Sr2* is favored in wheat breeding program too (Afzal et al., 2016).

1.5.8 Resistance to common bunt

Common bunt, incited by *Tilletia tritici (Bjerk.) Wint. (Tilletia caries (DC.) Tul.)* and *T. laevis Kuhn (T. foetida (Wallr.) Liro),* has been a serious disease of wheat, causing yield and quality losses in both spring and winter wheat *(Triticum spp.*) (Laroche, et al., 2000). On the Canadian prairies, common bunt has been controlled genetic resistance in wheat cultivars, and through seed treatment. Multi-genic resistance to common bunt has been reported, but very few specific genes have been identified. According to studies conducted by Galaev et al. (2006), Fofana et al. (2008) and Wang et al. (2009), trait loci (QTL) on chromosomes 1B and 7A have been identified. Another study by Hiebert et al. (2011) identified the location of *Bt10* on chromosome 6D using a segregating population of 185 doubled haploid spring wheat lines derived from the cross RL4452 x AC Domain. Fofana et al. (2008) detected three QTLs associated with common bunt resistance, of which two were located on chromosome 1B and one on chromosome 7A. Using a doubled haploid mapping population of a cross between Trintella and the susceptible variety Piko, Dumalasova et al. (2012) fond a gene conferring common bunt resistance on chromosome 1B, near to the centromere and closest to marker Xgwm273 on the short arm.

1.5.9 Resistance to tan spot

Tan spot, caused by *Pyrenophora tritici-repentis* is an economically significant disease that has been reported worldwide. At least eight races of *P. tritici-repentis* have been identified according to different toxins they produce and their ability to induce necrosis and/or chlorosis on differential wheat lines. (Strelkov and Lamari, 2003). The most characterized host-selective toxin

(HST) is *Ptr ToxA,* which is produced by race 1. This toxin can cause necrotic symptoms in susceptible wheat cultivars. *Ptr ToxB* and *Ptr ToxC,* which are isolated from race 5 and race 1 respectively, are able to cause chlorosis symptoms. (Orolaza et al., 1995; Friesen and Faris, 2004). A number of studies have been conducted to investigate the genetic structure of tan spot resistance. Chu (2009) detected five resistance QTLs on chromosome arms3AS, 3BL, 5AL and 7BL using a tetraploid wheat DH population derived from the cross between the durum wheat cultivar 'Lebsock' and the Accession PI 94749 of T. turgidum. Based on evaluating a population of recombinant inbred lines derived from a cross between the common wheat varieties Grandin and BR34, Faris and Friesen (2005) identified QTLs on the short arm of chromosome 1B and the long arm of chromosome 3B that were significantly associated with resistance to tan spot caused by Ptr races 1–3 and 5.

1.6 Conclusion

Wheat breeders in Canada primarily aim to develop cultivars with favored agronomic traits such as short stature, early maturity, high yield, high protein content, and resistance to priority diseases, such as stem rust, leaf rust, stripe rust, tan spot, and common bunt (http://www.pgdc.ca). With a few notable exceptions (particularly adult plant resistance genes) almost all the traits mentioned above are quantitatively inherited, and therefore controlled by many genes of small effect and can be affected by the environment (Sham et al. 2002, Koinange et al. 1996, White and Doebley 1998). With the development of QTL mapping facilitated by molecular markers, it is now possible to better understand the genetic architecture of quantitative traits, including the number of loci controlling a trait, their locations, their phenotypic effect and the interactions among these genes (Lee et al. 2005, Mackay 2001).

1.7 Overall Thesis objectives and hypotheses

Objectives:

Using SNP markers to map QTLs associated with agronomic traits such as time to flowering and maturity, tillering ability, plant height, thousand kernel weight, test weight, grain yield and grain protein content, and disease resistance, such as resistances to tan spot, common bunt, leaf rust, and stripe rust in a RIL mapping population derived from the cross between Attila and CDC Go.

Map QTLs associated with agronomic traits and grain protein content under conventional and organically managed environments.

To investigate if the use of the 90K SNP could detect more precise QTLs than DArT markers that we previously used in the 'Attila' × 'CDC Go' recombinant inbred line population (RIL).

Compare the QTL mapping studies with 8 testing environments and with 3 testing environments, to examine whether more testing environments improves QTL detection.

Hypotheses:

There are genomic regions controlling some of the variation in agronomic traits such as plant height, grain yield, tillering, kernel weight, test weight, and grain protein content, and resistance / susceptiblity to common bunt, tan spot, leaf rust and stripe rust in the population of 163 recombinant inbred lines (RIL) obtained from the cross Atilla × CDC Go.

There are differences in the results of QTL mapping with low density DArT markers and High Density SNP markers.

There are differences in the results of QTL mapping with different numbers of testing environments.

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Chapter 2 Mapping QTLs controlling agronomic traits in the Attila x CDC Go spring wheat population under organic management using 90K SNP array [1]

2.1 Introduction

Organic production (organic management system) may be defined in several ways, but here it refers to a method of crop production without the use pesticides and mineral fertilizers. It relies on crop rotation, mixed cropping, biological pest control, and fertilizers of organic origin, such as compost, manure, and green manure (www.intechopen.com). Over 95% of organic production is based on crop cultivars bred for conventional management systems (Lammerts van Bueren et al., 2011). Although most traits of interest in breeding for organic management are similar to conventional management, the expression of the traits under an organic system may be different from conventional management. In conventional management, breeders often select the best cultivars for optimal production under high inorganic fertilizer and high pesticides and herbicides, which is not the case for organic management. Therefore, some of the traits relevant to high input conventional farming may have negative effects in organic systems. For example, most wheat breeders aim to develop semi-dwarf cultivars which have resulted in (i) reduced root systems, (ii) increased reliance on N fertilizers to attain satisfactory protein content, and (iii) decreased competitiveness against weeds (Zerner et al., 2008). Semi-dwarf cultivars can produce

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high grain yield in conventional management, but they often produce significantly lower yield in organic management due to weaker weed competitiveness.

Taller plants exhibit better competitive ability against weeds than shorter ones, mainly due to better light interception that directly alters the photosynthetic activity of crop plants (Cudney et al., 1991; Mason et al., 2007a; Mason et al., 2007b). Wheat breeders may, therefore, need to consider developing relatively taller cultivars for organic agriculture than semi-dwarfs; without significantly affecting plant maturity, lodging resistance and other qualitative traits. Alternatively, organic wheat breeders need to develop short statured cultivars that have erect leaves with higher leaf area to maximize light interception (Watson et al., 2006) and increase photosynthetically active radiation, biomass, and tillering capacity.

Although the literature on mapping genes and QTLs under organic management is extremely limited (Asif et al., 2015), several studies have been conducted to map genomic regions associated with agronomic traits under conventional management. QTLs associated with yield and other agronomic traits have been reported on all 21 chromosomes of bread wheat (Huang et al., 2004; Cuthbert et al., 2007; Kumar et al., 2007; Bennett et al., 2012). The number of QTLs and the proportion of phenotypic variance explained by each QTL is highly variable depending on the type of population, population size, the number of environments and the management conditions. The University of Alberta wheat breeding program has been evaluating the performance of Canadian wheat cultivars and breeding lines under both conventional and organic management systems in Alberta, Canada (Mason and Spaner, 2006; Mason et al., 2007a; Mason et al., 2007b; Kaut et al., 2008; Kaut et al., 2009; Reid et al., 2009a; Reid et al., 2009b; Reid et al., 2011; Kubota et al., 2015). As part of this work, a recombinant inbred line (RIL) population developed from a cross between spring wheat cultivars Attila (CM85836-50Y-0M-

0Y-3M-0Y) and CDC Go was evaluated during 2008-2010 under both conventionally and organically managed field conditions, and genotyped with 579 diversity arrays technology (DArT) and *Rht-B1*gene specific markers (Asif et al., 2015). A total of 5 QTLs associated with four agronomic traits, averaged over three years of organic management were detected, which includes QTL for plant height on 4B, grain protein content on 6A, test weight on 1B, and kernel weight on both 4A and 6A. Those QTLs explained between 8.3 and 18.7% of the total phenotypic variance for plant height, grain protein content, test weight and kernel weight (Asif et al., 2015), which is equivalent to 24.4 to 42.5% of the genetic variance. No QTL was identified for flowering time, maturity, grain yield and number of tillers (tillering ability) averaged over three years. Although several factors might have contributed to the failure to detect QTLs that explained most of the phenotypic or genetic variance in the Attila x CDC Go RIL population, low marker density (low genome coverage) and uneven marker distribution in the linkage maps are possible reasons, which is the basis for the present study.

DArT 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), but the dominant inheritance of DArT markers is one of the drawbacks. In the absence of human error (e.g., contamination, labeling error), however, dominant inheritance of DArT markers may not be a major limitation for mapping in highly homozygous populations, such as RILs, doubled haploid (DH) lines and near isogenic lines (NILs). It would be a major limitation for marker-assisted selection (MAS). SSR markers are widely used by wheat researchers for different reasons, including wide availability, co-dominant inheritance, multiallelism, high polymorphism, uniform distribution, and high polymorphic information content (Gupta et al., 2002; Boopathi et al., 2013). The main limitations of SSRs include lower throughput as compared with the highly

multiplexed single nucleotide polymorphism (SNP) genotyping platforms and genotyping by sequencing (Elshire et al., 2011), stuttering, and plus or minus which generate spurious bands or peaks (Hu, 1993; Ginot et al., 1996). There is also discrepancy in the literature regarding the repeatability of the SSR markers, which primarily depends on marker repeat length and the large variety of automatic sequencing machines used for fragment analyses, each providing different migration, fluorescent dyes, and allele calling software (Vignal et al., 2002). SNPs have emerged as a marker of choice for various applications due to their low assay cost, high genomic abundance, locus-specificity, co-dominant inheritance, high throughput analysis and relatively low genotyping error rates (Rafalski, 2002; Schlotterer, 2004). Currently, a total of 81,587 (90K) gene-associated SNPs are available for wheat researchers through the iSelect platform (Wang et al., 2014), which has provided a tremendous opportunity for wheat researchers conducting studies requiring high marker density. The objectives of the present study were to reanalyze the phenotyic data (flowering time, maturity, plant height, test weight, kernel weight, number of tillers, grain yield and grain protein content) generated under organic management in our previous study (Asif et al., 2015) using novel genotypic data to (1) investigate if the 90K SNPs have potential to improve QTL detection in the Attila and CDC Go RIL population; and (2) compare the results with our previous study conducted using 580 DArT and *Rht-B1* markers.

2.2 Materials and Methods

2.2.1 Plant materials and phenotyping

We used a mapping population of 167 RILs developed from a cross between two spring wheat cultivars, Attila (CM85836-50Y-0M-0Y-3M-0Y) and CDC Go. Attila is an awned, medium yielding, semi-dwarf and early maturing cultivar developed by the International Maize and Wheat Improvement Center from CM85836-50Y-0M-0Y-3M-0Y and released in several

countries with different local names (Tadesse et al., 2010). Attila is grown on millions of hectares throughout the world and used as a slow-rusting donor in international spring wheat breeding programs (Rosewarne et al., 2008). CDC Go is a Canadian Western Red Spring wheat cultivar registered in 2003 and characterized by strong straw, hollow stem, medium height, relatively late maturity, high yield, high test weight and thousand kernel weight, resistant to bunt, with moderate resistance to leaf and stem rust (http://www.agric.gov.ab.ca). In 2011, CDC Go was grown on 5.5% of the wheat production area in the Prairies regions (http://www.growwinterwheat.ca/documents/CWB2011VarietySurvey.pdf). The RIL population and parents were phenotyped under organically managed field conditions three times between 2008 and 2010 at the Crop Research facility of the University of Alberta South Campus (53°19'N, 113°35'W), Edmonton, Canada. Phenotypic evaluation for each environment was conducted in randomized incomplete block designs with three replications. Each RIL was evaluated for flowering time, maturity, tillering ability, plant height, thousand kernel weight, test weight, grain yield, and grain protein content. Details on population development, phenotyping protocols, and agronomic practices have been described in our previous paper (Asif et al., 2015).

2.2.2 DNA extraction and genotyping

Genomic DNA was extracted from three weeks old seedlings using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1987). DNA concentration was measured using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, USA) and normalized to about 100 ng/µL. DNA samples were genotyped at the University of Saskatchewan Wheat Genomics lab, Saskatoon, Canada, with the 90K Illumina iSelect SNP array that consisted of 81,587 SNPs (Wang et al., 2014). Alleles were called with the Illumina Genome Studio Polyploid Clustering version 1.0 software (Illumina, San Diego, USA) using

default clustering parameters. Additional filtering was then done as described in one of our previous study (Perez-Lara et al., 2016) to select only SNPs that segregated in a biallelic pattern based on data from multiple mapping populations available to our programs. We also screened the two parents for polymorphism for photoperiod response (*Ppd-B1* and *Ppd-D*) (Beales et al., 2007), vernalization response (*Vrn-A1* and *Vrn-B1*) (Chen et al., 2013) and height reducing *Rht-B1* (Ellis et al., 2002) gene specific markers at the Agricultural Genomics and Proteomics lab, University of Alberta, Edmonton, Canada. *Ppd-D1*, *Vrn-A1,* and *Rht-B1* were polymorphic between Attila and CDC Go, while *Ppd-B1* and *Vrn-B1* were monomorphic. The RILs and the two parents were then genotyped with *Ppd-D1*, *Vrn-A1* and *Rht-B1* gene specific markers as described elsewhere (Perez-Lara et al., 2016).

2.2.3 Statistical analyses

For each trait, least squares mean, analysis of variance and heritability were obtained using PROC MIXED and PROC IML in SAS version 9.3 (SAS Institute Inc. Cary, USA). The phenotypic analyses were conducted for each environment separately and then combined across all environments. Genotypes (RILs) were considered fixed, while years, replications and blocks within replications were considered as random effects. For each trait, both test for normality and the frequency distribution were done using MiniTab v14. All SNPs that were monomorphic between the two parents and those with >20% missing data were excluded from linkage mapping. Linkage maps for the remaining SNPs and the three gene specific markers were constructed as described in another study (Perez-Lara et al., 2016). Linkage groups were assigned to individual chromosomes based on existing high density SNP maps of wheat (Cavanagh et al., 2013; Maccaferri et al., 2014; Wang et al., 2014).

Inclusive composite interval mapping (ICIM) was performed on the least squares means of each trait for individual years and combined across three years using QTL IciMapping v4.0 (Li et al., 2007; Meng et al., 2015) with the following options: a missing data point being replaced by the trait mean value, 1 cM walking distance, a minimum LOD of 2.5 and a model to determine additive effects at individual QTL and additive × additive epistatic interactions. QTL mapping was conducted after excluding all SNPs that mapped at exactly the same position. QTL names were designated following the International Rules of Genetic Nomenclature (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm), which consisted of trait acronym, lab designation (dms = Dean Michael Spaner), and chromosome. In this study, QTLs that explained <10%, 10-20% and >20% of the total phenotypic variation (R^2) were arbitrarily classified into minor, moderate and major effect QTLs, respectively. Genetic maps and QTL graphs were drawn using MapChart v2.1 (Voorrips, 2002). The extent of linkage disequilibrium (LD) between the *Rht-B1* and *Vrn-A1* gene specific markers and all SNPs that mapped on chromosomes 4B and 5A, respectively, was evaluated by computing the r^2 values using TASSEL version 5.2.28 (Bradbury et al. 2007).

2.3 Results

2.3.1 Summary of phenotypic traits and markers

Detailed results of the phenotypic evaluation were presented in our previous paper (Asif et al. 2015). In the three years (environments) combined data under organic management, CDC Go matured 4.3 days earlier, was 10.5 cm taller, produced 3.3 more tillers per m^{-2}, had 4 mg heavier kernels, 1.8 kg hL^{-1} higher test weight, and yielded 893.2 kg ha^{-1} more grain than Attila. Each RIL on average produced 94 tillers, required 53 days to flowering and 90 days to maturity, were 70 cm tall, produced 3.1 t ha^{-1} grain yield and had 13% grain protein content. The

distribution of least squares means estimated from the combined phenotypic data of all three environments was normal (p > 0.05) for all traits, except maturity (Appendix 1). The Shapiro-Wilk test rejected the hypothesis of normality (p = 0.010) for maturity, with 60.5% of the RILs maturing earlier than 90 days, which is the mean for the population. Broad sense heritability varied from 0.15 for tillering to 0.72 for flowering. Genotypes differed for all traits (p < 0.001) (Appendix 1).

Of the 81,587 SNPs used for genotyping the CDC Go and Attila RIL population, approximately 93% were discarded due to lack of polymorphism between the two parents, high (>20%) missing data, very high segregation distortion, and/or lack of linkage with other markers. Only 5,665 SNPs, which accounts for 7% of the 90K SNPs were incorporated into 27 linkage groups and 19 chromosomes; there were no linkage maps for both chromosomes 3D and 4D. The number of initially mapped markers per chromosome varied from 31 on chromosome 7D to 908 on 6B. However, many SNPs co-segregated (mapped at exactly the same position), so they were excluded from the final dataset. This reduced the final number of markers retained for QTL analyses to 1203, which includes 1200 SNPs and three functional markers (*Ppd-D1*, *Vrn-A1a* and *Rht-B1*) (Table 2-1), which is equivalent to 1.5% of the number of markers used for genotyping and 21.2% of the markers that were integrated into the initial genetic linkage map. The number of markers retained in the final linkage map varied from 4 on chromosome 1D to 150 on 2B, with an overall average of 63 markers per chromosome (Table 2-1). The total map length for the 19 chromosomes was 3442 cM, with each chromosome varying between 14.3 cM on 1D to 324.8 cM on 5B. Map distance between adjacent markers varied from 0.6 to 48.8 cM (Fig. 2-1) and the overall average was 2.9 cM.

2.3.2 QTL analyses

Table 2-2 summarize the QTLs associated with individual and combined environments. In the combined phenotypic data across the three environments, we found a total of 12 QTLs associated with the seven traits, which included one QTL for each of flowering, tillering, maturity, grain yield and plant height; Two QTLs for test weight, and five QTLs for thousand kernel weight. All QTLs associated with each trait exhibited mainly additive effects and QTL by QTL interactions were negligible ($R^2 < 1\%$). The QTL for flowering mapped at 297 cM on 5A (*QFlt.dms-5A.2*) and explained 17.2% of the phenotypic variance over three years. RILs that had the CDC Go alleles at the two flanking markers for *QFlt.dms-5A.2* flowered 2.2 days earlier than those RILs that had the Attila alleles. When the phenotypic data of the individual years were considered, we identified a single QTL for flowering on 5A in both 2009 and 2010 environments, but its position in 2010 shifted by 35 cM (262 cM instead of 297 cM). In addition, the LOD score and proportion of phenotypic variance (R^2) were lower in 2009 and 2010 environments as compared with the combined three years data (Table 2-2). The QTL for maturity mapped at 298 cM on 5A (*QMat.dms-5A*) and accounted for 17.2% of the phenotypic variance across the three environments. The favorable alleles for *QMat.dms-5A* originated from CDC Go. RILs that had the favorable alleles at the two flanking markers on average matured three days earlier than those RILs that had the unfavorable alleles. When individual environments were considered, *QMat.dms-5A* was detected both in 2009 and 2010 environments.

A QTL for plant height was mapped at 81 cM on 4B (*QPht.dms-4B*) that accounted for 18.8% of the phenotypic variance over 3 years. RILs carrying the CDC Go alleles at the two flanking markers for *QPht.dms-4B* were significantly different from those containing the Attila alleles for plant height (p < 0.012), but not for other traits (Table 3). On average, RILs with the CDC Go alleles at the two flanking markers were 7.2 cm shorter than those RILs with the Attila

alleles. In individual environments, *QPht.dms-4B* was detected at the same position only in 2010, but its effect was 9.0% smaller than that for the combined data across three years (Table 2-2). The QTL for tillering ability was mapped at the proximal tip of chromosome 4AS (*QTil.dms-4A*), which accounted for 9.3% of phenotypic variation for this trait across the three environments. RILs that had the CDC Go alleles at the two flanking markers for *QTil.dms-4A* produced 5.3 more tillers per m^2 than those RILs with the Attila alleles. However, this QTL was not detected in any of the individual environments. One QTL for grain yield were mapped at 37 cM on 5B (*QYld.dms-5B*) explained 7.5% of the phenotypic variance across three environments, (Table 2-2). RILs that had Attila alleles at the two flanking markers of *QYld.dms-5B* and *QYld.dms-7A* produced 287.3 kg ha^{-1} more grain yield than those RILs with the CDC Go alleles. When individual environments were considered, *QYld.dms-5B* was detected at the same position only in 2010 and had approximately the same R^2 value.

Two QTLs associated with test weight were mapped at 81 cM on 1A (*QTwt.dms-1A*) and at 204 cM on 5B (*QTwt.dms-5B*) that altogether explained 16.1% of the phenotypic variance across three environments. Each QTL for test weight individually explained 7.5 and 8.6% of the phenotypic variance across combined environments. RILs that were homozygous for the Attila alleles at the two flanking markers of all four QTLs for test weight were 0.6 to 0.8 kg hL^{-1} greater than those RILs with the CDC Go alleles (Table 2-2).

The five QTLs associated with thousand kernel weight mapped at 16 cM on 1B (*QTkw.dms-1B*), at 120 cM on 4A (*QTkw.dms-4A*), at 79 cM on 6A (*QTkw.dms-6A*), at both 6 cM (*QTkw.dms-6B.1*) and 37 cM (*QTkw.dms-6B.2*) on 6B, which altogether accounted for 39.4% of the phenotypic variance over three environments. Each QTL for thousand kernel weight explained between 5.5 and 12.2% of the phenotypic variance across combined environments.

The favorable alleles for *QTkw.dms-4A*, *QTkw.dms-6A* and *QTkw.dms-6B.2* originated from CDC Go, while those for *QTkw.dms-1B* and *QTkw.dms-6B.1* originated from Attila. RILs that were homozygous for the favorable alleles at the two flanking markers of each QTL had up to 2.1 mg heavier kernels than those RILs homozygous for unfavorable alleles. When individual environments were considered, both *QTkw.dms-4A* and *QTkw.dms-6A* were detected in 2009 and 2010 environments, respectively. For grain protein content, we only found two environment specific QTLs on 6B (*QGpc.dms-6B*) and 7A (*QGpc.dms-7A*) associated with the 2010 and 2008 environments, respectively, but none across the combined three environment data (Table 2-2). Although the RIL population exhibited transgressive segregates for grain protein content and a number of lines that were superior or inferior to the parents were identified, Attila had only 0.2% higher grain protein content than CDC Go (Appendix 1).

The coincidental QTL is the earliness *per se* QTL (Kamran et al., 2013) that is associated with both flowering time (*QFlt.dms-5A.2*) and maturity (*QMat.dms-5A*). This coincidental QTL on 5A is referred as *QEps.dms-5A* and explained 17.2% of the phenotypic variance for earliness across three environments. RILs carrying the CDC Go alleles at the two flanking markers for *QEps.dms-5A* were significantly different ($p \leq 0.026$) from those possessing the Attila alleles for flowering time, maturity, test weight and plant height, but not for other traits (Table 2-3). On average, RILs with the CDC Go alleles at the two flanking markers of the *QEps.dms-5A* flowered/matured 3 days earlier, were 3 cm shorter and exhibited 0.4 kg hL^{-1} greater test weight than those RILs that had the Attila alleles.

2.4 Discussion

In our previous QTL mapping study conducted in the Attila × CDC Go RIL population genotyped with 579 DArT and *Rht-B1* markers (Asif et al., 2015), we reported a QTL for plant

height on chromosome 4B, test weight on 1B, grain protein content on 6A, and kernel weight on both 4A and 6A, but none for flowering time, maturity, tillering and grain yield. The QTLs associated with each trait altogether explained from 8.3 to 18.7% of the total phenotypic variance across the combine data of the three organic environments (Asif et al., 2015), which is equivalent to 24.4 to 42.5% of the genetic variance. As heritability for the different traits in our previous study varied from 0.16 to 0.70, most of the genetic variance remained unexplained. Given the high heritability estimated for some of the traits, such as flowering time (0.70), our inability to detect QTLs explaining most of the phenotypic or genetic variance across the combined environments was unexpected. The use of high density polymorphic markers provides greater saturation of genetic linkage maps, which in turn improves the chance of identifying QTLs (Zych et al., 2015). However, QTL mapping studies in plant species with large genome size, such as hexaploid wheat, have been challenging due to the difficulty of finding sufficient number of polymorphic markers distributed across the genome. Our previous study was based on 579 markers covering a total map length of 2045 cM; the overall average map distance among adjacent markers (inter-marker interval) was 3.5 cM. We thought that the low marker density in the previous study might have restricted our ability to identify QTLs accounting for most of the phenotypic variance across three environments. The present study was based on a subset of 1200 out of 5,667 polymorphic markers and 3 gene specific markers, which resulted in a total map length of 3,442 cM and an overall average inter-marker interval of 2.9 cM. As compared to our previous study. Therefore, marker density and genome coverage increased over two fold and 1397 cM, respectively, while average inter-marker interval decreased by 0.6 cM. Such improvement of marker density and genome coverage gave us a better opportunity to uncover a total of sixteen QTLs associated with seven out of the eight traits evaluated across three

environments, which is three-fold more than the number of QTLs reported in our previous study. The number of QTLs identified in the present study varied from one for flowering, plant height and tillering to five for thousand kernels weight (Table 2). We still failed to identity a single QTL for grain protein content. The total phenotypic and genetic variance explained by the QTLs associated with the combined phenotype data varied from 9.3 to 39.4% and from 24.6 to 96.8%, respectively, which is much greater than our previous study. With regard to chromosome distribution of QTLs, only the QTL for plant height on 4B and the two QTLs associated with kernel weight on 4A and 6A were common between the two studies, but direct comparisons of their positions was not possible due to lack of consensus map of the different types of markers and physical position. The remaining thirteen out of the sixteen QTLs identified in the present study were not reported in our previous study.

In regions where the growing season is short and days are long, the development of early maturing cultivars is important to avoid frost damage, which can affect both yield and grain quality (Iqbal et al., 2007; Randhawa et al., 2013). In the present study, we found one earliness *per se* QTL (*QEps.dms-5A*) associated with both flowering (*QFlt.dms-5A.2*) and maturity (*QMat.dms-5A*) that mapped between 293 and 300 cM interval on chromosome 5A (Table 2). Several previous studies reported genes and/or QTLs for both flowering time and maturity on homoeologous group 5 (Law and Worland, 1997; Yan et al., 2003) chromosomes. In bread wheat, the three vernalization response genes (*Vrn-1, Vrn-2* and *Vrn-3*) directly influence both flowering and maturity (Galiba et al., 1995; Dubcovsky et al., 1998; Iwaki et al., 2002), of which *Vrn-A1* mapped to the long arm of chromosome 5A (Preston and Kellogg, 2008; Chen et al., 2013). In the present study, *Vrn-A1a* mapped at 295.1 cM and falls within the *QEps.dms-5A* confidence interval. Such tight linage between the *QEps.dms-5A* and the *Vrn-A1* gene suggests

that the QTL may be the same as the vernalization gene on chromosome 5A. However, *QEps.dms-5A* may be different from the *Vrn-A1* gene for two reasons. First, LD values between the *Vrn-A1a* and the SNP markers that mapped within the QTL confidence interval on 5A ranged from 0.70 to 0.75 (Fig. 2-3), which is not as high as expected. Second, the proportion of phenotypic variance explained by *QEps.dms-5A* was only 17.2%, equivalent to a reduction in flowering time and maturity by 2-3 days, which we think is not typical of a major gene such as *Vrn-A1*. Using the draft sequence of the hexaploid bread wheat, a recent study reported 124,201 genes within 17 mega base pairs (Lukaszewski et al., 2014), with an average of 137 kb per gene. The total genetic map in the Attila x CDC Go population was 3,442 cM and the ratio between the physical (17×10^6 kb) and the genetic position is 4,939 kb per cM. *Vrn-A1a* mapped about 2 cM proximal to one of the flanking markers for *QEps.dms-5A*, which is equivalent to 9,878 kb that could harbor clusters of as many as 72 genes. In another study conducted on *Arabidopsis thaliana* (Kroymann and Mitchell-Olds, 2005), the authors fine mapped a QTL within 1 cM genetic interval and found two growth rate QTLs exhibiting epistasis; the two genes were located within a 210 kb physical interval. Based on the reasons, we think that the *Vrn-A1* gene is tightly linked with *QEps.dms-5A*, but the statistical method failed to discriminate them, which may be resolved by screening large numbers of recombinants to break the linkage (Kolb et al., 2001).

Tillering ability is one of the most important agronomic traits affecting biomass and grain yield potential in cereals (Yan et al., 1998; Wang et al., 2016). Moisture and nitrogen fertilizer increase grain yield to a large extent by stimulating the development of more tillers. However, excessive tillering may lead to yield reduction because young tillers consume nutrients from the main shoot during vegetative growth, leading to senescence before the plant reaches maturity (Kebrom et al., 2012). In the present study, *QTil.dms-4A* was the only QTL associated with

number of tillers, explaining 9.3% of the total phenotypic variance for tillering ability across the three environments (Table 2). In our previous study (Asif et al., 2015), we reported a single environment specific QTL on 4A that explained 7% of the phenotypic variance for tillering ability in the Attila × CDC Go RIL population. In another Canadian western red spring wheat RIL population derived from a cross between CDC Teal and CDC Go, our group recently reported a QTL associated with tillering on chromosome 4A accounting for 6.7% of the phenotypic variance for tillering across three environments (Chen et al., 2015). Our results, together with others, clearly suggest the presence of gene clusters on chromosome 4A, which are likely associated with tillering ability in spring wheat.

We found one QTL on chromosomes 5B (*QYld.dms-5B*) associated with grain yield across the three environments, which explained 7.5% of phenotypic variance across three environments. In a RIL population derived from two spring wheat cultivars, Cutler and AC Barrie, our group reported two environment specific QTLs on chromosome 5B that increased grain yield between 290 and 310 kg ha^{-1} (Kamran et al., 2013). In a DH population derived from RAC875 and Kukri (Bennett et al., 2012), the authors reported nine QTLs associated with grain yield under drought, heat and irrigated environments, which included *QYld.aww-5B* on chromosome 5B. Our results, together with others, suggest that there are probably gene clusters on chromosome 5B regulating grain yield in spring wheat.

Test weight is an important trait for wheat millers due to its positive correlation with flour yield, whereas kernel weight is associated with both grain and flour yield (Ramya et al., 2010; Asif et al., 2015). In our previous study using DArT markers, we reported (i) a single QTL associated with test weight on 1B that explained 8.3% of the phenotypic variance across three environments; and (ii) two QTLs associated with kernel weight on chromosome 4A and 6A

that together explained 18.7% of the phenotypic variance across three environments (Asif et al., 2015). In the present study using SNPs, we uncovered (i) two QTLs associated with test weight (*QTwt.dms-1A*and *QTwt.dms-5B*) that individually explained 7.5% and 8.6% of the phenotypic variance, and (ii) five QTLs associated with kernel weight (*QTkw.dms-1B*, *QTkw.dms-4A, QTkw.dms-6A, QTkw.dms-6B.2 and QTkw.dms-6B.1*) that individually explained 5.5-12.2% and together 39.4% of the phenotypic variance across three environments. For both traits, therefore, the SNP markers allowed us not only to identify 1-3 additional QTLs but also increased the percentage of phenotypic variance accounted for by 21-25%. Two of the QTLs associated with kernel weight on chromosomes 4A and 6A were common between the present and previous studies, but none of the QTLs associated with test weight were common between the two studies. QTLs for test weight were previously reported on several chromosomes, including chromosomes 1A, 1B, 1D, 2D, 3B, 3D, 4A, 4D, 5A, 5D, 6B, and 7A (Elouafi and Nachit, 2004; Huang et al., 2006; McCartney et al., 2006; Narasimhamoorthy et al., 2006). In a RIL population derived from Chuan 35050 × Shannong 483, four QTLs were reported for kernel weight, which includes a consistent QTL on chromosome 6A (*QTkw.sdau-6A*) explaining between 6.1 and 13.2% of the phenotypic variance across three different environments (Sun et al., 2009).

2.5 Conclusions

In the combined phenotypic data across three environments, the SNP-based high density genotypic provided us a better opportunity to uncover four moderate- and eight minor-effect QTLs, which altogether accounted for 9.3 to 39.4% of the phenotypic and from 24.6 to 96.8% of the genetic variance across three organic environments combined. One of the moderate-effect QTLs was coincidental for both flowering time (*QFlt.dms-5A.2*) and maturity (*QMat.dms-5A*) and mapped within the same confidence interval as the *Vrn-A1* gene, which may be due to tight

linkage. This coincidental QTL on 5A explained 17.2% of phenotypic variance for both traits, which is equivalent to a reduction in flowering time and maturity by 2-3 days. It may be considered for further study for possible use in marker-assisted selection. Results from this study provide valuable information to wheat researchers developing early maturing and short stature cultivars for organic management systems.

2.6 Tables and Figures

Table 2-1. Summary of the SNP and gene specific markers used in the present study.

Chromosome	Initial number of mapped markers	Final number of markers used for QTL mapping	Total map length (cM)	Map to marker ratio (cM)
1A	581	54	168.1	0.3
1B	171	48	161.3	0.9
1D	38	4	14.3	0.4
2A	331	70	236.8	0.7
2B	638	150	305.1	0.5
2D	93	36	90.0	1.0
3A	265	93	316.4	1.2
3B	141	41	144.6	1.0
4A	452	68	166.7	0.4
4B	96	35	116.5	1.2
5A	157	71	311.9	2.0
5B	523	141	324.8	0.6
5D	53	8	18.6	0.4
6A	492	62	129.0	0.3
6B	908	128	322.3	0.4
6D	87	26	38.1	0.4
7A	340	86	270.8	0.8
7B	271	67	242.0	0.9
7D	31	15	64.5	2.1
Total	**5,667**	**1,203**	**3,442**	

Table 2-2. Summary of QTLs associated with eight traits on 167 recombinant inbred lines evaluated across three environments (2008-2010) under organic management system.

Trait	QTL*	Environment	Chrom	Position (cM)	Confidence interval (cM)	Left marker	Right marker	LOD	R^2 (%)	Additive effect**	Phenotypic difference***
Flowering	QFlt.dms-5A.1	2010	5A	262	260.5-263.5	wsnp_Ex_c2526_4715978	BobWhite_c14689_172	5.1	13.1	-0.9	
Flowering	QFlt.dms-5A.2	Combined	5A	297	293.5-297.5	Kukri_c12384_430	wsnp_Ex_c22727_31934296	6.8	17.2	-1.1	-2.2
Flowering	QFlt.dms-5A.2	2009	5A	298	296.5-298.5	wsnp_Ex_c22727_31934296	wsnp_Ex_rep_c66689_65010988	3.2	8.5	-0.7	
Maturity	QMat.dms-4B	2010	4B	79	78.5-79.5	BobWhite_c5694_1201	wsnp_Ra_c3790_6990678	3.8	9.0	1.3	
Maturity	QMat.dms-5A	2009	5A	297	293.5-297.5	Kukri_c12384_430	wsnp_Ex_c22727_31934296	3.8	10.1	-1.0	
Maturity	QMat.dms-5A	2010	5A	298	297.5-299.5	wsnp_Ex_c22727_31934296	wsnp_Ex_rep_c66689_65010988	3.4	7.9	-1.2	
Maturity	QMat.dms-5A	Combined	5A	298	297.5-299.5	wsnp_Ex_c22727_31934296	wsnp_Ex_rep_c66689_65010988	7.0	17.2	-1.4	-2.7
Plant height	QPht.dms-4B	2010	4B	80	79.5-80.5	RAC875_c103110_275	RAC875_c24550_1150	4.0	9.8	-2.9	
Plant height	QPht.dms-4B	Combined	4B	81	79.5-80.5	RAC875_c3790_429	Tdurum_contig29054_113	7.5	18.8	-3.7	-7.2
Protein content	QGpc.dms-6B	2010	6B	275	273.5-276.5	Tdurum_contig75763_930	Tdurum_contig30932_168	3.0	7.9	-0.2	
Tillering	QTil.dms-4A	Combined	4A	0	0-1.5	Excalibur_c82040_91	wsnp_Ra_rep_c70233_67968353	3.5	9.3	2.7	5.3
Grain yield	QYld.dms-5B	Combined	5B	37	36.5-38.5	TA002682-0717	BobWhite_c26082_80	3.2	7.5	-153.5	-287.3
Test weight	QTwt.dms-1A	Combined	1A	81	79.5-81.5	Kukri_c82555_88	wsnp_Ex_c31983_40709607	3.2	7.5	-0.3	-0.6
Test weight	QTwt.dms-4B	2009	4B	16	14.5-17.5	BS00073084_51	Kukri_rep_c78644_408	3.2	7.7	0.3	
Test weight	QTwt.dms-5B	Combined	5B	204	201.5-205.5	Kukri_c43972_367	Kukri_c46932_65	3.7	8.6	-0.3	-0.6
Test weight	QTwt.dms-6A	2010	6A	90	84.5-98.5	IACX2250	wsnp_Ku_c44079_51438574	3.0	8.2	0.5	
Test weight	QTwt.dms-7B	2009	7B	154	153.5-154.5	wsnp_Ku_c60707_62509051	wsnp_Ex_c10550_17231658	3.3	8.1	-0.3	
Kernel weight	QTkw.dms-1B	Combined	1B	16	14.5-17.5	Ku_c1932_1583	Excalibur_rep_c103592_565	3.1	5.5	-0.7	-1.3
Kernel weight	QTkw.dms-4A	Combined	4A	120	118.5-120.5	wsnp_Ex_c7899_13416443	wsnp_Ex_rep_c97236_84366722	3.8	6.8	0.7	1.8
Kernel weight	QTkw.dms-4A	2009	4A	121	120.5-121.5	wsnp_Ex_rep_c66706_65037564	wsnp_Ex_rep_c101638_86971861	3.2	8.7	0.9	
Kernel weight	QTkw.dms-6A	Combined	6A	79	77.5-80.5	wsnp_Ku_rep_c112734_95776957	BS00036878_51	6.5	12.2	1.0	2.1
Kernel weight	QTkw.dms-6A	2010	6A	84	79.5-84.5	BS00066623_51	BobWhite_c10342_117	3.6	9.6	1.2	
Kernel weight	QTkw.dms-6B.1	Combined	6B	6	4.5-12.5	wsnp_Ex_c56091_58346859	wsnp_JD_c23373_19987039	4.7	8.5	-0.8	-1.0
Kernel weight	QTkw.dms-6B.2	Combined	6B	37	29.5-42.5	Excalibur_c35713_106	RAC875_c6837_468	3.6	6.4	0.7	1.5

Table 2-2 Comparisons of recombinant inbred lines that had the CDC Go or Attila alleles at the flanking markers of the coincident QTL on eight traits evaluated at three (2008-2010) environments under organic management system. RILs with recombinant genotypes at the flanking markers of each coincident QTL were excluded from analysis.

Trait	Chrom	Coincident QTL name	Confidence interval (cM)	Attila type alleles	CDC Go type alleles	Difference*	F statistics	p value
Flowering time (days)	5A	*QFlt.dms-5A.2 vs QMat.dms-5A*	*293.5-299.5*	55.0	52.9	-2.1	33.940	0.001
Maturity (days)	5A			92.8	90.1	-2.7	28.980	0.001
Plant height (cm)	5A			72.9	70.2	-2.7	6.240	0.014
Grain yield (kg ha^{-1})	5A			3146.7	3256.7	110.0	1.510	0.222
Test weight (kg hL^{-1})	5A			76.0	76.4	0.4	5.040	0.026
1000 kernel weight (g)	5A			38.8	38.8	0.0	0.000	0.905
Grain protein content (%)	5A			13.0	12.8	-0.2	2.710	0.102
Number of tillers per m^2	5A			96.8	95.4	-1.4	1.000	0.319
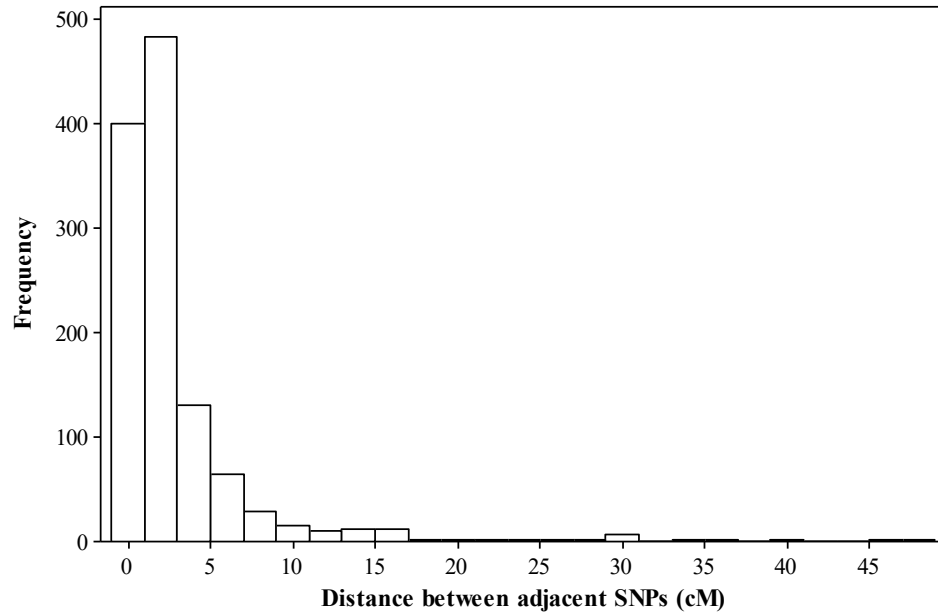
Figure 2-1. Observed frequency distribution of linkage map distances between adjacent loci based on the 1200 single nucleotide polymorphism (SNP) and three gene-specific markers mapped to the 19 hexaploid wheat chromosomes.
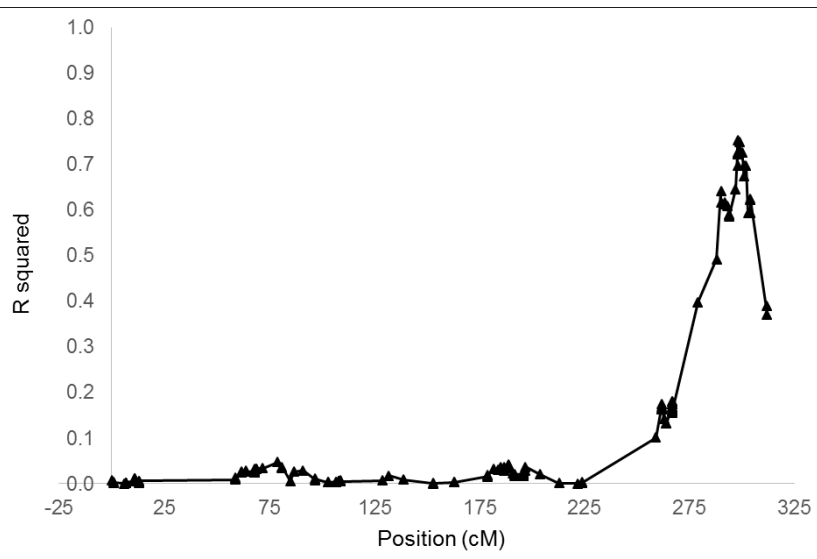
Figure 2- 2. The linkage disequilibrium (LD) plot between the Vrn-A1 gene- specific marker and all single nucleotide polymorphisms that mapped on chromosome 5A.

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Chapter 3 Effect of marker density and number of testing environments in mapping QTLs for agronomic traits in spring wheat [2]

3.1 Introduction

Average wheat productivity in Canada has increased from 2.7 Mg ha^{-1} in 2005 to 3.1 Mg ha^{-1} in 2014 (http://faostat3.fao.org), and Canada is now the sixth largest wheat producing and the second largest wheat exporting country (http://apps.fas.usda.gov/psdonline/circulars/production.pdf). Wheat breeders in western Canada primarily aim to develop short stature cultivars that are early maturing, high yielding with high protein content and elevated dough strength, with at least intermediate resistance to the five priority diseases of leaf, stem and yellow rust, fusarium head blight and common bunt (http://www.pgdc.ca). As most of these traits are quantitatively inherited, phenotypic performance only partially reflects the genetic value of individuals, which is due to the cumulative action of many genes or quantitative trait loci (QTL) and their interaction with the environment (Sham, et al. 2002). The availability of well-validated and fine mapped genes or major effect QTLs for a target trait and the associated technology (user friendly, high throughput, and low cost molecular markers) offers alternative methods for marker-assisted selection (Lin and Chen 2009; Singh, et al. 2007). Both linkage-based analysis and association mapping have been extensively used to map and characterize genes and QTLs associated with traits of economic importance in wheat (Huang, et al. 2006; Liakat Ali, et al. 2011;

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[2]A version of this Chapter has been submitted to PLOS ONE and is under review now. (Jun Zou, Kassa Semagn, Muhammad Iqbal, Hua Chen, Mohammad Asif, Amidou N'Diay, Alireza Navabi, Enid Perez-Lara, Curtis Pozniak, Rong-Cai Yang, Harpinder Randhawa, and Dean Spaner. Effect of marker density and number of testing environments in mapping QTLs for agronomic traits in spring wheatl)

Mergoum, et al. 2013; Mora, et al. 2015; Wang, et al. 2009; Yao, et al. 2009; Yu, et al. 2014). The success in identifying genes and major effect QTLs depends on several factors, including population type and size, reliability of the phenotypic data, trait complexity (heritability), the type and number of molecular markers (marker density) used for genotyping, and the statistical methods used for mapping (Bernardo 2008; Collard, et al. 2005; Semagn, et al. 2010).

Diversity Arrays Technology (DArT), simple sequence repeats (SSRs) and single nucleotide polymorphism (SNP) markers have been commonly used for QTL mapping. However, SNPs have become popular for many genetic applications due to 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; Schlotterer 2004). As reviewed elsewhere (Semagn, et al. 2014), there are numerous SNP genotyping platforms (Chen and Sullivan 2003; Gut 2001; Sobrino, et al. 2005; Syvanen 2001) that combine a variety of chemistry, allele discrimination techniques, detection methods, and reaction formats. In wheat, a total of 81,587 gene-associated SNPs (90K) is available through the Illumina iSelect SNP array platform (Wang, et al. 2014) of which at least 5 to 13% could be polymorphic in a given bi-parental mapping population (Babiker, et al. 2015; Liu, et al. 2016; Perez-Lara, et al. 2016; Zou, et al. 2016). Although the final number of SNPs retained from the 90K SNP array for QTL mapping maybe less than 2000, due to cosegregation (mapping at exactly the same position), they provide better opportunities to identify new QTLs that may not have been identified using other genotyping platforms, such as DArTs (Perez-Lara, et al. 2016; Zou, et al. 2016). The Cornell University-based genotyping by sequencing (GBS) (Elshire, et al. 2011; Poland and Rifeb 2012) has become a very popular SNP genotyping method that could generate high density genotype data (up to a million in crops, such as maize) at lower cost than most of the chip-based

genotyping platforms, but the current GBS technology has some limitations which include the following: (i) it often generates a relatively large proportion of missing data that requires reliable imputation methods (Beissinger, et al. 2013; Nazzicari, et al. 2016); (ii) GBS methodology is still evolving and may require re-analysis and re-examination of old data using improved computational tools to ensure allele calls have not changed; and (iii) GBS allele calls are still less accurate for heterogeneous and highly heterozygous germplasm as compared with highly homozygous lines (Semagn, et al. 2015). The DArTseq (Sansaloni, et al. 2011)-based GBS platform is an alternative method that generates a lower density of markers (50,000 to 350,000 SNPs) but with better coverage and lower levels of missing data than the Cornell-based GBS method (Chen, et al. 2016).

The Wheat Breeding group at the University of Alberta has been evaluating the performance of wheat cultivars and breeding lines under both conventional and organic management systems in Alberta, Canada (Asif, et al. 2015; Chen, et al. 2015; Kamran, et al. 2013; Kaut, et al. 2009; Kaut, et al. 2008; Mason, et al. 2007a; Mason, et al. 2007b; Mason, et al. 2007c; Reid, et al. 2009a; Reid, et al. 2011; Reid, et al. 2009b). In one of the recent studies (Asif, et al. 2015), we evaluated a recombinant inbred line (RIL) population developed from a cross between Attila (CM85836-50Y-0M-0Y-3M-0Y) (Tadesse, et al. 2010) and CDC Go in 2008, 2009 and 2010 under conventionally and organically managed field conditions and genotyped the population with 579 DArT and *Rht-B1* markers. Using the averaged phenotypic data across three environments, (i) we uncovered three QTLs under conventional management on chromosome 6A for grain yield, 4B for plant height and 1A for test weight, but none for the other five traits (number of tillers, kernel weight, grain protein content, days to flowering and maturity); (ii) we found five QTLs under organic management on 4B for plant height, 6A for grain protein content, 1B for test weight and both 4A and 6A for kernel weight, but none for four other traits (flowering

time, maturity, tillering and grain yield). The QTL for plant height on chromosome 4B that mapped close to the *Rht-B1* gene was the only common genomic region between the conventional and organic management systems.

The QTLs identified under organic and conventional management systems explained between 8.3 and 19.2% of the total phenotypic variance (Asif, et al. 2015), which is equivalent to 24.4 to 60.6% of the genetic variance. In both organic and conventional management systems, no QTL was identified for flowering time, maturity and number of tillers (tillering ability) across the three environments. As heritability for each trait under organic and conventional management average over three environments varied from 0.15 to 0.76, most of the genetic variance remained unexplained irrespective of the management system (Asif, et al. 2015). Although several factors might have contributed to our failure to identify QTLs explaining most of the genetic variance in the Attila x CDC Go RIL population, low marker density, and uneven marker distribution in the linkage maps are possible reasons. In order to investigate if an increase in marker density improves QTL detection, we reanalyzed the same phenotype data averaged over three organic management system with a subset of 1200 high quality SNPs out of the 90K SNP array and three gene specific markers (*Ppd-D1*, *Vrn-A1*, and *Rht-B1*). That study identified five moderate- and eleven minor-effect QTLs distributed across 10 chromosomes of which 13 QTLs were not reported using the DArT-based low-marker-density (Zou, et al. 2016). We found between one and five QTLs per trait (except grain protein content where we found none), which individually explained from 5.5 to 18.8% of phenotypic variance. For each trait under organic management, the total phenotypic and genetic variance explained by all detected QTLs varied from 9.3 to 39.4 and from 24.6 to 96.8%, respectively (Zou, et al. 2016). For some of the traits, such as flowering time, maturity and plant height, however, over 50% of the genetic variance still remained unexplained. This may have been due to inadequate number of testing (phenotype)

environments, and this is the basis for the present study. The objective of the present study was to investigate if the combined use of more phenotyping (testing) environments with high marker density improves QTL detection in the Attila × CDC Go RIL population genotyped with 90K SNP array and evaluated at seven environments grown in conventional management systems.

3.2 Materials and Methods

The present study was conducted on a mapping population of 167 RILs developed from a cross between two spring wheat cultivars - Attila (CM85836-50Y-0M-0Y-3M-0Y) and CDC Go. As described in our previous studies (Asif, et al. 2015; Zou, et al. 2016), Attila is a semi dwarf, early maturing and medium yielding cultivar from the International Maize and Wheat Improvement Center (CIMMYT), while CDC Go is a medium height, relatively late maturing and high yielding Canadian Western Red Spring wheat cultivar (Tadesse, et al. 2010). The RIL population and the parents were initially phenotyped under conventionally managed field conditions thrice between 2008 and 2010 at the Crop Research facility of the University of Alberta South Campus (53°19'N, 113°35'W), Edmonton, Canada (Asif, et al. 2015). Additional phenotypic data were obtained for four years by phenotyping the population between 2011 and 2014 at the same location. Each field experiment was conducted in a randomized incomplete block design with two to three replications depending on seed availability. Each entry was evaluated for flowering and maturity time, tillers, plant height, test weight (grain volume weight), thousand kernel weight (grain weight), grain yield, and grain protein content, as described in our previous study (Asif, et al. 2015).

The RIL population and the two parents were genotyped with the Wheat 90K Illumina iSelect SNP array (Wang, et al. 2014) and three gene specific markers (*Ppd-D1*, *Rht-B1* and *Vrn-A1*) as described in our previous studies (Perez-Lara, et al. 2016; Zou, et al.

2016). Linkage analysis was performed as described in the Cutler × AC Barrie mapping population (Perez-Lara, et al. 2016), while all other statistical analyses, including descriptive statistics, test for normality, F statistics, heritability and inclusive composite interval mapping were conducted as described in one of our recent studies (Zou, et al. 2016). The extent of linkage disequilibrium (LD) between the *Ppd-D1*, *Rht-B1* and *Vrn-A1* gene specific markers and all SNPs that mapped on chromosomes 2D, 4B, and 5A, respectively, was evaluated by computing the r^2 values using TASSEL version 5.2.28 (Bradbury, et al. 2007). QTL names were designated following the International Rules of Genetic Nomenclature (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm), which consisted of three letters for trait acronym, lab designation (dms = Dean Michael Spaner), and chromosome. Genetic maps and QTL graphs were drawn using MapChart v2.1 (Voorrips 2002). QTLs explaining <10%, 10-20% and >20% of the total phenotypic variation (R^2) were arbitrarily classified into minor, moderate and major effect QTLs, respectively.

3.3 Results

3.3.1 Phenotypic traits

Table 3-1 provides a summary of the descriptive and F statistics of the 167 RILs evaluated under conventional management across three (2008-2010) and seven (2008-2014) years (environments). In the averaged data across seven environments, CDC Go matured about 3 days earlier, produced 13 more tillers m^{-2}, with kernels 2 g heavier and 0.6% higher grain protein content, but was 2 cm taller and yielded 277 kg ha^{-1} less grain than Attila. The 167 RILs varied in height from 63 to 102 cm, required 48-60 days to flowering and 93-105 days to maturity, and yielded 3.5 to 5.9 Mg ha^{-1} grain. The distribution of least squares means estimated from the seven environments average phenotypic data was normal (P > 0.050) for all traits, except test weight. The Shapiro-Wilk test

rejected the hypothesis of normality (P = 0.018) for test weight (data not shown). Averaged across all seven environments, genotypes differed (p < 0.001) for all traits (Table 3-1). Broad sense heritability across the three and seven environments varied from 0.28 to 0.76 and from 0.26 to 0.73, respectively (Table 3-2).

3.3.2 QTLs associated with seven environments

We first conducted inclusive composite interval mapping (ICIM) on the least squares means estimated from the combined phenotypic data across the seven environments and also on individual environments. The analyses conducted using the combined data of the seven environments uncovered a total of 14 QTLs (Fig 3-1 and Table 3-3) associated with the eight traits, which included one QTL each for tillering per m^2 (*QTil.dms-6A.1*), flowering time (*QFlt.dms-5A*), plant height (*QPht.dms-4B*) and grain yield (*QYld.dms-2D.2*); two QTLs each for maturity (*QMat.dms-4B* and *QMat.dms-5A.2*), grain protein content (*QGpc.dms-2D.2* and *QGpc.dms-4B*) and test weight (*QTwt.dms-5A* and *QTwt.dms-5B.3*); and four QTLs for kernel weight (*QTkw.dms-4A*, *QTkw.dms-6A.1*, *QTkw.dms-6D.2,* and *QTkw.dms-7B.1*). The QTL for tillering mapped at 70 cM on chromosome 6A (*QTil.dms-6A.1*) and accounted for 11.2% of the phenotypic variance across the eight environments. RILs that had Attila alleles at the two flanking markers for *QTil.dms-6A.1*, on average, had 3.4 more tillers than those RILs that were homozygous for CDC Go alleles. However, this QTL was not detected in any of the individual environments; instead, we found 6 other environment specific QTLs on 4A (QTil.dms-4A), 5A (QTil.dms-5A), 6A (*QTil.dms-6A.2)* and 7A (*QTil.dms-7A.1*, *QTil.dms-7A.2* and *QTil.dms-7A.3*) that were associated with tillering in 2009, 2013 and/or 2014 environments (Appendix 4).

The QTL for flowering time mapped at 296 cM on chromosome 5A (*QFlt.dms-5A*), flanked by the *Vrn-A1* gene, and explained 16.8% of the phenotypic variance across the seven environments (Table 3-3). RILs that had CDC Go alleles at the two flanking markers for *QFlt.dms-5A* flowered 2.5 days earlier than those RILs that were homozygous for Attila alleles. When individual environments were considered, *QFlt.dms-5A* was detected at the same confidence interval in four environments (2009, 2010, 2011 and 2014), and explained between 12.6 and 13.0% of phenotypic variance of the individual environments (Appendix 4). In addition, three environment specific QTLs for flowering time were also detected on 4A (*QFlt.dms-4A*), 4B (*QFlt.dms-4B*) and 6B (*QFlt.dms-6B*), which individually explained between 7.6 and 9.0% of the phenotypic variance. The two QTLs for maturity mapped at 80 cM on chromosome 4B (*QMat.dms-4B*) and at 297 cM on 5A (*QMat.dms-5A.2*), which individually explained 15.9 and 14.0%, respectively, and altogether accounted for 29.9% of the phenotypic variance across the seven environments. The favorable alleles for *QMat.dms-4B* and *QMat.dms-5A.2* originated from Attila and CDC Go, respectively. RILs that were homozygous for the favorable alleles at the two flanking markers of each QTL matured about two days earlier than those RILs that were homozygous for the unfavorable alleles. When individual environments were considered, *QMat.dms-4B* and *QMat.dms-5A.2* were detected at the same confidence interval in five (2009 to 2014 except 2011) and four (2009, 2010, 2013 and 2014) out of the seven environments, respectively (Appendix 4). The proportion of phenotypic variance explained by *QMat.dms-4B* and *QMat.dms-5A.2* in individual environments varied from 7.4 to 19.4% and from 6.2 to 12.7%, respectively. Furthermore, we also found 6 environment specific QTLs for maturity on chromosomes 2D, 5B, 6B and 7A that individually explained between 0.8 and 13.3% of the phenotypic variance of the individual environments (Appendix 4).

The QTL associated with plant height across seven environments mapped at 82 cM on chromosome 4B (*QPht.dms-4B*) and explained 18.4% of the phenotypic variance. *Rht-B1* gene mapped 34.5 cM distal to *QPht.dms-4B and* 27 cM distal to one of the flanking SNP marker, wsnp_Ra_c1146_2307483. RILs that had the CDC Go alleles at the two flanking markers for *QPht.dms-4B* were 7.7 cm shorter than those RILs that were homozygous for Attila alleles (Table 3-3). When individual environments were considered, *QPht.dms-4B* was consistently detected at the same position in five (2010 to 2014) of the seven environments, but the proportion of phenotypic variance explained by *QPht.dms-4B* was variable, ranging from 11.9 to 23.9% (Table 3-3). We also found four environment specific QTLs for plant height on chromosomes 2D (*QPht.dms-2D.2*), 5A (*QPht.dms-5A*) and 6B (*QPht.dms-6B.1* and *QPht.dms-6B.2*), which individually explained between 3.2 and 13.3% of the phenotypic variance in the 2009, 2010 and 2013 environments, respectively (Appendix 4). We found one QTL for grain yield at 66 cM on 2D (*QYld.dms-2D.2*) that explained 9.3% of the phenotypic variance across the seven environments. The photoperiod response *Ppd-D1* gene mapped 66 cM distal to *QYld.dms-2D.2*. RILs with Attila alleles at the two flanking markers for *QYld.dms-2D.2* produced 375.7 kg ha^{-1} more grain yield than those RILs homozygous for CDC Go alleles. When individual environments were considered, *QYld.dms-2D.2* was detected at the same confidence interval in four (2010, 2011, 2013 and 2014) of the seven environments, explaining between 6.0 and 11.1% of the phenotypic variance at individual environments. In addition, five environment-specific QTLs associated with grain yield were also identified on chromosomes 3A, 6B, 7A and 7D explaining between 6.3 and 10.9% of the phenotypic variance (Appendix 4).

The two QTLs associated with test weight across the seven environments were located at 12 cM on chromosome 5A (*QTwt.dms-5A*) and at 239 cM on 5B (*QTwt.dms-5B.3*), and they explained 6.1 and 10.1% of the phenotypic variance, respectively

(Table 3-3). The favorable alleles for *QTwt.dms-5A* and *QTwt.dms-5B.3* originated from CDC Go and Attila, respectively. RILs homozygous for the favorable alleles at the two flanking markers for *QTwt.dms-5A* and *QTwt.dms-5B.3* had 0.5 kg hL^{-1} higher test weight than those RILs with unfavorable alleles. Neither *QTwt.dms-5A* nor *QTwt.dms-5B.3* were detected in any individual environments; instead, we found two environment specific QTLs at 109 cM on 2B and at 163 cM on 5B that individually explained 7.0 and 9.9%, respectively, of the phenotypic variance for test weight at individual environments. For kernel weight, we found four QTLs at 120 cM on 4A (*QTkw.dms-4A*), at 79 cM on 6A (*QTkw.dms-6A.1*), at 4 cM on 6D *(QTkw.dms-6D.2)* and at 158 cM on 7B *(QTkw.dms-7B.1)*. Each QTL individually explained between 6.7 and 12.1% and altogether accounted for 35.4% of the phenotypic variance across seven environments (Table 3-3). RILs homozygous for the CDC Go alleles at the two flanking markers of each QTL were from 0.9 to 1.1 mg heavier per kernel than those with Attila alleles. When individual environments were considered, both *QTkw.dms-4A* and *QTkw.dms-6A.1* were detected in 2009 environment; all other QTLs were not detected in any of the individual environments. We also found four additional environment specific QTLs associated with kernel weight on 2B, 3A, 5B and 6A explaining between 3.0 and 10.5% of the phenotypic variance (Appendix 4).

The two QTLs associated with grain protein content across seven environments mapped at 62 cM on 2D (*QGpc.dms-2D*) and at 80 cM on 4B (*QGpc.dms-4B*). *QGpc.dms-2D* and *QGpc.dms-4B* explained 13.4 and 6.3%, respectively, and together accounted for 19.7% of the phenotypic variance across seven environments (Table 3-3). The favorable alleles for *QGpc.dms-2D* and *QGpc.dms-4B* originated from CDC Go and Attila, respectively. RILs homozygous for the favorable alleles at the two flanking markers of each QTL showed 0.5% higher grain protein content than those RILs homozygous for unfavorable alleles. When individual environments were

considered, *QGpc.dms-2D* was detected in two (2009 and 2010) environments, while *QGpc.dms-4B* was detected only in 2010. In addition, three environment-specific QTLs on chromosomes 2B, 3A and 5B were detected that individually explained between 6.8 and 8.3% of the phenotypic variance at a single environment (Appendix 4).

3.3.3 Coincidental QTLs

The first coincidental QTL was mapped on chromosome 5A and was associated with both flowering time (*QFlt.dms-5A*) and maturity (*QMat.dms-5A.2*) in the combined data across seven environments plus plant height (*QPht.dms-5A*) in 2013 environment (Fig 1, Table 3). This coincidental QTL explained 14.0-16.8% of the phenotypic variance for flowering time and maturity across seven environments and 8.4-14.6% of the phenotypic variance for plant height in two environments (Appendix 4). RILs carrying the CDC Go alleles at the two flanking markers of the QTL on 5A were different (p ≤ 0.03) from those possessing Attila alleles for flowering time, maturity, plant height and test weight, but not for the other four traits (Table 3-4). On average, RILs with the CDC Go allele at the two flanking markers of the coincidental QTL on 5A flowered/matured 2 days earlier, were 4 cm shorter and had 0.3 kg hL^{-1} higher test weight than those RILs with Attila alleles. The second coincidental QTL mapped on 4B (Fig 1, Table 3) was associated with maturity (*QMat.dms-4B*), plant height (*QPht.dms-4B*) and grain protein content (*QGpc.dms-4B*). RILs with Attila alleles at the two flanking markers for this QTL on 4B were different (p ≤ 0.008) than those with CDC Go alleles for maturity, plant height and grain protein content, but not for the other five traits (Table 4). RILs carrying the Attila alleles at the two flanking markers matured 2.2 days earlier and had 0.3% higher grain protein, but were 7.6 cm taller than those homozygous for CDC Go alleles (Table 3-4). The third coincidental QTL mapped on 2D (Fig 3-1, Table 3-3) was associated with both grain yield (*QYld.dms-2D*) and grain

protein content (*QGpc.dms-2D*). RILs carrying the CDC Go alleles at the two flanking markers for the QTL on 2D were different (p ≤ 0.001) from those possessing Attila alleles for both grain yield and grain protein content, but not for the other six traits (Table 3-4). RILs carrying the Attila alleles at the two flanking markers yielded 335.9 kg ha^{-1} more grain with 0.4% lower grain protein content than those homozygous for CDC Go alleles (Table 3-4).

3.3.4 Comparison of QTLs by number of testing environments

To investigate if an increase in the number of testing (phenotyping) environments improved QTL detection, we compared QTL results obtained for three environments (2008-2010) used in our previous study (Asif, et al. 2015) with seven environments (2008-2014) that consisted of 4 years additional phenotypic data generated from 2011 to 2014. Using the averaged phenotypic data across three and seven conventional environments, we uncovered a total of 18 QTLs of which 8 QTLs were common between the three and seven environments, while the remaining 11 QTLs were detected either in the three (5) or seven (6) environments (Fig 1, Table 3). The 8 common QTLs between the two data sets were one for flowering time on 5A (*QFlt.dms-5A*), two for maturity on 4B and 5A (*QMat.dms-4B* and *QMat.dms-5A.2*), one for plant height on 4B (*QPht.dms-4B*), one for test weight on 5B (*QTwt.dms-5B.3*), two for kernel weight on 4A and 6A (*QTkw.dms-4A* and *QTkw.dms-6A.1*) and one for grain protein content on 2D (*QGpc.dms-2D*). In six of the eight common QTLs identified at the same position in the two data sets, the percentage of phenotypic variance explained by each QTL in the seven site years was up to 4.2% greater when compared with three site years of data. The remaining two QTLs (*Qmat.dms-5A.2* and *QTkw.dms-6A.1*) showed a reduction by 5.6 and 11.4% in the seven environments as compared to the original three environments (Table 3-3). For tillering, we found a single QTL associated with the seven environments, but not with the three

environments. For plant height, an additional QTL on 2D (*QPht.dms-2D.2*) was identified in the three environments that was not identified in the seven environments. For grain protein content, there was an additional QTL on 4B (*QGpc.dms-4B*) in the seven environments, but not in the three environments. For grain yield, we found QTLs on 6B and 2D in both data sets. For kernel weight, there were four QTLs each associated with the three and seven environments of which two QTLs were common between the two datasets. For test weight, we found two QTLs associated with each data set of which one was common and the second one was different between the three and seven environments (Table 3-3).

3.4 Discussion

3.4.1 Effect of marker density

In one of our previous studies in the Attila and CDC Go RIL population (Asif, et al. 2015), we genotyped the population with 579 DArT markers and *Rht-B1*, and phenotyped them at three environments grown under conventional management. That study identified a total of three QTLs associated with the averaged data over three environments, which included a QTL for grain yield on chromosome 6A, plant height on 4B and test weight on 1A. Each QTL accounted for 10.9 to 17.0% of the phenotypic variance (Asif, et al. 2015) and 18.9 to 60.0% of the genetic variance. However, no QTL was identified for the other 5 traits recorded averaged over three environments. Our previous study was based on a total map length of 2045 cM, with an overall average map distance among adjacent markers (inter-marker interval) of 3.5 cM (Asif, et al. 2015), while the present study was based on 1203 informative SNP and gene specific markers, which resulted in a total map length of 3442 cM and an overall average inter-marker interval of 2.9 cM. As compared with our previous study, genome coverage increased over two fold, while average inter-marker interval decreased by 0.6

cM. To explore if doubling marker density improves QTL detection in the Attila x CDC Go RIL population, we recently reanalyzed the phenotypic data generated across three organically managed environments using a subset of 1200 informative SNPs out of the 90K SNP array and three gene specific (*Ppd-D1*, *Rht-B1* and *Vrn-A1*) markers (Zou, et al. 2016). Using phenotypic data averaged across three organic environments, we found a total of 16 QTLs associated with all traits except grain protein content of which 13 QTLs were not detected using the DArT-based low marker density (Asif, et al. 2015). For each trait, the total phenotypic and genetic variance explained by the detected QTLs under organic management varied from 9.3 to 39.4% and from 24.6 to 96.8%, respectively, which was much greater than our previous study (Asif, et al. 2015). In the present study conducted using phenotypic data combined across three conventionally managed environments, we found a similar trend to that of the organic management system (Zou, et al. 2016); not only the number of detected QTLs increased, but also the percentage of phenotypic variance explained per trait. We found a total of 13 QTLs associated with all traits except tillers averaged over three conventionally managed environments of which only the QTL for plant height on chromosome 4B was common between the present and previous studies. The QTLs identified in the present study included one QTL each for flowering time, grain yield and grain protein content; two QTLs each for maturity, plant height and test weight, and four QTLs for kernel weight (Table 3-3). Although the use of high marker density improves QTL detection (Perez-Lara, et al. 2016; Zou, et al. 2016; Zych, et al. 2015) and the recent development of the 90K Wheat SNP array (Wang, et al. 2014) has provided good opportunity for QTL discovery, the D-genome is still poorly represented. In the present study, for example, there were no SNPs for both chromosomes 3D and 4D. Given the medium to high heritability for flowering time, plant height and grain yield that varied from 0.37 to 0.76, over 50% of the genetic variance for these traits, still remained unexplained. This could be due to lack of

82

sufficient number of evenly distributed polymorphic markers on some chromosomes or smaller number of testing (phenotyping) environments, which is discussed below.

3.4.2 Effect of number of testing environments

In order to explore if the increase in the number of phenotyping environments improves trait heritability, QTL detection and the proportion of phenotypic or genetic variance explained by the QTLs, we compared results obtained from three and seven environments. As compared with the three environments, heritability in the seven environments basically remained the same for all traits except grain protein content, which showed a reduction by 0.38 (Table 3-2). QTL analyses conducted using the averaged phenotypic data across three and seven environments with the 1203 markers identified a total of 19 QTLs of which 8 QTLs were common between the three and seven environments (Fig 3-1, Table 3-3). The 8 common QTLs between the three and seven environments were one for flowering time (*QFlt.dms-5A*), plant height (*QPht.dms-4B*), test weight (*QTwt.dms-5B.3*) and grain protein content (*QGpc.dms-2D*); two for maturity (*QMat.dms-4B* and *QMat.dms-5A.2*) and kernel weight (*QTkw.dms-4A and QTkw.dms-6A.1*). In six of the eight common QTLs, the percentage of phenotypic variance explained by each QTL in the seven environments was up to 4.2% higher than the three environments. The remaining two common QTLs (*QMat.dms-5A.2* and *QTkw.dms-6A.1*) showed a reduction by 5.6 and 11.4% in the seven environments as compared with the three environments (Fig 3-1, Table 3-3). Our results from the three and seven environments data sets, therefore, suggest three points. First, approximately 44% of the total QTLs detected in the two data sets mapped at exactly the same position, while 56% of the QTLs were detected either in the three or seven environments. Such detection of more QTLs that differed between the three and seven environments made comparison complicated. Second, we

think that the combined phenotypic data across the seven environments neither improved new QTL detection nor their effect. Third, some QTLs had higher LOD and R^2 values in the three environments, while others in seven environments. Overestimation of QTL effects is very common (Beavis 1998; Utz, et al. 2000; Wurschum and Kraft 2014; Xu 2003) for different reasons, including number of testing environments and population size (Melchinger, et al. 2004; Schon, et al. 2004).

Some QTL mapping studies compared the effect of population size (number of progenies) and number of testing environments (Melchinger, et al. 1998; Melchinger, et al. 2004; Schon, et al. 2004). Schon *et al.* (2004) evaluated 976 F_5 maize testcross progenies at 19 environments and studied the effect of population size and number of testing environments on the number of detected QTLs, their effect, and the corresponding bias on the estimated effect. The number of detected QTLs and the proportion of variance explained by the detected QTLs generally increased more with increasing sample size than with increasing number of environments. A substantial bias on QTL effect was found even with population size as high as 976, but the average bias generally showed reduction with an increase in both population size and number of testing environments. Melchinger *et al.* (2004) partitioned their entire dataset for maize testcross progenies (N = 976 progenies and E = 16 environments) into smaller datasets (N = 488, 244, 122 and E = 16, 4, 2) and reported improved QTL detection in larger sample size evaluated at more environments. In another study, Melchinger *et al.* (1998) compared results from QTL mapping conducted on two independent sample size of the same F_2 population (344 vs. 107 F_2s) and reported almost triple number of QTLs in the smaller population as compared with the larger population; only about half of the QTLs were common between 344 and 107 F_2 population. In the present study, the QTL effects estimated from the seven environments would be less biased (more reliable) than those estimated from the three environments, but most of the genetic variance for some of

the traits still remained unexplained by the detected QTLs. We think that the cost of the additional five testing environments did not outweigh the benefit in improving QTL detection. Our results, together with others (Melchinger, et al. 1998; Melchinger, et al. 2004; Schon, et al. 2004), demonstrated the need in increasing the population size that will in turn improve the power of QTL detection, the proportion of genetic variance explained by them, and reduce the bias than increasing only the number of phenotyping environments for relatively smaller population size.

3.4.3 Comparisons of QTLs detected in seven environments with other studies

In western Canada where the growing season is short and days are long, the development of early maturing wheat cultivars is important to avoid frost damage, which can affect both yield and grain quality (Iqbal, et al. 2007; Randhawa, et al. 2013). In the present study, we found one coincident QTL associated with both flowering (*QFlt.dms-5A*) and maturity (*QMat.dms-5A.2*) between 294 and 298 cM interval on chromosome 5A, which accounted for 14.0-16.8% of the phenotypic variance for both traits across seven environments (Table 3-3). RILs homozygous for the CDC Go alleles at the two flanking markers of this coincident QTL flowered/matured 2-3 days earlier than those homozygous for the Attila alleles. In bread wheat, *Vrn-A1* on the long arm of chromosome 5A (Chen, et al. 2013; Preston and Kellogg 2008) is one of the vernalization response genes that directly influence both flowering time and maturity (Dubcovsky, et al. 1998; Galiba, et al. 1995). In the present study, the *Vrn-A1* gene is either one of the flanking markers for*QFlt.dms-5A* and *QMat.dms-5A.2* or mapped 1-2 cM proximal to the coincident QT. Such tight linkage between the coincident QTL and the *Vrn-A1* gene suggests two possibilities. The first possibility is that the QTL may be the same as the *Vrn-A1* gene. LD values between the *Vrn-A1* gene specific marker and the SNP markers that mapped within the QTL confidence interval

on 5A ranged from 0.70 to 0.75 (data not shown), which indirectly supports the possibility of the QTL to be the same as *Vrn-A1* gene; however, the LD values were not high enough to confidently suggest that the QTL is the same as the *Vrn-A1* gene. The alternative scenario is that the QTL is different from the *Vrn-A1* gene, but it is tightly linked with the vernalization response gene due to low number of recombinants in the Attila x CDC Go RIL population to break up the linkage, which have been discussed in detail in our previous study (Zou, et al. 2016). Hexaploid bread wheat consisted of 124,201 genes within 17×10^6 kb genome (Lukaszewski, et al. 2014), which is an average of 137 kb per gene. The total genetic map in the Attila x CDC Go population was 3,442 cM and the ratio between the physical and the genetic position would be 4,939 kb per cM. If *Vrn-A1* mapped 1-2 cM proximal to the coincident QTL for flowering time and maturity, it is equivalent to 4,939 to 9,878 kb that could harbor clusters of 36 to 72 genes. A fine mapping study conducted within 1 cM genetic and 210 kb physical interval in *Arabidopsis thaliana* (Kroymann and Mitchell-Olds 2005) found two growth rate QTLs within such short physical interval that showed epistasis. The results from *Arabidopsis* clearly suggest the second scenario that the *Vrn-A1* gene is most likely tightly linked with the coincident QTL, but the statistical methods used for linkage analysis and QTL mapping failed to discriminate them, which may be resolved by screening large number of recombinants to break up the linkage (Kolb, et al. 2001).

One of the QTLs for maturity (*QMat.dms-4B*) mapped at 80 cM on chromosome 4B and coincided with the QTL for plant height (*QPht.dms-4B*) at 82 cM and grain protein content (*QGpc.dms-4B*) at 80 cM, which is also very evident from the significant differences observed between all RILs with the Attila alleles at the flanking markers compared with those with CDC Go alleles (Table 3-4). The confidence interval for the coincident QTL on 4B ranges between 79 and 86 cM, flanked by Tdurum_contig29054_113 and

wsnp_Ra_c1146_2307483 (Table 3-3). Coincident QTLs have been reported in several other studies (Babu, et al. 2003; Bai, et al. 2013; Lanceras, et al. 2004; Pushpendra, et al. 2007; Quarrie, et al. 2006), which could be due to either (i) tight linkages between genes or QTLs that regulate the expression of separate traits, or (ii) pleiotropic effect, the same gene or QTL may have an effect on two or more traits simultaneously (Tuberosa, et al. 2002). In hexaploid wheat, dwarfing has been achieved mainly through the introduction of *Rht-B1b* on chromosomes 4B and *Rht-D1b* on chromosomes 4D, which have been introduced in many varieties grown worldwide (Ellis, et al. 2002; Evans 1998; Pearce, et al. 2011). In a RIL population derived from Cutler and AC Barrie, our group has recently reported a very consistent major effect QTL adjacent to *Rht-D1b* gene on chromosome 4D that accounted for 38% of the phenotypic variance for plant height across five environments; RILs that had the Cutler alleles at the flanking markers were 13 cm shorter than those with the AC Barrie (Perez-Lara, et al. 2016). In the Attila x CDC Go RIL population, we found moderate effect QTL for plant height on 4B that accounted for 18% and 10% of the phenotypic variance across 8 conventionally and three organically managed environments, respectively. In both organic and conventional management conditions, however, this plant height QTL exhibited either strong linkage or pleiotropic effect with a QTL for maturity plus grain protein content or test weight. In the conventional management, RILs with the CDC Go alleles at the flanking markers were 7.6 cm shorter but matured two days later and had 0.3% less grain protein content than those with the Attila alleles (Table 3-4). The coincident QTL on 4B mapped adjacent to *Rht-B1* gene in our previous study (Asif, et al. 2015) and 33.5 cM proximal to the *Rht-B1* in the present study. Pairwise LD values between the *Rht-B1* gene specific marker and all SNP markers that mapped on chromosome 4B varied from 0.03 to 0.17 (data not shown), which is very low to suggest any association between the coincident QTL and the *Rht-B1* gene.

Tillering ability is one of the important agronomic traits affecting biomass and grain yield potential in cereals (Wang, et al. 2016; Yan, et al. 1998). Moisture and nitrogen fertilizer increase grain yield to a large extent by stimulating the development of more tillers, but excessive tillering is undesirable because young tillers consume nutrients from the main shoot during the vegetative growth stage that leads to leaf senescence before the plant reaches maturity. In the present study, we found a single QTL from Attila (*QTil.dms-6A.1*) at 70 cM on chromosome 6A that accounted for 11.2% and 44.9% of the phenotypic and genetic variance, respectively. In spring wheat, QTLs associated with tillering ability have been reported near *Gli-A2* (Xpsr10) on the short arm of chromosome 6A and several other chromosomes. For grain yield, we found a single QTL on chromosome 2D (*QYld.dms-2D.2*) that explained 9.3% and 22.1% of the phenotypic and genetic variance across seven years, respectively. RILs with the Attila alleles at the flanking marker of *QYld.dms-2D.2* produced 376 kg ha^{-1} more grain yield than those with the CDC Go alleles (Table 3). The photoperiod sensitivity gene (*Ppd-D1*) on chromosome 2D has been the focus in breeding for early maturing wheat cultivars to better adapt to their environments (Quarrie, et al. 2005; Wu, et al. 2012). Different studies have reported QTLs associated with grain yield on chromosome 2D (Breseghello and Sorrells 2007; Perez-Lara, et al. 2016; Wu, et al. 2012). In another study using RIL population derived from a cross between Cutler and AC Barrie, our group has recently reported a major coincident QTL associated with flowering time, maturity and grain yield on 2D, flanked by *Ppd-D1* gene, which resulted in a reduction in maturity up to 5 days, but showed a yield penalty of 436 kg ha^{-1} (Perez-Lara, et al. 2016). In the present study, however, the QTL associated with grain yield across the seven environments mapped 66 cM distal to the *Ppd-D1* gene, which is genetically far. Pairwise LD values between the

Ppd-D1 gene and all SNP markers that mapped on 2D varied from 0.002 to 0.035 (data not shown), which is extremely small to suggests any association between the QTL and the *Ppd-D1* gene.

Test weight is a very important trait to wheat millers due to its positive correlation with flour yield, whereas kernel weight is associated with both grain and flour yield (Asif, et al. 2015; Ramya, et al. 2010). In our previous study using DArT markers, we reported (i) a single QTL associated with test weight on 1B (*QTwt.dms-1B*) that explained 8.3% of the phenotypic variance across three environments; and (ii) two QTLs associated with kernel weight on chromosome 4A and 6A that together explained 18.7% of the phenotypic variance across three environments (Asif, et al. 2015). In the present study using SNPs and the seven environments combined data, we uncovered (i) *QTwt.dms-5A and QTwt.dms-5B.3* for test weight that individually explained 6.1 and 10.1%, respectively (Table 3-3) and altogether accounted for 16.2% of the phenotypic variance and 46.4% of the genetic variance (Table3- 2), and (ii) four QTLs associated with kernel weight (*QTkw.dms-4A*, *QTkw.dms-6A.1*, *QTkw.dms-6D.2* and *QTkw.dms-7B.1*) that individually explained 6.7-12.1% of the phenotypic variance (Table 3-3), and altogether accounted for 35.4% of the phenotypic and 90.8% of the genetic variance (Table 3-2). None of the QTLs for test weight and two of the four QTLs associated with kernel weight on chromosomes 4A (*QTkw.dms-4A*) and 6A (*QTkw.dms-6A.1*) were common between the present and previous (Asif, et al. 2015; Zou, et al. 2016) studies using the same mapping population. QTLs for test weight have also been reported on several chromosomes, including chromosomes 1A, 1B, 1D, 2D, 3B, 3D, 4A, 4D, 5A, 5D, 6B, and 7A (Elouafi and Nachit 2004; Huang, et al. 2006; McCartney, et al. 2006; Narasimhamoorthy, et al. 2006). In a RIL population derived from Chuan 35050 x Shannong 483, four QTLs

have been reported for kernel weight, which includes a consistent QTL on chromosome 6A (*QTkw.sdau-6A*) that explained between 6.1 and 13.2% of the phenotypic variance across three environments (Sun, et al. 2009).

Grain protein content is one of the important traits that determines the end-use and nutritional quality (Li, et al. 2015). The development of wheat cultivars with high grain protein content or high proportion of the essential amino acids have been one of the target traits by wheat breeders (Prasad, et al. 2003). In the present study, we found two QTLs associated with grain protein content on 2D (*QGpc.dms-2D)* and 4B (*QGpc.dms-4B*) that individually explained 13.4% and 6.3% of the phenotypic variance, respectively, and altogether accounted for 18.7% of the phenotypic variance (Table 3-3) and 71.7% of the genetic variance across seven environments (Table 2). *QGpc.dms-2D* was detected not only across the seven environments combined data, but also in 2009 and 2010 environments 61-65 cM distal to the *Ppd-D1* gene. Neither *QGpc.dms-2D* nor *QGpc.dms-4B* identified in the present study were reported in our previous studies in the Attila x CDC Go population evaluated across three conventionally and organically managed environments (Asif, et al. 2015; Zou, et al. 2016). As discussed above, the QTL for grain protein content on 4B coincided with maturity and plant height, which could be due to tight linkage or pleiotropic effect. The QTL for grain yield on 2D (*QYld.dms-2D.2*) mapped 5 cM distal to the QTLs for grain protein content (*QGpc.dms-2D*), but the genetic confidence interval between *QYld.dms-2D.2* (62-70 cM) and *QGpc.dms-2D* (59-63 cM) showed an overlap; both QTLs mapped 61-66 cM distal to the *PPd-D1* gene. RILs with the Attila alleles at the two flanking markers of *QYld.dms-2D.2* produced 375.7 kg ha^{-1} more grain yield but 0.3% lower grain protein content than those with the CDC Go alleles (Table 3-2). However, neither *QYld.dms-2D.2* nor *QGpc.dms-2D* are associated

with *Ppd-D1* gene. QTLs for grain protein content have been previously reported on several chromosomes (Peleg, et al. 2009; Suprayogi, et al. 2009) and were negatively correlated with grain yield and other yield-related traits (Wang, et al. 2012).

3.5 Conclusions

The present study explored the effects of marker density and number of testing environments in improving QTL detection in the Attila × CDC GO RIL population evaluated across three and seven conventionally managed environments. An increase in marker density was highly useful in detecting several new QTLs that were not detected using the DArT-based low marker density. However, over 50% of the genetic variance at least for four traits still remained unexplained even after an increase in marker density. We thought that an increase in the number of testing environments from three to seven would further improve detection of new QTLs in some of the traits where over 50% of the genetic variance remained unexplained by higher marker density alone. Using the averaged phenotypic data across three and seven conventionally managed environments, we uncovered a total of 19 QTLs of which 8 QTLs were common between the three and seven environments, while the remaining 11 QTLs were detected either in the three or seven environments. Although direct comparison of the QTL results between the three and seven environments was not straightforward, we think that the increase in the number of testing environments did not show clear pattern in improving QTL detection. As the total number of detected QTLs were basically the same in the three and seven environments, it was hard to tell the number of testing environments that provided convincing results. Overestimation of QTL effects is very common for different reasons, including number of testing environments and we expected to see consistent reduction on QTL effects in the seven than the three environments. In the present study, some QTLs had higher effect in the three environments, while others in the seven environments. We, therefore,

do not think that the benefits of the four additional testing environments in the RIL mapping populations outweighed the additional time and cost incurred for generating phenotypic data. Results from this study may provide valuable information to researchers involved on QTL detection across different species.

3.6 Tables and figures

Table 3-1. Summary of descriptive and F statistics for 167 recombinant inbred lines (RILs) evaluated at three (2008-2010) and seven (2008-2014) environments under conventional management system in Edmonton, Canada.

		Parents		RILs (descriptive statistics)					RILs
Trait	No. of environments	Attila	CDC Go	Min	Max	Mean	SD	CV (%)	F value*
No. of tillers (m^{-2})	3 environments	96.3	119.6	92.2	148.5	119.3	11.4	9.6	3.1
Flowering time (days)	3 environments	53.9	50	47.1	60.6	53.1	2.7	5.1	13.2
Maturity time (days)	3 environments	99.1	94	90.2	106.6	97.0	3.7	3.8	3.8
Plant height (cm)	3 environments	71	72.5	57.4	96.3	76.3	7.9	10.4	7.4
Grain yield (Mg ha^{-1})	3 environments	4.5	4.7	2.8	6.0	4.7	6.7	14.3	7.5
Test weight (kg hL^{-1})	3 environments	77.4	78.6	74.8	80.3	77.5	1.1	1.4	5.9
1000-kernels weight (g)	3 environments	39.2	41.5	35.6	46.4	41.4	2.3	5.5	3.0
Grain protein content (%)	3 environments	12.6	13.9	11.1	14.8	12.9	0.8	6.5	8.5
No. of tillers (m^{-2})	7 environments	94.5	107.4	88.7	125.8	107	6.5	6.1	2.7
Flowering time (days)	7 environments	54.4	50.6	48.4	60.1	53.3	2.5	4.7	20.7
Maturity time (days)	7 environments	99.3	96.1	92.8	105.1	97.9	2.7	2.7	5.0
Plant height (cm)	7 environments	75.1	77.1	62.5	102.0	81.5	8.3	10.2	24.4
Grain yield (Mg ha^{-1})	7 environments	5.4	5.1	3.5	5.9	4.7	5.4	11.4	10.8
Test weight (kg hL^{-1})	7 environments	77.6	77.7	74.7	79.1	77.0	0.8	1.1	3.6
1000-kernels weight (g)	7 environments	38.2	39.9	34.8	42.6	39.0	1.7	4.4	2.6
Grain protein content (%)	7 environments	12.3	12.9	10.9	13.7	12.4	0.6	4.5	4.3

*All F-values were significant at p < 0.001.

Table 3-2. Comparisons of QTLs associated with the combined phenotypic data across three (2008-2010) and seven (2008-2014) environments under conventional management system using 579 DArT (Asif et al. 2015), 1200 SNPs and 3 gene specific markers.

Trait*	Heritability (%)		No. of QTLs detected			R² (%)			Difference in R² (%)			Genetic variance (%)		
	(3 env)	(7 env)	(Asif et al. 2015)	(3 env)	(7 env)	(Asif et al. 2015)	(3 env)	(7 env)	(3 env - Asif et al. 2015)	(7 env - Asif et al. 2015)	(seven - three env)	(Asif et al. 2015)	(3 env)	(7 env)
Flt	0.76	0.73	0	1	1	0.0	18.9	16.8	18.9	16.8	-2.1	0.0	24.9	23.0
Mat	0.38	0.45	0	2	2	0.0	33.6	29.9	33.6	29.9	-3.7	0.0	88.4	66.5
Til	0.32	0.25	0	0	1	0.0	0.0	11.2	0.0	11.2	11.2	0.0	0.0	45.0
Pht	0.58	0.62	1	2	1	19.2	19.5	18.4	0.3	-0.8	-1.2	33.1	33.7	29.7
Tkw	0.37	0.39	0	4	4	0.0	35.0	35.4	35.0	35.4	0.4	0.0	94.7	90.8
Twt	0.28	0.35	1	2	2	10.9	20.5	16.2	9.6	5.3	-4.3	38.9	73.2	46.4
Yld	0.37	0.44	1	1	1	7.0	8.6	9.7	1.6	2.7	1.1	18.9	23.4	22.1
Gpc	0.64	0.26	0	1	2	0.0	12.1	18.6	12.1	18.6	6.6	0.0	18.9	71.7

*Flt: flowering time (days); Mat: maturity (days); Til: number of tillers (m^{-2}); Pht: plant height (cm); Tkw: thousand kernels weight (g); Twt: test weight (kg hL^{-1}); Yld: grain yield (Mg ha^{-1}); Gpc: grain protein content (%).

Table 3-3. Summary of QTLs associated with eight traits based on 167 recombinant inbred line population evaluated across three (2008-2010) and seven (2008-2014) environments under conventional management system.

QTL	Trait*	No. of environments	Chrom.	Position (cM)	Confidence interval (cM)	LOD	R^2 (%)	Additive effect	Difference**
QFlt.dms-5A	Flt	3 environments	5A	296	294.5-296.5	7.5	18.9	-1.2	-2.5
QGpc.dms-2D	Gpc	3 environments	2D	61	58.5-62.5	4.6	11.8	-0.3	0.6
QMat.dms-4B	Mat	3 environments	4B	80	78.5-80.5	7.2	13.9	1.5	2.8
QMat.dms-5A.2	Mat	3 environments	5A	297	295.5-297.5	9.9	19.7	-1.7	-3.2
QPht.dms-2D.2	Pht	3 environments	2D_LG2	0	0-0.5	2.7	5.4	2.0	3.8
QPht.dms-4B	Pht	3 environments	4B	83	80.5-86.5	6.1	14.1	-3.3	-6.4
QTkw.dms-3A.2	Tkw	3 environments	3A	238	231.5-248.5	2.9	4.7	-0.6	-1.0
QTkw.dms-4A	Tkw	3 environments	4A	120	118.5-120.5	5.8	7.9	0.8	1.5
QTkw.dms-6A.1	Tkw	3 environments	6A	78	77.5-79.5	10.7	16.0	1.1	1.6
QTkw.dms-6A.2	Tkw	3 environments	6A	114	112.5-115.5	4.7	6.4	-0.7	-0.3
QTwt.dms-5B.1	Twt	3 environments	5B	138	128.5-150.5	4.0	13.6	-0.5	-0.9
QTwt.dms-5B.3	Twt	3 environments	5B	239	237.5-239.5	4.0	6.9	-0.3	-0.8
QYld.dms-6B	Yld	3 environments	6B	229	228.5-230.5	3.2	8.6	194.2	395.7
QFlt.dms-5A	Flt	7 environments	5A	296	294.5-296.5	6.6	16.8	-1.0	-2.5
QGpc.dms-2D	Gpc	7 environments	2D	61	59.5-62.5	9.1	13.4	0.2	0.3
QGpc.dms-4B	Gpc	7 environments	4B	80	79.5-80.5	3.4	6.3	-0.2	-0.5
QMat.dms-4B	Mat	7 environments	4B	80	79.5-80.5	7.6	15.9	1.1	2.2
QMat.dms-5A.2	Mat	7 environments	5A	297	295.5-297.5	6.8	14.0	-1.0	-1.9
QPht.dms-4B	Pht	7 environments	4B	82	80.5-85.5	7.3	18.4	-3.8	-7.7
QTil.dms-6A.1	Til	7 environments	6A	70	67.5-71.5	3.3	11.2	2.0	-3.4

QTL	Trait	Environments	Chr	Peak	Interval				
QTkw.dms-4A	Tkw	7 environments	4A	120	119.5-120.5	5.8	12.1	0.6	1.1
QTkw.dms-6A.1	Tkw	7 environments	6A	79	77.5-79.5	4.0	8.5	0.5	1.1
QTkw.dms-6D.2	Tkw	7 environments	6D_LG2	4	3.5-5.5	3.2	6.7	0.4	0.9
QTkw.dms-7B.1	Tkw	7 environments	7B	158	156.5-158.5	4.0	8.1	0.5	0.9
QTwt.dms-5A	Twt	7 environments	5A	12	10.5-12.5	2.5	6.1	0.2	0.5
QTwt.dms-5B.3	Twt	7 environments	5B	239	237.5-239.5	4.4	10.1	-0.3	-0.5
QYld.dms-2D.2	Yld	7 environments	2D	66	62.5-69.5	2.9	9.3	-153.3	-375.7

*Flt: flowering time (days); Mat: maturity (days); Til: number of tillers (m^{-2}); Pht: plant height (cm); Tkw: thousand kernels weight (g); Twt: test weight (kg hL^{-1}); Yld: grain yield (kg ha^{-1}); Gpc: grain protein content (%). ** Difference in phenotypic performance of all RILs that had the CDC Go alleles at both flanking markers of every QTL from those having the Attila alleles. Positive and negative additive effect and differences for grain yield, grain protein content, test weight and kernel weight indicate that the favorable alleles originated from CDC Go and Attila, respectively; for flowering, maturity and plant height, positive and negative values indicate the opposite (the favorable alleles originated from Attila and CDC Go, respectively), because selection is made against higher values (late flowering, late maturity and taller plants).

Table 3-4. Comparisons of recombinant inbred lines that had the CDC Go or Attila alleles at the flanking markers of three coincident QTLs on eight traits evaluated under seven (2008-2014) conventional management systems.

Trait	Chromosome	Coincident QTL name	Attila type alleles	CDC Go type alleles	Difference*	F statistics	p value
Flowering time (days)	2D	*QGpc.dms-*	53.90	52.90	-1.00	3.00	0.087
Maturity (days)	2D	*2D vs*	98.40	97.70	-0.70	1.00	0.317
Number of tillers (m^{-2})	2D	*QYld.dms-*	107.00	106.90	-0.10	0.01	0.938
Plant height (cm)	2D	*2D.2*	82.90	80.70	-2.20	1.50	0.217
1000 kernel weight (g)	2D		38.90	39.20	0.30	0.81	0.369
Test weight (kg hL^{-1})	2D		77.00	77.00	0.00	0.03	0.869
Grain yield (Mg ha^{-1})	2D		4.95	4.61	-0.34	16.00	0.001
Grain protein content (%)	2D		12.10	12.50	0.40	23.10	0.001
Flowering time (days)	4B	*QGpc.dms-*	53.20	53.80	0.60	1.00	0.324
Maturity (days)	4B	*4B vs*	97.10	99.20	2.10	25.00	0.001
Number of tillers (m^{-2})	4B	*QMat.dms-*	106.60	107.80	1.20	0.50	0.473
Plant height (cm)	4B	*4B vs*	83.90	76.30	-7.60	30.10	0.001
1000 kernel weight (g)	4B	*QPht.dms-*	39.10	38.70	-0.40	1.90	0.240
Test weight (kg hL^{-1})	4B	*4B*	77.10	77.00	-0.10	1.20	0.280
Grain yield (kg ha^{-1})	4B		4.74	4.78	0.04	0.40	0.538
Grain protein content (%)	4B		12.50	12.25	-0.25	7.10	0.008
Flowering time (days)	5A	*QFlt.dms-*	54.50	52.25	-2.25	32.50	0.001
Maturity (days)	5A	*5A vs*	99.00	97.00	-2.00	23.80	0.001
Number of tillers (m^{-2})	5A	*QMat.dms-*	108.20	106.20	-2.00	3.60	0.061
Plant height (cm)	5A	*5A.2 vs*	83.60	79.60	-4.00	9.80	0.002
1000 kernel weight (g)	5A	*QPht.dms-*	39.00	39.00	0.00	0.20	0.694
Test weight (kg hL^{-1})	5A	*5A*	76.90	77.20	0.30	4.80	0.030
Grain yield (Mg ha^{-1})	5A		4.77	4.73	-0.04	0.27	0.605
Grain protein content (%)	5A		12.35	12.40	0.05	0.22	0.402

*The difference was calculated by subtracting values for Attila type alleles from those of CDC Go type alleles. Positive and negative values for grain yield, grain protein content, test weight, kernel weight and number of tillers indicate that the favorable alleles originated from CDC Go and Attila, respectively; for flowering, maturity and plant height, positive and negative values indicate the opposite (the favorable alleles originated from Attila and CDC Go, respectively), because selection is made against higher values (against late flowering, late maturity, and taller plants).
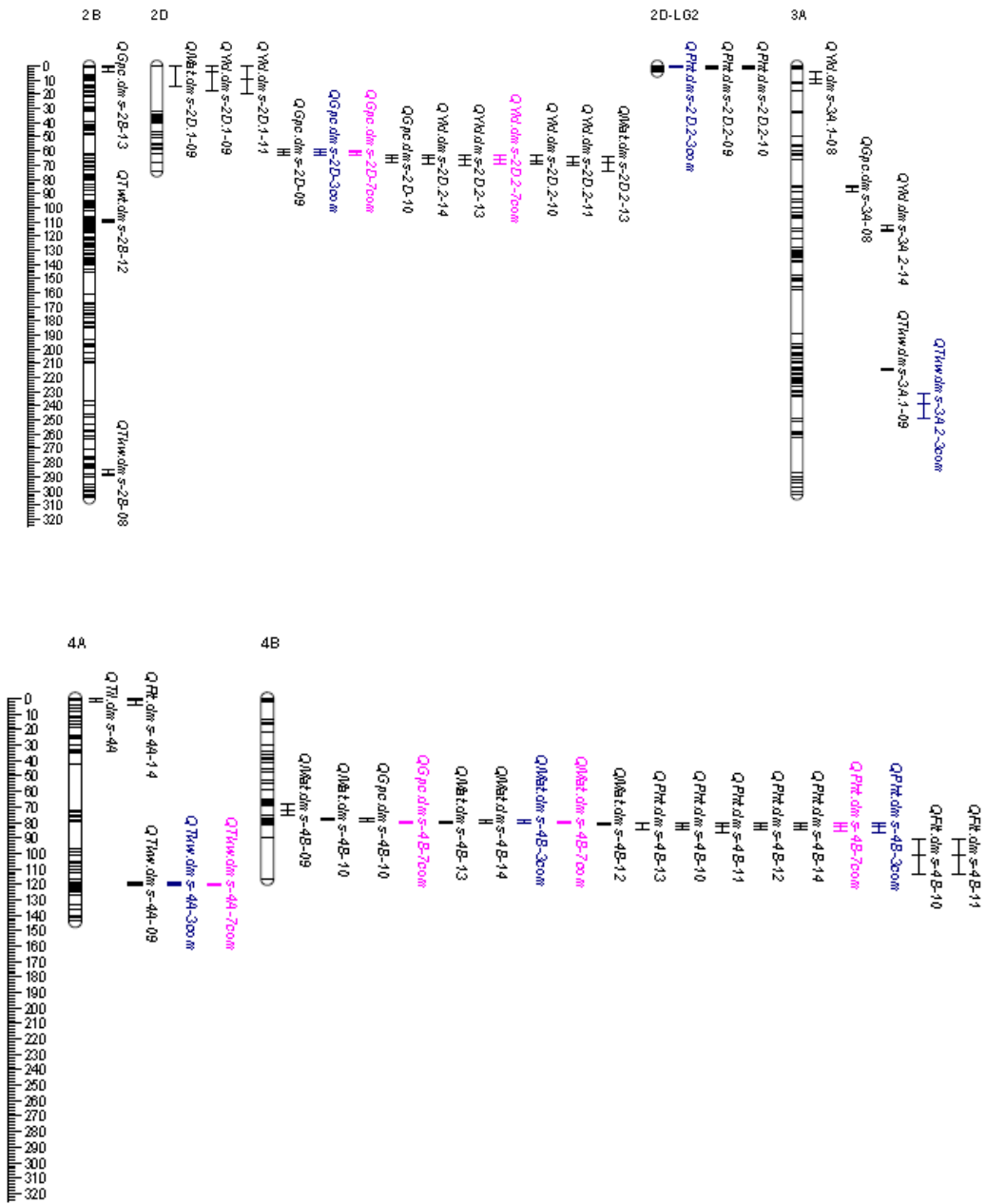
Figure 3-1. The distribution of QTLs associated with eight traits evaluated in a single environment (black) or averaged across three (red) or seven (pink) environments.
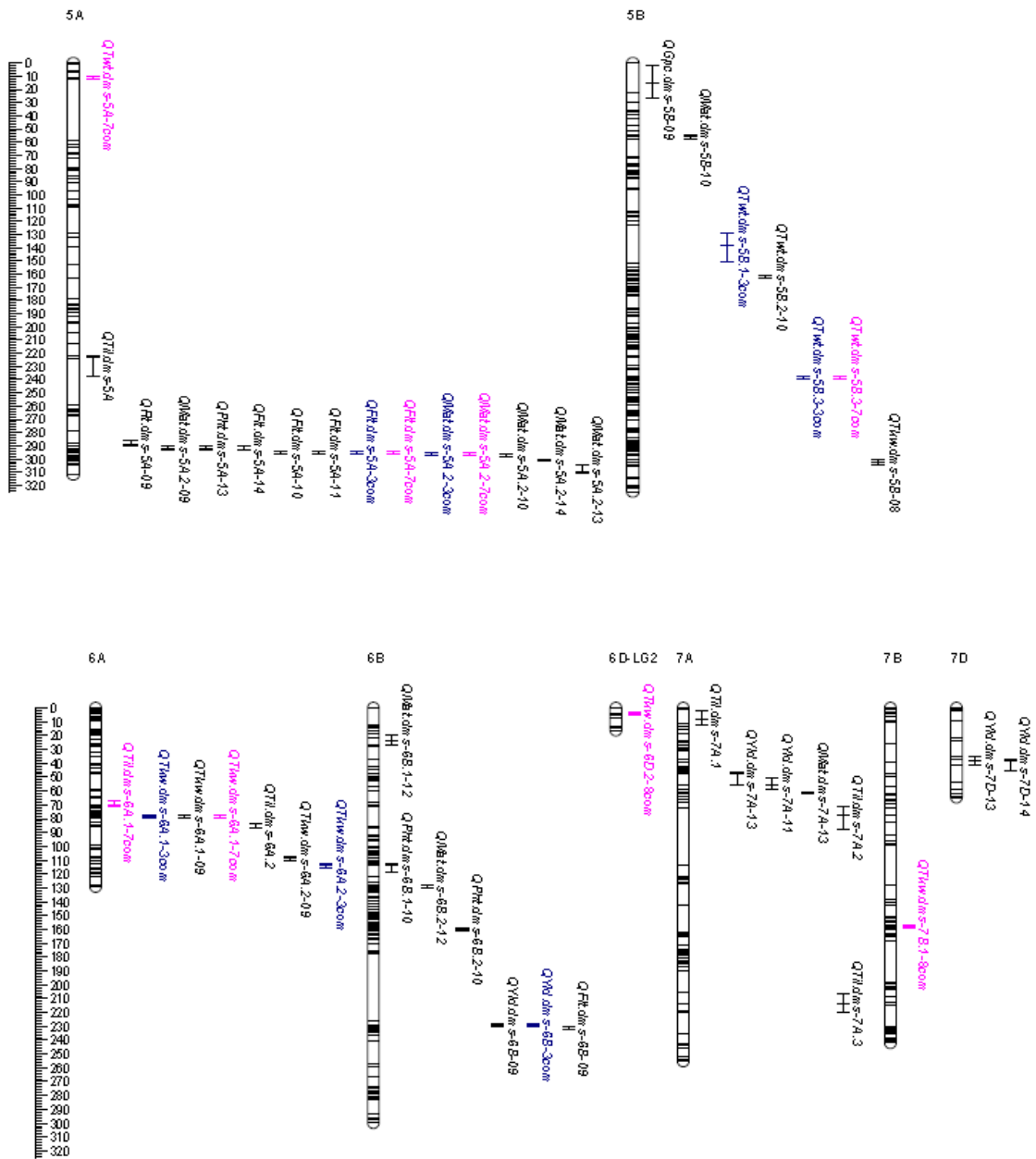
Figure 3-1 (Continued) The distribution of QTLs associated with eight traits evaluated in a single environment (black) or averaged across three (red) or seven (pink) environments.

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Chapter 4: QTL mapping of disease resistance in a RIL population derived from a cross of wheat cultivars Attila and CDC Go

4.1 Introduction

Over 85% of spring wheat in Canada is produced in the western Canadian prairie provinces of Manitoba, Saskatchewan, and Alberta, with a very small proportion in British Columbia and eastern Canada (McCallum and DePauw, 2008). Currently, cultivars to be released in the region must be at least intermediately resistant to stem rust (*Puccinia graminis* f. sp. *tritici*), leaf rust (*Puccinia triticina*), yellow (stripe) rust (*Puccinia striiformis* f. sp. *tritici*), and common bunt (caused by two very closely related fungi, *Tilletia tritici* and *Tilletia laevis*). Breeding for resistance to diseases involves (i) identification of sources of resistance, (ii) introgressing the new sources of resistance from the resistant parents in to the genetic background of other parents to develop segregating populations, and (iii) selecting progenies showing acceptable combinations of resistance to diseases and other agronomic traits. Breeding for disease resistance is often challenging for at least two reasons. First, breeders often need to pyramid different sources of resistance to multiple diseases in to the same genetic background. Second, the inheritance of each disease is both qualitative and quantitative (Faris et al., 1996, Faris et al., 1997, Faris and Friesen, 2005, Singh et al., 2007, Chu et al., 2008, Chu et al., 2010, Singh et al., 2016), which complicates the selection process. Qualitative resistance is controlled by a single gene with a major effect, but resistance regulated by most single genes lose their effectiveness over time due to changes in pathogen populations. On the other hand, quantitative resistance is controlled by minor effect genes or quantitative trait loci (QTLs) with small additive effects, which are more durable (Singh et al., 2008), but require the introgression of multiple genes or QTLs that confer resistance for a given disease.

Over 70 leaf rust (*Lr*) and 65 yellow rust (*Yr*) resistance genes have been reported in the literature in almost every hexaploid wheat chromosome (McIntosh et al., 2012, Dakouri et al., 2013). Several gene combinations have been reported in contributing some level of resistance to rusts in many of the cultivars grown in western Canada (McCallum et al., 2007, McCallum et al., 2012, Randhawa et al., 2012). However, only few gene combinations (pyramids) have provided good level of durable resistance in spring wheat cultivars released since mid-1980s, which includes the slow-rusting *Lr34*/*Yr18* gene on chromosome 7DS (Suenaga et al., 2003, Spielmeyer et al., 2005, Lagudah et al., 2009), the *Lr46*/*Yr29* on 1BL (Singh et al., 1998, William et al., 2003), *Sr2/Yr30* on 3BS (Singh et al., 2005), and *Lr67/Yr46* on 4DL (Hiebert et al., 2010, Herrera-Foessel et al., 2011).

At least fifteen monogenic and race specific genes (named from *Bt1* to *Bt15*) that conferred resistance to common bunt (Goates, 1996), also known as stinking smut and covered smut (Gaudet and Puchalski, 1989), have also been reported in wheat. Both incidence and severity of common bunt have been controlled largely by introgressing resistance genes, such as *Bt10* and *Bt8* (Menzies et al., 2006, McCallum and DePauw, 2008, Hiebert et al., 2011). The resistance to common bunt has remained generally stable against shifts in virulence in the pathogen, but the vulnerability of such major genes through intense selection pressure on the pathogen is a concern (Wang et al., 2009). QTLs associated with resistance to common bunt have also been reported on some chromosomes, including 1B and 7A (Galaev et al., 2006, Fofana et al., 2008, Wang et al., 2009). Tan spot, caused by *P. tritici-repentis*, is the most destructive leaf spotting diseases of wheat in Canada and other major wheat growing countries (Faris et al., 1997, Friesen and Faris, 2004). In the wheat–P. tritici-repentis system, the host interactions with necrotrophic pathogens involve the recognition of HSTs by dominant host

sensitivity genes. This recognition results in a compatible interaction, which leads to susceptibility. While the lack of HST recognition by the host leads to an incompatible interaction which will result in resistance. If the pathogen does not produce the HST or if the host does not have the sensitivity gene, there will be a resistance response. Isolates of *P .tritici-repentis* (Ptr) are classified into 8 races based on their ability to cause necrosis and/or chlorosis in differential wheat lines, which is determined by the production of host specific toxins (HSTs), such as Ptr ToxA, Ptr ToxB, and Ptr ToxC (Ciuffetti et al., 1998, Lamari et al., 2003, Ciuffetti et al., 2010). Fungal isolates of races 2, 3, and 5 produce Ptr ToxA, Ptr ToxC, and Ptr ToxB, respectively (Strelkov and Lamari, 2003, Lamari and Strelkov, 2010). Isolates of races 1, 6, and 7 each produce two HSTs, with race 1 producing Ptr ToxA and Ptr ToxC, race 6 producing Ptr ToxB and Ptr ToxC, and race 7 producing Ptr ToxA and Ptr ToxB. Race 8 isolates produce all the three HSTs (Faris et al., 2013). Fungal isolates producing both Ptr ToxA and Ptr ToxC are highly abundant in the Canadian prairies, while those producing Ptr ToxB are extremely rare in this region (Lamari et al., 1998, Lamari et al., 2003, Aboukhaddour et al., 2013).

The wheat breeding group at the University of Alberta has been evaluating the performance of wheat cultivars and mapping populations for a wide range of agronomic traits and diseases. One of the mapping populations was a recombinant inbred lines (RILs) population derived from a cross between two spring wheat cultivars, 'Attila' and 'CDC Go'. 'Attila' is an awned, medium yielding, semi-dwarf and early maturing cultivar from developed by the International Maize and Wheat Improvement Center from CM85836-50Y-0M-0Y-3M-0Y (Tadesse et al., 2010). It has been released in several countries with different local names and grown on millions of hectares throughout the world. 'Attila' carries at least two additive genes for slow rusting resistance to leaf rust and three for stripe rust, such as *Yr27* (Rosewarne et al.,

2008) and *Lr46/Yr29* (Rosewarne et al., 2006, Datta et al., 2009), showed moderate to high levels of field resistance to both leaf and stripe rusts , and has been frequently used as a slow-rusting donor parent in international spring wheat breeding programs (Rosewarne et al., 2008). 'CDC Go' is a Canadian Western Red Spring wheat cultivar characterized by strong straw, medium height, relatively late maturity, high yield, high test weight and thousand kernel weight (Asif et al., 2015) and resistant to bunt, and exhibited moderately resistant to resistant to both leaf and stripe rust (Randhawa et al., 2012, Perez-Lara et al., 2016). 'CDC Go', however, did not have the *Lr34/Yr18* resistance allele (Randhawa et al., 2012). The 'Attila' × 'CDC Go' RIL population has been used to map QTLs associated with agronomic traits both under conventional and organic management systems (Asif et al., 2015, Zou et al., 2016, Zou et al., 2016). This RIL population has also shown good segregation for different wheat diseases, but information on the genetics of disease resistance in this population has not been previously investigated. Here, we present QTLs associated with common bunt, tan spot, leaf rust, and stripe rust resistance in the 'Attila' × 'CDC Go' RIL population using the Wheat 90K SNP array.

4.2 Materials and methods

4.2.1 Plant materials and phenotyping

The present study was conducted on a mapping population of 167 RILs developed from a cross between two spring wheat cultivars - 'Attila' (CM85836-50Y-0M-0Y-3M-0Y) and 'CDC Go'. The RIL population was developed as described in one of our previous papers (Asif et al., 2015). The RIL population and the two parents, along with two susceptible ('AC Barrie' and 'AC Crystal') and resistant ('Lillian' and 'Carberry') checks were evaluated eight times between 2012 and 2015 for their reaction to stripe rust in disease screening nurseries. The stripe rust field studies were conducted at Creston, British Columbia (49.06° N, 116.31° W) in 2011, 2013 and

2014; at the Agriculture and AgriFood Canada Lethbridge Research Centre, Alberta (49.7° N, 112.83° W) between 2012 and 2015, and at Ellerslie research station, Edmonton, Alberta in 2015. Plots consisted of 5 m long rows, one row per genotype, arranged in a randomized complete block design with two to three replicates per trial depending on seed availability. Ten seeds of each genotype were planted per hill with a spacing of 25 cm between hills or rows. Infection resulted from natural inoculum as described elsewhere (Randhawa et al., 2012).

The RIL population, the two parents, and checks also were evaluated for three years (2012-2014) for reaction to leaf rust, common bunt and tan spot at the Crop Research Facility of the University of Alberta, South Campus (53°19'N, 113°35'W), Alberta, Canada. Each trial was conducted in a randomized complete block design as described above. The following cultivars were used as susceptible/moderately susceptible and moderately resistant/resistant checks: (i) leaf rust nurseries: 'AC Barrie' and 'Park' as moderately susceptible to susceptible checks, and 'Peace' and 'Carberry' as moderately resistant to resistant checks; (ii) tan spot nurseries: 'AC Barrie', 'Unity' and 'Glenlea' as moderately susceptible checks, and 'Neepawa' as moderately resistant check; and (iii) for common bunt nurseries: 'Glenlea' and 'Neepawa' as moderately susceptible checks; 'AC Barrie' and 'Unity' as moderately resistant and resistant checks, respectively. All susceptible checks were used as spreader rows. To create homogeneous disease epidemics within each trial, spreader rows of susceptible checks were planted every three rows. For leaf rust epidemic initiation, spreader rows were sprayed with an equal mixture of urediniospores of the prevalent races in the region using hand sprayer; the urediniospores were collected from spreader rows of the previous year in the nursery and suspended in mineral oil. Both leaf and yellow rust severity ratings were recorded using a modified Cobb scale (Peterson et al., 1948) when the spreader rows reached maximum infection. Visual disease assessment was

done on a scale of 1 (no visible sign or symptom = resistant) to 9 (leaf area totally covered with spores = highly susceptible) on each hill plot basis.

Tan spot evaluation was conducted by spraying spore suspension consisting of an equal mixture of two isolates (AB7-2 and AB50-2) belonging to race 1 of *P. tritici-repentis* (Ptr), which is predominant in Alberta (Aboukhaddour et al., 2013). When wheat grains were at the milk-stage, disease reaction was recorded on a scale of 1 to 9 in the same manner as described for the leaf and yellow rusts. For common bunt screening, 10 seeds of each genotype were mixed with common bunt spores that consisted of a combination of race L16 of *T. laevis* and race T19 of *T. tritici* in an envelope (Sukhwinder et al., 2003). At the dough stage, all heads of each cultivar in a hill plot were examined for common bunt infection and recorded in percentages as the ratio of the number of infected heads to the total number of head per hill plot.

4.2.2 DNA extraction and genotyping

DNA extraction and genotyping was done as described in our previous studies (Perez-Lara et al., 2016, Zou et al., 2016). Briefly, DNA was extracted from three weeks old seedlings using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method and genotyped at the University of Saskatchewan Wheat Genomics lab, Saskatoon, Canada, with the 90K Illumina iSelect SNP array that consisted of 81,587 SNPs (Wang et al., 2014). SNP alleles were called with the Illumina Genome Studio Polyploid Clustering version 1.0 software (Illumina, San Diego, USA) using default clustering parameters and filtered as described in our previous study (Perez-Lara et al., 2016).

4.2.3 Data analyses

Linkage analysis was performed as described in one of our previous studies (Perez-Lara et al., 2016) Briefly, draft' linkage maps were first generated using the minimum spanning tree

map (MSTMap) software (Wu et al., 2008) and 'then refined using the MapDisto version 1.7.5 software (Lorieux, 2012) using a cut off recombination value of 0.35, a minimum LOD score of 3.0, and Kosambi mapping function (Kosambi, 1944). Linkage groups were assigned to chromosomes based on existing high density SNP maps of wheat (Cavanagh et al., 2013, Maccaferri et al., 2014, Wang et al., 2014). Least square means, F statistics, and heritability were computed for each year (environment) separately and then combined across all environments using PROC MIXED and PROC IML in SAS version 9.3 (SAS Institute Inc. Cary, USA). Genotypes (RILs) were considered fixed, while environments, replications, and blocks within replications were considered as random effects. Both test for normality and the frequency distribution were computed using MiniTab v14. Inclusive composite interval mapping (ICIM) was performed on the least square means of each trait for individual environment and combined across all environments with QTL IciMapping v4.1 (Li et al., 2007, Meng et al., 2015) using a mean replacement for missing data, 1 cM walking distance, a minimum logarithm of odds (LOD) score of 2.5 and a model to determine additive effects at individual QTL, and additive × additive epistatic interactions. QTL names were designated following the International Rules of Genetic Nomenclature (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm), which consisted of three letters for trait acronym, lab designation (dms = Dean Michael Spaner), and chromosome. Genetic maps and QTL graphs were drawn using MapChart v2.1 (Voorrips, 2002).

4.3 Results

4.3.1 Diseases evaluation

Least square means diseases scores of the susceptible and resistant checks across the combined environments were first examined to get an insight on the magnitude of leaf rust, stripe rust, tan spot, and common bunt severity in our trials. Tan spot, leaf rust and stripe rust mean

119

disease scores among the checks varied from 1.1 to 3.8 for the resistant checks and from 5.3 to 7.4 for the susceptible checks. For common bunt, mean scores varied from 1.8 to 3.8% for the resistant checks and from 7.4 to 34.3% for susceptible checks (data not shown). Table 1 shows summary of the descriptive and F statistics. For tan spot, stripe rust and leaf rust, RILs with mean disease scores ≤ 3.0 were considered resistant, 3.1–5.0 moderately resistant, 5.1-7.0 moderately susceptible, and 7.1–9.0 susceptible. Based on such category, both parents ('Attila' and 'CDC Go') showed moderate resistance to leaf rust (4.2 for both parents) and stripe rust (3.3-3.5), but moderately susceptible to tan spot (5.4-5.8). However, common bunt infection in 'CDC Go' was 9%, which is half of the 18% observed for 'Attila'.

The 167 RILs showed highly variable reaction to the four diseases, which varied from 4.0 to 8.5 for tan spot, from 2.5 to 5.8 for leaf rust, from 1.8 to 7.2 for stripe rust, and from 1.8 to 37.2% for common bunt (Table 1). Overall, the RIL population exhibited transgressive segregation for all four diseases and a number of RILs that were superior or inferior to the parents were observed (Figure 1). A total of 11 and 33 RILs were found to be resistant with a disease score rating of ≤3.0 for leaf rust and stripe rust, respectively, but only 6 RILs were resistant to both rusts. Pearson correlation among the four diseases was very low, ranging from 0.11 between common bunt and stripe rust to 0.42 between tan spot and leaf rust; the correlation between leaf and stripe rust scores was 0.35. RILs differed (p <0.0001) for their reactions to all four diseases. Broad sense heritability varied from 0.15 for leaf rust to 0.48 for common bunt (Table 1). The distribution of least square means of disease scores averaged across all environments was normal (P > 0.050) for both tan spot and leaf rust, but it deviated from normality (P ≤ 0.018) both for common bunt and stripe rust.

4.3.2 Linkage and QTL analyses

Detailed results on the genotypic data and linkage maps were presented in our previous study (Zou et al., 2016). Briefly, a total of 5,665 of the 81,587 SNPs initially used for genotyping the RIL population were incorporated into 27 linkage groups that covered all chromosomes except chromosomes 3D and 4D (Supplementary material 1). However, many SNPs cosegregated (mapped at exactly the same position) and they were excluded from the final dataset. This reduced the final number of markers retained for QTL analyses to 1203, which included 1200 SNPs and three functional markers (*Ppd-D1*, *Vrn-A1a*, and *Rht-B1*). The total map length for the 19 chromosomes was 3442 cM, with each chromosome varying between 14.3 cM on 1D to 324.8 cM on 5B (Zou et al., 2016).

Because of the skewed distribution on common bunt and stripe rust disease scores, we performed QTL analyses on the original (untransformed) and Log_{10} transformed data. However, the QTL results obtained from the untransformed and transformed datasets were basically similar, so we only presented results from the untransformed data. Using composite interval mapping and the average diseases scores across all environments, we identified a total of 11 QTLs (Table 2, Figure 2) associated with resistances to tan spot (3), common bunt (2), leaf rust (3) and stripe rust (3). The three QTLs for resistance to tan spot mapped at 263 cM on 2B (*QTs.dms-2B*), at 3 cM on 2D *QTs.dms-2D*) and at 27 cM on 6B (*QTs.dms-6B*), which individually explained between 7 and 10% and altogether accounted for 24.0% of the phenotypic and 52.2% of the genetic variance (Table 3) for tan spot across all combined environments. RILs that consisted of the 'CDC Go' alleles at the two flanking markers of all three QTLs for tan spot showed between 0.6 and 0.9 (in 1 to 9 scale) less tan spot scores than those RILs that had the 'Attila alleles'.

ICIM uncovered two QTLs associated with common bunt resistance that mapped at 52 cM on the second linkage group of chromosome 1B (*QCbt.dms-1B.2*) and at 202 cM on

chromosome 3A (*QCbt.dms-3A*) that altogether accounted for 26.5% of the phenotypic and 55.2% of the genetic variance (Table 3) across all combined environments. *QCbt.dms-1B.2* is a moderate effect QTL that mapped between BS00086854_51 and wsnp_Ex_c5679_9976893 on chromosome 1B, had a LOD score of 7.2 and individually explained 18.7% the phenotypic variance. *QCbt.dms-3A* was a minor effect QTL that mapped between RAC875_c17453_896 and RAC875_c57584_240 on 3A and explained 7.9% of the phenotypic variance across all combined environments. On average, RILs with the 'CDC Go' alleles at the two flanking markers for *QCbt.dms-1B.2 and QCbt.dms-3A* had 7.2% and 2.9%, respectively, less common bunt disease score than those RILs with the 'Attila' alleles.

Three QTLs associated with resistance to leaf rust were mapped at 39 cM on 2D (*QLr.dms-2D*), at 2 cM on 2D second linkage group (*QLr.dms-2D.2*) and at 17 cM on 3A (*QLr.dms-3A*) that altogether explained 21.5% of the phenotypic and 86.0% of the genetic variance (Table 3) across all the environments. Each QTL for resistance to leaf rust individually explained between 5.9 and 8.6% of the phenotypic variance across the combined environments. RILs with the 'CDC Go' alleles at the flanking markers of all three QTLs for leaf rust resistance scored from 0.3 to 0.5 less leaf rust disease score than those with the 'Attila' alleles. The three QTLs associated with resistance to stripe rust were mapped at 300 cM on 3A (*QYr.dms-3A*), at 123 cM on 4A (*QYr.dms-4A*) and at 191 cM on 5B (*QLr.dms-5B*). Each QTL for stripe rust resistance individually explained between 6.7% and 8.5% and altogether accounted for 23.1% of the phenotypic and 82.5% of the genetic variance across all combined environments (Table 3). The resistant alleles for *QYr.dms-3A* and *QYr.dms-4A* originated from 'CDC Go', while that of *QYr.dms-5B* from 'Attila'. RILs with the resistance alleles at the two flanking markers of every

QTL for stripe rust had between 0.5 and 0.7 less stripe rust disease score than those with the susceptible alleles.

4.4 Discussion

4.4.1 Resistance to common bunt and tan spot

The present study uncovered a minor effect QTL on chromosome 3A (QCbt.dms-3A) that accounted for 8% of the phenotypic variation (Table 2). We are not aware of any mapping studies that reported either single major effect gene(s) or QTL on chromosome 3A that confers resistance to common bunt in wheat. It is possible that QCbt.dms-3A may be a novel minor effect QTL that has not been reported elsewhere. In addition, we also identified a moderate effect QTL on chromosome 1B (QCbt.dms-1B.2), which accounted for 19% of the phenotypic variation for common bunt disease reaction. Several previous studies have reported genes and QTLs associated with resistance to common bunt on chromosome 1B (Fofana et al., 2008, Wang et al., 2009, Dumalasová et al., 2012, Singh et al., 2016). One of the studies used a doubled haploid (DH) spring wheat population derived from a cross between 'RL4452' × 'AC Domain' and reported three QTLs associated with resistance to common bunt (Fofana et al., 2008). In that study, the authors observed a continuous frequency distribution and transgressive segregation for common bunt and reported two QTLs on chromosome 1B (QCbt.crc-1B.1 and QCbt.crc-1B.2) that together explained 29% and another QTL on chromosome 7A that accounted for 3% of the phenotypic variation. QCbt.crc-1B.1 mapped on the short arm of chromosome 1B between XGwm374.1 and XWmc818, had a LOD score of 9.0 and accounted for 21% of the phenotypic variance, while QCbt.crc-1B.2 mapped between GluB1 and XGwm274 on the long arm of chromosome 1B, had a LOD score of 3.6 and explained 8% of the phenotypic variance. Our results, together with others suggest the presence of a moderate to major effect QTL associated

with common bunt resistance on chromosome 1B irrespective of the genetic background of the mapping populations, the type of markers and marker density.

At least fifteen monogenic and race specific genes (named from Bt1 to Bt15) that conferred resistance to common bunt have also been reported in wheat (Goates, 1996), of which Bt4, Bt5, and Bt6 mapped on chromosome 1B (Sears et al., 1960, Scmidt et al., 1969). In a winter wheat DH population derived from a cross between 'Blizzard' × '8405-JC3C', a single gene associated with common bunt resistance has been reported on the short arm of chromosome 1B (Wang et al., 2009). Using a DH population derived from a cross between 'Trintella' and 'Piko', another group mapped a single major effect gene that confers resistance to common bunt around the centromere region on chromosome 1B, flanked by Xgwm273 (Dumalasová et al., 2012). That gene had a LOD score of 38 and explained up to 30% of the phenotypic variance for common bunt disease severity. Common bunt disease severity in the 'Trintella' × 'Piko' DH population showed bimodality distribution, which clearly support the presence of major effect gene or QTL segregating in that population. In the present study, common bunt disease scores in the 'Attila' × 'CDC Go' RIL population showed a continuous and an approximately bimodial distribution, which supports the presence of moderate effect QTL on chromosome 1B, but not single major effect genes.

For tan spot, we identified three minor effect QTLs on chromosomes 2B (QTs.dms-2B), 2D (QTs.dms-2D) and 6B (QTs.dms-6B), which individually explained between 6.9 and 10.0% of the phenotypic variance for tan spot disease reaction. Previous studies have reported genes and QTLs associated with tan spot resistance on several chromosomes, including 2B (Friesen and Faris, 2004, Gurung et al., 2011), 2D (Gurung et al., 2011) and chromosome 6B (Singh et al., 2016). The virulence of the tan spot causing P. tritici-repentis depends on the production of three

host-selective toxins (HSTs), namely Ptr ToxA, Ptr ToxB and Ptr ToxC (Lamari and Strelkov, 2010). Ptr ToxB is a proteinaceous host-selective toxin produced primarily by race 5 isolates (but also both by race 6 and race 8) and induces chlorosis in wheat lines harboring the dominant Tsc2 gene (Strelkov et al., 1999, Lamari et al., 2003). The Tsc2 gene is located on the short arm of chromosome 2B (Friesen and Faris, 2004, Abeysekara et al., 2010). In addition, three minor effect QTLs associated with Ptr ToxB were reported on the short arm of chromosomes 2A, the long arms of both 2B and 4A (Friesen and Faris, 2004). Using a DH population derived from a cross between 'CPI133872' and 'Janz', another mapping study reported a major effect QTL on chromosome 3D, the recessive tsn1 gene on 5BL that confers insensitivity to Ptr ToxA, and five environment specific QTLs on chromosomes 2B, 2D, 3A, 4B and 5A (Zwart et al., 2010). Based on an association mapping study conducted on 567 spring wheat landraces from the USDA-ARS National Small Grains Collection, several genomic regions, including chromosomes 2B and 2D, that individually explained between 1.3 and 5.9% of the phenotypic variance for race 1 and/or race 5 isolates have been reported (Gurung et al., 2011). A recent associating mapping study conducted on a set of CIMMYT bread wheat germplasm has also reported 9 genomic regions associated with tan spot resistance, including chromosome 6B (Singh et al., 2016). Therefore, the QTLs that we identified for tan spot on chromosomes 2B, 2D and 6B may be on the same genomic regions with those genes and QTLs reported in previous studies. However, direct comparisons among the different studies was not possible due to differences on the types of markers used and lack of consensus or physical maps that shows the positions of the flanking markers reported in the different studies.

4.4.2 Resistance to leaf and stripe rust

'Attila' was reported having moderate to high level of field resistance to both leaf and stripe rusts (Rosewarne et al., 2006, Datta et al., 2009), and has been frequently used as a slow-rusting donor parent in international spring wheat breeding programs (Rosewarne et al., 2008, Datta et al., 2009). 'CDC Go' has also been reported to be moderately resistant to leaf rust, and resistant or moderately resistant to stem rust (http://www.agric.gov.ab.ca) and stripe rust (McCallum et al., 2012, Randhawa et al., 2012). In our disease evaluation nurseries, both 'Attila' and 'CDC Go' showed moderate level of resistance to both leaf and stripe rusts, which is in agreement with previous studies. Using ICIM, we identified 3 QTLs associated with resistance to leaf rust on the short arms of both chromosomes 2D (QLr.dms-2D.1 and QLr.dms-2D.2) and 3A (QLr.dms-3A), which individually explained between 5.9 and 8.6% of the phenotypic variance (Table 2). Previous mapping studies have reported several single genes associated with leaf rust resistance on chromosome 2D, which includes Lr2a, Lr2b and Lr2c (Dyck and Samborski, 1974), Lr15 (Luig and McIntosh, 1968), Lr22a, Lr22b and Lr22c (Rowland and Kerber, 1974, Dyck, 1979), Lr39 and Lr41 (Singh et al., 2004) and Lr54 (Marais et al., 2005). However, the observed continuous leaf rust disease scores distribution with a single peak in the 'Attila' × 'CDC Go' RIL population does not support the presence of major effect single genes. Several QTLs associated with leaf rust resistance have also been reported on chromosome 2D. One of the QTLs located on chromosome 2D was QLrid.osu-2D, which explained between 21.5 and 26.4% of the phenotypic variance for leaf rust infection in a RIL population derived from 'CI13227' × 'Suwon92' (Xu et al., 2005). A major effect QTL on the short arm of chromosome 2A (QYr.ufs-2A), along with three minor effect QTLs on chromosomes 2D (QYr.ufs-2D), 5B (QYr.ufs-5B) and 6D (QYr.ufs-6D) have been reported (Agenbag et al., 2012). QYr.ufs-2D was located on the short arm of chromosome 2D that is believed to be the position of Yr16 and explained between

4.7 and 10.3% of the phenotypic variance for strip rust on individual experiments. An environment specific QTL associated with leaf rust has also been reported on the short arm of chromosome 2D distal to Xwmc25.2 (Buerstmayr et al., 2014). Three other minor effect QTLs for leaf rust resistance were reported on chromosomes 1B, 2A, and 2D (Rosewarne et al., 2012). A recent meta-analysis compiled 144 QTLs reported in 19 studies conducted between 1999 and 2015 using 20 mapping populations (Soriano and Royo, 2015). That study reported a total of 35 meta-QTLs associated with leaf rust resistance, of which 3 meta-QTLs mapped on chromosome 2D (MQTL9, MQTL10, and MQTL1) and two meta-QTLs mapped on 3A (MQTL12, MQTL13 and MQTL14), with each meta-QTL consisting of clusters of two to six QTLs (Soriano and Royo, 2015). MQTL14 mapped around Xmwg570 on chromosome 3A, and the individual QTLs explained between 19 and 30% of leaf rust resistance (Maccaferri et al., 2008). Overall, results from the various studies, together with ours, clearly revealed the presence of several genomic regions on chromosome 2D that confer resistance to leaf rust, which do not seem the case for chromosome 3A.

For stripe rust resistance, we identified three QTLs at 300 cM on 3A (QYr.dms-3A), at 123 cM on 4A (QYr.dms-4A), and at 191 cM on 5B (QLr.dms-5B (Table 2). Each QTL associated with stripe rust resistance individually explained between 6.7% and 8.5% of the phenotypic variance (Table 2). Although we are not aware of stripe rust resistance gene assigned to chromosome 3A, few minor effect QTLs for stripe rust have been reported on chromosome 3A. Using CIM in the Avocet × Saar population, for example, a QTL associated with resistance to stripe rust was reported on the short arm of 3A, which colocalized with a QTL for powdery mildew resistance (Lillemo et al., 2008). In another study, a minor effect QTL (QYr.ifa-3AS) that explained 5.6% of the phenotypic variance for stripe rust severity was reported on the short

arm of chromosome 3A in three of five experiments in 'Capo' × 'Arina' population (Buerstmayr et al., 2014). Another study (Rosewarne et al., 2012)has also reported minor effect QTLs for stripe rust resistance on several chromosomes, including 3A.

Yr51 (Randhawa et al., 2014) and Yr60 (Herrera-Foessel et al., 2015) are the two genes reported on chromosome 4A that confers resistance to stripe rust. Yr60 is located on the long arm of 4A and conferred moderate levels of resistance at both seedlings and adult plant stages against two Mexican races of P. striiformis (Herrera-Foessel et al., 2015). The distribution of stripe rust disease score in the present study, however, was continuous with a single peak, which did not support the segregation of single gene with major effect. Recently, two QTLs on chromosomes 4A (QYrel.wak-4A) and 6B (QYrfi.wak-6B) that explained between 15 and 16% of the phenotypic variance for stripe rust disease severity in a RIL population derived from 'Eltan' and 'Finch' have been reported (Klarquist et al., 2016). Yr19 (Chen et al., 1995) and Yr47 that mapped distal to Lr52 (Bansal et al., 2011) are the single genes that confer resistance to stripe rust resistance on chromosome 5B. Some QTLs that confer an adult stage stripe rust resistance have also been reported on chromosome 5B, which includes Yrco.wpg-5B and QYrbr.wpg-5B (Case et al., 2014).

4.5 Conclusions

Using the combine disease scores across three to eight environments, we identified a total of eleven QTLs associated with resistance to four wheat diseases, which included two for common bunt and three each for tan spot, leaf rust, and stripe rust. Each QTL showed either minor or moderate effect and individually explained between 5.9 and 18.7% of the phenotypic variance, and altogether accounted from 21.5 to 26.5% of phenotypic and from 52.2 to 86.0% of the genetic variance. Even though the identified QTLs are of minor to moderate effect, they

provide useful information to spring wheat breeders that aim in pyramiding such types of genomic regions for developing wheat cultivars with durable level of disease resistance. Some of the QTLs identified in the present study were novel, while others were located on the same regions as previously reported QTLs, but direct comparisons on QTL positions across multiple studies was difficult due to differences in marker platforms, lack of common set of markers and/or physical positions of flanking markers reported in different studies.

4.6 Tables and figures

Table 4-1. Summary of descriptive and F statistics for 167 recombinant inbred lines (RILs) evaluated for common bunt, tan spot, leaf rust and stripe rust under field conditions between 2011 and 2015 in Canada.

Trait	LS mean score of parents		RILs Descriptive statistics					F statistics)*
	No. of trials (environments)	Attila	CDC Go	Min	Max	Mean	SD	CV (%)	F value
Tan spot	3	5.8	5.4	4.0	8.5	6.3	1.1	17	2.4
Leaf rust	4	4.2	4.2	2.5	5.8	4.1	0.8	19.0	1.8
Stripe rust	8	3.5	3.3	1.8	7.2	3.9	1.1	17.9	4.3
Common bunt	3	18.8	9.0	1.8	37.2	17.0	7.8	15.6	1.8

*All F-values were significant at $p < 0.001$.

Table 4-2. Summary of QTLs associated with four wheat diseases based on 167 recombinant inbred line (RIL) lines evaluated at 3-8 environments between 2011 and 2015 in field nurseries.

Trait	QTL	Chrom	Position (cM)	Confidence interval (cM)	LOD	R^2 (%)	Additive effect	Difference*
Tan spot	QTs.dms-2B	2B	264	262-270	3.0	7.0	-0.3	-0.6
Tan spot	QTs.dms-2D	2D	3	0-15	3.6	10.0	-0.3	-0.9
Tan spot	QTs.dms-6B	6B	27	22-29	3.2	6.9	-0.3	-0.7
Common bunt	QCbt.dms-1B.2	1B-LG2	52	47-55	7.7	18.7	-3.2	-7.1
Common bunt	QCbt.dms-3A	3A	202	200-206	3.5	7.9	-2.0	-2.9
Leaf rust	QLr.dms-2D.1	2D	39	37-42	3.2	6.9	-0.2	-0.3
Leaf rust	QLr.dms-2D.2	2D-LG2	2	0-4	3.9	8.6	-0.2	-0.5
Leaf rust	QLr.dms-3A	3A	17	13-19	2.7	5.9	-0.2	-0.4
Stripe rust	QYr.dms-3A	3A	300	298-304	3.3	8.0	-0.3	-0.7
Stripe rust	QYr.dms-4A	4A	123	121-126	3.4	8.5	-0.3	-0.5
Stripe rust	QYr.dms-5B	5B	191	189-194	2.8	6.7	0.3	0.6

Table 4-3. Summary of the number of QTLs identified, heritability and total phenotypic and genetic variance.

Trait	No. of environments	Heritability	Number of QTLs identified	Total phenotypic variance explained (%)	Total genetic variance explained (%)
Common bunt	3	0.48	2	26.5	55.2
Tan spot	3	0.46	3	24.0	52.2
Leaf rust	3	0.25	3	21.5	86.0
Stripe rust	8	0.28	3	23.1	82.5
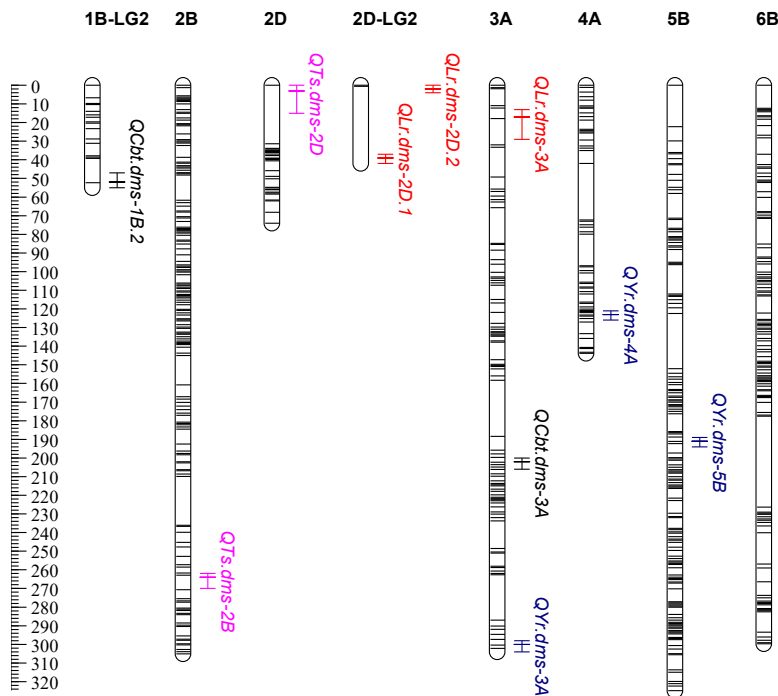
Figure 4-1. The distribution of the quantitative trait loci (QTLs) associated with the four disease resistances evaluated in a single environment (pink, blue or red) or combined across all environments (black).

4.7 Reference

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Chapter 5 General discussion and conclusions

5.1 Introduction

Bread wheat (Triticum aestivum L.) is one of the most important staple crops feeding around 35% of the world population. The average wheat productivity in Canada has increased from 2.7 t ha^{-1} in 2005 to 3.1 t ha^{-1} in 2014, which makes Canada the sixth largest wheat producing and the second largest wheat exporting country.

Wheat breeders in Canada primarily aim to develop cultivars with favored agronomic traits such as short stature, early maturing, high yielding, preferred end-use quality such as high protein content, and at least moderately resistant to priority diseases, such as leaf rust, stem rust, yellow rusts, fusarium head blight and common bunt. As most of these traits are quantitatively inherited, phenotypic performance only partially reflects the genetic value of individuals, which is due to the cumulative action of many genes or quantitative trait loci (QTL) and their interaction with the environment. The availability of well-validated and fine mapped genes or major effect QTLs for a target trait and the associated technology (user friendly, high throughput, and low cost molecular markers) offers alternative methods for marker-assisted selection. linkage-based analysis has been extensively used to map and characterize genes and QTLs associated with traits of economic importance in wheat.

Single nucleotide polymorphism (SNP) have emerged as powerful tools for QTL mapping due to their low assay cost, high genomic abundance, locus-specificity, co-dominant inheritance, simple documentation, high throughput analysis and relatively low genotyping error rates. Currently, a total of 90,000 (90K) gene-associated SNPs are available for wheat researchers through the iSelect platform (Wang *et al.*, 2014), which has provided a tremendous opportunity for wheat researchers conducting research requiring high marker density.

The goal of this thesis was to explore and understand the genetic control of agronomic traits and resistances to diseases in Canadian spring wheat. The specific objectives were: 1) Using SNP markers to map QTLs associated with agronomic traits such as time to flowering and maturity, tillering ability, plant height, thousand kernel weight, test weight, grain yield and grain protein content, and disease resistance, such as resistances to tan spot, common bunt, leaf rust, and stripe rust in a RIL mapping population derived from the cross between Attila and CDC Go. 2) To investigate if the use of the 90K SNP could detect more precise QTLs than DArT markers that we previously used in the 'Attila' × 'CDC Go' recombinant inbred line population (RIL). 3) Compare the QTL mapping studies with 8 testing environments and with 3 testing environments, to see if more testing environments improves QTL detection.

5.2 Contribution to knowledge

5.2.1 Mapping QTLs controlling agronomic traits in the Attila x CDC Go spring wheat population under organic management using 90K SNP array

Our group previously reported five quantitative trait loci (QTL) associated with plant height, test weight, thousand kernel weight and grain protein content in a recombinant inbred line (RIL) population derived from Attila and CDC Go, evaluated across three environments (2008-2010) under organic management, and genotyped with 579 diversity arrays technology (DArT) and *Rht-B1* markers. No QTL was identified for flowering time, maturity, grain yield, and number of tillers across all three environments. In this study, we reanalyzed the same phenotypic data with a subset of 1200 informative single nucleotide polymorphic (SNP) markers out of the 90K SNP array, and three gene specific markers (*Ppd-D1*, *Vrn-A1,* and *Rht-B1*), to investigate if high marker density improves QTL detection. Here, five moderate- and eleven minor-effect QTLs were detected across all three organic environments using the new genotypic data,

including 13 QTLs that were not previously detected. Up to five QTLs were detected for each trait, except grain protein content, which individually accounted for 5.5 to 18.8% of phenotypic variance. For each trait, the total phenotypic and genetic variance explained by all detected QTLs varied from 9.3 to 39.4 and from 24.6 to 96.8%, respectively, which was much greater than our previous study. One of the moderate-effect QTLs on 5A was coincidental for flowering time and maturity, and mapped close to the *Vrn-A1* gene, while the second moderate effect coincidental QTL on 4B was associated with both plant height and maturity, but it was 27 cM from the *Rht-B1* gene. Results from this study provide additional information for wheat researchers and organic wheat breeders.

5.2.2 Effect of marker density and number of testing environments in mapping QTLs for agronomic traits in spring wheat

Previous efforts on QTL detection in spring wheat, including those conducted by our group, have been limited either to genotyping mapping populations at a lower marker density or to phenotyping in few environments. In a previous study, we evaluated a mapping population of 167 recombinant inbred lines derived from a cross between two spring wheat cultivars, Attila and CDC Go, for eight traits (number of tillers, flowering time, maturity, plant height, test weight, kernel weight, grain yield, and grain protein content) at three conventionally managed environments and genotyped with 579 diversity arrays technology (DArT) and *Rht-B1* markers. Using composite interval mapping on averaged phenotypic data across three environments and 580 DArT-based low density markers, we previously identified a total of three QTLs associated with plant height, test weight, and grain yield. The objective of this study was to investigate if an increase in both marker density and the number of testing environments improves the power of detecting QTLs on the eight agronomic traits in the Attila × CDC Go RIL population. Here, we

evaluated the RIL population at seven conventionally managed environments and genotyped with the Wheat 90K single nucleotide polymorphic (SNP) array. Inclusive composite interval mapping was conducted using average phenotypic data of three and seven environments and a subset of 1200 informative SNPs out of the Wheat 90K SNP array and three gene specific (*Ppd-D1*, *Rht-B1,* and *Vrn-A1*) markers. Using the combined phenotypic data across three environments, an increase in marker density from 580 to 1203 resulted in the detection of a total of 13 QTLs as compared with only 3 QTLs identified using the DArT-based low density markers. The high marker density was highly useful in detecting several new QTLs that altogether accounted for 8.6-35.0% of the phenotypic and 18.9-94.7% of the genetic variance per trait. When the averaged phenotypic data across three and seven environments were considered, we uncovered a total of 19 QTLs of which 8 QTLs were common between the three and seven environments, while the remaining QTLs were detected either in the three (5) or seven (6) environments. Although direct comparison of the QTL results between the three and seven environments was not simple, we think that the increase in the number of testing environments neither improved new QTL detection nor their effect. For the combined phenotypic data across seven environments, we found a total of 6 minor- and 8 moderate-effect QTLs which individually explained 6.1-18.4% of the phenotypic variance. Overall, the QTLs associated with each trait averaged across seven environments accounted for 9.7-35.4% of the phenotypic and 22.1-90.8% of the genetic variance. Three QTLs on chromosomes 2D, 4B and 5A were coincident for two to three traits, which could be either due to tight linkage or pleiotropic effect.

5.2.3 QTL mapping of disease resistance in a RIL population derived from a cross of wheat cultivars Attila and CDC Go

In this study, we used a mapping population of 167 RILs developed from a cross between two spring wheat cultivars, 'Attila' and 'CDC Go' in our study, and evaluated the RIL population of resistances to diseases to leaf rust, stripe rust, tan spot and common bunt in the field. Inclusive composite interval mapping was conducted using phenotypic data obtained from 2012 to 2014, and a subset of 1200 informative SNPs out of the Wheat 90K SNP array. After analysis, In the combined phenotypic data across all the environments, we uncovered a QTL for resistance to common bunt (*QCbt.dms-1B.2)* mapped at 52 cM on 1B.2 (LOD score 8.1) and flanked by SNP markers *BS00086854_51* and *wsnp_Ex_c5679_9976893*. For tan spot, our study identified three QTLs mapped at 263 cM on 2B (*QLs.dms-2B*), 200 cM on chromosome 5B (*QLs.dms-5B*) and at 27 cM on 6B (*QLs.dms-6B*). Three QTLs associated with resistance to leaf rust were mapped at 16 cM on 2D (*QLr.dms-2D*), at 2 cM on 2D.2 (*QLr.dms-2D.2*), and at 18 cM on 3A (*QLr.dms-3A*) altogether explaining 22% of the phenotypic variance across all three years. For resistance to leaf rust and stripe rust, altogether six QTLs have been identified in the combined data across three years, and 17 QTLs have been detected in individual years and in all the combined years.

5.3 Future research

As indicated in our study, the high-density 90K SNPs are able to help wheat breeders to better understand the genetic background of Canadian wheat and can be used in the future mapping studies.

Our study showed that differences existed between the organic and conventional systems for selection response, heritabilities, and correlations. As indicated in the present studies and the previous studies, most of the identified and mapped QTL were either specific to organic or conventional management systems. Even for the consistant QTLs identified in both

environments, their phenotypic variation and additive effect were different. Recently, the domestic organic market demand is increasing, therefore it is important to further study and explore cultivars under organic conditions for better agronomic traits and end-use quality.

we found some QTLs/regions associated with agronomic traits (grain yield, test weight, thousand kernel weight, time to flowering and maturity, tillering ability, plant height), end-use quality (protein content), and disease resistances in wheat. Some of those locations should be confirmed in the future genetic study.

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Appendices

Appendix 1 Summary of descriptive statistics, F statistics, and heritability for the 167 RILs evaluated at 3 environments under organic management system.

Trait	df	F value	P value	H	SE	CDC Go	Attila	Minimum	Maximum	Mean	StDev	CV
	F statistics (RIL)			Heritability		Parents		Descriptive statistics (RILs)				
Flowering (days)	166	12.69	<.0001	0.72	0.03	51.0	54.0	43.0	65.0	52.8	4.9	9.2
Maturity (days)	166	6.2	<.0001	0.44	0.04	87.6	91.9	76.0	114.0	89.8	8.4	9.4
Number of tillers per m^{-2}	166	2.15	<.0001	0.15	0.03	88.1	84.8	48.0	171.0	93.7	23.1	24.7
Plant Height (cm)	166	4.22	<.0001	0.33	0.03	71.3	60.8	32.0	110.0	70.2	17.0	24.2
Test weight (kg hL^{-1})	166	4.23	<.0001	0.35	0.04	77.6	75.8	68.0	82.0	76.5	2.4	3.2
Thousand kernel weight (g)	166	4.67	<.0001	0.40	0.04	40.9	36.9	26.0	50.0	38.9	4.0	10.3
Grain yield (t ha^{-1})	166	4.06	<.0001	0.28	0.03	3.6	2.7	0.3	6.9	3.1	1.2	39.8
Grain protein content (%)	166	3.12	<.0001	0.21	0.03	13.2	13.4	9.0	17.0	13.0	1.3	9.9

Appendix 2 Frequency distribution of least squares means of 167 RILs and parents evaluated for 8 traits across three environments under organic management system. The arrows indicate values of the two parents: CDC Go (C) was 10.5 cm taller, produced 3.3 more tiller.
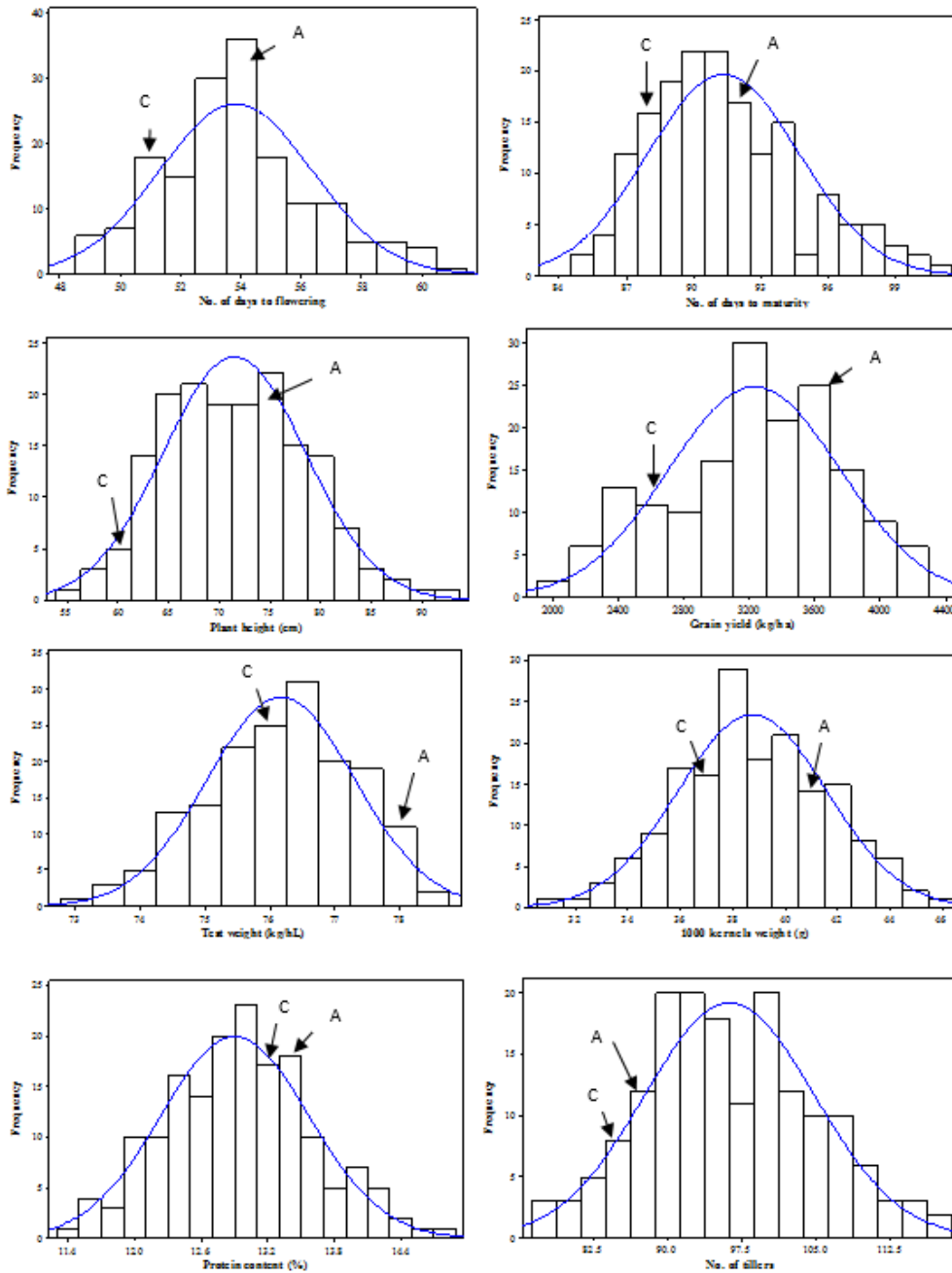
Appendix 3 Comparison of the phenotypic and genetic variance explained by QTLs identified in our previous study using DArT (Asif et al. 2015) and present studies using SNPs and functional markers.

Trait*	Heritability (Asif et al. 2015)	No. of QTLs (Asif et al. 2015)	No. of QTLs (present study)	R^2 (%) (Asif et al. 2015)	R^2 (%) (present study)	Genetic variance (%) (Asif et al. 2015)	Genetic variance (%) (present study)
FLT	0.70	0	1	0.0	17.2	0.0	24.6
MAT	0.47	0	2	0.0	23.2	0.0	49.4
NT	0.16	0	1	0.0	9.3	0.0	58.1
PH	0.43	1	1	18.1	18.8	42.1	43.7
TKW	0.44	2	5	18.7	39.4	42.5	89.5
TW	0.34	1	4	8.3	32.9	24.4	96.8
GY	0.18	0	2	0.0	13.3	0.0	73.9
GPC	0.27	1	0	10.9	0.0	40.4	0.0

Appendix 4 Summary of QTLs associated with the different traits based on 167 recombinant inbred lines population evaluated in a single environment and averaged across three (2008-2010) and seven (2008-2014) environments under conventional management system.

QTL	Trait*	Environment	Chrom	Position (cM)	Confidence interval (cM)	Left Marker	Right Marker	LOD	R^2 (%)	Additive effect	Difference**
QFlt.dms-4A	Fllt	2014	4A	1	0-3.5	Excalibur_c82040_91	wsnp_Ra_rep_c70233_67968353	3.0	8.5	-0.6	
QFlt.dms-4B	Fllt	2010	4B	101	90.5-113.5	wsnp_Ra_c1146_2307483	Rht-B1	2.5	9.0	0.9	
QFlt.dms-4B	Fllt	2011	4B	101	90.5-113.5	wsnp_Ra_c1146_2307483	Rht-B1	2.5	9.0	0.9	
QFlt.dms-5A	Fllt	2009	5A	289	285.5-290.5	Ra_c3966_2205	Tdurum_contig10843_745	5.2	13.0	-1.0	
QFlt.dms-5A	Fllt	2014	5A	293	290.5-293.5	wsnp_Ex_rep_c101994_87256479	Excalibur_c26671_282	4.4	12.6	-0.8	
QFlt.dms-5A	Fllt	2010	5A	296	294.5-296.5	Vrn-A1	Kukri_c12384_430	6.8	13.0	-1.1	
QFlt.dms-5A	Fllt	2011	5A	296	294.5-296.5	Vrn-A1	Kukri_c12384_430	6.8	13.0	-1.1	
QFlt.dms-5A	Fllt	3 environments	5A	296	294.5-296.5	Vrn-A1	Kukri_c12384_430	7.5	18.9	-1.2	-2.5
QFlt.dms-5A	Fllt	7 environments	5A	296	294.5-296.5	Vrn-A1	Kukri_c12384_430	6.6	16.8	-1.0	-2.5
QFlt.dms-6B	Fllt	2009	6B	232	230.5-232.5	wsnp_Ex_c18632_27501906	Tdurum_contig32579_121	3.3	7.6	0.8	
QGpc.dms-2B	Gpc	2013	2B	1	0-4.5	IAAV7130	wsnp_BG274584B_Ta_2_1	2.5	6.8	0.2	
QGpc.dms-2D	Gpc	2009	2D	61	58.5-62.5	BS00011109_51	Excalibur_c24307_739	4.4	8.9	-0.3	
QGpc.dms-2D	Gpc	3 environments	2D	61	58.5-62.5	BS00011109_51	Excalibur_c24307_739	4.6	11.8	0.3	0.6
QGpc.dms-2D	Gpc	7 environments	2D	61	59.5-62.5	BS00011109_51	Excalibur_c24307_739	9.1	13.4	0.2	0.3
QGpc.dms-2D	Gpc	2010	2D	65	62.5-68.5	RAC875_rep_c73531_335	wsnp_Ex_c8303_14001708	6.0	12.0	-0.4	-375.7
QGpc.dms-3A	Gpc	2008	3A	86	84.5-88.5	RAC875_c6006_105	Tdurum_contig34075_98	2.7	7.2	-0.2	-422.2
QGpc.dms-4B	Gpc	2010	4B	79	77.5-79.5	RAC875_c47018_72	Tdurum_contig29054_113	5.4	10.8	-0.4	
QGpc.dms-4B	Gpc	7 environments	4B	80	79.5-80.5	Tdurum_contig29054_113	wsnp_Ra_c3790_6990678	3.4	6.3	-0.2	-0.5
QGpc.dms-5B	Gpc	2009	5B	15	1.5-26.5	BS00062618_51	wsnp_Ex_c3874_7036132	3.1	8.3	0.3	
QYld.dms-2D.1	Yld	2009	2D	4	0-17.5	Ppd-D1	GENE-0787_85	2.7	8.4	-178.2	
QYld.dms-2D.1	Yld	2011	2D	9	0-19.5	Ppd-D1	GENE-0787_85	3.9	11.7	-313.1	
QYld.dms-2D.2	Yld	2014	2D	65	62.5-69.5	RAC875_rep_c73531_335	wsnp_Ex_c8303_14001708	3.7	9.2	197.7	
QYld.dms-2D.2	Yld	2013	2D	66	62.5-70.5	RAC875_rep_c73531_335	wsnp_Ex_c8303_14001708	4.1	10.5	212.3	
QYld.dms-2D.2	Yld	7 environments	2D	66	62.5-69.5	RAC875_rep_c73531_335	wsnp_Ex_c8303_14001708	2.9	9.3	-153.3	-375.7
QYld.dms-2D.2	Yld	2010	2D	67	62.5-69.5	RAC875_rep_c73531_335	wsnp_Ex_c8303_14001708	4.2	11.1	253.0	
QYld.dms-2D.2	Yld	2011	2D	68	63.5-70.5	RAC875_rep_c73531_335	wsnp_Ex_c8303_14001708	3.7	6.0	224.9	
QYld.dms-3A.1	Yld	2008	3A	9	3.5-12.5	wsnp_Ex_c15475_23757972	IACX6065	2.9	7.4	260.6	
QYld.dms-3A.2	Yld	2014	3A	116	112.5-116.5	BS00048633_51	RAC875_rep_c109228_400	4.3	10.3	202.8	
QYld.dms-6B	Yld	2009	6B	229	228.5-230.5	BS00067388_51	Excalibur_c7713_272	4.1	10.9	197.8	
QYld.dms-6B	Yld	3 environments	6B	229	228.5-230.5	BS00067388_51	Excalibur_c7713_272	3.2	8.6	194.2	395.7
QYld.dms-7A	Yld	2013	7A	48	46.5-55.5	Kukri_c18440_92	Kukri_rep_c75743_357	3.2	7.5	-179.3	
QYld.dms-7A	Yld	2011	7A	56	50.5-58.5	Kukri_c18440_92	Kukri_rep_c75743_357	2.8	6.3	-194.0	
QYld.dms-7D	Yld	2013	7D	38	34.5-41.5	Excalibur_c4508_1959	wsnp_Ex_c17914_26681837	3.3	7.7	181.9	
QYld.dms-7D	Yld	2014	7D	38	37.5-45.5	Excalibur_c4508_1959	wsnp_Ex_c17914_26681837	3.4	7.7	176.6	
QMat.dms-2D.1	Mat	2009	2D	0	0-14.5	Ppd-D1	GENE-0787_85	2.5	0.8	-0.5	
QMat.dms-2D.2	Mat	2013	2D	68	63.5-74	RAC875_rep_c73531_335	wsnp_Ex_c8303_14001708	2.8	4.7	0.9	
QMat.dms-4B	Mat	2009	4B	72	68.5-75.5	tplb0026o15_1634	BobWhite_c4311_148	3.4	7.6	0.7	
QMat.dms-4B	Mat	2010	4B	78	77.5-78.5	wsnp_Ex_c35910_43971560	RAC875_c47018_72	9.4	19.7	2.2	
QMat.dms-4B	Mat	2013	4B	80	79.5-80.5	Tdurum_contig29054_113	wsnp_Ra_c3790_6990678	9.7	17.7	1.8	
QMat.dms-4B	Mat	2014	4B	80	78.5-80.5	Tdurum_contig29054_113	wsnp_Ra_c3790_6990678	5.1	11.4	0.8	
QMat.dms-4B	Mat	3 environments	4B	80	78.5-80.5	Tdurum_contig29054_113	wsnp_Ra_c3790_6990678	7.2	13.9	1.5	2.8
QMat.dms-4B	Mat	7 environments	4B	80	79.5-80.5	Tdurum_contig29054_113	wsnp_Ra_c3790_6990678	7.6	15.9	1.1	2.2
QMat.dms-4B	Mat	2012	4B	81	80.5-81.5	RAC875_c103110_275	RAC875_c24550_1150	3.7	7.4	1.0	
QMat.dms-5A.2	Mat	2009	5A	292	290.5-293.5	Tdurum_contig10843_745	wsnp_Ex_rep_c101994_87256479	4.7	9.7	-0.8	
QMat.dms-5A.2	Mat	3 environments	5A	297	295.5-297.5	Kukri_c12384_430	wsnp_Ex_c22727_31934296	9.9	19.7	-1.7	-3.2
QMat.dms-5A.2	Mat	7 environments	5A	297	295.5-297.5	Kukri_c12384_430	wsnp_Ex_c22727_31934296	6.8	14.0	-1.0	-1.9
QMat.dms-5A.2	Mat	2010	5A	298	296.5-298.5	wsnp_Ex_c22727_31934296	wsnp_Ex_rep_c66689_65010988	3.3	6.3	-1.2	
QMat.dms-5A.2	Mat	2014	5A	301	300.5-301.5	wsnp_Ex_c2702_5013188	Excalibur_rep_c111129_125	5.6	12.7	-0.9	
QMat.dms-5A.2	Mat	2013	5A	310	304.5-311	BS00044408_51	wsnp_Ex_c27046_36265198	3.5	6.2	-1.0	
QMat.dms-5B	Mat	2010	5B	56	54.5-57.5	BS00048316_51	IAAV8455	2.6	5.0	-1.1	
QMat.dms-6B.1	Mat	2012	6B	24	19.5-26.5	Ex_c20409_854	wsnp_Ex_c19082_27999258	6.0	13.3	1.3	

QTL	Trait	Year/Env	Chr	Pos	Interval	Left marker	Right marker				
QMat.dms-6B.2	Mat	2012	6B	130	128.5-130.5	wsnp_BM134512B_Ta_2_1	wsnp_Ra_c14498_22667649	2.9	5.8	-0.8	
QMat.dms-7A	Mat	2013 3	7A	61	60.5-61.5	wsnp_Ra_rep_c105976_89839782	BobWhite_c911_127	3.4	5.7	-1.0	
QPht.dms-2D.2	Pht	environments 3	2D_LG2	0	0-0.5	RAC875_c29241_165	wsnp_CAP7_rep_c5643_2537213	2.7	5.4	2.0	3.8
QPht.dms-2D.2	Pht	2009	2D_LG2	1	0-1.5	wsnp_CAP7_rep_c5643_2537213	D_F5XZDLF02HWOJZ_227	3.1	8.5	2.1	
QPht.dms-2D.2	Pht	2010	2D_LG2	1	0-1.5	wsnp_CAP7_rep_c5643_2537213	D_F5XZDLF02HWOJZ_227	2.7	3.2	2.2	
QPht.dms-4B	Pht	2013	4B	81	80.5-84.5	RAC875_c103110_275	RAC875_c24550_1150	8.2	20.0	-5.2	
QPht.dms-4B	Pht	2010	4B	82	80.5-84.5	RAC875_c24550_1150	wsnp_Ra_c1146_2307483	8.5	11.2	-4.2	
QPht.dms-4B	Pht	2011	4B	82	80.5-86.5	RAC875_c24550_1150	wsnp_Ra_c1146_2307483	5.2	13.4	-3.4	
QPht.dms-4B	Pht	2012	4B	82	80.5-84.5	RAC875_c24550_1150	wsnp_Ra_c1146_2307483	9.0	22.6	-4.5	
QPht.dms-4B	Pht	2014 7	4B	82	80.5-84.5	RAC875_c24550_1150	wsnp_Ra_c1146_2307483	10.3	23.9	-5.0	
QPht.dms-4B	Pht	environments 3	4B	82	80.5-85.5	RAC875_c24550_1150	wsnp_Ra_c1146_2307483	7.3	18.4	-3.8	-7.7
QPht.dms-4B	Pht	environments	4B	83	80.5-86.5	RAC875_c24550_1150	wsnp_Ra_c1146_2307483	6.1	14.1	-3.3	-6.4
QPht.dms-5A	Pht	2013	5A	292	290.5-293.5	Tdurum_contig10843_745	wsnp_Ex_rep_c101994_87256479	3.7	8.4	-3.3	
QPht.dms-6B.1	Pht	2010	6B	113	112.5-118.5	wsnp_Ex_c3101_5719964	wsnp_Ra_rep_c73731_71807419	5.3	6.5	-3.0	
QPht.dms-6B.2	Pht	2010	6B	160	158.5-161.5	Kukri_c59960_211	Ku_c59960_1939	10.2	13.3	4.4	
QTil.dms-4A	Til	2009	4A	0	0-1.5	Excalibur_c82040_91	wsnp_Ra_rep_c70233_67968353	3.3	10.2	3.9	
QTil.dms-5A	Til	2013 7	5A	223	221.5-237.5	Excalibur_c47920_249	RAC875_c2061_292	4.8	6.4	6.3	
QTil.dms-6A.1	Til	environments	6A	70	67.5-71.5	wsnp_BG262421A_Ta_2_2	wsnp_Ku_c38451_47086066	3.3	11.2	-2.0	-3.4
QTil.dms-6A.2	Til	2013	6A	84	83.5-86.5	BS00066623_51	BobWhite_c10342_117	3.0	3.8	-5.0	
QTil.dms-7A.1	Til	2013	7A	8	1.5-12.5	wsnp_Ra_rep_c69620_67130107	GENE-5000_606	3.9	5.9	-6.1	
QTil.dms-7A.2	Til	2013	7A	77	71.5-87.5	RAC875_c29361_70	Excalibur_c49272_174	6.8	12.9	9.2	
QTil.dms-7A.3	Til	2014	7A	214	206.5-219.5	BS00068575_51	wsnp_Ku_c28104_38042857	3.7	9.8	-4.0	
QTkw.dms-2B	Tkw	2008	2B	288	284.5-289.5	Kukri_c15782_491	Tdurum_contig54649_798	2.6	6.5	0.8	
QTkw.dms-3A.1	Tkw	2009 3	3A	214	213.5-214.5	wsnp_Ex_c16864_25440739	RAC875_rep_c113506_409	2.8	3.0	-0.6	
QTkw.dms-3A.2	Tkw	environments 3	3A	238	231.5-248.5	Ra_c6118_450	wsnp_Ra_c9738_16174002	2.9	4.7	-0.6	-1.0
QTkw.dms-4A	Tkw	2009 3	4A	120	118.5-120.5	wsnp_Ex_c7899_13416443	wsnp_Ex_rep_c97236_84366722	9.3	10.7	1.2	
QTkw.dms-4A	Tkw	environments 7	4A	120	118.5-120.5	wsnp_Ex_c7899_13416443	wsnp_Ex_rep_c97236_84366722	5.8	7.9	0.8	1.5
QTkw.dms-4A	Tkw	environments	4A	120	119.5-120.5	wsnp_Ex_c7899_13416443	wsnp_Ex_rep_c97236_84366722	5.8	12.1	0.6	1.1
QTkw.dms-5B	Tkw	2008 3	5B	303	300.5-304.5	Kukri_rep_c106832_790	wsnp_JD_c38123_27754848	4.1	10.5	-1.0	
QTkw.dms-6A.1	Tkw	environments	6A	78	77.5-79.5	TA005366-0788	wsnp_Ku_rep_c112734_95776957	10.7	16.0	1.1	1.6
QTkw.dms-6A.1	Tkw	2009 7	6A	79	77.5-79.5	wsnp_Ku_rep_c112734_95776957	BS00036878_51	15.2	19.3	1.6	
QTkw.dms-6A.1	Tkw	environments	6A	79	77.5-79.5	TA005366-0788	wsnp_Ku_rep_c112734_95776957	4.0	8.5	0.5	1.1
QTkw.dms-6A.2	Tkw	2009 3	6A	108	107.5-110.5	Kukri_c669_259	Excalibur_rep_c104696_400	6.4	7.0	-1.0	
QTkw.dms-6A.2	Tkw	environments 7	6A	114	112.5-115.5	Tdurum_contig77175_248	Tdurum_contig60549_397	4.7	6.4	-0.7	-0.3
QTkw.dms-6D.2	Tkw	environments 7	6D_LG2	4	3.5-5.5	wsnp_Ex_c14691_22765150	wsnp_Ex_c14691_22763609	3.2	6.7	0.4	0.9
QTkw.dms-7B.1	Tkw	environments	7B	158	156.5-158.5	wsnp_Ku_c17161_26193994	BS00003350_51	4.0	8.1	0.5	0.9
QTwt.dms-2B	Twt	2012	2B	109	108.5-110.5	RAC875_c85927_269	Kukri_c6973_344	2.6	7.0	-0.5	
QTwt.dms-5A	Twt	environments 3	5A	12	10.5-12.5	wsnp_Ex_rep_c107017_90850230	RAC875_c9617_395	2.5	6.1	0.2	0.5
QTwt.dms-5B.1	Twt	environments	5B	138	128.5-150.5	Tdurum_contig5017_993	RAC875_c30011_426	4.0	13.6	-0.5	-0.9
QTwt.dms-5B.2	Twt	2010 3	5B	163	160.5-163.5	Tdurum_contig53072_1935	Kukri_rep_c69276_59	3.7	9.9	-0.4	
QTwt.dms-5B.3	Twt	environments 7	5B	239	237.5-239.5	wsnp_Ra_c26091_35652620	RAC875_c12552_233	4.0	6.9	-0.3	-0.8
QTwt.dms-5B.3	Twt	environments	5B	239	237.5-239.5	wsnp_Ra_c26091_35652620	RAC875_c12552_233	4.4	10.1	-0.3	-0.5

Appendix 5 Frequency distribution of least squares means of 167 RILs evaluated for 8 traits across seven environments (2008-2015) under conventional management system. The arrows indicate values of the parents: CDC Go (C) and Attila.