Mapping QTLs for different traits in conventional and organic management systems and evaluating the effects of *Lr34/Yr18* and *Lr37/Yr17* in a Canadian western hard spring wheat population

by

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Abstract

Canadian western red spring wheat (CWRS) has been predominantly cultivated class in Western Canada, because of its premium quality attributes and excellent adaptability to the relatively short growing season. Early maturity, short plant stature, higher grain yield, protein content and dough strength, and moderate to high levels of resistance to stem rust, leaf rust, stripe rust, common bunt, and fusarium head blight are important breeding objectives in western Canada. In the first study, we evaluated a mapping population of 168 recombinant inbred lines derived from a cross between two CWRS cultivars 'Peace' and 'CDC Stanley' for agronomic and quality traits under organic and conventional managements from 2016 to 2017. Days to heading and maturity, grain yield and protein content, thousand kernel weight (TKW) and test weight expressed high broad-sense heritability across two management systems. The population was genotyped with 90K single nucleotide polymorphism (SNP) array and quantitative trait loci (QTL) analysis was performed. However, only six of 50 QTLs could be detected across two management systems. The phenotypic variance explained for each trait varied from 0.5 – 23.3 % in conventional and 1.3 – 25.9 % in organic environment. A QTL on chromosome 2D was associated with multiple traits (plant height, grain yield, grain protein content and test weight in conventional and days to maturity, grain yield and plant height in organic environment), which is possibly due to tight linkage of multiple loci on this chromosomal segment, whereas another coincidental QTL on 4B for grain yield and protein content in conventional management system could be due to pleiotropic effect. We also validated a major pre-harvest sprouting (PHS) resistance QTL *Qphs-usask-4A* in the mapping population. PHS resistant genotypes possessed significantly higher falling number, however standardized methods are required to examine the effect of *Qphs-usask-4A*.

The second study was to investigate the combined effects of *Lr34/Yr18* and *Lr37/Yr17* genes on disease resistance in the same mapping population. Lines with only *Lr34/Yr18* expressed reduced plant height, SDS sedimentation and yield penalty, possibly due to genetic linkage or pleiotropic effects of co-expression of leaf tip necrosis on flag leaf and rust resistance. The presence of *Lr37/Yr17* was not associated with reduction in grain yield or end-use quality. In the

breeding practice, we failed to integrate *Lr34/Yr18* and *Lr37/Yr17* genes with considerable grain yield, protein content, dough strength and early maturity, which was most likely due to insufficient population size. Thus, whole-scale dependence on markers in a marker-assisted selection program will likely eliminate desirable genotypes. Nonetheless, five lines with substantial disease resistance conferred by the *Lr34/Yr18* and/or *Lr37/Yr17* resistance alleles and improved agronomic and quality characters remain in the breeding process that has potential to become parental materials.

Preface

The development of Canadian western hard spring wheat cultivar in breeding programs primarily focuses on improving grain yield and quality attributes such as grain protein content and dough strength while shortening plant stature and days to maturity. Moderate to high disease resistance is also desirable against newly emerged races of stem rust, leaf rust, stripe rust and tan spot and is a necessity to common bunt pathogen. Competitivity traits including taller plant height and early maturity that are favored when breeding for organic agriculture. Therefore, a better understanding of the underlying genetics for these traits helps with accelerating the breeding process.

A version of Chapter 2 of this dissertation has been submitted to the journal *Crop Science* as "Evaluating the phenotypic performance and associated QTLs of 'Peace' × 'CDC Stanley' RIL population under conventionally and organically management and the effect of *Qphs.usask-4A* pre-harvest sprouting resistance QTL on traits of breeding significance", authored by Xiang, R., Chen, H., Iqbal, M. Pozniak, C. and Spaner, D. The recombinant inbred lines population derived from a cross between Canadian western hard spring wheat cultivars was developed before I joined the wheat breeding program in 2016. The phenotypic data was collected by the wheat breeding group. I carried out phenotypic data collection at F_8 generation and molecular marker screening (*Vrn-A1, Vrn-B1, Vrn-D1, Rht-B1, Rht-D1, Ppd-A1, Ppd-D1,* DuPw004, barc170 and wms650) in the population from 2016 to 2017 and participated in the extraction and shipping of the DNA samples to the University of Saskatchewan, where the samples were further genotyped by using iSelect Illumina SNP Array. Dr. Hua Chen provided predominantly assistance and interpretation in statistical analyses and population mapping. The manuscript composition was done by me while Dr. Dean Spaner, Dr. Muhammad Iqbal and Dr. Hua Chen provided extensive support and help in manuscript editing and interpretation of the results.

A version of the third chapter has been submitted to the journal *Crop Science* as "Effect of *Lr34/Yr18* and *Lr37/Yr17* on phenotypic traits of breeding interest and associated marker assisted selection for these two genes in the Canadian spring wheat 'Peace' × 'CDC Stanley' mapping

population", authored by Xiang, R., Chen, H., Iqbal, M., Randhawa, H., Ciechanowska, I. and Spaner, D. I was responsible for disease ratings for leaf rust and tan spot in diseases nursery at the Edmonton Research Center, University of Alberta in 2017, whereas Dr. Hua Chen scored disease for leaf rust and tan spot in 2016. Rating of stripe rust infection severity in Edmonton, Lethbridge and Creston were recorded by Drs. Harpinder Singh Randhawa and Dean Spaner, respectively. The Selection study was guided by Dr. Dean Spaner and Dr. Hua Chen, I did the data analysis with significant help from Dr. Hua Chen. All planting and plot maintenance were done by the wheat breeding group at the University of Alberta. Drs. Dean Spaner and Muhammad Iqbal provided sufficient assistance during thesis writing and considerable editorial of this manuscript.

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Chapter 1 Literature review

1.1 Wheat breeding in Canada

Wheat is the most prevalently grown crop globally, with an extensive cultivation range from 67˚N in Russia to 45˚S in Argentina, including tropical areas and elevated topographic regions (Feldman, 1995). Wheat provides significant food calories and 20% of daily protein to humanity (Lucas, 2012). In 2016, global wheat production reached 749 million tonnes (FAOSTAT, 2016). Wheat has existed in the farming system for over 10 000 years following the transition from gathering and hunting to farming as part of the development of sedentary human civilization (Shewry, 2009). A diploid genome form of Einkorn (*Triticum monococcum*, AA) and a tetraploid genome form of Emmer (*Triticum turgidum* subspecies (ssp.) *dicoccon*, AABB) are the earliest form of the domesticated wheats that were selected by farmers from natural populations based on characteristics such as high yield, no shattering and free-threshing (Shewry, 2009). However, the evolution of modern bread wheat involves in several steps of independent hybridization of domesticated emmer wheat with a diploid wild grass *Triticum tauschii* (or *Aegilops tauschii,* DD) (Dubcovsky and Dvorak 2007). Then, the selection of greater agronomic traits had been conducted to further develop the hexaploid wheat (*Triticum aestivum* L., genomes AABBDD), which belongs to the Poaceae family (Feldman, 2001). This genetic complexity contributes to the superior adaptability of bread wheat, which allows it to achieve substantial production under diverse temperate environments in comparison with other temperate crops (Feldman et al., 1995). Wheat is commonly classified as a caloric source because of 60-70% of the whole grain is composed of starch (Shewry, 2009). Although only 8 -15% of the grain consists of storage proteins that interact with each other together to form a gluten protein matrix, this matrix determines the baking properties of the wheat flour and end use products (Shewry, 2009). Additionally, wheat can also be regarded as a source of minerals. In the UK particularly, it contributes 44% of the daily intake of iron and 25% of the daily intake of zinc (Handerson et al., 2007), yet modern wheat cultivars are claimed to contain lower mineral levels compared to older cultivars (Shewry, 2009).

Triticum aestivum L. (common wheat) and *Triticum turgidum* L. var. *durum* Desf. (durum wheat) are the most prevalent wheat species grown in Canada and globally (Randhawa et al., 2013). The winter growth habit of common wheat that is planted in the Fall and harvested in the summer of the following year, has been limited in western Canada due to the harsh winter and often lack of adequate snow cover. The spring growth habit of common wheat has a much shorter growth cycle is more suitable for the climate in the western Canadian Prairies (Randhawa et al., 2013). Common wheat is classified into different milling classes based on characteristics such as growth habit, kernel color and hardness, protein content as well as gluten strength to meet the domestic and export demands (McCallum and DePauw 2008). These critical quality factors determine end-use functionalities of the wheat flour. There are nine milling classes of western Canadian wheat, including Canada Northern Hard Red (CNHR), Canada Prairie Spring Red (CPSR), Canada Prairie Spring White (CPSW), Canada Western Amber Durum (CWAD), Canada Western Extra Strong (CWES), Canada Western Hard White Spring (CWHWS), Canada Western Red Spring (CWRS), Canada Western Red Winter (CWRW), Canada Western Soft White Spring (CWSWS) (Canadian Grain Commission, 2017).

The adaptation of CWRS cultivars to many growing conditions and their high protein and advanced milling properties result in this class having a premium price in international trade and is preferred by a wide range of domestic manufacturers (McCallum and DePauw 2008). Red Fife was the first Canadian awnless red spring wheat with excellent end-use quality bred and was found on David Fife's farm in Ontario, Canada in 1841 (Dickenson 1976; Campbell and Shebeski 1986). This cultivar was brought to western Canada in 1871, and resulted in the threefold increase of the wheat production in Manitoba following its adoption (Dickenson 1976). Agronomic limitations of Red Fife included in susceptibility to lodging, stem rust, frost damage and prone to shatter (Newman 1928). It was later replaced by the cultivar Marquis cultivar derived from a cross between Hard Red Calcutta and Red Fife (Morrison 1960). As an improved cultivar, Marquis not only retained superb bread-making quality but was also less susceptible to stem rust, lodging and shattering because of the shorter statue. It also matured earlier and had higher grain yield than Red

Fife (Morrison 1960). Marquis was then replaced by Thatcher as it was particularly subject to frost damage in the northern growing regions of the Prairies which reduced baking quality (Geddes et al., 1932). Thatcher had a higher level of resistance to stem rust and was the result of a complex pedigree, i.e. Marquis/Iumillo Durum//Marquis/Kanred winter wheat (Knott 2000). However, the major leaf rust resistance gene *Lr22b* in Thatcher was not durable on its own and was then vulnerable to isolate mutations of *P. triticina* in the leaf rust epidemic growing areas (Dyck 1979). Nonetheless, the excellent baking quality from Marquis and early maturity made it an excellent cultivar and it became a major component in the pedigree of subsequent CWRS cultivars to this day (McCallum and DePauw 2008). Manitou, Neepawa and Katepwa were later developed for early maturity, lodging resistance, enhanced rust resistance and high yield (Campbell 1970). The cultivar Columbus was also regarded as an excellent genetic platform for future cultivar development as it shows strong resistance to pre-harvest sprouting. It also is related to Red Fife (McCallum and DePauw 2008).

AC Barrie was adopted in the Prairies in 1998 and remained the leading cultivar until 2005 because of its relative shortness and high yielding attribute in comparison with contemporary cultivars and more of importance, higher protein content (McCaig et al., 1996; McCallum and DePauw 2008). More cultivars were subsequently developed, including AC Domain, CDC Teal, McKenzie, AC Splendor and Superb that are capable of producing higher yield than the early cultivars.

The economic loss caused by wheat stem sawfly (*Cephus cinctus* Nort.) can be very serious as the adult sawfly lays eggs in the stem. Larva feeds inside of the stem that results in reduced grain yield and quality or it gridles the wheat stem that becomes lodged and unharvestable (Michaud, 2013). Lilian was a solid stem cultivar resistant to wheat stem sawfly, which also has desirable grain yield and protein concentration integrated with moderate maturity (DePauw et al., 2005; DePauw et al., 2007). Therefore, it soon became the leading CWRS cultivar in 2007 four years following its registration (McCallum and DePauw 2008).

Over 90 years of CWRS cultivar development in Western Canada has improved the yield potential 6-9 kg/ha per year on average (McCaig and Depauw, 1994) and a net 20 kg/ha in comparison between the first released cultivar Red Fife and the contemporary cultivars registered up to 1990 (Hucl and Graf 1995). This rate of genetic gain has increased 0.74% since 1990 as a result of the combination of accelerated and more advanced breeding activities and breeding strategies (DePauw et al., 2007). A single leading cultivar used to dominate vast areas of the Prairie in the past. Now, growers can select the most suitable wheat cultivars to meet their needs from a great range of modern cultivars in the market expressing elite traits (McCallum and DePauw 2008).

The hard kernels of Canada Western Extra Strong (CWES) cultivars have very strong gluten yet medium protein concentration, which result in significantly different end-use qualities mostly used in flour blends from CWRS cultivars (McCallum and DePauw 2008). Glenlea is a high yielding cultivar that used to be the most popular CWES cultivar on the prairies from 1994 to 2002 and from 2005 to 2006 (McCallum and DePauw 2008). It is the first major Canadian wheat cultivar with the durable wheat disease resistance gene *Lr34* conferring various degree of resistance against three common rust diseases and powdery mildew (Sucher et al., 2017). Disease resistance genes expressed by this cultivar protect the crop from losing yield potential in stem rust and leaf rust prevailing areas (Dyck et al., 1985). In terms of the end-use products, extremely strong gluten derived from Glenlea is due to the over expression of glutenin subunit *Glu-B1al*, which help to improve baking quality of wheat that has weaker gluten strength by blending with Glenlea flour (Bushuk 1980). CWES flour is widely used in frozen dough products as the specific gluten property is capable to tolerate the freezing and thawing cycles while maintaining proper baking qualities (McCallum and DePauw 2008). Nevertheless, the significant decline of CWES production areas indicates the reduced international demand for very strong gluten properties in making frozen dough products, which is possibly due to changes in processing technologies in North America (McCallum and DePauw 2008).

The development of the Canada Prairie Spring Wheat (CPS) class targets on improving end-use quality and genetic resistance to common bunt and loose smut (McCallum and DePauw

2008). HY320 is in the pedigree of most red kernel CPS cultivars (McCallum and DePauw 2008) and in a cross with the white seeded NB402 to develop the first white seeded CPS cultivar Genesis (DePauw et al., 1989). The intermediate kernel hardness of CPS cultivars falls between CWRS and CWRW and the flour can be utilized for different end-use purposes. In addition, some high yielding cultivars have potential as a feed stock for the bio-ethanol industry (McCallum and DePauw 2008).

Most Canada Western Soft White Spring (CWSW) wheat is produced in Southern Alberta under irrigation (McCallum and DePauw 2008). CWSW cultivars have low protein content, which is an ideal baking characteristic to make pastries, cookies and some noodles (Canadian Wheat Commission, 2017). Researchers and industry are also interested in the value of involving CWSW cultivars in bio-ethanol production by taking advantage of its high starch yield potential (McLeod et al., 2010). Canada Western Hard White Spring cultivars on the other hand have excellent end-use quality, and this class was developed to target the Asian noodle and pan bread market. However, cultivars of this class are not widely grown in the prairies despite of their acceptable disease resistance and agronomic performance (Randhawa et al., 2012). While spring wheat is predominantly grown in the prairies, the demand for winter wheat production has been recently increasing. Canada Western Red Winter Wheat (CWRW) cultivars have better disease resistance along with superior milling quality that is ideal for ethanol production as an alternative market (McCallum and DePauw 2008); in addition to making French bread and noodles (Canadian Grain Commission 2017).

Durum wheat (*Triticum durum* L.ssp.*durum* (Desf.) Husn.) is the raw material to make Italian premium quality pasta because of its high semolina content and recognized protein content, which is primarily correlated with pasta cooking quality (Marchylo et al., 2001). It is also an important food source (particularly in Mediterranean countries) of couscous, bulgur and various of baking goods (Quaglia 1988). Additionally, durum wheat can be treated as an alternative to bread wheat in blends with high-quality baking flour, which in turn expand the end-use product market for durum farmers (Marchylo et al., 2001; Boggini and Pogna 1989). Durum wheat has

been produced in western Canada since the early stage of wheat cultivation in Canada (McCallum and DePauw 2008). The development of the Canada Western Amber Durum (CWAD) milling class started with cultivar introductions from other countries until the 1960s (Knott 1995). Mindum as the end-quality standard for Canadian durum wheat cultivars and Carleton from USA were the leading cultivars from the 1940s (Knott 1995). Subsequent stem rust resistant cultivars including Stewart 63 and Hercules were released and cultivated predominantly in the prairies between the 1960s and 1970s (McCallum and DePauw 2008). Hercules specifically was more lodging resistant, matured earlier and exhibited improved end-use quality (Leisle 1970). It replaced Mindum to become the standard of CWAD quality in 1972 (Irvine 1983). During the period between 1976 and 1986, strong gluten cultivar Wakooma was registered to fit the export market. Its flour was also blended with Wascana that has a higher yellow pigmentation to supplement the gluten strength and yellow pigment preferred by the Italian premium market (Hurd et al., 1973; Irvine 1983). One significant achievement during the evolution of durum breeding in Canada was the registration of AC Strongfield in 2004. This cultivar expressed remarkably higher grain yield, protein concentration and yellow pigment concentration integrated with greater straw strength and shorter stature; and more substantially, reduced uptake of the heavy metal cadmium in relation to the previous leading cultivars (Clarke et al., 2005). All CWAD cultivars registered afterwards expresses the trait of cadmium uptake reduction and significantly higher gluten index (McCallum and DePauw 2008).

Improvement of agronomic traits in western Canadian wheat breeding programs includes yield potential, lodging resistance, and early maturity to avoid frost damage (McCallum and DePauw 2008). In addition, effective weed control can be achieved through the genetic approach of incorporating *Imi1* resistance gene into CWRS cultivar CDC Imagine in order to retain stable yield potential under routine application of imidazolinone class of herbicides (Pozniak and Hucl 2004). Cultivars expressing a higher tolerance to toxic concentrations of aluminum in acidic soils is a minor breeding objective (Briggs et al., 1991). End-use quality traits that require improvement include higher protein content, gluten strength, milling yield, bread-making quality, resistance to

pre-harvesting sprouting and specific criteria enhancement corresponding to each milling class (McCallum and DePauw 2008).

Molecular markers have been applied in wheat breeding programs in Canada to accelerate the selection process and to improve the selection precision of desirable traits. In contrast to traditional wheat breeding, where the specific traits selections are solely dependent on phenotype; marker assisted breeding involves desirable genotype selection and introgressing multiple genes of interest into a single germplasm line (Babu et al., 2004). Marker assisted breeding is primarily applied at the early generations after the initial cross to maximize diversity of desirable genes in the population through allelic segregation (Randhawa et al., 2013). Critical factors to enable successful marker assisted breeding include (i) co-segregation between markers and the desired genes, (ii) availability of cost-effective and time-efficient screening methodology that can be duplicated across laboratories (Randhawa et al., 2013), (iii) validation of markers to be linked with respective traits in the desired genotypes under target growing conditions (Sharp et al., 2001). As of 2012, 97 functional markers have been released to recognize the 93 alleles related to gene sequences in relation to 30 discovered loci that are responsible for traits involving disease resistance, agronomic performance and end-use quality (Liu et al., 2012). Integration of molecular markers into breeding programs have effectively developed elite wheat cultivars that are able to maintain desirable grain yield, diseases resistance and end-use quality in the presence of biotic and abiotic stress (Randhawa et al., 2013).

1.2 Common rusts of spring wheat

1.2.1 Stripe Rust (*Puccinia striiformis*)

Of three destructive rust diseases, stripe rust has become an annual problem in Southern Alberta since the late 1990s (Su et al., 2003). In this region irrigation is commonly used for late maturing soft white wheat production. Since 2000, this disease has been prevalent on the western prairies, thought to be triggered by high spring precipitation. This disease reduces grain yield and quality and there was an extensive epidemic in central Saskatchewan in 2005 (Tran and Kutcher,

2015). This has led the research and farming community to search for effective control strategies. The fungal pathogen *Puccinia striiformis* causing stripe rust is an obligate biotrophic parasite, i.e. a living host is needed to consistently absorb water and nutrients to support sporulation (Chen et al*.,* 2014). Continuous sporulation spreads urediospores to adjacent fields, increasing disease severity. Urediospores travel by wind across the USA - Canada border from the Pacific Northwestern region of the USA to Southern Alberta. Nevertheless, the teliospore stage is capable of overwintering in Alberta (Conner et al*.,* 1988). Infection occurs any time after landing on green leaves and interferes with the photosynthetic efficiency of the wheat plant. Long and narrow stripes along the leaf veins are initiated from the accumulation of tiny yellow to orange-colored rust pustules on leaves two weeks after infection. Necrosis occurs if infection occurs at the adult stage, generally after stem elongation (Chen, 2005; McCallum et al., 2006). Pre-maturing ripening of wheat heads resulting from infection is directly associated to grain yield loss. The pathogen further infects wheat seeds during grain filling, which will not be suitable for seeding the next year due to poor performance after emergence. Disease severity can be 100% if infection of a susceptible cultivar occurs at the seedling stage without effective control management during the growing season (Chen 2005).

Moisture, temperature and wind are three major environmental factors affecting stripe rust development. In general, the development of stripe rust favors cooler temperature and humid conditions in comparison with other rusts. It has mainly been found in temperate regions and areas of high elevation in tropical climates (Chen, 2005). High moisture promotes urediniospore germination and infection while it adversely affects spore survival due to reduced viability and spore dispersal, as clusters increase in size with increased relative humidity (Rapilly 1979). Accordingly, frequent occurrence of stripe rust would be predicted in a region favouring dew formation. Temperature plays an important role at almost all stages of disease development, including urediniospore germination and infection, latent period, sporulation, spore survival and host resistance (Chen, 2005). Infection occurs more frequently at night because of the relatively lower temperature and associated dew formation. In addition, new races adapted to warmer

climates have been identified based on doubled spore germination rate and shortened latent period at increased temperatures from 12˚C to 18˚C (Milus et al., 2006). Although prevailing wind patterns affects the spread patterns of stripe rust, it is not a limiting factor on spore dispersal within a small area with respect to the presence of local inoculum. More importantly, wind reduces relative humidity, which in turn extends the duration of viability of urediniospores during widespread epidemics (Chen 2005).

P. striiformis is assumed to be an autoecious microcylic pathogen, despite similarities with other cereal rust fungi (Chen et al., 2014). Therefore, the dikaryotic (n+n) urediniospore is the dominant asexual stage of pathogen populations on its primary hosts - including common wheat (*Triticum aestivum* L), durum wheat (*T. turgidum* var. *durum* L.), cultivated emmer wheat (*T. dicoccum* Schrank), wild emmer wheat (*T. dicoccoides* Korn) and triticale (*Triticosecale*) (Chen, et al., 2014). Urediniospores are responsible for infection and epidemic spread, which will turn into the overwintering stage as teliospores later in the season (Chen et al., 2014). The alternative host of *P. striiformis* has never been identified until Jin *et al.* (2010) observed the telial infection on *Berberis* species and the successful infection on wheat leaves by resultant aeciospores in June 2009. This valuable finding suggests that pathogen variability could possibly be contributed by sexual hybridization, in addition to stepwise mutation and somatic hybridization reported in previous studies (Stubbs, 1985). The presence of *Berberis spp.* allows the pathogen to complete its sexual cycle by undergoing genetic recombination, which accelerates the development of new races that will soon be capable of rendering current resistance genes ineffective in available wheat cultivars (Jin et al., 2010).

A better understanding of race virulence along with the distribution and frequency of virulent pathotypes is critical for successful management of stripe rust. An initial study of pathogen specialization and virulence in Canada was documented by Newton et al. (1933), who collected two physiological races from the east border region of Saskatchewan. Virulence of *P. striiformis* in western Canada has been studied by Su et al. (2003). Thirty nine of 57 isolates (isolates collected before 2000 originated from Creston B.C whereas isolates collected during 2000 and 2001 were

from central Alberta, southern Saskatchewan and southern Manitoba) collected from 1984 to 2002 were identified as having virulence on 24 spring wheat lines (17 differential series containing *Yr* genes plus 7 supplemental lines of local interest or possessing resistance genes that are different from those of world and European differentials) (Su et al., 2003). As only races collected from southern British Columbia (Creston) before 2000 are virulent on 'Lee' (*Yr7, Yr22, Yr23*) and 'Owens' (*Yr*7), which were reported in the Pacific Northwest in 1996, 1998 and 2000 (Line and Chen, 1997; Line et al., 1999; Chen et al., 2002) researchers suggested that races of stripe rust identified in BC may originate from common inoculum for certain races identified in the Washington basin (Line and Qayoum, 1991). The virulence spectra of races on the Canadian prairies since 2000 shares much similarity with races found in the Rocky Mountains in the USA, but has consistently less variation (Chen et al., 2002). Specifically, new races revealed in this study have virulence on 'Compare' (*Yr8, Yr19*) and 'Clement' (*Yr9, YrCle*).

More recent studies on the virulence dynamics of *P. striiformis* primarily in central Alberta provides valuable resources that potentially could be useful for selecting effective resistance genes in breeding. Of 38 isolates of *P striiformis* collected in fields from 2007 to 2008 on one hand, which was further grouped into 13 pathotypes subject to wheat differentials. Wheat lines containing any of *Yr1, Yr5, Yr15* and *YrSP* show complete resistance to all 13 pathotypes among R genes used in this study; *Yr10, Yr24* and *Yr28* were avirulent to around 90% of the isolates, while wheat lines having *YrA, Yr2, Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr31* and *Yr32* (*YrCV)* are susceptible to 71-100% of the isolates (Kumar et al. 2012). The low frequency and instability of virulence to *Yr1* and *YrSP* suggests that these races are not prevalent in western Canada, and this may be related to lack of selection pressure (Brar and Kutcher, 2016). The disease resistance of *Yr24,* also known as *Yr26* (Li et al., 2006), is achieved by restricting the uredial growth on leaves. Most races are avirulent to *Yr10* with exception of races detected in southern Alberta in 2010 and 2011. This gene has been deployed in the winter wheat 'Radiant' (Kutcher et al., 2012). Increased selection pressure on this gene could result in larger populations of virulent races. On the other hand, recombinant races are capable of overcoming race-specific adult plant resistance genes,

including *Yr2*, *Yr6, Yr7, Yr8, Yr9, Yr17, Yr32* (*YrCV),* resulting in high disease level (Hovmøller et al., 2016). Additionally, the effectiveness of *Yr17*, a seedling resistance gene is dependent on external temperature. The increased virulence frequency of this gene has been attributed to high temperatures and the presence of high selection pressure possibly due to the tight linkage of stem rust and leaf rust resistance genes *Sr38* and *Lr37* (McCallum and DePauw, 2008). The breakdown of the *Yr27* gene, which was deployed in Western Asian and African wheat cultivars, contributed to the severe epidemics in 2010 in those regions (Xi *et al.,* 2015). A follow-up study of Kumar et al. (2012) on virulence phenotyping of *P. striiformis* on wheat was conducted by Holtz *et al.,* (2013) with more isolates collected from 2009 to 2011 and more host resistance genes examined (Holtz et al., 2013). In their study, 67 isolates were classified into 12 pathotypes by 25 wheat differentials. Wheat lines containing *Yr5* and *Yr15* were resistant to all pathotypes. Resistance genes *Yr1*, *Yr10*, *Yr24*, *Yr28*, *YrTyee* and *YrSP* protected crops from 80–98% of the isolates. These results pointed to the rapid erosion of seedling resistance genes due to new evolved virulence, and the authors suggested that gene pyramiding could be an effective to strategy to provide durable crop protection against this pathogen (Chen et al., 2010).

Current cultivars carry resistance genes that are classified into two main classes: race specific resistance genes and race non-specific resistance genes. Race specific resistance genes are effective at all stages of plant growth and are prone to breakdown by the emergence of new races owing to host selectivity (Singh et al., 2014). In contrast, resistance conferred by race non-specific genes is durable over a longer period due to its slow rusting mechanism (Caldwell, 1968). As these genes only exhibit resistance in the adult plant stage, it is also known as adult plant resistance gene (APR) (Singh et al., 2014). However, environmental conditions including external temperature and soil temperature affects the effectiveness of some APR genes (Randhawa et al., 2011). According to Fetch et al., (2011), most Western Canadian cultivars are susceptible to partially resistant to stripe rust. Adult plant resistance (APR) gene *Yr18*, which was originally deployed in the Brazilian cultivar Frontana (Kolmer et al., 2008; McCallum and DePauw, 2008), appears to be the primary stripe rust resistance source in these cultivars by increasing the latent period and

reducing infection frequency and stripe length (Singh et al., 2004). In field conditions, the intermediate to moderate resistance level (i.e. the average disease severity of cultivars having only *Yr18* varies from 1% to 15%) contributed by this gene is not 100% effective in protecting crops from losing grain yield under high disease pressure. Breeding programs started incorporating multiple slow rusting genes (equal or greater than three) into cultivars with the *Yr18* gene to extend the resistance longevity as well as to achieve adequate resistance levels. In terms of available resistance genes in CWRS wheat germplasm, *Yr17* and *Yr36* in addition to the predominant *Yr18* gene, are present in a few cultivars and do result in greater resistance levels (Randhawa et al., 2012). Moreover, the CWRS cultivar 'Lilian' carrying both *Yr18* and *Yr36* shows excellent resistance to stripe rust with an average disease severity of 0.5%, while the slightly higher average disease severity of cultivars like 'Muchmore' with only *Yr18* is 1.5% (DePauw et al., 2011). Strong additive interaction would be expected to improve resistance against stripe rust in a CWRS wheat cultivar carrying all three *Yr18, Yr17* and *Yr36* genes.

Incorporation of alien slow rusting genes or genes from close relatives into adapted backgrounds is considered as an effective strategy to improve genetic variability. In one study, the dual APR genes *Lr53/Yr15* were identified in the tetraploid wheat relative *Triticum dicoccoides* that conferred seedling resistance to leaf rust and stripe rust (Marais et al., 2005). Interestingly, homozygous resistant plants derived from the cross between tetraploid emmer wheat and common bread wheat have a normal phenotype without losing reproductive capability. Similar to *Lr34/Yr18*, slow rusting genes *Lr67/Yr46* have also been identified from wheat lines derived from Thatcher's genetic background ((Herrera-Foessel et al., 2014) and thus becomes a valuable source for combination with other minor R genes to develop high levels of durable resistance to stripe rust (Herrera-Foessel et al. 2011). Additionally, the challenging approach of transferring APR genes from wild relatives including *Aegilops neglecta* (three awn goat grass) to a common wheat chromosome through conventional breeding has been used in resistance breeding programs. Marais et al., (2009) reported the *Yr42* gene in this wild relative, which provides effective seedling resistance to South African races of stripe rust when transferred onto wheat chromosome 6A

(Marais et al., 2009). Adapted cultivars carrying this gene introgressed from *Aegilops neglecta* are expected to maintain yield potential under disease pressure of new virulent races.

The combination of slow rusting genes, however, does not always improve the effectiveness of resistance. One study indicated that the combination of *Yr18, Yr29* and *Yr46* did not exhibit additive effect against rust infection (Herrera-Foessel et al., 2011). A possible explanation is that the low disease intensity is primarily contributed by the presence of *Yr18* so that the resistance effect of other APR genes could not be detected. It is necessary to characterize other slow-rusting resistance loci to enable further decisions on which genes in combination would give rise to a stronger synergistic effect against rust infection, prior to conducting APR gene incorporation.

1.2.2 Stem Rust (*Puccinia graminis* f. sp. *Tritici*)

Stem rust (*Puccinia graminis* f. sp. *Tritici*) caused a series of severe epidemics in spring wheat fields in Manitoba, Saskatchewan and east-central Alberta in the early 1900s, resulting in grain losses greater than 5.5 million t each year from 1953 to 1955 (Peturson, 1958). Abundant rust spores overwinter in the south-central U.S. The long-distance dispersal mechanism allows spores to rapidly spread northward on winds and eventually enter the Canadian prairies during the cropping season. Infection occurs once environmental conditions are conductive to rust development. Complete crop loss can happen to a seemingly healthy-looking susceptible cultivar three weeks prior to harvesting in the presence of this pathogen (Peturson, 1958). Obvious disease symptoms don't appear on plant stems immediately. They appear one to two weeks after the initial infection when explosive buildup of reddish brown color urediniospores underneath the host epidermis finally rupture the surface layer and forms oblong pustules throughout the whole stem. Elongated pustules interrupt the translocation of water and photosynthate flow from roots and leaves to the developing heads during filling period, resulting in shrivelled kernels. Additionally, infected plants are prone to collapse owing to weakened stem strength and enormous water loss (Roelfs et al., 1992; Fetch et al., 2011).

The fungus *Puccinia graminis* is the causal agent of stem rust of wheat. This obligate biotroph is heteroecious and requires a primary host from the *Poaceae* family and an alternative host from the *Berberis* genes to complete its full life cycle (Leonard and Szabo, 2005). In temperate regions like western Canada, the main inoculum source is urediniospores that are either dispersed over great distances via wind currents from south-central U.S. or produced on infected volunteer wheat from previous years (Fetch et al., 2011; Schumann and Leonard, 2000). Successful establishment of the obligate relationship between urediniospores and the host, followed by accelerated regeneration of urediniospores under warmer conditions (between 25˚C -30˚C), enables wide spread of this disease to adjacent wheat fields in the same growing season. When temperatures start to decrease at the end of growing season, urediniospores are replaced by black resting spores known as teliospores produced from blister-like pustules on the primary host, which are capable of maintaining viability by undergoing dormancy under mild winter conditions. The following spring, when entering the sexual stage of the lifecycle, mature haploid basidiospores are the end product of teliospore germination, and they can be carried by prevailing wind to infect the alternative host *Berberis* spp (Leonard and Szabo, 2005). Infection takes place on the upper epidermis of barberry leaves, producing flask shaped pycnia. Pycniospored are self-incompatible male gametes. Hence, they have to fuse with pycniospores from a different pycnium in order to generate a cup-shaped dikaryotic aecium that breaks open from the lower surface of barberry leaves when mature. Aeciospores produced from aecium serve as initial spore inoculum that exclusively infect the primary host at early growth stages (Roelfs, 1985). Dark orange lesions on wheat stem are uredia. Subsequent infection caused by urediospores dispersed from uredia from this point onwards is the asexual stage of the pathogen lifecycle.

The presence of *Berberis spp.* is crucial for the sexual stage of the *P. graminis* lifecycle due to three facts: (i) it allows the pathogen to survive the following year by providing a host for basidiospores produced from overwintering telia, (ii) it enables the development of local initial inoculum through the production of aeciospores that can directly infect wheat seedlings, resulting in large yield losses, and (iii) it plays a key role in generating new virulent races through

hybridization between strains (Fetch et al., 2011; Leonard and Szabo, 2005). Therefore, the eradication of this alternative host is critical to control this disease - not only by eliminating genetic variation of the pathogen but also by reducing the population of primary spore inoculum to minimize the risk of initiating early, regional epidemics. In the United States, a barberry eradication program funded by federal-state governments was efficiently carried out between 1918 and 1980. After over 500 million barberry bushes were destroyed from the proximity of wheat fields in major wheat-producing states, a 10-day delay in the onset of disease has been reported, along with the reduction of pathogen genetic viability. This has resulted in the stabilization of pathogen races over the last half century (Roelfs, 1982). A study on the 'effect of Barberry eradication on changes in populations of *Puccinia graminis* in Minnesota' conducted by Peterson et al. (2005) suggested that the diversity of aerial collection has greatly declined during the Barberry eradication program and has remained constant for over 90 years. Thus, the elimination of much of the barberry population diminishes the potential of developing uncontrollable epidemics or an outbreak of new pathogen races in North America. Despite the successful achievement of supressing the primary inoculum of stem rust, an active sexual population composed of 16 races in a relatively small area between the states of Washington and Idaho was collected from a single wheat field in 2009 (Jin 2011). The differentiation of these races was conducted by evaluating their virulence/avirulence response to six resistance genes: *Sr5, Sr7b, Sr8a, Sr9a, Sr9d* and *SrMcN* (Jin, 2011).

Growing disease resistant cultivars is an important strategy to protect yield potential from disease epidemics. The evolution of breeding for stem rust resistance in Canadian wheat started with the release of 'Thatcher' in 1939, a cultivar carrying complex stem rust resistance genes, including *Sr5, Sr9g, Sr12*, and *Sr16* and other unidentified genes (Knott, 2000). The buildup of intense selection pressure by extensively growing 'Thatcher' in western Canadian prairies led to the breakdown of durable resistance against race 15B (Peturson, 1958). The cultivar 'Selkirk' with *Sr2, Sr6, Sr7b, Sr9d, Sr17* and *Sr23* was released to replace 'Thatcher', which had been predominantly grown in commercial wheat fields on the eastern prairies where the rust epidemics

were most severe. Stem rust populations have decreased in terms of race diversity and remained asexual; this can be attributed to growing resistant cultivars in combination with barberry eradication in the United States and Canada (Roelfs, 1982). In addition, all current cultivars in the dominant class Canada Western Red Spring (CWRS) are resistant to stem rust, yet the genetic combinations remained unknown in most cultivars (McCallum *et al.,* 2007). One exception is 'Neepawa' (with *Sr5, Sr7a, Sr9b, Sr12* and *Sr16*) (Kolmer et al., 1991), a cultivar derived from 'Thatcher', which is present in the pedigree of most of the current wheat cultivars. Another major resistance gene *Sr6* identified in some CRWS wheat cultivars is effective against all races including RCRSK, QFCSR, and QCCJN in the current rust population predominantly present in Western Canada (Fetch and Dunsmore, 2004).

Stem rust remains a devastating threat worldwide to wheat production; even though epidemics of other two rust diseases (leaf and stripe rusts) have occurred more frequently. The new race *Ug99* of stem rust that emerged in Uganda in 1998 (Pretorius et al., 2000) has a broad spectrum of virulence to most resistance genes of wheat origin including gene *Sr38*, which has been introduced into several European, Australian and new CIMMYT wheat germplasm lines from *Triticum ventricosum* (Jin *et al.,* 2007). *Ug99* is also commonly known as TTKS, designated by Wanyera et al., (2006) using the North American nomenclature system (Roelfs and Martens, 1988), and was recently updated to TTKSK after the fifth differential has been characterized (Jin et al., 2008). The unique virulence combination of *Ug99* becomes more complex as new resistance genes have continuously been detected to lose effectiveness against this race, raising concern of the potential wide spread of this race that may put susceptible wheat germplasm worldwide at great risk. Therefore, precise prediction of the migration path of race *Ug99*, given the pathogens dispersal mechanism, is of vital importance, in addition to improving genetic resistance (Jin et al. 2008). Studies into the presence of race *Ug99* have been conducted in other East African countries surrounding Uganda, and reports suggest that this race has successfully established on east African highlands, also regarded as "hot-pots" (Singh et al., 2006). Given that the availability of susceptible wheat cultivars all year coupled with optimum environmental conditions promotes

fungal development as well as rapid buildup of pathogen population, Singh et al., (2006) hypothesized that one potential route of urediniospores movement is from East Africa to Middle East to West Asia then to South Asia following the direction of prevailing air currents. It's clear that pathogen movement prediction studies involve variable levels of considerable uncertainty, and the establishment of improved spatial modelling is also required for accurate prediction of detailed pathogen movement. By acting as an early warning, results from prediction alerts local researchers collaborating with governments to develop accurate monitoring in advance, in order to alleviate great economic losses (Singh et al., 2006).

In Western Canada, the *SrCad* gene has been identified in the CWRS cultivar 'Peace' that confers strong seedling resistance level to race *Ug99*/TTKSK with the presence of *Lr34/Yr18* (Hiebert et al., 2011). This gene is located on chromosome 6DS, closely linked with *Bt10* in coupling mapped by the PCR marker FSD_RSA for common bunt resistance (Ghazvini et al., 2012). 'Peace' was registered as an early maturing cultivar in 2002 that is suitable to grow in areas with short growing seasons (Humpherys et al., 2014). It carries the gene *Lr34/Yr18* that is resistant to leaf and stripe rust. It also has the gene *Bt10* that is resistant to common bunt (Humphreys et al., 2014). According to a study conducted by Hiebert et al., (2010), the lowest disease severity was attributed to the combination of *SrCad* and *Lr34*. Additionally, this study revealed that *Lr34* was strongly correlated with stem rust resistance at both the seedling and adult plant stages. It specifically conferred seedling resistance to races TPMK and RKQQ. *Lr34* is a major leaf rust resistance gene that also confers partial resistance to a broad spectrum of diseases including powdery mildew (Spielmeyer et al., 2005). One hypothetical explanation is that *Lr34* acts like an inhibitor to a suppressor of stem rust resistance on chromosome 7D (Vanegas et al., 2008; Kerber and Aung 1999). This suppressor is commonly found on the D genome of hexaploid wheat, which is possibly donated by *Triticum tauschii* (Kerber 1983). The superior stem rust resistance is only detected when the suppressor locus is mutated or eliminated. The expression of *Lr34* as a gene of suppressor allows the expression of rust resistance possessed by other genes, which is similar to the elimination or mutation of the suppressor locus (Dyck 1987). Overall, the discovery of *SrCad*

in ‘Peace’ represents valuable genetic information that could be used for developing an *Ug99*-resistant elite cultivar.

In addition, race-specific resistance genes originating from close relatives of wheat are available for incorporation into adapted cultivars with premium agronomic traits. Although *Sr25* has been observed to express a sufficient level of disease resistance in few genotypes, the virulence to gene *Sr25* has not been discovered yet (Singh et al., 2006). This gene is tightly linked with *Lr19*, translocated on chromosome 7DL from *Thinopyrum elongatum* (Singh et al., 2006). *Sr25* exhibits adequate resistance against stem rust especially when *Sr2* is present, with the advantage of yield potential enhancement. It has not been widely accepted in breeding programs because of its association with the accumulation of yellow pigment on the plant (Singh et al., 1998; Reynolds et al., 2001). *Sr26*, another slow-rusting gene also originating from *Thinopyrum elongatum,* has been transferred into Australian germplasm to achieve greater protection from stem rust. Yet its large-scale deployment may be associated with a yield penalty (The et al., 1988). Systematically incorporating race-specific genes into susceptible yet important cultivars is the fastest way to combat the race *Ug99* (Singh et al., 2006). The dilemma in current breeding programs is to combine more than two translocations into a single genotype by being aware of the undesirable impact on grain yield and quality associated with these genes (Singh et al., 2006). A potential solution is to repeatedly backcross with adapted cultivar at each generation, followed by selection of outperforming progenies while targeting resistance genes with the assistance of molecular markers (Singh et al., 2006).

1.2.3 Leaf Rust (*Puccinia triticina* Eriks)

Leaf rust is the most widely distributed of the three rust diseases and it has become an annual production problem caused by *Puccinia triticina* Eriks in western Canada. The damage caused by leaf rust tends to be less destructive in comparison with that of stem or stripe rust, yet the higher frequency and widespread occurrence of this pathogen results in more significant economic losses over greater geographic areas. This disease is prevalent, especially in eastern

Saskatchewan and southern Manitoba, resulting in 5-25% annual yield loss from 22% flag leaf infection (Huerta-Espino et al., 2011; Fetch et al., 2011). The epidemic severity varies from year to year, depending on three factors: (i) environmental conditions favored for rust development, (ii) the growing stage of the crop at the time of disease onset, and (iii) susceptibility of the cultivar. The leaf rust pathogen *Puccinia triticina* attacks mainly the foliar region of the crop, forming ovoid uredinia on both upper and lower surface of the leaf without developing chlorosis or necrosis (Bolton et al., 2008), to interfere with photosynthesis efficiency. Reduced amount of photosynthate produced from leaves hence is not adequate to support grain filling, resulting in shriveled kernels and decreased number of kernels per head at harvest.

Puccinia triticina is a macrocyclic and heteroecious rust fungus requiring a primary host (primarily wheat) and a taxomically unrelated alternative host (*Thalictrum speciosissimum* or *Isopyrum fumaroides)* to complete its lifecycle through five spore stages (Bolton et al., 2008). The host range of this pathogen is fairly narrow due to a high degree of telia host specificity. More specifically, as infection on wild grasses by primary inoculum of leaf rust has never been identified, hexaploid wheat becomes the major primary host for pathogen to complete the asexual cycle (Roelfs et al., 1992). In terms of alternative host, the pathogen rarely undergoes the sexual stage of its lifecycle as the native species in North America are relatively resistant to basidiospore infection (Jackson and Mains, 1921; Saari et al., 1968). Therefore, lack of suitable alternative host makes it impossible for the pathogen to complete the sexual cycle, suggesting that the evolution of new virulent races of *Piccinia triticina* is more likely contributed by mutation with/without the presence of selection pressure, rather than genetic recombination. Nevertheless, the general lifecycle of leaf rust is not different than the other two rust diseases. The production of asexual urediniospores takes place on a primary host (Dolton et al., 2008). Mature urediniospores are able to re-infect healthy leaves from adjacent crops in the presence of free water at a temperature range of 10˚C to 25˚C. Black dikaryotic teliospores replace urediniospores produced from uredinia as infected crops approach maturity and remain dormant over winter. Production of basidiospores resumes when conditions are suitable, followed by subsequent pycnial and aecial development on

the alternative host. Once the aecium erupts through the surface layer of host leaves, aeciospores are disseminated by wind to infect hexaploid wheat. The asexual stage (i.e., production of urediniospores) cycles multiple times throughout the growing season.

It is clear that knowledge of dynamic shifts in the virulence of *P. triticina* populations to resistance genes as well as new virulence phenotypes of the pathogen helps breeders make informed decisions for developing wheat cultivars with effective resistance to leaf rust (Kolmer 1996; McCallum et al. 2010). McCallum et al. (2010) conducted a virulence survey to monitor changes in the *P. triticina* population in Canada. Of 381 single-pustule isolates collected in the wheat fields across from Canada, five virulent phenotypes (MBDS, MBBJ, MBGJ, MLDS and TDBJ respectively) were identified from 11 isolates collected from British Columbia and Alberta and 12 virulence phenotypes were identified out of 323 isolates collected from Saskatchewan and Manitoba. MLDS was the new virulent phenotype detected in this survey, exhibiting avirulence to *Lr9*. Corresponding primary inoculum with this virulent phenotype could have blown from the northern United States to Canada (Samborski 1985). Overall, TDBG retained its status of the most common virulent phenotype in western Canada with a frequency of 61%, followed by TDBJ (15.2%), MDPS (5.3%) and MDLS (5.3%) as the most isolated virulent phenotypes. Resistance genes *Lr1, Lr2a, Lr2c, Lr3, Lr24* and *Lr10* have been ineffective against leaf rust in this region due to substantially increased frequency of virulence (over 80%) in the pathogen population over a decade. Nonetheless, the resistance genes including *Lr18, Lr19, Lr21, Lr22a, Lr25, Lr29, Lr32, Lr34 and Lr35* exhibit effective resistance in controlling this disease.

The evolution of leaf rust resistance breeding in Canada started with the release of the initial resistant cultivars ‘Renown’, ‘Regent’ and ‘Redman’ in the 1930s. The resistance exhibited by these cultivars is attributed to the gene *Lr14a*, derived from the cultivar ‘Hope’ (Martens and Dyck, 1989). ‘Selkirk’ was another cultivar carrying resistance genes to both stem rust and leaf rust, and was a dominant cultivar when severe epidemics occurred in the mid 1900s. It carried *Lr10, Lr14a* and *Lr16* that protected the crop from severe disease damage (Samborski 1985; Martens and Dyck, 1989). Virulence to these three resistance genes developed very quickly due to

the intensive use of this single cultivar over time. From 1967 to 1993, the predominant cultivars 'Manitou', 'Neepawa', and 'Katepwa' replaced 'Selkirk' in wheat fields. The presence of *Lr13* in these cultivars only confers partial resistance against leaf rust. 'AC Barrie', was developed thereafter and it became the predominant cultivar because of its excellent agronomic characteristics (McCaig et al. 1996). It also carried *Lr13* and *Lr16*, yet virulence on both resistance genes has been detected. Increased frequency of virulence to *Lr16* made 'AC Barrie' moderately susceptible to leaf rust. The moderate resistance level conditioned by *Lr16* is still useful in Western Canadian wheat breeding programs; particularly being used in combination with other resistance genes, regardless of the existence of virulence for this gene remained in the pathogen population (McCallum et al., 2007).

Among effective resistance genes, *Lr34* is especially of importance as it not only provides adequate level of leaf rust control, but also exhibit effective resistance to a broad spectrum of biotrophic pathogens (Spielmeyer et al., 2005). This resistance gene is completely linked with a resistance gene Y*r18* to stripe rust (Singh 1992; McIntosh 1992), and is present in approximately half of the spring wheat cultivars in western Canada (Fetch et al., 2011). The origination of *Lr34* has been determined by Kolmer et al. (2008) in a study characterizing the resistance region of *Lr34/Yr18* in wheat germplasm on a molecular basis. The Brazilian cultivar 'Frontana', derived from a cross between a European cultivar 'Mentana' and a south American cultivar 'Frontiera' (Borghi, 2001) has been identified as the primary donor of *Lr34/Yr18* in wheat germplasm of CIMMYT and the United States. Subsequent cultivars arising from crosses with 'Frontana' serve as key founders to pass this major APR gene to modern high quality CWRS wheat cultivars (Kolmer et al., 2011).

Lr34/Yr18 confers durable resistance in a non-hypersensitive response manner through latent period extension and reduction in intercellular hyphal development after pathogen infection (Rubiales and Niks, 1995; Chen et al., 2016). While *Lr34* has been considered as race non-specific due to the predominant expression at the adult crop stage, plants with this gene have also been reported to have low disease severity at the seedling stage when the temperature is between 4˚C to

8˚C (Rubiales and Niks, 1995). Detection of the presence of *Lr34* can be achieved through phenotyping or with the assistance of molecular markers. The strong association with leaf tip necrosis owing to the tight linkage with *Ltn1* acts as a morphological marker to identify cultivars with *Lr34* in the field. Yet the accuracy of merely relying on this method is highly dependent on environmental conditions. As opposed to morphological markers, the application of molecular markers is independent of environmental variation (Collard and Mackill, 2008), efficiently facilitating selection of wheat lines carrying desirable target genes. Several options are available to detect the presence/absence of *Lr34.* Flanking SSR markers were initially applied to narrow down the position of this gene from the whole genome to chromosome 7DS. Lagudah et al. (2009) later developed a combination of dominant markers with extensive Restriction Fragment Length Polymorphism (RFLP) analysis to reveal the monomorphic patterns of the presence of the gene based on the variant size of the insertion in an intron. The codominant sequence tag site is 0.4 cM away from the *Lr34* locus. In addition, SNP markers are available to analyze the full *Lr34* sequence (Chen et al., 2016), which enables further studies on the allelic variations and haplotypes in wheat germplasm.

Singh and Rajaram (1993) stated that the durable resistance attributed by *Lr34/Yr18* has not been overcome by current virulence phenotypes of *P. triticina* and could be enhanced through combinations with 3 - 4 minor genes to protect grain yield from future genetic shifts in the pathogen. The incorporation of *Lr34/Yr18* with *Lr46/Yr29* has been observed to have a substantial enhancement of resistance on selected lines (Kuchel et al., 2007). Similar results occur with the combination of *Lr34* and *Lr13,* producing progeny with stronger resistance than either parent (German and Kolmer, 1992). The interaction among genes in a cultivar with multiple minor genes contributes to a synergistic effect on resistance improvement. Moreover, *Lr21*, a resistance gene originated from *A. tauchii* has been fully effective against all known races of leaf rust since the cultivar 'AC Cora' that has been released (Huang et al., 2003; Huang et al., 2009). However, the virulence to this gene has been recently discovered in Canada at a low frequency of 3.8% (McCallum et al., 2017). Another adult-plant leaf rust resistance gene *Lr22a* confers strong

resistance due to the low exposure to the virulent races of *P. triticina* in Canada and the United States (Hiebert et al., 2007). However, Canadian cultivars with this gene have only occupied a niche portion of wheat production areas from 1998 to 2006 (Canadian wheat board, http://www.cwb.ca). Therefore, the stacks of effective resistance genes such as *Lr21* and *Lr22* with *Lr34* promise the effective resistance of most modern cultivars to combat wheat leaf rust.

1.3 Pre-harvest sprouting

Wheat production contributes a large portion of overall annual farm value in Canada by supporting domestic flour, pastry, confectionary and pasta industries in addition to the export market (Clarke et al., 2005). The suitability for end-use application is not guaranteed when the premium quality of the wheat is lost due to biotic and abiotic stress. For example, bleaching impacts flour color resulting in increased hydrolytic enzymes activity, which is highly correlated with the negative alternation of functionality of the starch protein matrix for end-use product processing (Clarke et al., 2005). Therefore, damaged kernels will be downgraded or devaluated dependent on the predominant factor or multiple stress factors. The presence of abiotic stress during the wheat growing season and storage period can reduce test weight, increase bleaching and or result in pre-harvest sprouting (Clarke et al., 2005). Bread made from sprouted wheat flour shrinks in size and has a compact interior (Mansour 1993). An additional crop value of $34 million per year can be achieved (based on 2000-2001 crop year prices) by improving 20% of grade 2 Canada Western Red Spring wheat to grade 1. The estimated annual economic loss due to pre-harvest sprouting is around $100 million (DePauw et al., 2012).

As one of the most limiting factors for wheat grain quality, pre-harvest sprouting is common in wheat-growing regions and strongly associated with the climatic conditions (Shorinola et al., 2016). The term represents a physiological change inside the grain kernel at a relatively high humidity, i.e. the physiologically mature grain starts spouting as a result of dramatically increased α-amylase activity when extended rainfall occurs prior to harvesting (Martynov and Dobrotvorskaya, 2016). Falling Number indirectly measure the α-amylase activity that damages

starch quality during germination (Reddy et al., 1983), which assists in selecting genotypes expressing PHS resistance. Another factor critically influencing pre-harvest sprouting after seed maturity is seed dormancy at a very low level. Seed dormancy at very high level, on the other hand, prevents seeds from germination after being sown. Hence the degree of seed dormancy should be controlled to a desirable degree for wheat genotype development (Mares 1987; Kumar et al., 2015). In fact, measurements such as germination index, germination resistance and percent germination relied on dormancy level are commonly applied as phenotypic traits characterization to determine pre-harvest sprouting resistance (Rasul et al., 2009; Singh et al., 2010; Knox et al., 2005, 2012). Other phenotypic traits affecting the sprouting of wet seeds include presence of awns, erectness of spikes, openness of florets and concentration of biochemical compounds that regulate or inhibit germination (Paterson et al., 1989).

Many studies suggest a strong relationship between PHS and seed coat color (Groos et al., 2002), where red kernel wheat on average appears to resist PHS resistance better than white kernel wheat (DePauw and McCaig 1983; Himi et al., 2002). Wu et al. (1996) reported that the higher germination rate of white wheat kernels is due to oligo proanthocyanidin deficiency in the seed coat, which results in faster water absorption. On the contrary, greater amount of anthocyanin in red wheat cultivars may contribute a lower germination rate (Gao et al., 2013). The major difference between the two seed coat colors is the pleiotropic characteristics of the three genes controlling this trait, *R-A1, R-B1* and *R-D1* located cytogenetically on chromosomes 3A, 3B and 3D respectively (Sears 1944; Allan and Vogel 1965; Metzger and Silbaugh 1970). One or more of the three red alleles (*R-A1b, R-B1b* and *R-D1b*) are found in red kernel wheats whereas white kernel wheats carry homozygous recessive alleles at all loci represented as *R- A1a, R-B1a* and *R-D1a* (McIntosh et al., 1998; Flintham 2000). The biochemical mechanism of grain color in response to PHS resistance hasn't been completely understood as some studies have revealed that the three R genes also regulate the level of seed dormancy (Warner et al., 2000; Flintham 2000), whereas others claimed that the mechanism of seed dormancy is independent of grain color (DePauw and McCaig 1983). In addition to the coloration of the seed coat, the arrangement of

epidermal cells of the seed coat also affects resistance to PHS. Cultivars that have loosely arranged epidermal cells in the seed coat are prone to PHS in the presence of external water (Gao et al., 2013).

There are many other genes and quantitative trait loci (QTL) responsible for regulation and controlling of the seed dormancy and PHS resistance in addition to environmental factors and environment × genotype interactions. For instance, one transcription factor *VIVIPAROUS* (*Vp*) has homologs *Vp-1A*, *Vp-1B* and *Vp-1D* located on 3A, 3B and 3D homologous wheat chromosomes (Nakamura and Toyama, 2001). The transcription factor is expressed in the cytoplasm during kernel development, not only to influence embryo sensitivity to abscisic acid and seed dormancy, but more importantly to accelerate the process of seed desiccation and to suppress germination, expressed by other genes at the transcriptional level (Hoecker et al., 1995; Paek et al., 1998; Wilkinson et al., 2002; Divashuk et al., 2012; Gao et al., 2013). Divashuk et al. (2012) elucidated the correspondence of the allelic states and the expression level of *Vp-1B* to PHS tolerance in their study. According to the results taken from geographically different wheat germplasm, six allele types of *Vp-1B* are revealed as *Vp-1Ba*, *Vp-1Bb*, *Vp-1Bc*, *Vp-1Bd*, *Vp-1Be* and *Vp-1Bf*, respectively (Yang et al. 2007; Chang et al. 2010; Divasguk et al., 2012). More specifically, *Vp-1Bf* amplified as 616 base pairs in length, which is 84 bp longer than the wild allele *Vp-1Ba* (Chang et al., 2010*a).* A 109bp deletion was detected in *Vp-1Bf* relative to *Vp-1Bb,* whereas a four bp insertion was observed in *Vp-1Be* compared to *Vp-1Bc* (Chang et al., 2010*b*). Moreover, researchers observed lower germination index (GI) and greater PHS tolerance in Chinese wheat cultivars containing *Vp-1Ab* and *V-1Ad* (Chang et al., 2010*b*). Some studies also have confirmed the consistent correlation of two novel alleles *Vp-1Be* and *Vp-1Bf* with high seed dormancy and lower GI, and also suggested the potential of using *Vp-1B* as a molecular marker for PHS detection with respect of its rich allelic variation and association with white grain in Chinese wheat germplasm (Yang et al. 2007; Chang et al. 2010*a*; Chang et al. 2010*b*).

QTL/genes related to PHS tolerance/dormancy across mapping populations around the world have been detected with different types of molecular markers (Kulwal et al., 2010). QTLs

tend to share the same chromosomal locations across different populations indicating that similar QTLs are responsible for the PHS resistance (Ogbonnaya et al., 2008). However, the complex interactions between QTLs (Q x Q) and between QTLs and environment (Q x Q x E) become a major dilemma in genetic control of PHS (Kumar et al., 2015). Therefore, measuring the phenotypic expression under a wide spectrum of environmental conditions is necessary to identify the effects of corresponding QTLs. Phenotypic expression was well described in a study of identification of QTLs associated with PHS resistance (Kumar et al., 2015). Five to ten winter wheat spikes at physiological maturity were harvested per plot of a doubled haploid population that was developed from a cross between the Canada Prairie Spring AC Karma (PHS resistant) and the white-seed germplasm parent (PHS moderate susceptible). Germination index, germination resistance and germination percentage were then evaluated on these seeds under seven environments. After treated with 4.0–6.0 ml of 0.5 mM gibberellic acid (GA_3) solution for one hour, seeds failed to germinate were regarded as unviable and were excluded from the experiment (Kumar et al., 2015).

Genome-wide association study (GWAS) is an innovative approach that detects multiple genetic recommendation events which may be useful for wheat functional genomic research studies (Jia et al. 2018). GWAS has been conducted in common wheat studies by applying markers with diversity arrays technology (DArT). QTLs that have been identified in 717 Chinese natural populations (i.e. landraces) across ten various agroclimatic growing environments are associated with PHS resistance. The incorporation of these QTLs integrated with artificial selection has potential to maintain high levels of PHS resistance for sustainable crop improvement in the world (Zhou et al., 2017).

1.4 Organic agriculture

Organic production has been one of effective approaches to achieve agricultural sustainability in the long term, which strives to promote a balance among crop cultivation, animal welfare, social justice and the health of the ecosystem (Snyder and Spaner, 2010). Agriculture and

Agri-Food Canada summarizes the evaluation and definition of sustainability into four general principles: (i) biodiversity conservation and the protection of the natural resources including soil, water and air quality from degradation, (ii) economic and social well-being contribution, (iii) an ensured safe supply chain of high quality agricultural products, (iv) the security of agribusiness success as well as the well-being of the workers and their families (Snyder and Spaner, 2010). As an alternative to conventional production, organic agriculture supports crop production without the use of pesticides and the supplement of synthetic fertilizers. Instead, manure or compost application integrated with cultural and mechanical practices provide a relatively healthy environment favored by crop development. Reduced yield in comparison with conventional production systems is one the major undeniable disadvantages of organic production. Wheat grain production is 21-31% on average lower under organic than conventional management (Kitchen et al., 2003). Farmers are more likely to experience great yield loss in the transitional and early years of organic production (OACC 2008). In the Canadian prairies, soil phosphorous depletion along with the long-term impacts of intense tillage from organic production may lead to more severe yield loss and reduced grain quality (Entz *et al.,* 2001; Nelson et al., MacRae et al., 2007). Despite inherent limitations, the number of certified organic farms and market demand in Canada has been increasing steadily over the past few decades (Statistics Canada, 2016). It is important for researchers to focus on evaluating the sustainability contributed by organic management in the context of its agronomic and environmental aspects. Research may also help plant breeders make selection decisions by attempting to understand the needs of the whole organic management system (Wolfe et al., 2008).

Weed control, soil fertility and crop rotations are the major concerns in organic production as this production system relies on non-chemical agronomic techniques. Without herbicide application, the presence of weeds especially at early crop growing stage can be very competitive against crop establishment, due to its greater abundance and species diversity in an organic field (Mason and Spaner, 2006). Two major weeds that appear on organic farms on the Canadian prairies are wild mustard (*Sinapis arvensis* L.) and Canada thistle (*Cirsium arvense* L.) (Entz et

al., 2001). In addition, nutrient deficiency more like caused by existing weeds reduces grain yield as the soil nutrient level is overall lower at the beginning of the growing season in organic systems (Barberi 2002, Entz et al., 2001). One of the common mechanical weeding methods is pre-seeding tillage. This application is able to control small-seed weeds prior to crop emergence (Snyder and Spaner, 2010). However, due to the heavy disruption of soil structure from tillage, the organically managed land is prone to lowered moisture retention and a higher risk of soil erosion (Lafond and Derksen, 1996). Additionally, the prevalence of weed populations is correlated to tillage intensities, i.e. more biennial and perennial weeds are found in the land with reduced tillage while annual weeds are mostly observed in conventional managed lands with no-tillage or directly-seeding systems (Blackshaw et al., 2005). Increasing the recommended seeding rate is another common cultural practice in organic management system. It not only enhances the crop competitiveness but also results in approximately 10% of grain yield increase, on average (Mason et al., 2007).

Soil fertility can limit production in organic systems. Maintenance or build-up of adequate soil organic matter is important to supply nutrients and promote healthy microbiological activity in the soil (Canadian General Standards Board, 2018). Phosphorous deficiency has been reported in organic farms in the Canadian prairies with sufficiency of other nutrients including nitrogen, potassium and sulphur (Entz et al., 2001). Therefore, effective soil management favors mycorrhiza fungi populations, which establishes mycorrhizal colonization of plant roots to enhance phosphorous availability as soil phosphorous is not available for direct crop uptake (Douds et al., 1997). More specifically, minimal tillage intensity lowers the risk of destruction of the mycelial network (Nelson and Spaner, 2010), which maintains sufficient level of mycorrhizal colonization for inorganic phosphorous uptake. Crop rotations are also extremely important for soil fertility. Including forage crops to extend crop diversity does not directly improve P availability, it improves the productivity of the subsequently growing crop by supressing weeds population, enhancing nitrogen fixation and through carbon sequestration (Entz et al., 2002).

The improvement of food systems through plant breeding programs involves three major steps: (i) the identification of farmer's needs, (ii) superior genotype selection, (iii) successful field

adoption of advanced cultivars. Participatory plant breeding methods can be applied prior to organic production (Kucek et al., 2017). It involves incorporation of clients' participation in the breeding process and decentralization. The former aids in the identification of needs in crop improvement by controlling the flexibility in the selection program, i.e. to allow changes of the length of selection process but also the cultivar adoption (Kucek et al., 2017). Clients play an important role in trait evaluation as the difference of clients' traits priority in comparison with that of plant breeders (Ashby 2009) assists in re-orientation of the breeding objectives to ensure relevant end products (Kucek et al., 2017). Decentralization indicates the use of multiple selection sites across broad environmental gradients. The objective is to select a cultivar that has consistent superior performance among a broad array of environments. The inconsistency of genotypic performance between selection and specific environment that has ideal growing conditions leads to inefficient or inconsistent results for farmers' fields (Ceccarelli 2015).

Organic wheat flour seems to have different nutritional, technological and functional properties compared to conventional wheat flour (Pontonio et al., 2016). Organic wheat kernels tend to have more micronutrients contributed by the application of organic fertilizers such as organic manure. Organic wheat specifically has higher levels of K, Zn and Mo while conventional wheat has significant higher amounts of Ca, Mn and Fe (Vrček et al., 2014). Macronutrients such as storage proteins are not accumulated as much in organic wheat flour due to possible nitrogen deficit or lack of fast-effect synthetic fertilizers. In particular, researchers have reported that organically grown wheat contains significantly lower protein and gluten content, but higher gluten strength and starch content, lower sedimentation and flour water absorption values, shorter dough stability time and lower loaf value in comparison with conventional wheat. White flour protein yield was significantly higher in conventionally grown wheat. However, moisture content, tenacity/extensibility index and falling numbers for detection of pre-harvest sprouting did not show any significant difference between two cropping systems (Mazzoncini et al., 2015; Rizzello et al., 2015). Another important parameter to assess nutritional quality is the protein digestibility of the wheat grain (Pontonio et al., 2016). Vrcek et al. (2014) reported that organic wheat has higher

protein digestibility than conventional wheat (Vrček et al., 2014). Moreover, organic wheat has a higher concentration of defence-related secondary metabolites to biotic and abiotic stresses as a result of the synthesis of carbon-containing compounds, possibly due to nitrogen limitations and higher micronutrient content of organic fertilizer (Brandt and Mølgaard, 2001; Rembiałkowska, 2007, Ibrahim et al., 2013).

Spring wheat is the most commonly grown crop under organic management across the Canadian prairies, although limited cultivars are developed exclusively for organic farming and haven't been used for large scale commercial production ((Macey 2010; Wiebe et al., 2016). Studies have suggested that directly selecting cultivars for organic farming systems results in 5-31% yield improvement compared to indirect cultivar selection from conventional farming systems, which potentially eliminates high-yielding lines (Murphy et al., 2007; Reid et al., 2009; Kirk et al., 2012). In addition to direct yield assessment after harvesting, common measures of yield physiology involving kernel number per unit area, kernel number and grain yield per unit of biomass at anthesis and harvest index provide a deeper insight of crop physiological responses to the organically farming conditions (Fischer and Kohn 1966; Doyle and fisher 1979). Among all the measures of yield physiology, kernel number per unit of biomass reflecting kernel production is the most significant in wheat as it is a sink-limited crop (Entz and Fowler 1990; Fischer and Edmeades 2010). In a combined analysis study of performance evaluation of advanced genotypes directly selected from organic breeding program, Wiebe et al. (2016) identified higher harvest index and grain yield, a higher yield efficiency determined by kernel number per unit of crop biomass at anthesis and greater kernel mass in organic lines while the conventional commercial cultivars have a higher average grain protein content. Yield improvement under organic management is a result of better assimilate partitioning at anthesis and crop maturity (Wiebe et al., 2016). Although organic wheat breeding programs are slowly making progress, resolving the main dilemma of organic farming, which refers to enhancement of the use of nutrient efficiency and weed competitiveness is a priority.

1.5 Research Objectives and null hypotheses

Three major objectives were involved in this thesis as described below:

1. Assess the field performance and post-harvest quality traits of a recombinant inbred line (RIL) population derived from the cross between two Canadian Western Hard Red Spring (CWRS) wheat cultivars 'Peace' and 'CDC Stanley' under conventionally and organically growing conditions and uncover the genomic regions associated with selected phenotypic traits that are management system specific.
2. Validate the presence of a major pre-harvest sprouting resistance quantitative trait loci (QTL) (*Qphs.usask-4A*) and its effects with phenotypic traits of breeding interest by screening 'Peace' x 'CDC Stanley' RIL population with three Simple sequence repeats markers including 'DuPw004', 'barc170' and 'wmc650'.
3. Examine the segregation of disease resistance genes *Lr34/Yr18* and *Lr37/Yr17* in the RIL population, evaluate their effects on disease resistance and phenotypic traits of breeding interest. Superior genotypes from marker assisted selection for multi-environment yield trial prior to official registration.

The null hypotheses tested corresponding to objectives were:

1. There is no difference for heritability and correlation in terms of agronomic and quality traits in tested RIL population between conventional and organically managed systems, therefore fail to conduct direct selection of suitable RILs for organic agriculture. No management specific quantitative trait loci could be identified for ten phenotypic traits.
2. The presence of pre-harvest sprouting resistance QTL (*Qphs.usask-4A*) has no effect on important agronomic traits in tested RIL population.
3. RILs carrying *Lr34/Yr18* and/or *Lr37/Tr17* do not differ in important agronomic traits or disease resistance of leaf rust, stripe rust, common bunt and tan spot compared with the lines lacking resistance genes, thus there is no outstanding lines eligible to enter official registration trial.

Chapter 2 Evaluating the phenotypic performance and associated QTLs of 'Peace' × 'CDC Stanley' RIL population under conventionally and organically management and the effect of *Qphs.usask-4A* pre-harvest sprouting resistance QTL on traits of breeding significance

2.1 Introduction

Agronomic traits requiring improvement in western Canadian wheat breeding programs include yield potential, lodging resistance, and early maturity to avoid frost damage (McCallum and DePauw 2008). End-use quality traits requiring improvement include protein content, gluten strength, milling yield, bread-making quality, resistance to pre-harvesting sprouting and specific criteria enhancement corresponding to each milling class (McCallum and DePauw 2008). Of nine milling classes in western Canada, Canada Western Red Spring (CWRS) CWRS is the most prevalently grown because of its adaptation to many growing conditions, high protein and advanced milling properties. As a result, cultivars of this class command premium prices in international trade and this class is preferred by a wide range of domestic manufacturers (McCallum and DePauw 2008). Over 90 years of CWRS cultivar development in Western Canada yield potential has increased 6-9 kg/ha per year on average (McCaig and Depauw, 1994). This rate of genetic gain has increased 0.74% since 1990 as a result of a combination of accelerated and more advanced breeding activities and breeding strategies (DePauw et al., 2007).

As an alternative to conventional production, organic agriculture supports crop production without the use of pesticides and the supplement of synthetic fertilizers (IFOAM-Organics International, 2017). Instead, manure or compost application, integrated with cultural and mechanical practices, provides a relatively healthy environment favored for crop development. Organic production has been an effective niche approach to achieve agricultural sustainability in the long term, as it strives to promote a balance among crop cultivation, animal welfare, social justice and the health of the ecosystem (Snyder and Spaner, 2010). In terms of the benefits associated with wheat production, organic wheat flour appears to have different nutritional,

technological (i.e. for bread making) and functional properties compared to conventional wheat flour (Mason and Spaner, 2006; Pontonio et al., 2016). Organic wheat kernels were reported to have higher levels of K, Zn and Mo than conventional wheat; and significantly less amounts of Ca, Mn and Fe (Vrček et al., 2014). Macronutrients such as storage proteins are not accumulated as much in organic wheat flour due to possible nitrogen deficit or lack of fast-effect synthetic fertilizers. However, reduced yield in comparison with conventional production systems is one the major disadvantages of organic production (Kamran et al., 2014a; Mason and Spaner, 2006). In particular, wheat grain production is 21-31% on average lower under organic than conventional management (Kitchen et al. 2003; Asif et al., 2015; Reid et al., 2009). Indirect selection for yield improvement and substantial quality attributes integrated with superior agronomic performance has been the most common breeding practice for organic management in western Canada; i.e. direct selection of target traits in a conventional environment other than in an organic environment in order to achieve higher selection efficiency (Asif et al., 2015; Reid et al., 2011).

Pre-harvest sprouting (PHS) refers to the in-spike germination of kernels at physiological maturity in response to relatively high humidity prior to harvest (Singh et al., 2012). As a major downgrading trait, the occurrence of PHS often leads to economic loss from a substantial reduction of grain yield, end-use quality and possibly seed variability for planting the following year (Derera 1989). Sprouted grains generally have low kernel weight and bread loaves made from the flour of germinated grain exhibit reduced functional quality; having sticky crumb, dark colored crust, large air bubble holes in the bread and such bread is difficult to slice (Singh et al., 2010). Therefore, breeding for elite wheat cultivars with PHS resistance is needed to save approximately $100 million annual loss for grain producers in western Canada under PHS favorable weather conditions (Clarke et al., 2005). Marker assisted selection based on the genetic information of identified quantitative trait loci that are associated with PHS resistance would improve the efficiency and reliability of conventional breeding methodologies (DePauw et al., 2012).

Linkage mapping is an effective population genetic tool for hexaploid wheat genomic studies, marker assisted selection and map-based cloning (Maccaferri et al., 2015). It exploits

markers tightly linked with effective quantitative trait loci (QTL) or genomic regions that are associated with traits of economic importance and adaptive improvements (Wen et al., 2017). Genetic mapping of hexaploid wheat has been improved through the use of single nucleotide polymorphism (SNPs) markers, due to low genotyping assay cost, abundant distribution across the genome, minor allele frequency (MAF), codominant inheritance with a low level of linkage disequilibrium, and locus specificity along with relatively low genotyping error rates (Cavanagh et al., 2013; Ganal et al., 2011; Rafalski, 2002; Schlötterer, 2004). High-density SNP chips that provide a better coverage of especially small chromosomal regions in the wheat genome have been developed from sets of informative SNPs, and to improve the detection of complex trait- associated QTLs (Cavanagh et al., 2013; Wang et al., 2014). Currently, the wheat iSelect platform comprising a sum of eighty-one thousand eight hundred fifty-seven (90K) gene-associated SNPs has been developed by Wang et al. (2014) to dissect the phenotypic from the genetic perspective in polyploid wheat populations (Maccaferri et al., 2015; Zou et al., 2017*a*). The wheat breeding program at University of Alberta has reported 14 and 16 minor- to moderate-effect QTLs associated with eight traits of agronomic importance in a recombinant inbred lines (RILs) population developed from a cross between 'Attila' x ''CDC Go', including tillering, days to flowering and maturity, grain yield, grain protein content, kernel weight and test weight in both conventionally and organically managed systems (Zou et al., 2017*a*; Zou et al., 2017*b*).

The first objective of the present study was to assess the field performance and post-harvest quality attributes of a recombinant inbred line (RIL) population derived from a cross between two Canadian Western Hard Red Spring (CWRS) wheat cultivars 'Peace' and 'CDC Stanley' under conventionally and organically managed growing conditions. Subsequently, we mapped genomic regions with a 90 K SNPs array associated with traits of breeding interest and investigated the presence of management system-specific QTLs in this population. Lastly, we screened the 'Peace' x 'CDC Stanley' RIL population with a QTL-specific simple sequence repeats (SSR) markers including 'DuPw004', 'barc170' and 'wmc650' to validate the presence of a major pre-harvest

sprouting resistance quantitative trait loci (QTL) (*Qphs.usask-4A*) and to examine its effects on phenotypic traits of breeding interest.

2.2 Materials and Method

2.2.1 Field phenotypic evaluation

The population of 168 RILs from the 'Peace' x 'CDC Stanley' mapping population, the two parents and six check CWRS wheat cultivars including AC Splendor (Fox et al., 2007), 'CDC Kernen', 'Parata' (Spaner et al. 2016), 'Go Early', 'Glenn' and 'PT472' were tested in conventionally and organically managed fields less than 500 m apart with the same weather conditions at the University of Alberta South Campus Crop research facility, Edmonton, AB Canada (53°29' N, 113°32'W) in 2016 and 2017. The organic land has been managed as such for the last 20 years using 3-year crop rotations consisting of experimental wheat (*Triticum astivium* L.), followed by cereal legume mixtures and peas (*Pisum sativum* L.) in sequence. All experiments were conducted as randomized complete block designs with sub-blocks nested in two blocks of replications. The experimental plots were four m long and 1.14 m wide consisting of six rows spaced 22.5 cm between rows. Viable seeds harvested from the previous year were sown at a rate of 300 seeds m^{-1} in May in both years with banded fertilizer (N-P_2O_5-K_2O: 11-52-0) applied at a rate of 40 kg ha^{-1} for the conventional trials. No chemical fertilizer was applied to the organically managed site. The soil nutrient levels of the organically managed soils were Nitrogen (N) 59 mg Kg^{-1}, Phosphorus (P) greater than 60 mg Kg^{-1}, Potassium (K) 310 mg Kg^{-1} and Sulphur (S) 14 mg Kg^{-1}. The organically managed soil also had a neutral soil pH of 6.6; 0.75 dS m-1(good) electric conductivity, 12.9% (high) organic content. Precipitation and temperature data during the growing season for each year were obtained from Environment Canada during growing seasons for both years (http://climate.weather.gc.ca/climate_data) (Figure 2-1). Chemical herbicide was only sprayed at the conventional site and followed standard local recommendations as described by Asif et al. (2015). We harvested both trials at the beginning of September in both years.

Phenotypic data was recorded for plant height, lodging scoring, days to heading and maturity, grain yield, thousand kernel weight and test weight. Days to heading was assessed as the day when 50% of the spikes in the plot were visible beyond the flag leaf. Days to physiological maturity was recorded from the day of sowing to the day when 50% of peduncles had turned yellow or brown (Mason et al. 2007). Plant height was measured from the base of plants to the tips of the heads excluding the length of the awns when stem elongation was complete. Grain yield on a plot basis was evaluated by converting into kg ha^{-1} after weighing the clean seeds that had been air dried at 76˚C for at least 24 hours. To calculate test weight, one pint (473ml) of clean grain samples from each plot was measured using a hopper and stand (Seedburo Equipment Co. Des Plaines, USA) and then weighted. Two hundred clean grains from each plot were counted using a digital seed counter (Agriculex Inc. Guelph, Canada) and thousand kernel weight was calculated as five times of the measured grain weight.

2.2.2 Post-harvest quality evaluation

The post-harvest quality tests we conducted in this study included grain protein content, sodium dodecyl sulfate (SDS) sedimentation, and falling number. Grain protein content was estimated by using Near Infrared Reflectance spectroscopy from a SpectraStar RTW apparatus (Unity Scientific SpectraStar^{TM} 2500, Unity Scientific Asia Pacific, Australia). Grain samples were first ground to flour on a 14 % moisture basis by using the Udy cyclone mill (UDY Corporation, Fort Collins, USA) fitted with a standard 0.8mm sieve in order to conduct SDS sedimentation and falling number tests. 2.5 ± 0.020 g of wheat flour per sample was soaked in 25 ± 0.2 ml of deionized water for 15 minutes following by 2.5ml of a stock solution of 30 g L^{-1} SDS in ~ 0.012 mol L^{-1} lactic acid for 20 minutes to read SDS sedimentation volume by following the standard SDS-lactic acid method 56 -70 (AACC 2000). 7 ± 0.020 g of wheat flour per sample was dispensed in 25 ± 0.2 ml of deionized water and thoroughly shaken to slurry by using the Shakematic automatic sample mixer (Shakematic 1905). Tubes with sample slurry were then transferred to the falling number apparatus (FN 1700, Perten Instruments) to be immersed in

boiling-water bath. The total time that the stirrer reached to the bottom of the tubes including the 60 seconds stirring time was recorded as the falling number value.

2.2.3 DNA extraction and Genotyping

Wheat leaf samples of the 168 RILs and parents for genomic DNA extraction were collected at the seedling stage, three weeks after greenhouse sowing. DNA extraction was conducted by following a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1987), and the DNA concentration was normalized to approximately 100ng μL^{-1} after being assessed with a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, USA). Genotyping of DNA samples with the 90K Illimina iSelect SNP platform (Wang et al., 2014) was done in the Wheat Genomics laboratory at the University of Saskatchewan, Saskatoon, Canada. Default clustering parameters from the Illumina Genome Studio Polyploid Cluster software version 1.0 (Illumina, San Diego USA) were initially applied to call allelic variants, where SNPs with more than three clusters were excluded from scoring. In regard to the complexity of wheat's polyploidy nature, the possibility of the observed polymorphism in the 90K due to off-target variants instead of actual nucleotide variants is sometimes difficult to determine according to the source sequence file (Perez-Lara et al., 2016). Therefore, additional filtering was required to select segregated SNPs in a biallelic pattern that were also recognized to be allelic from several mapping populations available to the programs (Perez-Lara et al., 2016).

The population was genotyped with three microsatellite markers that were linked to a major QTL (*Qphs.usask-4A*) for pre-harvest sprouting (PHS) resistance at the Agricultural Genomics and Proteomics Lab, University of Alberta, Edmonton, Canada. Polymerase chain reaction (PCR) analysis took place in 96 - well plates performed in a Veriti® Thermal Cycler (Applied Biosystems, Foster City, USA). The 12 µl of reaction mixture for PHS resistance QTL markers (DuPw004, barc170 and wmc650, respectively) (Singh et al., 2012) composed of 1X PCR buffer, 0.2 mM of dNTP, 0.2 µM of each of the forward and reverse primers, 1 unit GoTaq® Flexi DNA polymerase (QiaGen Toronto Canada) and 1µl of template DNA. The PCR condition started with 4.15 minutes

initial denaturation at 95˚C, followed by 30 cycles of 45 seconds denaturation at 95˚C, 20 seconds of annealing at 65˚C for DuPw004 (XDuPw004_F: 5'- GGT CTG GTC GGA GAA GAA GC -3'; XDuPw004_R: 5'- TGG GAG CGT ACG TTG TAT CC -3'), at 60˚C for barc170 (XBarc170_F: 5'- CGC TTG ACT TTG AAT GGC TGA ACA -3'; XBarc170_R: 5'- CGC CCA CTT TTT ACC TAA TCC TTT TGA A -3') and wmc650 (WMC650_F: 5'- AAA GCA AGA GCA GAC TGG C -3'; WMC650_R: 5'- GCA CAT CAG TAA CGC ATC TC -3'), 1.5 minute of extension at 72˚C, and finished with 10 minutes final extension at 72˚C (Singh et al., 2012). All amplified products were stored at 4˚C prior to visualization. The separation of amplified products was conducted on 2 % agarose gel containing 1.5% SYBR® Safe DNA Gel Stain (QiaGen Toronto, Canada). The amplification of gene expression was visualized under the fluorescent by using Typhoon Trio (GE Healthcare Life Science Quebec, Canada).

The two parents were also screened for polymorphism with genetic specific markers that were responsible for photoperiod response (*Ppd-B1* and *Ppd-D1*) (Beales et al., 2007), vernalization response (*Vrn-A1* and *Vrn-B1*) (Chen et al., 2013), and height-reduction (*Rht-B1*) (Ellis et al., 2002). All tested markers were monomorphic between 'Peace' and 'CDC Stanley'.

2.2.4 Statistical analysis

We conducted analyses of variance (ANOVA) for all traits, computed broad-sense heritability of each phenotypic trait and the phenotypic correlations among traits via multivariate restricted maximum likelihood (REML) using PROC MIXED and PROC CORR in SAS version 9.3.1 (SAS Institute Inc. Cary, USA). In the model of randomized incomplete block design, individual RIL line/genotype and organic or conventional management were considered as fixed effects to calculate the mean of each trait; whereas year, replication and blocks nested within each replicate were considered random for yearly data (1). In the two-year combined data analyses, year was also considered random (2). The linear models were:

For each year under each management:

$$Y_{ijkm} = \mu_i + G_{mi} + R_{ij} + R(B)_{ijk} + \varepsilon_{ijkm} \quad (1)$$

Where μ_i is the mean effect of the phenotypic trait i, G_{mi} is the effect of genotype/RIL line m on phenotypic trait i, R_{ij} is the random effect of block (replication) j on phenotypic trait i, $R(B)_{ijk}$ is the random effect of sub-block k nested within replication j on phenotypic trait i. ε_{ijkm} is the error term.

For two-year combined data under each management:

$$Y_{ijkmn} = \mu_i + G_{mi} + T_{ni} + G_{mi}*T_{ni} + T_n(R)_{ij} + T_n[R_j(B)]_{ki} + \varepsilon_{ijkm} \quad (2)$$

In addition to the symbols mentioned above, T_{ni} is the effect of year n on trait i, $T(R)_{ijn}$ is the effect of block (replication) j on trait i in year n, and $T_n[R_j(B)]_{ki}$ is the sub-block k nested within block (replication) j in year n.

For each year with combined management:

$$Y_{ijkmp} = \mu_i + G_{mi} + M_{pi} + (G*M)_{mpi} + R_{ij} + R(B)_{ijk} + \varepsilon_{ijkmp} \quad (3)$$

Where μ_i is the mean effect of the phenotypic trait i, G_{mi} is the effect of genotype/RIL line m on phenotypic trait i, M_{pi} is the mean effect of management (conventional or organic) p on trait i, $(G*M)_{mpi}$ is the interaction between genotype m and management p on trait i, R_{ij} is the random effect of block (replication) j on phenotypic trait i, $R(B)_{ijk}$ is the random effect of sub-block k nested within replication j on phenotypic trait i. ε_{ijkm} is the error term.

Broad-sense heritability was estimated by the following equation:

$$H = \sigma^2_G / (\sigma^2_G + \sigma^2_{GE} + \sigma^2_\varepsilon);$$

Where σ^2_G was the genetic variance among RILs, σ^2_{GE} was the variance of the interaction between RILs and environments of different years and σ^2_ε represented the error variance.

2.2.5 QTL detection and analysis

SNPs that presented monomorphic between 'Peace' and 'CDC Stanley' and those with greater than 20 % missing data including heterozygous calls were dropped from linkage mapping. The linkage map was constructed in two steps according to the statistical analysis described by Hussain et al. (2017). The first step was the binning of initial 7883 markers with an identical segregation pattern from adjacent bins by a single recombination event, constructed by

implementing the BIN tool algorithm in ICI mapping software v 4.1. Markers were then grouped with minimum logarithms of odds (LOD) scores ≥ 4. Linkage groups (LGs) were assigned to individual wheat chromosomes in accordance with existing consensus high density SNP maps of hexaploid wheat, where LGs from the same chromosome were merged and LGs with less than five markers were excluded for subsequent map construction. Marker ordering within LGs was done by using the RECORD (Recombination Counting and ORDering) algorithm, and Kosambi mapping function was applied to convert the recombination frequencies between markers into centiMorgans (Kosambi, 1943). The correction of genotyping errors and elimination of low-quality markers were performed with a recombination cut-off value of 0.35 along with a minimum LOD score of 3 by using MapDisto version 1.7.5 software (Lorieux, 2012) prior to the second step of linkage mapping, known as the refinement of linkage map. 4107 remaining SNPs were used for the construction of the final linkage map by using ICiMapping v 4.1.

Inclusive Composite Interval Mapping (ICIM) was generated according to the least square means of each trait, where the trait mean value was substituted for the missing value using a software QTL iciMapping version 4.0 (Meng et al., 2015), with the following specifications: a minimum LOD threshold of 2.5, a model to determine additive effects at individual QTL and additive x additive epistatic interactions automatic cofactor selection, F-to-Enter value of ten with scaning intervals of 1 cM (Semagn et al., 2007). QTL mapping thereafter was performed with the exclusion of all co-segregated SNPs that were mapped at the same location. The nomenclature of identified QTLs was assigned by following the International Rules of Genetic Nomenclature (http://wheat.pw.usda.gov/ggpages/ wgc/98/Intro.htm), which was comprised of trait acronym, lab designation (dms = Dean Micheal Spaner), and chromosme. In this study, the effect size of QTLs was classfied into three catogries (i.e. minor, moderate and major respectively) on an arbitrary basis corresponding to their proportional explaination (i.e. less than 10 %, 10 - 20% and greater than 20 %, respectively) of the total phenotypic variation (R^2) . MapChart v 2.1 (Voorrips, 2002) was used to construct genetic maps and QTL graphs.

2.3 Results

2.3.1 Analysis of phenotypic traits

On average over two years under conventional management 'Peace' matured 3 days earlier, was slightly (6 cm) taller, more lodging resistant, with greater thousand kernel weight (TKW) (2.4 g), with 0.2 t ha^{-1} less grain yield than 'CDC Stanley' (Table 2-1). There were no significant differences between the two parents for test weight in both years. In addition, 'Peace' had 4 mm higher SDS sedimentation volume, 0.4 % greater grain protein content and 35 seconds higher falling number than 'CDC Stanley'. On average over two years under organic management 'Peace' matured 1 day earlier, was 7 cm taller, with greater grain yield, TWT and TKW than 'CDC Stanley' (Table 2-1). 'CDC Stanley' produced 3 mm and 0.5 % lower SDS sedimentation volume and grain protein content, yet it expressed 12 seconds higher falling number than 'Peace' under organic management.

All agronomic traits in the RIL population exhibited transgressive segregation as the phenotypic values of some RIL lines were greater than these of the two parents. The broad-sense heritability estimates (Table 2-1) in the population for days to heading, days to maturity, grain yield, TKW, TWT and grain protein content were relatively high over two years under conventional management. Phenotypic variability under organic management was attributed to environmental factors to a greater degree. When expressed in broad-sense heritability, only days to heading, lodging resistance and TKW represented a comparatively higher fraction of variance components to phenotypic variance. The SDS sedimentation levels exhibited the lowest broad-sense heritability estimate in both management systems. Individual lines of the RIL population significantly differed ($P<0.0001$) for grain yield across the two farming managements over two years (Table 2-2). Trials were all moderately and positively correlated; a slightly higher correlation coefficient of 0.62 for grain yield occurred between the two conventional trials in 2016 and 2017, while the lowest correlation coefficient of yield (0.41) occurred between the two organic trails.

2.3.2 Correlation among phenotypic traits

Under conventional management over two years, days to heading was strongly positively associated with days to maturity and negatively correlated with plant height and grain protein content (Figure 2-2). There was a strong negative correlation between plant height and protein content. Earliness of heading and maturity were negatively correlated with TWT and falling number. Test weight was positively correlated with grain yield, while grain yield was negatively associated with grain protein content (Figure 2-2). Under organic management over two years, days to heading and maturity were negatively correlated with test weight (Figure 2-3). Plant height was negatively correlated with grain yield and TKW. Test weight and TKW were positively correlated in organically managed system.

2.3.3 Marker detection and QTL analyses

There were 1839 final polymorphic SNPs in total identified on all 21 wheat chromosomes in the 'Peace' x 'CDC Stanley' mapping population of 165 RILs (Table 2-3). The total length of the high-density linkage map constructed was 3970.2 cM. Map distance between adjacent markers varied between 0.1 cM to 25.2 cM; with an overall average distance of 2.2 cM (Figure 2-4). The number of filtered markers for QTL mapping ranged from 31 in chromosome 3D to 191 on 2A, with an overall average 87 markers per chromosome. Genotyping analysis (Figure 2-5) revealed a total of 51 QTLs associated with ten phenotypic traits in the six management system - year combinations. All QTLs responsible for individual traits were predominantly additive.

Under conventional management, one QTL (*QMat.dms-4A*) for maturity mapped at 87 cM on 4A and accounted for 8.8 % of phenotypic variance (Table 2-4). Six QTLs associated with plant height (*QHt.dms-1A, QHt.dms-1B, QHt.dms-2B, QHt.dms-2D, QHt.dms-4D and QHt.dms-6A*) were detected at 251 cM on 1A, 151 cM on 1B, 13 cM on 2D, 29 cM on 4B and 170 cM on 6A. A total of 48.6 % of phenotypic variance was explained by five protein content related QTLs (*QGpc.dms-4B, QGpc.dms-5B, QGpc.dms-6A, QGpc.dms-6B* and *QGpc.dms-6D*) at 60 cM, 137 cM, 20 cM, 108 cM and 149 cM on corresponding chromosomes 4B, 5B, 6A, 6B and 6D

respectively. There were four QTLs that explained accumulatively 31.7 % of grain yield variance, including *QYld.dms-2B* at 111 cM on 2B, *QYld.dms*-2D at 13 cM on 2D, *QYld.dms-4B* at 61 cM on 4B, and *QYld.dms-6D* at 150 cM on 6D with LOD scores of 3.4, 6.1, 8.7 and 3.0. One major QTL for test weight was identified at 13 cM on 2D, which accounted for 11% of phenotypic variance. In addition, six QTLs for thousand kernel weight were detected in the population with one major QTL (*QGwt.dms-2D*) mapped at 107 cM position on 2D that explained 24.4 % of the phenotypic variance. The three QTLs *QSv.dms-2B* and *QSv.dms-5B* associated with SDS sedimentation were individually located on 1B at 113 cM, the tip of 2B at 1 cM and 126 cM on 5B, with that each of QTLs explained 18.6 %, 7.8 % and 7.7 % of the phenotypic variance. One QTL (*QFn.dms-6A*) associated with falling number was mapped at 171 cM on 6A, accounting for 14.3 % of phenotypic variance. Of four QTLs detected for lodging, *QLdg.dms-2D* mapped at 13 cM on 2D with a LOD score of 14.1 and was responsible for 21.5 % of the phenotypic variance, while the phenotypic variance and the LOD scores explained by the other QTLs ranged from 3.5 to 4.9 % and 3.5 to 4.5.

Under organic management one QTL for days to heading *QHd.dms-4A* mapped to 88 cM on 4A with a LOD score of 3.5 (Table 2-5). Maturity QTL (*QMat.dms-2D*) mapped at 13 cM on 2D accounted for 13 % of phenotypic variance. One QTL for plant height was located at the distal end of 2D at 13 cM. *QGpc.dsm-4B* and *QGpc.dms-5D* explained 10.1 and 11.8 % of the phenotypic variance of grain protein content, with LOD scores of 4.5 and 3.7 respectively. The LOD score and the phenotypic variance explained by one QTL (*QYld.dms-2D*) for grain yield was 4.8 %, located at 13 cM on 2D. Among three QTLs detected on 1A and 1D, *QTwt.dms-1A* on chromosome 1A at 43 cM with a LOD score of 19.3 explaining 11.7 % of the phenotypic variance. Two additional QTLs together accounted for 11.7% of phenotypic variance, mapped at 4 cM and 65 cM on chromosome 1D. A thousand kernel weight associated QTL that was identified consistently in conventional management was mapped at 140 cM on 6B, and also explained 10.9 % of the phenotypic variance. 8.4 % of phenotypic variance was explained by one QTL (*QSv.dms-3A*) associated with SDS sedimentation volume. Among seven QTLs identified at 5 cM and 167 cM

on 2A, 145 cM on 2B, 30 cM on 4B, 110 cM on 5A, 81 cM and 170 cM on 6A, *QFn.dms-4B* with a LOD score of 37.6 explained a major portion of phenotypic variance of 25.9 %, followed by *QFn.dms-5A* with a LOD score of 23.3 that explained 12.9 % of the total phenotypic variance. Two QTLs for lodging were found at 13 cM on 2D and 150 cM on 6D, explaining 18.3 % of total phenotypic variance (Table 2-5).

Two of six QTLs in conventional and one of four QTLs in organic management were coincident with multiple phenotypic traits (Table 2-6). One important QTL mapped at 13 cM on chromosome 2D was coincident with plant height, grain yield, test weight and lodging resistance under conventional growing conditions. RILs carrying the 'Peace' favorable alleles of the coincidental QTL on 2D significantly improved grain yield and test weight by 0.9 t ha^{-1} and 2.3 kg hL^{-1} with 6.4 cm taller plant stature. RILs possessing 'Peace' favorable alleles were also 2.7 days later maturing, with 6 cm shorter plants and 1 t ha^{-1} less grain yield than those with 'CDC Stanley' alleles for these traits. Additionally, one stable QTL that was positioned at 29 cM on 4B was associated with both grain yield and grain protein content under conventional management.

2.3.4 Validation and Phenotypic correlation of diagnostic markers for *Qphs.usask-4A*

The RIL population was screened for a major pre-harvest sprouting resistance QTL *Qphs.usask-4A* (Table 2-7). Only 'CDC Stanley' carried all three diagnostic microsatellite marker alleles, DuPw004 (200 bp), wms650 (109 bp) and barc170 (158 bp) respectively reported to be associated with QTL *Qphs.usask-4A* (Singh et al. 2012). The proportion of RIL lines carrying dominant SSR marker alleles to RIL lines with recessive SSR marker alleles demonstrated no evidence of distorted segregation as it fitted the expected 1:1 ratio at all loci of interest (Dupw004: $\chi^2 = 0.389 < 3.84 = \chi^2_{0.05}$; barc170: $\chi^2 = 0.222 < 3.84 = \chi^2_{0.05}$; wms650: $\chi^2 = 3.455 < 3.84 = \chi^2_{0.05}$, respectively).

Heading days were greater in RIL lines with three dominant *Qphs.usask-4A* marker alleles than lines without these dominant marker alleles (Table 2-7). A significantly higher falling number of 11.4 seconds ($P<0.05$) occurred in lines carrying dominant DuPw004 allele and 13.5 seconds

(*P<0.01*) in lines with dominant barc170 allele. Days to maturity, height, TKW, grain yield, TWT and SDS sedimentation volumes did not differ between genotypes with or without dominant DuPw004 and barc170 alleles.

Lines with dominant barc170 allele had significantly greater test weight and falling number (*P<0.05*). The TKW and test weight of lines with dominant wms650 allele were 0.53 g (*P<0.05*) and 0.26 kg hL^{-1} (*P<0.05*) higher than lines with the recessive allele at wms650. However, the carriers of each of dominant DuPw004, barc170 and wms650 alleles did not alter days to maturity, height or SDS sedimentation volume.

2.4 Discussion

2.4.1 Phenotypic performance

We tested 168 recombinant inbred lines derived from a cross between 'Peace' and 'CDC Stanley' in both organic and conventional management systems over two years in Edmonton AB, Canada. Organically managed trials, on average, matured four days earlier with 19 % less grain yield and 12 % less grain protein and lower dough strength as determined by SDS sedimentation. Such results may partially result from stress caused by low nutrient availability in the organically managed system. Kamran et al. (2014) reported differential maturity dates for certain spring wheat cultivars grown in paired conventional and organic management systems. O'Donovan et al. (2011) also reported lower days to grain maturity coupled with lower protein concentration in malting barley cultivars at low nitrogen rates. With the limited nitrogen supply, organically produced wheat is expected to express improved nitrogen use efficiency resulting in higher grain protein content (Konvalina et al., 2014). We did not observe this in our mapping population.

Correlation analysis indicates the intensity of association among economically important traits (Steel and Torrie, 1984). Indirect selection for the higher heritable trait controlled by single major gene would more likely result in progress for all complex characters such as grain yield and protein quality that are positive correlated (Akinwale et al., 2011). Our results suggest a strong negative correlation between plant height and days to heading in both systems. Flowering and

maturity times of hexaploid wheat are controlled by certain genes that are responsible for vernalization response and photoperiod sensitivity plus earliness *per se,* (Kosner and Pankova, 1998; Kamran et al., 2012). A negative relationship between heading time and plant height in our population was possibly due to the absence of polymorphism at *Ppd-D1* gene in both parents, regardless of the environmental effects. In contrast, the positive correlation between the flowering date and plant height has been reported as a result of effects of gibberellin insensitive dwarfing allele *rht8* and the photoperiod response allele a of *Ppd-D1* on the same chromosome 2DS (Kowalski et al., 2016). Kamran et al. (2014) reported that shorten life cycle was associated with reduction in plant stature, number of tillers and spikelets per year under similar growing conditions as our study. A relative delay in maturity due to prolonged grain filling period combined with relatively shorter plant height would potentially contribute to greater grain yield (Chen et al., 2016).

Negative correlations between protein content and days to heading in the present study suggested RILs growing in conventional managed land that headed earlier tended to produce greater protein content. Previous studies have reported that 80% of grain protein content was ascribed to nitrogen assimilation from post-anthesis plant biomass and 20% of which was produced during grain-filling period (Austin et al., 1977; Sylvester-Bradley and Kindred, 2009). However, in organic land where soil nitrogen availability was finite for plant uptake, early heading lines had lower grain protein content. Osman et al. (2012) reported that bread wheat with improved baking qualities for organic agriculture exhibited delayed heading with increased grain protein content. They suggested that the nitrogen source for protein content accumulation in wheat grain under such conditions derived from plant biomass before heading or leaf senescence, during the active growing period. A prolonged period between heading and maturity may improve grain filling through the possible approach of delaying leaf senescence, which in turn would permit contribution of post-anthesis assimilates from extended active photosynthesis (Bidinger et al., 1977; Khan et al., 2002).

2.4.2 QTL analysis

Fifty stable marker-trait associations altogether distributed on 15 hexaploid wheat chromosomes were uncovered in both conventional and organic management systems. Among four grain yield QTLs observed on 2B, 2D, 4B and 6D in the present study, *QYld.dms-2D* was consistent in both management systems, which may be the same yield associated QTL (*QYld.dms-2D.2)* in a RIL population derived from 'Attila' x 'CDC Go', recently reported by our group (Zou et al., 2017*a*). One of the flanking markers tplb0049d24_996 linked to this QTL was positioned at 76.53 cM on a wheat reference map (SynOp) constructed with population sequencing (POPSEQ) on the long arm of chromosome 2D (Poland et al., 2012), which was only 3.45 cM distal to both of the flanking markers for previously reported *QYld.dms-2D.2*. This major coincidental QTL cluster (Table 2-6) was also consistently associated with traits of agronomic importance such as plant height and lodging across both combined management systems, as well as expressing management specific association to maturity and yield component traits including thousand kernel weight and test weight. Another major coincidental QTL for flowering time, maturity and grain yield on 2D, flanked by *Ppd-D1* gene, has been reported in a study of QTL mapping for agronomic traits in a RIL population derived from 'Cutler' and 'AC Barrie' (Perez-Lara et al., 2016), which shortened up to 5 days for plants to reach maturity with a yield penalty of 4.36 kg ha^{-1}.

Grain yield associated QTLs have been reported to be unevenly distributed on all 21 wheat chromosomes from 37 studies using 26 populations (Zhang et al., 2010). Specific studies have confirmed that chromosome 2D harbors major effect genomic regions for grain yield (Kumar et al., 2007; Wu et al., 2012). The coincidence of QTL/chromosomal clusters is most likely due to following reasons: (i) tight linkages among multiple genes or QTLs that govern the expression of individual traits, which was not distinguished by statistic methods (Perez-Lara et al., 2016); and (ii) pleiotropic effect induced by a single QTL, which concurrently regulates multiple traits (Buerstmayr et al., 2009). The genetic distance between the two flanking markers (tplb0049d24_996 and Excalibur_rep_c69755_624) for the coincident QTL on 2D in the present study was 65.91 cM. The large genetic distance suggests that the genetic composition harboured

by the chromosomal region between two flanking markers involves more QTLs, which could be uncovered by screening a greater number of recombinants in order to break linkages (Perez-Lara et al., 2016).

Breeding for new cultivars with higher levels of lodging tolerance is desirable as this trait effectively reduces the detrimental effects of harsh environments on grain yield loss (Tumino et al., 2017). One stable QTL for lodging performance across six different environments that was mapped at the same position on 2D as the coincidental QTL for grain yield mentioned above. The additive effect of this QTL did not differ in both management systems, and the 21.5 % (conventional) and 15.8 % (organic) of the phenotypic variance explained by this QTL is equivalent to 20 % less lodging. Short stature generally correlated with lodging tolerance and this reduced plant height is important to improve grain yield potential. This has been achieved in wheat breeding through incorporating dwarfing genes in many cultivars since Green Revolution (Evenson and Gollin, 2003). However, there was no allelic variation of major dwarfing genes of the gibberellic acid insensitive group including *Rht-B1* and *Rht-D1,* between the two parents in the present study. Nonetheless, the environment-specific QTL *QHt.dms-4B* expressed moderate effect under conventional management, resulting in RILs with 'CDC Stanley' alleles that were 6.9 cm shorter than RILs with homologous 'Peace' alleles. One of the flanking SNP markers BS00023035 was less than 1 cM away from wsnp_Ra_c1146_2307483,which is one of the flanking SNP markers of an environment-stable coincidental QTL *QPht.dms-4B* for plant height and maturity in the 'Attila' x 'CDC Go' RIL population (Zou et al., 2017*a*). This suggests that the two QTLs from different RIL populations may indicate the same genomic region. There was 33.5 cM between the reported QTL and the dwarfing gene *Rht-B1* in our previous study (Zou et al., 2017*a*).

Early maturity is a desirable agronomic trait to avoid potential loss of grain yield and quality from frost damage or post-harvest problems due to fairly short growing season in western Canada (Asif et al., 2015). In addition to the coincidental QTL for maturity mapped on 2D, a mild effect coincidental QTL mapped on chromosome 4A was observed in the present study. RILs that possessed homologous 'CDC Stanley' alleles matured 0.8 day earlier than RILs with 'Peace'

alleles under conventional management. Under organic growing conditions, this QTL also resulted in a 0.4 day reduction in days to heading in RILs with 'Peace' alleles. This QTL may be the previously reported *QMat.dms-4A* for maturity in 'Cutler' x 'AC Barrier' mapping population, which accounted for 12% of the phenotypic variance under field conditions (Perez-Lara et al., 2016). *QMat.dms-4A* was mapped at the tip of the short arm of chromosome 4A by one of the flanking markers CAP12_rep_c4000_432, which shared the same position with both flanking markers (i.e. 0 cM on 4AS detected by population sequencing methodology) linked to the coincidental QTL in the present study. Moreover, chromosome 4A has been reported to harbour important QTLs associated with earliness *per se* (Chen et al., 2015). In the 'CDC Teal' x 'CDC Go' CWRS mapping population, Chen et al. (2015) identified major effect QTLs coincident for date of heading, flowering, and maturity that consistently explained a significant portion of the phenotypic variance across multiple environments. Nevertheless, direct comparisons of target QTLs detected by variable types of functional markers may not be feasible without either a reliable physical map of common marker sets or a high-density consensus genetic map based on integrated high output marker types (Chen et al., 2017).

Grain protein content plays a vitally important role in the development of wheat cultivars in the CWRS class of Canadian wheat (Asif et al., 2015). We identified a stable protein content associated QTL *QGpc.dms-4B* at 69.86 cM flanked by SNP markers Kukri_c15910_159 and Ra_c3117_2098 that possessed an additive effect of 0.3 % of protein content in both management systems in the current study. This QTL was also coincident for grain yield in conventional management, having 0.3 t ha^{-1} higher grain yield and 0.3 % of higher grain protein content on average in RILs carrying favorable alleles originated from 'CDC Stanley'. Protein content is considerably influenced by nitrogen availability in the soil (Asif et al., 2015). In comparison with only one QTL on 5D identified in growing conditions where nitrogen is limited, we mapped four additional QTLs for protein content including one major effect genomic region on chromosome 6B in conventional management, which could possibly enhance the utilization ability of sufficient soil nitrogen for protein production. The inconsistency of QTL identification across environments

in our study is possibly due the expression of such trait with genetic complexity, easily influenced by environmental factors (Paul et al., 2002).

The SDS sedimentation test is commonly applied to evaluate the end-use quality parameters of wheat grains and serves as a proxy for mixograph or farinograph measures. In the current study, chromosomal regions responsible for sedimentation volume were identified to be management specific. Detected QTLs were distributed on 1B, 2B, 5B and 6A in conventional management system and on 3A in organic management system. In a population derived from spring wheat cross between 'RL4452' and 'AC Domain' used to map QTLs associated with quality traits, McCartney et al. (2006) mapped a novel QTL for SDS sedimentation adjacent to a major glutenin coding locus *Glu-B1* gene. This QTL accounted for 20.6 % of phenotypic variation and contributed up to 6.22 cm of sedimentation volume to those genotypes with 'RL4452' alleles (McCartney et al., 2006). However, the moderate effect *QSd-dms-1B* revealed in our study is not likely to be the same QTL due to a greater genetic distance of over 6 cM between linked flanking markers and Glu-B1 on the reference map (Poland et al., 2012). Apart from chromosome 1B, SDS sedimentation QTLs have also been earlier detected on 1A, 1D *(Glu-D1, Glu-D1x*), 2D, 3AS, 3BL, 3D, 5AL, 5B, 5D, 6A, 6D and 7BS (Blanco et al., 1998; Nelson et al., 2006; Kunert et al., 2007; Carter et al., 2012; Reif et al., 2011; Li et al., 2009; Sun et al., 2008).

Yield component traits including test weight and thousand kernel weight have been thoroughly studied yet not well understood owing to their complex genetic nature (Kumar et al., 2007). In the present study, one QTL for TKW mapped proximally at 134 cM on the long arm of 6B that was consistent over both management systems, despite the fact that it explained more phenotypic variance in the combined organic environments. Two stable QTLs located on 6B and 7A have also been reported by Raman et al. (2009) using a breeding population developed from Australian cultivars 'Chara'/'WW2449'. In conventional management, a moderate effect QTL *QGwt.dms-2B* for TKW resulted in greater TKW conferred by 'Peace' alleles. Zhang et al. (2010) reported major-effect QTL for TKW on 1B, 3B and 4AL. We did not map QTL consistent in both management systems for test weight. However, three QTLs were mapped on chromosome 1A and

1D under organic management conditions, which had variable additive effect, ranging from - 1 to 0.9 kg hL^{-1}. Our research group previously reported a consistent major effect QTL for test weight on 5B across two management systems, and three management dependent QTLs on 1B, 6A and 7A (Asif et al., 2015), suggesting a specific breeding value to breeders under certain growing conditions.

Falling number is a proxy measure of pre-harvest sprouting resistance as the measure of α-amylase activity from falling number test is be an indirect indicator of PHS (Simsek et al., 2014). One QTL associated with falling number mapped to 74.23 cM (Poland et al., 2012) on 6A under organic management and was also mapped under conventional management, with less effect. Another major effect QTL was flanked by two SNP markers (RAC875_c1918_101 and Tdurum_contig29960_214) at approximately 86.62 cM on the long arm of 4B. Molecular selection based on this management specific QTL may be suitable for organic wheat production in the areas where profound precipitation events occur frequently during the growing season. In a recent Genome-Wide Association mapping Study of PHS in wheat, significant QTLs for falling number were detected on 4A, 5A, 5D, 7A and 7B in specific environments (Martinez et al., 2018). However, a lack of strong correlation was observed between QTLs associated with falling number and PHS resistance based on sprouting scores, in spite of relatively close genetic distance to one another. One possible explanation could be the complexity of this trait that is controlled by different factors in addition to the environment. Therefore, combining complementary information of QTLs for falling number and traits of germination-based assays is necessary in MAS breeding for PHS resistant cultivars (DePauw et al., 2012).

2.4.3 The effects of *Qphs.usask-4A* on phenotypic performance

A major QTL *Qphs.usask-4A* for PHS resistance was identified in a mapping population derived from a cross between a white grain non-dormant cultivar 'Argent' and a red grain, dormant cultivar 'W98616' (Singh et al., 2010). Three diagnostic SSR markers closely associated with this QTL were validated across different germplasm for marker assisted selection of target genotypes

in previous studies (Singh et al., 2012; Singh et al., 2010). In our study, the effects of *Qphs.usask-4A* on target traits varied not only across conventionally and organically managed systems, but also based on the presence of different diagnostic marker alleles, regardless of the fact that all three markers are closely associated with this identical PHS resistance QTL. More specifically, all three markers indicated that the RILs with *Qphs.usask-4A* headed significantly earlier than the lines without only under conventional management. In addition to the association between PHS and heading date described by Yasue and Asai (1968), harvest-time seed dormancy has been reported as a major factor conferring potential PHS resistance (Singh et al., 2010). The inability of viable seeds to germinate in germination favorable environments was mainly regulated by the underlying metabolic balance of synthesis and responsiveness between abscisic acid (ABA) and gibberellic acid (GA) (Bewley, 1997; Walker-Simmons, 1987). The external factors induced breakage of seed dormancy in PHS resistant lines suggested an accelerated crop development to reach anthesis stage. Lin et al. (1998) also reported a significant negative correlation between one major QTL for seed dormancy and one QTL for early heading date on chromosome 3 in rice, possibly due to genetic linkage.

Hagberg falling number test serves as an indirect quantification of the PHS level in advance of any appearance of visible sprouting signs. The hydrolysis of endosperm starch from elevated level of enzyme activities represented as lower falling number values, which in turn indicates an low degrees of PHS resistance or seed dormancy (Cabral et al., 2014). In a study evaluating several methods for PHS resistance in wheat breeding, Paterson et al. (1989) noted that while the falling number test has been a widely accepted method of indirectly selecting sprout damage in wheat breeding programmes, the various components of PHS resistance cannot be merely reflected by high falling number. Additionally, falling number was proposed as an indirect measure of α-amylases activities, and it is more likely to quantify the amount of liquefaction of a sample (Johansson, 2002). Sprouted wheat kernels negatively affect the quantity and quality of the grain protein, which are critical factors of determining dough strength and flour baking quality (Simsek et al., 2014). Several researchers have reported a pronounced protein increase with a magnitude

ranged from 14 % to 40 % in several cereals when sprouted beyond from three days to 10 days (Chavan et al., 1989; Dalby and Tsai, 1976; Wu and Wall, 1980; Opuku et al., 1981). An improvement of crude protein, digestibility and solubility of the protein was also identified in sprouted chick peas (Khalil et al., 2007).

Due to the recombination of alleles of three *Qphs.usask-4A* associated markers DuPw004, barc170 and wms650, RILs consisting of corresponding haplotypes were further analyzed to detect the relationship among these three markers and their diagnostic effect towards considerable falling number. Lines with resistant haplotypes at all three markers had higher falling number values in comparison with those carrying susceptible haplotypes at all three markers across two managed systems over two years. Singh et al. (2010) reported that the genetic distance between DuPw004 and barc170 was 2 cM, between DuPw004 and wms650 was 3 cM, and between barc170 and wms650 was 1cM respectively on chromosome 4AS. Despite the low genetic distance between barc170 and wms650, our results showed no evidence of co-segregation between barc170 and wms650 (Table 2-8). Sorrells et al. (2003) reported barc170 and wms650 were located in separate deletion bins of 4AS4-0.63-0.76 and 4AS--.76-1.00. Balcarkova et al. (2017) further revealed in a map of wheat chromosome 4A that they were physically located at least 2.5k bp apart from each other, based on the sequences of these two markers using Basic Local Assignment Search Tool (BLAST) from Ensembl Plants database.

2.5 Conclusion

We tested 168 recombinant inbred lines derived from a cross between ‘Peace’ and ‘CDC Stanley’ in both organic and conventional management systems over two years in Edmonton AB, Canada. Organically managed trials, on average, matured six days earlier with 19 % less grain yield and 12 % less grain protein and lower dough strength as determined by SDS sedimentation. Such results may partially result from stress caused by low nutrient availability in the organically managed system. Of 30 QTLs for ten phenotypic traits identified in conventional management, six were consistent under organic management, suggesting the considerable influence of management

on the expression of most QTLs uncovered in the present study. The management specificity of major effect QTLs could offer a possibility of practical interest for cultivar development adapted to specific management systems. MAS relied on coincidental QTLs could be advantageous for introgression of target traits simultaneously with minimal adverse effect of undesirable linkage drag. However, the coincidence of multiple agronomic traits induced by the stable QTL on 2D that was detected in fairly large chromosomal segments is possibly regulated by other genes/QTL. The discovery of these unknown genes therefore could be conducted in a larger population with sufficient recombinants aiming to break the tight linkage through further segregation.

2.6 Tables and figures

Table 2-1. Summary of two - year (2016-2017) combined descriptive statistics of days to heading and maturity, plant height, lodging score, thousand kernel weight (TKW), grain yield, test weight (TWT), SDS sedimentation volume, falling number and grain protein content (WPRO) of check cultivars, parents and the 'Peace' × 'CDC Stanley' Recombinant Inbred Lines (RILs) population grown in conventional and organic management trials in Edmonton AB Canada.

Traits	Management	Parents		Parents difference	Checks n=7	Population (n=168)			Heritability (SE)
		Peace	CDC Stanley			Mean±SE	Range	F value	
Heading (day)	CON	51	52	-1	49	52 ± 4	44 - 63	7.8	0.64 (0.03)
	ORG	48	49	-1	47	49 ± 2	45 - 55	5.6	0.70 (0.04)
Maturity (day)	CON	90	93	-3	90	92 ± 4	82 - 100	6.2	0.62 (0.04)
	ORG	87	88	-1	88	88 ± 3	81 - 97	4.9	0.53 (0.04)
Plant height (cm)	CON	101	95	6	99	97 ± 14	62 - 132	2.9	0.42 (0.05)
	ORG	95	88	7	93	91 ± 9	62 - 130	4.5	0.51 (0.04)
Lodging	CON	3	2	2	3	3 ± 1	1 - 9	4.5	0.52 (0.05)
	ORG	2	2	1	2	2 ± 1	0 - 8	5.1	0.59 (0.04)
TKW (g)	CON	39.9	37.4	2.4	40.2	38.2 ± 2.8	26.4 - 44.8	10.7	0.77 (0.03)
	ORG	38.6	36.7	1.9	39.4	37.7 ± 3.2	20.2 - 46.2	6.7	0.60 (0.04)
Yield ($t\ ha^{-1}$)	CON	5.75	5.97	-0.22	5.72	5.60 ± 0.98	1.37 - 7.46	6.28	0.62 (0.04)
	ORG	4.9	4.82	0.08	4.41	4.52 ± 1.07	0.90 - 7.10	3.4	0.41 (0.05)
TWT ($kg\ hL^{-1}$)	CON	82.1	82.1	0	82.1	81.5 ± 1.6	72.8 - 84.5	10.5	0.75 (0.03)
	ORG	80.8	80.7	0.1	80.6	80.3 ± 2.3	71.1 - 85.6	4.4	0.55 (0.05)
SED (mm)	CON	80	76	4	83	78 ± 8	50 - 100	1.6	0.16 (0.05)
	ORG	62	58	3	65	62 ± 7	40 - 93	ns	0.10 (0.05)
Falling number (s)	CON	492	457	35	441	458 ± 70	165 - 699	1.7	0.20 (0.06)
	ORG	441	453	-12	411	463 ± 63	237 - 655	2.2	0.28 (0.05)
WPRO (%)	CON	14.8	14.4	0.4	14.8	14.6 ± 2.1	10.7 - 20.6	6.1	0.62 (0.04)
	ORG	13.1	12.6	0.5	13.1	13.0 ± 1.8	9.31 - 18.9	2.1	0.26 (0.05)

Table 2-2. Pearson correlation coefficients for grain yield of the 'Peace' × 'CDC Stanley' mapping population grown in organic and conventional management systems 2016 – 2017, Edmonton AB Canada.

Management		Conventional		Organic	
	Year	2016	2017	2016	2017
Conventional	2016		0.62	0.56	0.40
	2017			0.51	0.42
Organic	2016				0.41
	2017				

Table 2-3. Initial, final numbers of the Single Nucleotide Polymorphism (SNP) markers used for QTL mapping, total map length on all 21 chromosomes and average marker density based on 168 'Peace' × 'CDC Stanley' Recombinant Inbred Lines (RILs).

Chr.ł	Initial number of markers used for QTL mapping	Final number of markers used for QTL mapping	Total map length (cM)	Mean Map distance / markers
1A	276	139	255.9	1.8
1B	306	123	178.7	1.5
1D	119	74	152.9	2.1
2A	441	191	189.1	1.0
2B	469	168	221.9	1.3
2D	166	72	200.1	2.8
3A	144	60	324.5	5.4
3B	105	54	301.7	5.6
3D	72	31	164.8	5.3
4A	137	60	88.3	1.5
4B	147	62	135.4	2.2
4D	40	30	99.6	3.3
5A	179	94	238.1	2.5
5B	387	171	253.8	1.5
5D	133	70	137.1	2.0
6A	290	102	227.5	2.2
6B	160	88	191.8	2.2
6D	81	53	167.8	3.2
7A	220	92	241.3	2.6
7B	180	65	74.3	1.1
7D	55	40	125.6	3.1
Total	4107	1839	3970.2	2.2

ł Chr., chromosome

Table 2-4. Summary of identified quantitative trait loci (QTLs) associated with ten traits on 165 Recombinant Inbred Lines assessed in a two-year (2016-2017) combined environment under conventional management. QTL clusters are highlighted in bold.

Trait	QTL	Chr.ǂ	Position	Confidence interval	Left Marker	Right Marker	LOD¶	PVE	Additive effect*
				—cM—				%	
Maturity	*QMat.dms-4A*	4A	87	85.5-88	wsnp_Ex_c28429_37553452	wsnp_Ra_c14920_23225219	4.5	8.8	-0.8
Plant height	*QHt.dms-1A*	1A	251	250.5-251.5	Kukri_c8390_547	Kukri_c14149_462	44.6	16.1	7.9
Plant height	*QHt.dms-1B*	1B	151	148.5-151.5	BS00071289_51	BS00023105_51	3.5	0.6	1.5
Plant height	*QHt.dms-2B*	2B	211	210.5-212.5	BobWhite_c15453_678	Tdurum_contig53156_111	3.3	1.0	-3.4
Plant height	***QHt.dms-2D***	2D	13	12.5-13.5	tplb0049d24_996	Excalibur_rep_c69755_624	4.8	1.3	-6.4
Plant height	*QHt.dms-4B*	4B	29	28.5-29.5	Excalibur_c28734_435	BS00023035_51	40.3	12.6	-6.9
Plant height	*QHt.dms-6A*	6A	170	169.5-170.5	BS00054054_51	BS00010811_51	3.2	0.6	1.5
protein content	***QGpc.dms-4B***	4B	60	59.5-60.5	Kukri_c15910_159	Ra_c3117_2098	13.0	4.6	-0.3
protein content	*QGpc.dms-5B*	5B	137	135.5-139.5	wsnp_Ku_c19334_28808006	Ku_c1203_342	6.8	2.2	0.3
protein content	*QGpc.dms-6A*	6A	20	19.5-20.5	BS00041200_51	Kukri_c9940_659	33.7	16.1	-0.6
protein content	*QGpc.dms-6B*	6B	108	107.5-108.5	Excalibur_c37062_65	RAC875_c54671_199	42.5	23.2	0.8
protein content	*QGpc.dms-6D*	6D	149	148.5-149.5	Kukri_c55362_75	wsnp_Ex_c4789_8550135	7.5	2.5	0.2
Grain yield	*QYld.dms-2B*	2B	111	108.5-113.5	tplb0053o16_915	Kukri_c49647_151	3.4	4.6	220.7
Grain yield	***QYld.dms-2D***	2D	13	11.5-13.5	tplb0049d24_996	Excalibur_rep_c69755_624	6.1	11.1	-977.5
Grain yield	***QYld.dms-4B***	4B	61	60.5-61.5	BS00022194_51	BS00022809_51	8.7	12.2	359.8
Grain yield	*QYld.dms-6D*	6D	150	149.5-150.5	wsnp_Ex_c4789_8550135	wsnp_Ex_c14439_22426200	3.0	3.8	-203.1
Test weight	***QTwt.dms-2D***	2D	13	12.5-13.5	tplb0049d24_996	Excalibur_rep_c69755_624	7.4	11.0	-2.3
TKW	*QGwt.dms-1A*	1A	21	18.5-22.5	Tdurum_contig11106_264	RAC875_c7563_273	5.7	1.2	0.9
TKW	*QGwt.dms-1B*	1B	74	73.5-74.5	BS00022429_51	BS00065487_51	3.0	0.5	0.6
TKW	*QGwt.dms-2B*	2B	69	68.5-69.5	wsnp_Ex_c55735_58127324	Ex_c68194_1994	46.2	16.3	-3.2
TKW	*QGwt.dms-6A*	6A	0	0.0-0.5	wsnp_Ex_rep_c68915_67808431	BobWhite_c6527_222	3.5	0.6	0.6
TKW	*QGwt.dms-6B*	6B	140	139.5-141.5	Tdurum_contig55549_379	Tdurum_contig45478_176	3.5	0.8	1.4
Sedimentation	*QSv.dms-1B*	1B	113	112.5-113.5	wsnp_Ku_c8235_14030979	BS00001835_51	9.1	18.6	1.9
Sedimentation	*QSv.dms-2B*	2B	1	0.0-5.5	Excalibur_c17039_436	Kukri_c42244_809	3.8	7.8	1.2
Sedimentation	*QSv.dms-5B*	5B	126	122.5-128.5	RAC875_c53379_135	Excalibur_c23738_213	3.5	7.7	1.5

Falling number	*QFn.dms-6A*	6A	171	170.5-171.5	wsnp_BF483993A_Ta_2_1	Excalibur_c4152_1031	40.6	14.3	-57.1
Lodging	***QLdg.dms-2D***	2D	13	11.5-13.5	tplb0049d24_996	Excalibur_rep_c69755_624	14.1	21.5	1.5
Lodging	*QLdg.dms-2D.1*	2D	78	76.5-80.5	GENE-1067_598	Excalibur_c14933_154	3.5	3.5	-0.2
Lodging	*QLdg.dms-3A*	3A	24	22.5-26.5	Excalibur_c53004_130	Kukri_c46740_226	4.5	5.3	0.4
Lodging	*QLdg.dms-6D*	6D	152	150.5-152.5	wsnp_Ex_c14439_22426200	BobWhite_c7090_522	4.4	4.9	0.3

ł Chr. Chromosome

¶ LOD; Logarithm of odds

***** Additive effect is half the difference between the genotype values of 'Peace' and 'CDC Stanley'. For grain yield, grain protein content, test weight, TKW, sedimentation, falling number, positive and negative additive effects indicate that the favorable alleles are originated from 'Peace' and 'CDC Stanley', respectively. For heading, maturity, plant height, positive and negative additive effects indicated that the favorable alleles are originated from 'CDC Stanley' and 'Peace', because selection is conducted against longer duration to heading, maturity and taller plants.

Table 2-5. Summary of identified quantitative trait loci (QTLs) associated with 10 traits on 165 Recombinant Inbred Lines assessed in a two-year (2016-2017) combined environment under organic management. QTL clusters are highlighted in bold.

Trait	QTL	Chr.ǂ	Position	Confidence interval	Left Marker	Right Marker	LOD¶	PVE	Additive effect*
			—cM—					%	
Heading	*QHd.dms-4A*	4A	88	86.5-88	wsnp_Ra_c14920_23225219	BS00022177_51	3.5	8.7	-0.4
Maturity	***QMat.dms-2D***	2D	13	11.5-13.5	tplb0049d24_996	Excalibur_rep_c69755_624	7.4	13.0	2.7
Plant height	***QHt.dms-2D***	2D	13	12.5-13.5	tplb0049d24_996	Excalibur_rep_c69755_624	3.4	5.9	-5.5
Protein content	*QGpc.dms-4B*	4B	60	59.5-60.5	Kukri_c15910_159	Ra_c3117_2098	4.5	11.8	-0.3
Protein content	*QGpc.dms-5D*	5D	73	72.5-73.5	Kukri_c805_325	Ex_c2615_564	3.7	10.1	0.3
Grain yield	***QYld.dms-2D***	2D	13	12.5-13.5	tplb0049d24_996	Excalibur_rep_c69755_624	3.1	4.8	-1015.1
Test weight	*QTwt.dms-1A*	1A	43	42.5-43.5	Excalibur_c15357_432	BS00031066_51	19.3	11.7	-1.0
Test weight	*QTwt.dms-1D*	1D	4	0-6.5	BS00048032_51	RAC875_c51493_471	4.4	2.4	0.4
Test weight	*QTwt.dms-1D.1*	1D	65	64.5-67.5	Kukri_c25215_124	Ra_c1211_3306	17.2	9.3	0.9
TKW	*QGwt.dms-6B*	6B	140	137.5-140.5	Tdurum_contig55549_379	Tdurum_contig45478_176	4.7	10.9	1.7
Sedimentation	*QSv.dms-3A*	3A	63	55.5-66.5	Excalibur_c10366_109	RAC875_c35122_367	4.6	8.4	1.6
Falling number	*QFn.dms-2A*	2A	5	2.5-5.5	Excalibur_c18630_268	Kukri_c4790_923	3.2	1.4	-9.9
Falling number	*QFn.dms-2A.1*	2A	167	166.5-167.5	Excalibur_c36481_519	Ku_c15323_908	3.1	1.3	-9.7
Falling number	*QFn.dms-2B*	2B	145	144.5-146.5	BS00099469_51	wsnp_Ex_rep_c67619_66272209	3.5	1.4	-14.8
Falling number	*QFn.dms-4B*	4B	30	29.5-30.5	RAC875_c1918_101	Tdurum_contig29960_214	37.6	25.9	-42.5
Falling number	*QFn.dms-5A*	5A	110	109.5-110.5	RAC875_c104483_216	BS00012006_51	23.3	12.9	30.0
Falling number	*QFn.dms-6A*	6A	81	80.5-81.5	wsnp_Ex_rep_c70675_69579757	BobWhite_c5872_589	4.7	2.0	-11.7
Falling number	*QFn.dms-6A.1*	6A	170	169.5-170.5	BS00054054_51	BS00010811_51	4.1	1.7	-11.0
Lodging	***QLdg.dms-2D***	2D	13	12.5-13.5	tplb0049d24_996	Excalibur_rep_c69755_624	14.5	15.8	1.5
Lodging	*QLdg.dms-6D*	6D	150	148.5-151.5	wsnp_Ex_c4789_8550135	wsnp_Ex_c14439_22426200	3.4	2.5	0.2

ǂ Chr. Chromosome

¶ LOD; Logarithm of odds

* Additive effect is half the difference between the genotype values of 'Peace' and 'CDC Stanley'. For grain yield, grain protein content, test weight, TKW, sedimentation, falling number, positive and negative additive effects indicate that the favorable alleles are originated from 'Peace' and 'CDC Stanley', respectively. For heading, maturity, plant height, positive and negative additive effects indicated that the favorable alleles are originated from 'CDC Stanley' and 'Peace', because selection is conducted against longer duration to heading, maturity and taller plants.

Table 2-6. Comparisons of Recombinant Inbred Lines carrying 'Peace' or 'CDC Stanley' alleles at the flanking markers of three coincidental quantitative trait loci (QTLs) on 10 traits (2016-2017 combined environments) in both conventional and organic management systems. Traits that were bolded indicated QTLs identified in the common confidence interval.

Trait	Management	Chr.ŧ	Common confidence interval (cM)	Peace'-type alleles	CDC Stanley'-type alleles	Difference ǂ	t value	p-value
Flowering time, d		2D		51.6	52.5	0.9	1.6	0.36
Maturity, d		2D		92.1	93.2	1.1	3.03	0.21
Plant height, cm		2D		100.3	93.9	6.4	8.29	<0.001
Grain yield, t ha^{-1}		2D		5.6	4.7	0.9	11.93	<0.001
Test weight, kg hL^{-1}	**Conventional**	2D	11.5-13.5; [1] 12.5-13.5	81.6	79.3	2.3	18.2	<0.001
TKW, g		2D		38.6	38.2	0.4	0.52	0.61
Grain protein content, %		2D		14.5	14.5	0	0.06	0.95
Sedimentation, mm		2D		78.4	78.2	0.2	0.01	0.92
Falling number, s		2D		459.1	456.9	2.2	3.05	0.002
Flowering time, d		2D		-	-	-	-	-
Maturity, d		2D		87.3	90	2.7	7.83	<0.001
Plant height, cm		2D		94.1	88.1	6	10.9	<0.001
Grain yield, t ha^{-1}		2D		4.9	3.9	1	13.05	<0.001
Test weight, kg hL^{-1}	**Organic**	2D	11.5-13.5; [2] 12.5-13.5	81.4	81.5	0.1	0.4	0.68
TKW, g		2D		38.5	37.7	0.8	1.11	0.26
Grain protein content, %		2D		13	13.3	0.3	1.02	0.31
Sedimentation, mm		2D		61.7	63	1.3	0.81	0.42
Falling number, s		2D		464.1	461.2	2.9	3.95	0.003
Flowering time, d		4B		51.8	51.5	0.3	1.77	0.07
Maturity, d		4B		93.4	93.1	0.3	1.13	0.26
Plant height, cm		4B		97.2	95.6	1.6	2.62	0.0089
Grain yield, t ha^{-1}		4B		5.8	5.5	0.3	7.74	<0.001
Test weight, kg hL^{-1}	**Conventional**	4B	28.5-29.5	81.7	81.4	0.3	2.14	0.03
TKW, g		4B		36.9	36.8	0.1	0.84	0.4005
Grain protein content, %		4B		12.8	13.1	0.3	11.71	<0.001
Sedimentation, mm		4B		78	78.5	0.5	0.91	0.36
Falling number, s		4B		442.3	451.2	8.9	1.71	0.087

[1] [2] QTL for Lodging was also identified in this confidence interval

ǂ Chr. Chromosome

‡ The difference was calculated by subtracting values for 'Peace'-type alleles from those of 'CDC Stanley'-type alleles. For grain yield, grain protein content, test weight, TKW, sedimentation, falling number, positive and negative additive effects indicate that the favorable alleles are originated from 'Peace' and 'CDC Stanley', respectively. For heading, maturity, plant height, positive and negative additive effects indicated that the favorable alleles are originated from 'CDC Stanley' and 'Peace', because selection is conducted against longer duration to heading, maturity and taller plants.

Table 2-7. Effects of dominant allele of DuPw004, barc170 and wms650 that were associated with pre-harvest sprouting resistance QTLs on phenotypic traits of 'Peace' × 'CDC Stanley' Recombinant Inbred Lines (RILs) population in conventional and organic environments.

		DuPw004				Barc170				wms650			
Trait	**Man°**	Present	Absent	Diff	SE	Present	Absent	Diff	SE	Present	Absent	Diff	SE
Heading (day)	CON	51	52	-0.5**	0.14	52	52	-0.3*	0.15	52	52	-0.3*	0.15
	ORG	49	49	0.0	0.16	49	49	0.0	0.16	49	49	0.0	0.16
Maturity (day)	CON	92	92	0.0	0.22	92	92	0.0	0.22	92	92	0.0	0.22
	ORG	88	88	0.0	0.17	88	88	0.0	0.17	88	88	0.0	0.17
Plant height (cm)	CON	97	98	-1.0	0.60	97	98	-1.0	0.60	97	98	-1.0	0.60
	ORG	90	91	-1.0	0.51	91	91	0.0	0.52	90	91	-1.0	0.52
TKW (g)	CON	38.2	38.3	-0.1	0.21	38.0	38.5	-0.4	0.21	38.0	38.4	-0.4	0.21
	ORG	37.6	37.9	-0.4	0.22	37.5	38.0	-0.4	0.22	37.4	38.0	-0.5*	0.22
Yield (t/ha)	CON	5.8	5.7	0.1	0.74	5.7	5.7	0.0	0.75	5.7	5.7	0.0	0.75
	ORG	4.5	4.6	0.1	0.73	4.6	4.5	0.1	0.74	4.5	4.6	-0.1	0.74
TWT (kg/hL)	CON	81.5	81.6	-0.1	0.12	81.4	81.6	-0.2	0.12	81	82	0.0	0.12
	ORG	80.3	80.7	-0.4	0.11	80.3	80.6	-0.3**	0.11	80	81	-0.3*	0.11
SED (mm)	CON	78	78	0.0	0.57	78	79	-1.0	0.57	78	79	-1.0	0.57
	ORG	62	62	0.0	0.49	62	62	0.0	0.50	62	62	0.0	0.50
Falling number (s)	CON	452	464	-11.4*	4.96	452	465	-13.5**	5.03	453	463	-10.0	5.03
	ORG	463	464	0.0	4.75	459	469	-10.4*	4.75	462	464	-2.0	4.78
WPRO (%)	CON	14.6	14.5	0.1	0.35	14.7	14.4	0.3	0.35	14.6	14.5	0.1	0.35
	ORG	12.9	13.1	-0.3*	0.11	12.9	13.1	-0.2*	0.11	12.9	13.1	-0.2*	0.11

Man° Management
*Significant at *P <0.05*
** Significant at *P<0.01*

Table 2-8. Average falling number values in response to number of 'Peace' × 'CDC Stanley' Recombinant Inbred Lines with corresponding haplotypes at pre-harvest sprouting resistance QTL *Qphs.usask-4a*diagnostic markers DuPw004, barc170 and wms650 respectively across conventional and organic managed systems from 2016 to 2017.

Diagnostic Markers			RILs count	Falling number (s)			
				Conventional		Organic	
Dupw004	barc170	wms650		2016	2017	2016	2017
N[1]	N	N	67	425	480	452	470
N	N	Y[2]	8	423	470	450	459
N	Y	N	5	457	481	462	486
N	Y	y	5	426	474	503	481
Y	N	N	3	402	464	460	472
Y	N	Y	12	437	481	433	456
Y	Y	Y	68	435	498	458	475

N[1] indicates lines without dominant allele of the diagnostic markers
Y[2] indicates lines with dominant allele of the diagnostic markers

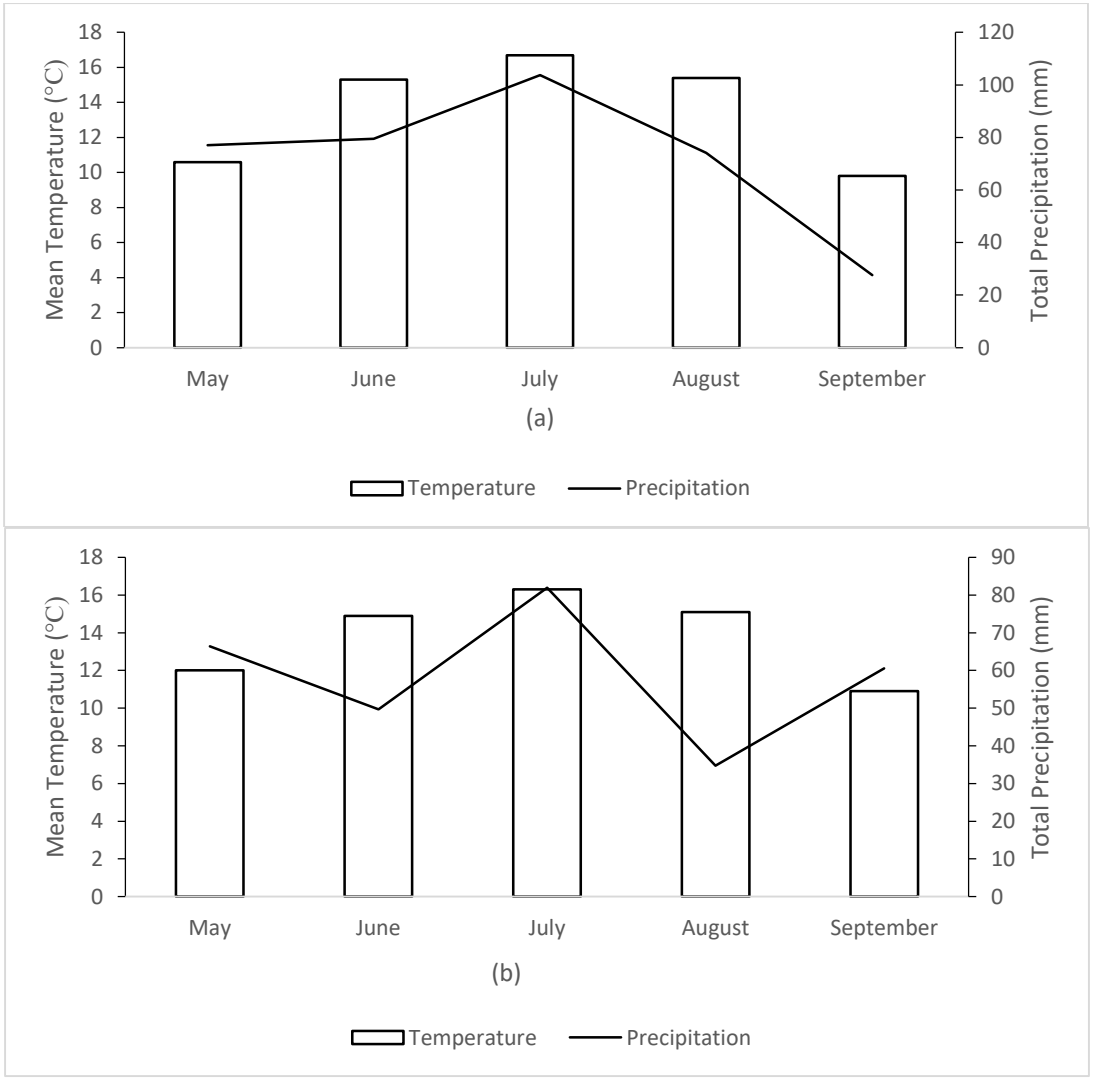


Figure 2-1. Monthly mean temperature and total precipitation recorded at the Edmonton international airport for 2016 (a) to 2017 (b). Weather data was retrieved from Environment Canada weather data achieve (Environment Canada, 2018).

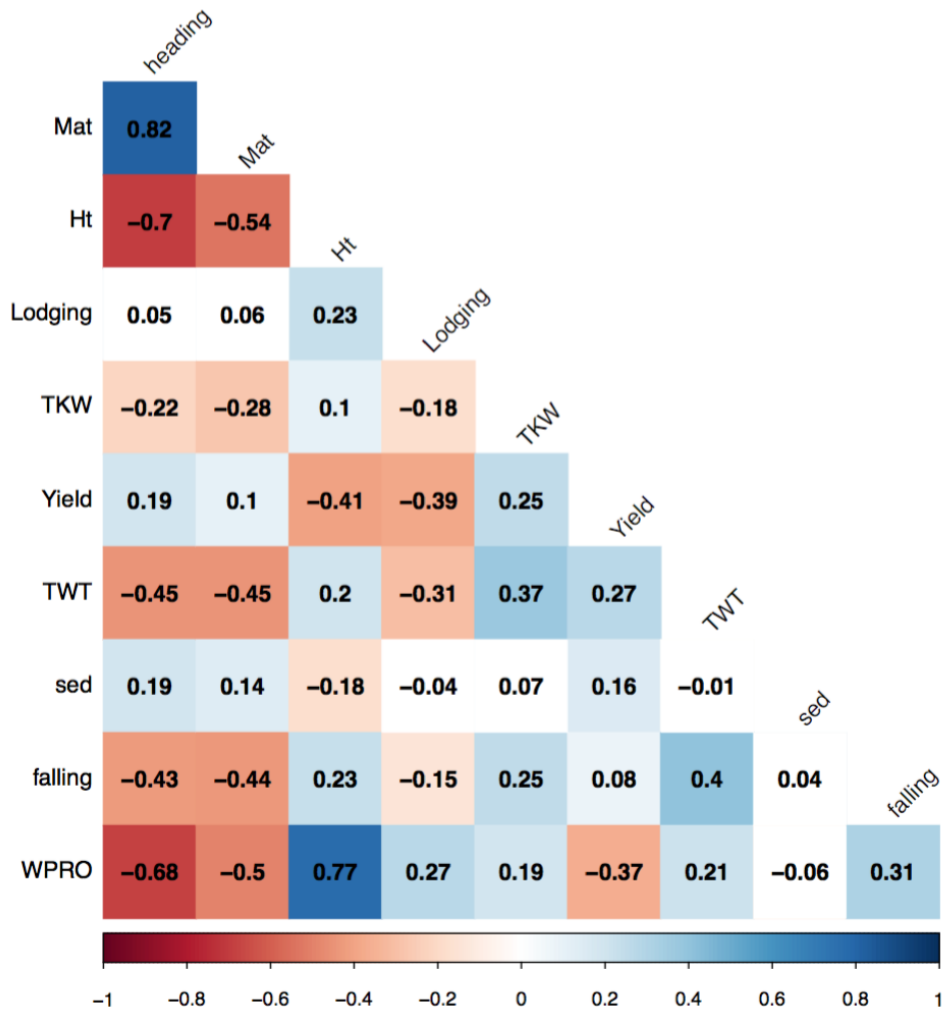


Figure 2-2. A correlogram of Pearson correlation coefficients for 10 phenotypic traits evaluated in 'Peace' × 'CDC Stanley' mapping population grown in conventional management system. Correlation coefficients with no statistically significance (P > 0.05) were indicated in white background.

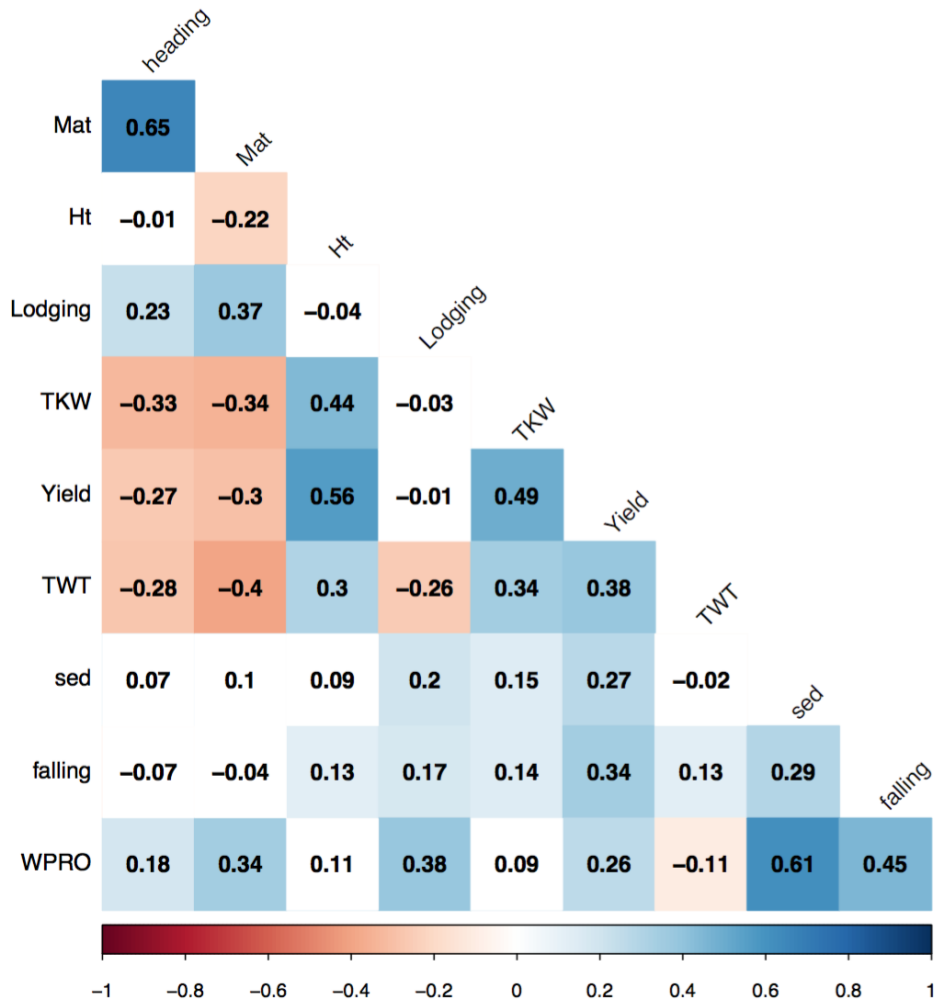


Figure 2-3. A correlogram of Pearson correlation coefficients for 10 phenotypic traits evaluated in 'Peace' × 'CDC Stanley' mapping population grown in organic management system. Correlation coefficients with no statistically significance (P > 0.05) were indicated in white background.

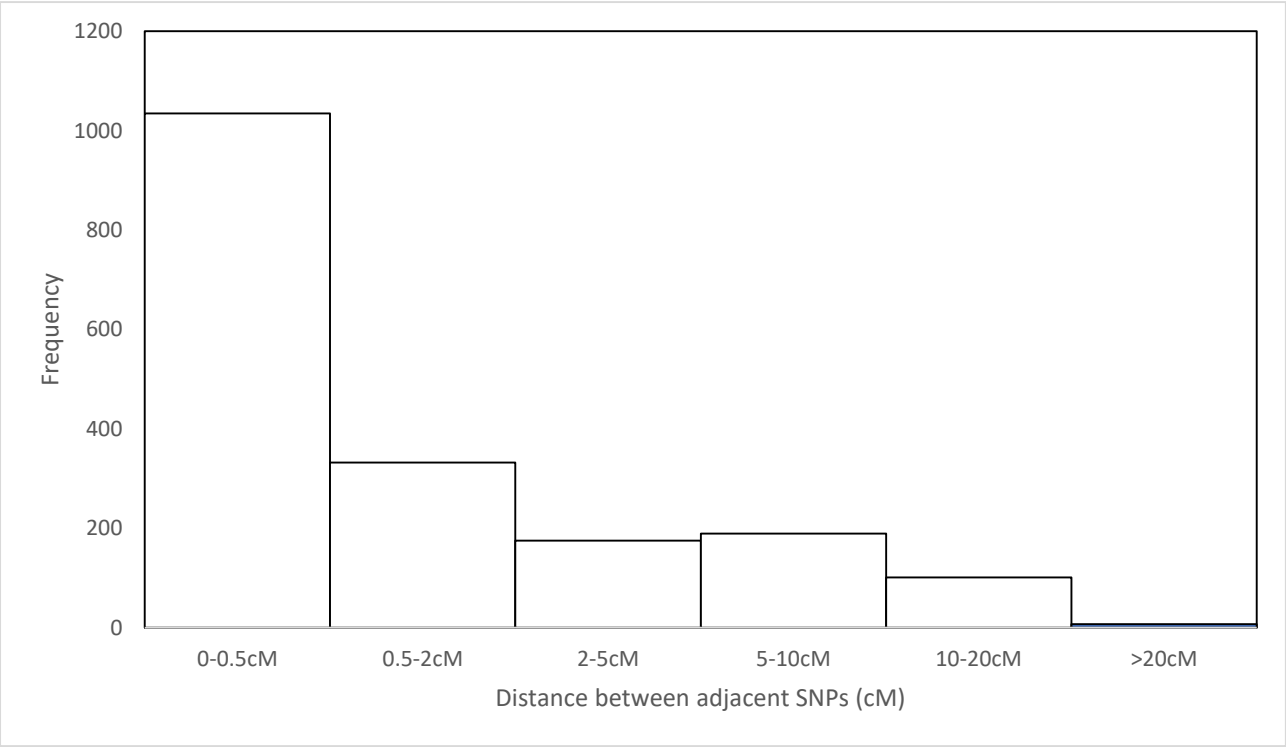


Figure 2-4. Identified frequency of genetic distance between adjacent SNP markers (cm) based on 1839 final markers mapped on all 21 wheat chromosomes.

Figure 2-5. The distribution of uncovered QTLs for target traits evaluated in combined environments on a linkage map of 15 wheat chromosomes. Map scale in centiMorgans (cM) is present on the left side of the map, with each horizontal line indicating the position of an individual SNP marker. The names of detected QTLs are shown on the right side of each chromosome, with bars indicating 95% confidence interval and a central line within the bar representing an estimated position of the QTL associated with traits uncovered in organic management systems are highlighted in red, QTL clusters highlighted in bold, consistent QTLs appeared across both management systems were underlined.

Chapter 3 Effects of *Lr34/Yr18* and *Lr37/Yr17* on phenotypic traits of breeding interest and associated marker assisted selection for these two genes in the Canadian spring wheat 'Peace' × 'CDC Stanley' mapping population

3.1 Introduction

Stem rust, leaf rust and stripe rust, caused by the airborne pathogens *Puccinia graminis, Puccinia triticina* and *Puccinia striiformis* respectively, are three important diseases in wheat globally. Leaf and stripe rust have been reported to be more destructive in western Canada in recent years. In southern Alberta Canada, grain yield loss induced by stripe rust has become an annual problem for farmers (McCallum et al., 2007). The ability to mutate and multiply rapidly, coupled with wind dispersal and the ability to travel long distances facilitates pathogen adaptation and active disease infestation during the growing season in western Canada (Singh et al., 2005). Most genes conferring resistance in disease resistant cultivars express race-specific resistance against the pathogen, which is not durable. Adult plant slow rusting genes exist in wheat and provide a more durable resistance pattern (Ellis et al., 2014). The gene cluster *Lr34/Yr18* located on chromosome 7DS and provides durable disease resistance which is not race specific (Lagudah, 2011). The occurrence of this gene cluster in historical and current spring wheat germplasm has been reported to condition moderate resistance to leaf rust, stem rust, stripe rust and powdery mildew; and also tolerance to barley yellow dwarf virus (Spielmeyer et al., 2005; Singh 1993). Leaf tip necrosis is a phenotypic response correlated to the presence of *Lr34* in the adult plant stage (Dyck 1987; Singh 1992). Another gene cluster *Lr37/Yr17* confers all-stage/seedling resistance and has been incorporated into advanced cultivars to protect yield from biotic stresses (Milus et al., 2015). It was originally located in *Triticum ventricosum* (Tausch) Cess. on chromosome 2AS and has been translocated to the short arm of hexaploid wheat 2A. This gene cluster confers substantial resistance against stripe rust, leaf rust and stem rust (Helguera et al., 2003). Pyramiding of several highly effective genes into one cultivar via marker assisted selection (MAS) may

prolong the longevity of rust disease resistances (Leonard and Szabo 2005), given that a new avirulent pathotype requires simultaneous mutations at multiple virulence loci (Pink 2002).

Single seed descent or modified bulk breeding with recurrent selection within bi- or tri-parental multi-families, along with doubled haploid (DH) breeding are among the most common wheat breeding strategies in western Canada (Reid et al., 2011; You et al., 2016). In early generations, moderate selection is conducted on rust reaction, earliness, body type and straw strength (Fox et al., 2007). Selection intensity increases to 5-10% twice a year starting at the F_4 generation, during the growing season in the Canadian summer and in a Southern-hemisphere winter nursery in the Canadian winter (Reid et al., 2011). The most advanced lines are then selected to undergo multi-location replicated yield trials for agronomic traits, disease evaluation and quality screening for at least 6 years in Canada prior to registration. The last step prior to cultivar registration is for Prairie Registration Recommending Committee for Grain (PRRCG) to vote on whether a given elite line meets agronomic, quality and decease characteristics of each grain class (Galushko and Gray, 2008). This occurs when a given line has agronomic characters (yield, height, lodging tolerance, maturity dates and test weight) and measured quality traits (protein of grain and flour, falling number, flour yield and other flour traits; farinograph, extensograph and bake traits) similar to or better than the check cultivars chosen a priori for each registration trial (Prairie Grain Development Committee 2016). The elite line must also exhibit at least intermediate resistance to stripe rust (caused by *Puccinia striiformis*), stem rust (caused by *Puccinia graminis*) and leaf rust (caused by *Puccinia triticina*), fusarium head blight (caused by *Fusarium graminearum*) and common bunt (caused by *Tilletia tritici*), as detected in multi-year, multi-replicate concurrent disease nursery trials (Prairie Grain Development Committee 2015).

The objective of this present study was to examine the segregation of disease resistance genes *Lr34/Yr18* and *Lr37/Yr17* in the biparental recombinant inbred line (RILs) population derived from the cross of CWRS cultivars ‘Peace’ and ‘CDC Stanley’, followed by the evaluation of their effects on disease resistance and phenotypic traits of breeding interest. Superior genotypes from marker assisted selection thereafter were tested in a multi-environment yield trial to

investigate the breeding result of integrating disease resistance genes *Lr34/Yr18* and *Lr37/Yr17* with elite phenotypic traits including high yielding, considerable quality attributes and desirable plant height in our breeding program, prior to official registration.

3.2 Materials and methods

3.2.1 Disease screening

A description of 'Peace' x 'CDC Stanley' RIL population development and phenotyping protocols is detailed in the previous chapter. The population consisted of 168 RILs and two parents 'Peace' and 'CDC Stanley' were arranged in a randomized complete block design with sub-blocks nested in two blocks of replications planted in leaf rust, common bunt and tan spot nurseries in Edmonton in 2016 and 2017. The population was evaluated at Lethbridge, AB (49.7˚N, 112.83˚W) for stripe rust reaction. Ten seeds of each genotype were sown in hills 25 cm apart from adjacent hills or rows. In the stripe rust disease nursery, 'Lilian' and 'Carberry' were used as resistant checks, with 'AC Barrie' and 'AC Crystal' used as susceptible cultivars. These disease reaction check cultivars were employed in addition to the check cultivars grown in the replicated field-based yield and agronomic evaluations. 'AC Barrie' and 'Park' were considered susceptible cultivars, with 'Peace' and 'Carberry' employed as resistant checks for the leaf rust screening nursery. Susceptible cultivars used for tan spot screening nursery were 'AC Barrie', 'Unity' and 'Glenlea', while moderate resistant check was 'Neepawa'. In our common bunt screening nursery, the cultivars 'Unity' and 'AC Barrie' were used as resistant checks whiles 'Glenlea' and 'Neepawa' were used as susceptible cultivars (Perez-Lara et al., 2017*a*). All susceptible checks were used as spreader rows. Susceptible spreader lines were planted every three rows in leaf rust, tan spot and common bunt nurseries; and every 6^{th} row in the Lethbridge stripe rust nursery to create homogeneous disease epidemics (Perez-Lara et al., 2017*a*).

A mixture of urediniospores of prevalent leaf rust races collected from the previous year and suspended in mineral soil was evenly sprayed on the spreader rows using a hand sprayer to initiate leaf rust epidemic inoculation. According to the modified Cobb scale (Peterson et al. 1948),

the host reaction type was scaled from 0 to 9 when spreader rows reached maximum infection, where 0 (resistant) stands for no pustules observed on the leaf area and 9 (susceptible) represents leaf area full of urediniospore pustules on each hill plot basis. Natural inoculum collected similarly the year prior, was sprayed to initiate stripe rust infection and stripe rust resistance scoring was assessed in reference to the average percentage of leaves being covered with urediniospores pustules at grain milk stage (Chen et al., 2016; Perez-Lara et al., 2017*a*; Perez-Lara et al., 2017*b*).

For tan spot, spore suspension mixed with even amount of *P. tritici-repentis* (Ptr) race 1 isolates AB7-2 and AB50-2 was sprayed to spreader rows at heading stage. Disease reaction was scored on a scale of 0 (resistant) to 9 (highly susceptible) where 0 represents no or little chlorosis on leaf area and 9 represents severe necrosis observed on most leaf area on a hill plot basis (Perez-Lara et al., 2017*b*). Race T-19 of *Telletia laevis* and race L-16 of *Tilletia tritici* were used for common bunt inoculation (Hua Chen et al., 2016). The bunt inoculum consisted of an equal mixture of these two races. All heads of each genotype in each hill plot was visually assessed for bunt infection at dough stage. The infection rate was examined based on the ratio of the number of the infected heads to the total number of heads per hill plot (Hua Chen et al., 2016).

Disease severity was examined at soft dough stage by ratio of infected heads to healthy heads within each hill plot. Lines with a disease rating of 0 to 2 were considered as resistant; lines with an average rating of 3 to 4 were considered as moderately resistant; lines with an average rating of 5 to 6 were considered as intermediate; lines with an average rating of 7 to 9 were considered as susceptible for all diseases (Perez-Lara et al., 2017*b*).

3.2.2 DNA extraction

Five seeds of each line were planted in 4 x 8 pot-trays filled with soilless media (Sunshine-LA4 Sun Grow Horticulture, Canada) in a University of Alberta growth chamber. Sixteen hours daylight, at 21˚C daytime and 19˚C night time were provided to maintain consistent plant growth. Approximately 200 mg leaf tissues were collected into a 2-ml tube at two-leaf stage, frozen with liquid nitrogen and immediately stored in a - 80˚C freezer. Genome DNA was extracted with the

procedure provided by Diversity Array Technology (http://www.diversityarrays.com) and then quantified by using the NanoDrop® ND-1000 Spectrophotomteter (Thermo Scientific, USA). The concentration of genome DNA was then normalized to 100ng μL^{-1} based on λ-DNA concentration according to the agarose gel test.

3.2.3 Marker analysis for Disease resistance genes

The population was genotyped with two resistance gene specific markers (*Lr34/Yr18/Pm38* and *Lr37/Yr17/Sr38*) at the Agricultural Genomics and Proteomics Lab, University of Alberta, Edmonton, Canada. Polymerase chain reaction (PCR) analysis took place in 96-well plates performed in a Veriti® Thermal Cycler (Applied Biosystems, Foster City, USA). A total 12µl of reaction mixture for *Lr34/Yr18/Pm38* was consisted of 1X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of dNTP, 0.1 µM of each of the forward and reverse primers (cssfr5: Lr34DINT9F: 5'- TTG ATG AAA CCA GTT TTT TTT CTA -3'; Lr34MINUSR: 5'-TAT GCC ATT TAA CAT AAT CAT GAA -3'; Lr34SPF: 5'- GGG AGC ATT ATT TTT TTC CAT CAT G -3'; Lr34DINT13R2: 5'- ACT TTC CTG AAA ATA ATA CAA GCA -3') (Lagudah et al., 2009), 0.5 unit GoTaq® Flexi DNA polymerase (QiaGen Toronto Canada) and 1µl of template DNA. The PCR condition was: 5 cycles of 1 minute initial denaturation at 94˚C, 1 minute of annealing at 58˚C and 2 minutes elongation at 72˚C, followed by 30 cycles of 30 seconds denaturation at 94˚C, annealing at 72˚C for 30 seconds and 30 seconds extension at 72˚C; finished with 5 minutes final extension at 72˚C.

The 12 µl of reaction mixture for *Lr37/Yr17/Sr38* gene marker VENTRIUP-LN2 was composed of 1X Magnesium chloride ($MgCl_2$) free PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of dNTP, 0.2 µM of each of the forward and reverse primers (VENTRIUP: 5'- AGG GGC TAC TGA CCA AGG CT -3'; LN2: 5'- TGC AGC TAC AGC AGT ATG TAC ACA AAA -3') (Helguera et al., 2003), 1 unit GoTaq® Flexi DNA polymerase (QiaGen Toronto Canada) and 1 µl of template DNA. The PCR condition started with 5 minutes initial denaturation at 94˚C, followed by 30 cycles of 45 seconds denaturation at 94˚C, 30 seconds of annealing at 65˚C, 1 minute of extension at 72˚C, and finished with 7 minutes final extension at 72˚C.

All amplified products were stored at 4˚C prior to visualization. The separation of amplified products was conducted on 2 % agarose gel containing 1.5 % SYBR® Safe DNA Gel Stain (QiaGen Toronto, Canada). The amplification of gene expression was visualized under the fluorescent by using Typhoon Trio (GE Healthcare Life Science Quebec, Canada).

3.2.4 Breeding selection experiment

Twenty-one lines were selected from the original 'Peace' x 'CDC Stanley' RIL population (≈ 12.5%), based on the ranking of yield and grain protein content as well as the adult leaf rust and stripe rust resistance genes *Lr34/Yr18* and *Lr37/Yr17*. These lines along with two parents and four check cultivars ('Carberry', 'Splendor', 'Parata' and 'Glenn') were grown in multi-location yield trials for advancing breeding selection. Trials were sown in randomized complete block designs with three replications at four sites representing wheat production climatic and soils of the western prairies including Edmonton AB, Ellerslie AB (53˚46'N, 112˚48'W), Lethbridge AB and Fort St John BC (54˚14'N, 120˚44'W) in 2017. Agronomic practices were applied following standard protocol to maintain consistent field performance during the growing season (Asif et al., 2015). Data collection of days to heading and maturity, plant height, test weight, thousand kernel weight, yield, grain protein content, sedimentation and falling number after harvesting coupled with the performance of leaf rust and stripe rust resistance were obtained to assist in subsequent selection of advanced lines as previously described in our group (Asif et al., 2015; Perez-Lara et al., 2016).

3.2.5 Statistical analyses

Statistical analyses for the RIL population have been described in the previous chapter. Analysis of variance (ANOVA) was applied for all phenotypic traits evaluated in twenty-one selected lines by using PROC MIXED in SAS version 9.4 (SAS Institute Inc., Cary, USA). The linear model applied for each trait to run the ANOVA is:

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

Where Y_{ijk} represents the observed value of each trait for the i^{th} genotype in k^{th} replication in the j^{th} environment; μ is the overall mean; G_i stands for the effect of the i^{th} genotype; E_j is the effect of the j^{th} environment; GE_{ij} represents the interaction effect between genotype G_i and E_j; $R(E)_{kj}$ is the effect of k^{th} replication within the j^{th} environment; and ε_{ijk} is the random error.

The difference of disease infection severity and reaction between genotypes with the combination of resistance/susceptibility *Lr34/Yr18* and *Lr37/Yr17* alleles was analyzed using a parametric t-test analysis (Hua Chen et al., 2016).

The description of statistical analyses for index selection has been previously detailed in our group (Chen et al. 2016), An index (*I*) was designed as a linear function of the presence of *Lr34* and/or *Lr37* (X_1) along with observed phenotypic values of grain yield (X_2), grain protein content (X_3), and plant height (X_4) where b are index coefficients:

$I = b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4$

3.3 Results

In the original mapping population grown in conventional management in 2016 and 2017, RIL lines with resistance *Lr34/Yr18* allele were shorter with lower SDS sedimentation volume than those with the susceptible *Lr34/Yr18* allele (Table 3-1). RIL lines carrying *Lr37/Yr17* resistance allele were taller with greater SDS sedimentation volume than lines without *Lr37/Yr17.* RIL lines carrying *Lr34/Yr18* also took longer time to reach heading and maturity than lines without *Lr34/Yr18*. Additionally, the presence of *Lr34/Yr18* resulted in lower kernel and test weight, and grain yield. Genotypes carrying *Lr37/Yr17* resistance allele were not significantly different from the lines without *Lr37/Yr17* for days to heading and maturity, grain yield, TWT and falling number (Table 3-1).

3.3.1 Disease resistance assessment

'CDC Stanley' expressed an intermediate to strong level of resistance to tan spot, common bunt and stripe rust in disease nurseries over two years (Table 3-2). 'Peace' was resistant to races

of common bunt pathogens. This cultivar was weakly to moderately resistant to tan spot and stripe rust with mean disease scores of 4.5 and 5, respectively, based on infection type. Transgressive segregation occurred in the RIL population for three disease reactions; RIL lines varied greatly from very susceptible to highly resistant (i.e. infection scores of tan spot and common bunt: 1-9; infection score of stripe rust: 1-9). Although 'CDC Stanley' was moderately resistant to leaf rust with a disease score of 3, 'Peace' was intermediate to leaf rust disease pressure and there was no significant difference in terms of visible leaf rust reaction in the RIL population.

3.3.2 Validation and Phenotypic correlation of molecular markers for *Lr34/Yr18* and *Lr37/Yr17*

A seven hundred fifty-one-base pairs allele of cssfr5 was detected only 'Peace' and RIL lines that carry *Lr34/Yr18* resistance allele whereas a 523-bp amplicon was amplified in 'CDC Stanley' and non-*Lr34* RIL lines. The appearance of the expected 259 bp amplicon in 'CDC Stanley' and RIL lines indicated the presence of dominant allele of *Lr37*. The proportion of RIL lines with dominant and recessive SSR marker alleles demonstrated no evidence of distorted segregation as it fit the expected 1:1 ratio at the loci of interest (*Lr34*: $\chi^2 = 0.030 < 3.84 = \chi^2_{0.05}$; *Lr37:* $\chi^2 = 6.521 < 6.68 = \chi^2_{0.05}$; respectively).

Genotypes with both *Lr34/Yr18* and *Lr37/Yr17* resistance alleles exhibited significantly greater leaf and stripe rust resistance overall than those carrying no resistance alleles (Table 3-3). On average, however, CDC Stanley expressed resistance to these two rust diseases similar to those with the two pooled resistance genes. In 2016, lines carrying only *Lr34/Yr18* resistance allele expressed lower tan spot and stripe rust infection compared with those with the *Lr37/Yr17* resistance allele (Table 3-3). Lines with either *Lr34/Yr18* or *Lr37/Yr17* allele exhibited significantly greater stripe rust resistance compared to lines without any resistance alleles over two years. The two genes appeared to have little impact on bunt expression.

3.3.3 Selection responses

Twenty-one lines were selected from the conventional trial of 'Peace' x 'CDC Stanley' RIL population based on their performance with respect to grain yield, grain protein content and SDS sedimentation volume in 2016; and the presence of any disease resistance alleles of *Lr34/Yr18* and *Lr37/Yr17* (Table 3-1). Selected lines are presented along RIL population bell curves for grain yield and protein to visualize the selection decision criteria in Figure 3-1.

All the selected RILs from the original population had higher average grain yield than the lowest yielding check cultivar Splendor (Table 3-4). There were 20 lines with greater grain yield than the lower yielding parent 'Peace' and three lines yielding more grain the high yielding parent 'CDC Stanley' (5.5 t ha^{-1}), including PS025, PS168 and PS102 (Table 3-4). Two RILs (PS025 and PS101) were taller than the tallest check 'Glenn'. Although only eight RILs were shorter than the taller parent 'Peace', all selected lines exhibited moderate to strong lodging resistance, mostly within the range of the check cultivars; except PS027 and PS064, PS101 and PS113, which lodged more than the checks.

Most selected lines had acceptable grain protein within the range of checks, with the exception of PS022, PS035 and PS122, which had lower protein content than 'Carberry' (Table 3-4). The highest grain protein content of 16.8% was in PS130. Additionally, PS025, PS064, PS076 and PS168 had greater grain protein content than the higher protein parent 'Peace'. SDS sedimentation is an indicator of dough strength. The highest SDS sedimentation level was PS064, which was significantly higher than all the checks. Lines including PS027, PS034 and PS087 exhibited acceptable grain protein content but with lower SDS sedimentation volume than the low protein / low dough strength check 'Carberry'.

Both parents were moderately resistant to tan spot while most other check cultivars were susceptible, except 'Glenn' (Table 3-4). The 21 selected lines exhibited strong to moderate resistance reactions to tan spot. Parents and most selected lines were highly resistant to common bunt with mean disease scoring ranged from 1 to 3 except PS035 and PS101, which exhibited intermediate resistance. Additionally, check cultivars were moderately resistant to leaf rust.

Although both parents were moderately leaf rust resistant, the leaf rust reactions of selected genotypes were variable from highly resistant to susceptible to this disease. Among leaf rust resistant lines, PS035 expressed a substantial degree of resistance to leaf rust pathogens. Other genotypes with improved leaf rust resistance included PS015, PS034, PS076, PS087 and PS135. All tested cultivars and RILs excluding PS022, PS104 and PS122 were highly resistant to the prevalent Canadian races of stripe rust. There was no visible infection/zero disease severity detected on PS015, PS027, PS064, PS087, PS088, and PS130.

In addition to providing substantial rust resistance, *Lr37/Yr17* conferred significant improvement on traits of breeding importance including lodging tolerance, test weight and grain yield, while *Lr34/Yr18* alone or integrated with *Lr37/Yr17* on the other hand was a potent locus that resulted in a higher SDS sedimentation volume in 21 selected RILs (Table 3-5). The presence of *Lr37/Yr17* resistance alleles in five genotypes induced better lodging tolerance (i.e. reduction of lodging score by 1) and 0.4 t ha^{-1} increased grain yield in comparison with four RILs with *Lr34/Yr18* resistance allele only. Five lines that carried *Lr37/Yr17* resistance allele also exhibited 0.3 t ha^{-1} greater grain yield ($P < 0.001$) than six lines without any of these two resistance alleles. The expression of the *Lr34/Yr18* resistance allele alone in four lines contributed 8.4-10 mm greater SDS sedimentation volume than lines without *Lr34/Yr18* and *Lr37/Yr17* resistance alleles and lines only carrying *Lr37/Yr17* resistance allele respectively. In contrast to the effect of *Lr34/Yr18* alone, the presence of both *Lr34/Yr18* and *Lr37/Yr17* induced 9 mm greater SDS sedimentation in six genotypes that carried both genes. The presence of additional resistance allele *Lr37/Yr17* increased test weight.

3.4 Discussion

3.4.1 The effects of Disease resistance genes *Lr34/Yr18* and *Lr37/Yr17*

'Peace' carries the resistance allele of the adult plant slow rusting disease gene cluster *Lr34/Yr18* and 'CDC Stanley' carries *Lr37/Yr17* (Randhawa et al., 2013a). In this study, the performance of a RIL population derived from a cross between 'Peace' x 'CDC Stanley' was tested

for disease reaction in stripe and leaf rust, tan spot and common bunt nurseries. We wished to undercover the potential enhancement of the disease resistance through the additive effects contributed by the integration of *Lr34/Yr18* and *Lr37/Yr17* against rust diseases. Lines with pooled *Lr34/Yr18* and *Lr37/Yr17* resistance alleles were significantly more resistant to leaf and stripe rust, but not tan spot or common bunt. Differences in tan spot and stripe rust infection severity were observed among several groups of genotypes carrying combinations of *Lr34/Yr18* and *Lr37/Yr17* resistance/susceptibility alleles (i.e. Absent-Absent, Absent-Present, Present-Absent and Present-Present, respectively) in the current study. Pyramiding resistance alleles of *Lr37/Yr17* and *Lr34/Yr18* into wheat cultivars enhances resistance durability as many recorded epidemics of stripe rust and leaf rust globally have been the consequence of lacking adequate race specific genes and/or non race-specific resistance genes (McCallum et al., 2007; Wan et al., 2007; Chen 2007).

Lines carrying the resistance allele of *Lr34/Yr18* reached the heading stage and matured earlier, with lower kernel weight and considerably reduced grain yield. Drijepondt et al. (1990) reported that 'Thatcher', a leaf rust susceptible cultivar normally expresses higher grain yield in a disease-free environments than the disease resistant line 'RL6508' with *Lr34/Yr18*. However, under high disease severity 'Thatcher' yielded 25% less grain with 16% of less kernel weight than 'RL6508'. Singh and Huerta-Espino (1997) reported reduced kernel weight and a 15% of a yield penalty associated with the presence of the resistance allele of *Lr34/Yr18* in certain disease absent areas. Lines with the resistance allele of *Lr34/Yr18* were shorter with lower SDS sedimentation volume. In a near-isogenic lines population derived from 'Thatcher' the leaf rust susceptible cultivar, and 'Karlee' the resistance genes carrier of *Lr29*, *Lr34*, *Lr35* and *Lr37*, Labuschagne et al. (2002) also reported that lines with *Lr34/Yr18* exhibited lower SDS-sedimentation volume but a higher grain protein content.

Lines with resistance allele of *Lr37/Yr17* were shorter with lower kernel weight and higher SDS sedimentation volume. Kloppers et al. (1995) reported no genetic association between deleterious quality characteristics and the presence of *Lr37/Yr17* in South African wheat breeding

lines. Genotypes with or without this rust resistance gene cluster did not differ for grain yield in our study, which implied that unlike *Lr34/Yr18*, this gene does not carry a yield penalty.

The relationship between grain yield reduction and the presence of *Lr34/Yr18* may be due to genetic linkage or pleiotropic effects. Breakage of genetic linkage under target disease-free conditions would be one possible way to address this breeding dilemma (Brown, 2002). A successful example was the *Pch1* gene translocated from the wild grass *Aegilops ventricosa* that confers resistance to a stem-base disease eyespot (*Tapesia* spp.). It has been reported to be no longer linked with the yield reduction gene in wheat recombinant lines. In contrast, the existing linkage between yield penalty and resistance attributed by other resistance genes including leaf rust resistance gene *Lr9* originated from *Aegilops umbellulata* (Ortelli et al., 1996), stem rust resistance gene *Sr26* from *Agropyron elongatum* (Latter et al., 1988) and wheat streak mosaic virus resistance gene *Wsm 1* from *Thinopyrum intermedium* have not yet been broken thus hampered the selection process (Sharp et al., 2002). There is a co-expression of leaf tip necrosis on the flag leaf and rust resistance attributed by *Lr34/Yr18* (Singh 1992). Because of the close association between leaf tip necrosis and *Lr34/Yr18*, it is often used as a phenotypic marker for the presence of *Lr34/Yr18* resistance allele (Mishra et al., 2005). However, the necrotic leaf area on leaf tips characteristic of the expression of this gene cluster, results in lower photosynthetic ability which ultimately impacts grain yield potential.

3.4.2 Marker assisted selection

A major objective of this study was to cross parents carrying resistance alleles of *Lr34/Yr18* and *Lr37/Yr17,* thereby attempting to pool resistance genes while selecting for improved grain yield potential, lodging resistance, quality attributes, and disease resistance in the CWRS class of wheat. We therefore selected 21 lines (or 12.5%) from the population, to undergo replicated multi-location yield trials in the next year. These lines were selected on the basis of yield and protein content, and also considering the allelic makeup of *Lr34/Yr18* and/or *Lr37/Yr17* resistance allele.

Among 21 selected lines, we selected five lines (PS015, PS034, PS064, PS102, and PS168) as having acceptable and desirable grain yield, lodging resistance, test weight, grain protein, sedimentation and falling number profiles, with heightened disease resistance. Of these five lines, one (PS034) carried resistance alleles at both gene clusters *Lr37/Yr17* and *Lr34/Yr18*. Three lines (PS015, PS102 and PS168) carry the resistance allele of *Lr37/Yr17* only and one line (PS064) carries the resistance allele of *Lr34/Yr18* only. Thus, whole-scale dependence on markers in a marker-assisted selection program will likely eliminate desirable genotypes such as quality attributes.

Kuchel et al. (2007*b*) reported a positive effect of *Lr37/Yr17* locus on grain yield gain across the range of stripe rust infection severities in a mapping population consisting of doubled haploid lines derived from a stripe rust resistant southern Australian wheat cultivar 'Trident' and susceptible cultivar 'Molineux'. The deployment of *Lr37/Yr17* has been successfully utilised in breeding programs for its resistance to several rust races but also for its disassociation with yield penalty or undesirable influence on grain yield components where disease is absent (Kuchel et al., 2007*b*). Our group previously reported a lack of significant difference between SDS sedimentation attributes and the presence of *Lr34/Yr18* resistance allele in a mapping population derived from 'CDC Teal' and 'CDC Go' (Hua Chen et al., 2016).

Population size is a key parameter to maintain the effectiveness of marker assisted selection for desirable traits, as random genetic drift may be caused by insufficient population size (Wang et al., 2016). As transgressive segregation for all phenotypic traits was identified in our population, a greater effective population size would be required for the breakage of the limits in artificial selection by breaking linkage of undesirable genes as well as maximizing genetic variability and additive genetic gain (Comeron et al., 2008; Hill and Robertson, 1968; Kimura, 1983). A single seed descent or doubled haploid derived population size required for a marker assisted selection for one target marker in combination with multiple phenotypic traits with quantitative inherence would include more than 500 lines to expect possible selection success (Chen et al., 2016). In rice breeding, two F_2 populations with 500 and 806 RILs evolved from two crosses between three

resistant germplasms were used to pyramid four bacterial leaf blight (BLB) resistance genes. 12 pyramided genotypes with three resistance genes from two populations exhibited considerably yielding and desirable agronomic potentials with 100% of marker assisted selection efficiency (Perumalsamy et al., 2010).

Bonnett et al. (2005) proposed that doubled haploid (DH) and backcrossing as two effective breeding strategies as better approaches for introgressing of target genes. Backcrossing allows great reduction of population size for recovery of target genotypes but only facilitated the introgression of one or two genes. DH involves an enrichment in F_2, which increases frequency of desirable alleles and enables screening in relatively small populations (Bonnett et al., 2005). A successful example was demonstrated in a practical development of a superior Australian wheat germplasm that carries glutenin genes *Glu-D1* and *Glu-A3* and multiple major rust resistance genes including *Lr34/Yr18*, *Lr46/Yr29* and *Lr24/Sr24* (Kuchel et al., 2007*a*). In their validation study, backcrossing was employed to develop a BC_1F_1 population of 72 heterogeneous individuals at *Glu-A3, Lr34/Yr18* and *Lr46/Yr29* loci. Marker assisted screening was then conducted to select 242 lines with target alleles, which were used to generate a DH population. As a result, advanced wheat lines were identified exhibiting improved dough resistance and extensibility along with enhanced rust resistance attributed by multiple gene complexes through the marker-assisted selection (Kuchel et al., 2007*a*).

In general, the RIL population expressed improved resistance of leaf rust and stem rust compared to both parents. The durability of rust resistance would be extended by the presence of both *Lr34/Yr18* and *Lr37/Yr17* resistance alleles. Despite the negative correlation between the presence of rust resistance genes and economically important traits especially grain yield, the agronomic and quality performance of 21 selected lines, on average, were better than most the check cultivars. Although there was no individual genotype represented advanced potential with all desirable traits, five lines with substantial disease resistance and improved agronomic and quality characters remain in the breeding process.

3.5 Conclusions

In this study, the performance of a RIL population derived from a cross between 'Peace' x 'CDC Stanley' was tested for disease reaction in stripe and leaf rust, tan spot and common bunt nurseries. We wished to undercover the potential enhancement of the disease resistance through the additive effects contributed by the integration of *Lr34/Yr18* and *Lr37/Yr17* against rust diseases. Lines with pooled *Lr34/Yr18* and *Lr37/Yr17* resistance alleles were significantly more resistant to leaf and stripe rust, but not leaf spots or common bunt. Differences in leaf spot and stripe rust infection severity were observed among several groups of genotypes carrying combinations of *Lr34/Yr18* and *Lr37/Yr17* resistance/susceptibility alleles (i.e. Absent-Absent, Absent-Present, Present-Absent and Present-Present, respectively). Despite the negative correlation between the presence of *Lr34/Yr18* rust resistance genes and economically important traits especially grain yield, the agronomic and quality performance of 21 selected lines, on average, were better than most the check cultivars. Although there was no individual genotype represented advanced potential with all desirable traits, five lines with substantial disease resistance and improved agronomic and quality characters remain in the breeding process. Of these five lines, one (PS034) carried resistance alleles at both gene clusters *Lr37/Yr17* and *Lr34/Yr18*. Three lines (PS015, PS102 and PS 168) carry the resistance allele of *Lr37/Yr17* only and one line (PS064) carries the resistance allele of *Lr34/Yr18* only. Thus, whole-scale dependence on markers in a marker-assisted selection program will likely eliminate desirable genotypes.

3.6 Tables and figures

Table 3-1. Effects of resistance alleles of *Lr34/Yr18* and *Lr37/Yr17* apart on 10 traits including days to heading and maturity, plant height, thousand kernel weight (TKW), grain yield, test weight (TWT), SDS sedimentation volume, falling number and grain protein weight (WPRO) for 168 'Peace' × 'CDC Stanley' Recombinant Inbred Lines in conventional environment.

Trait	*Lr34/Yr18/Pm38*				*Lr37/Yr17/Sr38*			
	Present	Absent	Difference	SE	Present	Absent	Difference	SE
Heading (day)	52	51	0.4 *	0.14	52	52	0	0.15
Maturity (day)	93	92	0.9**	0.22	92	92	0	0.22
Plant height (cm)	97	98	-1.2*	0.6	98	97	1.3*	0.61
TKW (g)	37.9	38.6	-0.7**	0.2	38.6	38	0.6*	0.21
Yield (t ha^{-1})	5.47	5.86	-0.4**	0.07	5.72	5.73	0	0.08
TWT (kg hL^{-1})	81.4	81.8	-0.4**	0.12	81.8	81.4	0.4	0.12
Sedimentation (mm)	78	79	-1.2*	0.57	79	78	1.4*	0.57
Falling number (s)	452	461	-8.7	5.00	457	457	0	5.06
WPRO (%)	14.7	14.5	0.2	0.06	13.2	12.9	0.3	0.18

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

Table 3-2. Summary of Least Square Mean score and significance for disease severity evaluation of tan spot, common bunt, stripe rust and leaf rust in the 'Peace' × 'CDC Stanley' Recombinant Inbred Lines population 2016 -2017 in western Canada.

	No. of	Parents		RIL population					
Disease	Trials	Peace	CDC Stanley	min	max	mean	SD	CV (%)	F value
Tan spot	2	4.5	2.9	1.0	9.0	3.8	1.1	33	1.9*
Common bunt	2	1.0	3.0	1.0	9.0	1.9	1.3	68	3.8*
Stripe rust	7	5.0	1.0	1.0	9.0	3.4	2.0	86	15.6*
Leaf rust	1	5.0	3.0	1.0	9.0	4.2	2.0	48	-

*Significant at $P < 0.05$

Table 3-3. Mean and significance of the disease infection rating difference for the Recombinant Inbred Lines with or without resistance allele(s) of *Lr34/Yr18* and/or *Lr37/Yr17* on leaf rust, tan spot, common bunt and stripe rust in the 'Peace' × 'CDC Stanley' mapping population planted in disease nurseries between 2016 and 2017.

Year	*Lr34/Yr18*	*Lr37/Yr17*	Leaf rust	Tan spot	Common bunt	Stripe rust[a]	Stripe rust[b]	Stripe rust[c]
			Mean	Mean	Mean	Mean	Mean	Mean
2016	Peace		-	4.2	1.8	3.3	7.3	4
		CDC Stanley	-	3.3	3.3	3.5	2.3	1.3
	Absent	Absent	-	4	2.1	5.5	6.9	7.9
	Absent	Present	-	3.7	2.4	3.8	4.4	3.6
	Present	Absent	-	4.3	1.8	3.7	6	5.6
	Present	Present	-	3.9	2.6	2.4	2.5	2
	P-value							
	AA vs. AP		-	┐	ns	***	***	***
	AP vs. PA		-	***	ns	**	┐	**
	PA vs. PP		-	ns	ns	***	***	***
	AA vs. PA		-	**	ns	***	**	*
	AP vs. PP		-	*	ns	**	ns	ns
	AA vs. PP		-	ns	ns	***	***	***
2017	Peace		3.2	4	1	0.7	-	-
		CDC Stanley	3.7	3	2.3	4.4	-	-
	Absent	Absent	4.6	3.5	2	34	-	-
	Absent	Present	4.5	3.3	1.6	12.1	-	-
	Present	Absent	4.1	3	1.7	9	-	-
	Present	Present	3.9	3.2	1.7	7.4	-	-
	P-value							
	AA vs. AP		ns	ns	┐	***	-	-
	AP vs. PA		ns	ns	ns	ns	-	-
	PA vs. PP		ns	ns	ns	ns	-	-
	AA vs. PA		*	ns	ns	***	-	-
	AP vs. PP		*	ns	ns	┐	-	-
	AA vs. PP		**	ns	ns	***	-	-

┐ Significant at P = 0.1; *Significant at *P* = 0.05; ** Significant at *P* = 0.001

[a] Stripe rust nursery grown in Lethbridge, AB
[b] Stripe rust nursery grown in Edmonton, AB
[c] Stripe rust nursery grown in Creston, BC

Table 3-4. Summary of the Least Squares Means for disease infection ratings of leaf rust, stripe rust, tan spot and common bunt, and for seven traits and yield with rank by yield for twenty-one selected Recombinant Inbred lines from the 'Peace' × 'CDC Stanley' mapping population grown as multiple-site yield trials in 2017 over three locations in Alberta Canada.

							Grain Yield									
Entry	*Lr34*/ Yr18	*Lr37*/ *Yr17*	Leaf rust (0-9)	Stripe rust[a] (0-100)	Tan spot (0-9)	Bunt (0-9)	t ha^{-1}	%Chk	Rank	WPRO (%)	SDS (mm)	Ht (cm)	Lodging (1-9)*	Maturity (day)	TWT (kg/ha)	FN (s)
Glenn	A[b]	A	4.0	0	2.0	1.5	4.89	104	23	15.5	86	97	2.6	96	84	416
Splendor	A	A	6.0	10	3.0	1.0	4.22	90	27	16.2	91	93	3.1	92	79.5	489
CDC Stanley	A	P	3.5	5	3.0	3.0	5.53	118	4	14.9	77	94	2.2	97	81.8	495
Peace	P	A	4.0	0	3.0	1.0	4.89	104	24	15.2	80	94	3.1	98	81.3	494
Carberry	P	A	-	-	4.0	-	5.00	106	21	14.4	76	90	3.1	94	82.5	426
Parata	P	A	4.5	0	3.0	1.0	4.72	100	25	15.7	85	89	3.2	91	81.6	552
PS022	A	A	4.0	50	2.0	3.0	5.30	113	13	14.1	70	94	2.6	96	82	503
PS025	A	A	3.5	3	3.5	1.0	5.68	121	1	15.4	78	100	2.8	101	80.2	400
PS027	A	A	3.0	0	3.5	1.0	5.24	111	15	14.9	71	94	3.2	94	82.4	505
PS104	A	A	3.5	70	3.5	1.0	5.26	112	14	15.1	77	96	2.6	99	81.5	449
PS122	A	A	4.0	30	3.5	1.0	5.05	107	20	14.2	79	95	3.1	99	81.8	469
PS130	A	A	6.5	0	4.0	1.0	4.68	99	26	16.8	83	93	3.0	94	80.6	513
PS015	A	P	2.5	0	4.0	1.0	5.44	116	8	14.4	71	93	2.3	97	82.8	442
PS047	A	P	6.5	4	4.0	1.0	5.47	116	6	14.7	79	95	2.5	97	82.3	586
PS102	A	P	3.5	3	3.5	1.0	5.63	120	3	14.7	85	95	2.8	99	82.3	428
PS117	A	P	5.0	2	3.5	2.0	5.33	113	11	14.7	80	91	2.4	95	83.2	580
PS168	A	P	4.5	2	3.5	1.5	5.66	120	2	15.9	82	96	2.5	98	80.9	527
PS045	P	A	4.0	10	2.5	2.0	5.14	109	18	14.8	84	97	2.9	98	82.3	499
PS064	P	A	3.0	0	2.5	1.0	5.15	109	17	15.7	102	95	3.4	98	81	509
PS101	P	A	5.5	3	4.5	4.0	4.90	104	22	14.6	87	98	3.6	96	80.8	466
PS135	P	A	3.5	5	3.5	2.0	5.43	115	9	14.5	77	93	2.8	96	82.1	447
PS034	P	P	2.0	0	3.5	2.5	5.49	117	5	15.2	74	92	2.9	94	82.2	498
PS035	P	P	1.0	2	4.0	4.0	5.20	110	16	13.9	74	94	2.5	94	82.4	490
PS076	P	P	5.0	10	2.0	2.0	5.11	109	19	15.3	83	96	2.5	94	83.2	615
PS087	P	P	2.5	0	3.0	1.0	5.30	113	12	15.1	73	95	3.0	102	82.9	442
PS088	P	P	3.0	0	3.0	1.0	5.37	114	10	15	86	95	2.7	96	82.2	548
PS113	P	P	4.5	2	4.5	1.5	5.47	116	7	14.5	81	96	3.4	99	83	531
Mean							5.30			14.9	80	95	2.8	97	82	498

CV (%)	6.50	3.8	5.5	3.8	23.8	2.9	1.1	15
LSD	0.32	1.0	7.6	4.3	0.6	2.7	1.6	126

[a] Stripe rust severity = percentage of rust infection on the canopy

[b] Absence (A) or Presence (P) of the resistance alleles of *Lr34/Yr18* and *Lr37/Yr17*

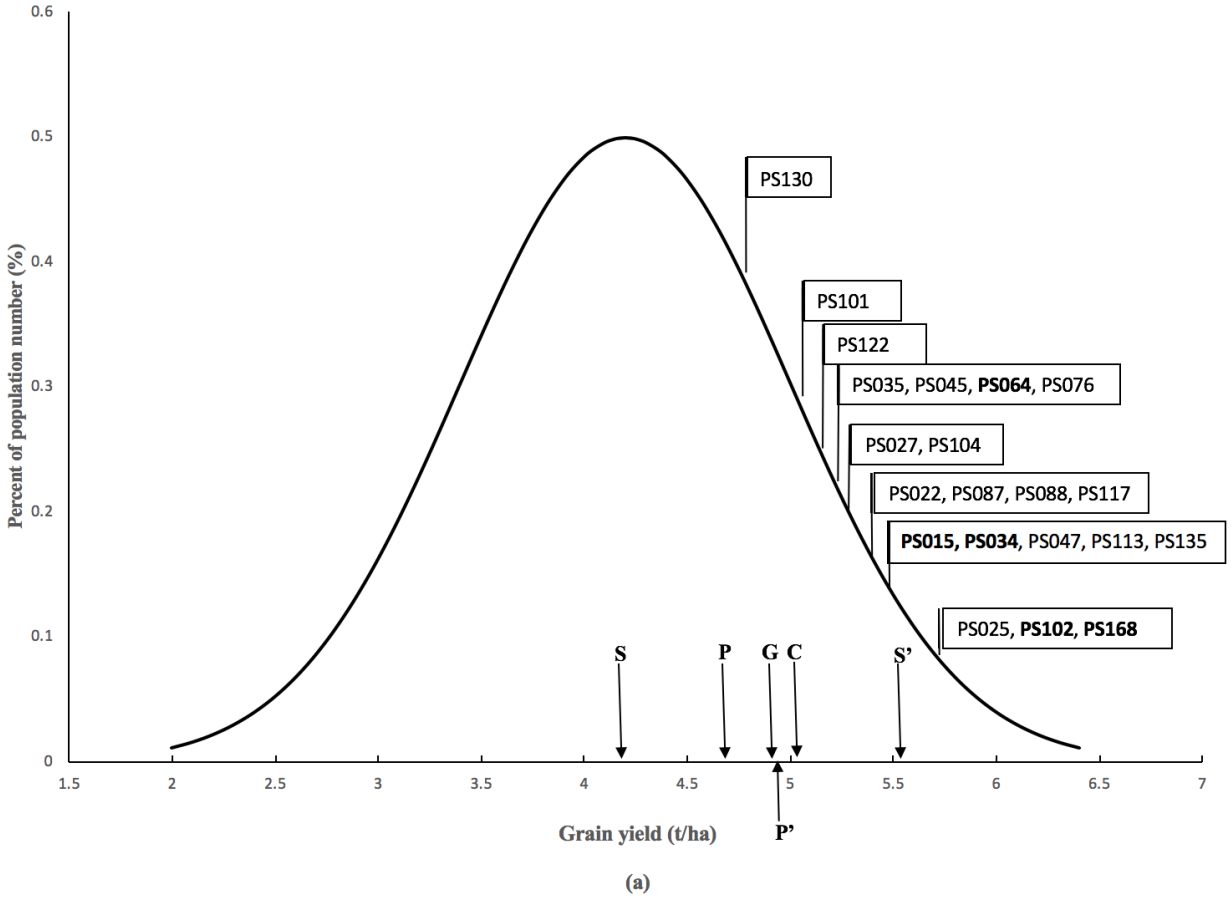
[*]Lodging (1-9) where scale 1 stands for strongly resistant and 9 stands for completely susceptible to lodging

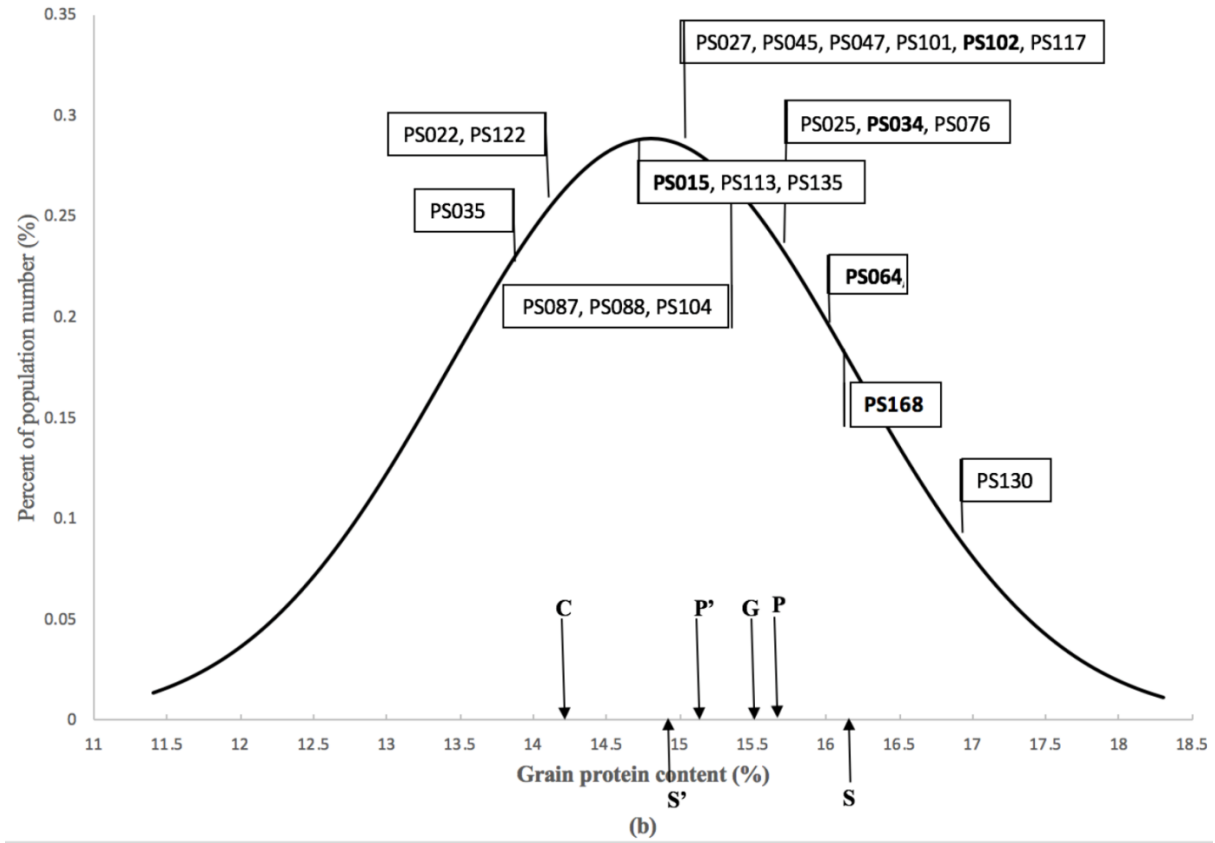
Table 3-5. Least Square Means estimates, significance test and contrasts of group effects of twenty-one selected lines from 'Peace' × 'CDC Stanley' mapping population grouped into four combinations of *Lr34/Yr18* and *Lr37/Yr17* resistance/susceptibility alleles (presence/absence) for days to maturity, plant height, lodging tolerance, test weight, grain yield, grain protein content (WPRO), SDS sedimentation volume and falling number over three locations in 2017.

Lr34/Yr18	*Lr37/Yr17*	No. of lines	Maturity (days)	Height (cm)	Lodging	Test weight (kg hL^{-1})	Yield (t ha^{-1})	WPRO (%)	SDS (mm)	FN (s)
A	A	6	97	95	2.9	81.5	5.2	15.1	78	482
A	P	5	97	94	2.6	82.4	5.5	14.9	80	520
P	A	4	97	95	3.2	81.2	5.1	14.9	88	468
P	P	6	96	95	2.8	82.6	5.3	14.9	79	521
Effect of Locus/loci										
Lr34/Yr18			1.44	0	6.39	0.08	3.91	0.2	5.89*	0.07
Lr37/Yr17			0.07	1.23	11.36**	17.95***	14.97**	0.29	3.689 ⅂	3.83 ⅂
Lr34 * Lr37			0	0.26	0.01	1.09	1.14	0.11	8.8*	0.1
Effect of AA vs. AP			0.19	1	0.35 ⅂	-0.8 ⅂	-0.3**	0.4	-2	-38
Effect of AA vs. PA			1	0.36	-0.28 ⅂	0.3	0.06	0.3	-10**	13
Effect of AA vs. PP			1	1	0.09	-1 ⅂	-0.1	0.4	-1	-39
Effect of AP vs. PA			0.59	-1	-1**	1.2 ⅂	0.4**	0.4	-8.4*	52
Effect of AP vs. PP			1	-0.34	-0.26 ⅂	-0.2	0.2 ⅂	0.3	1	-1
Effect of PA vs. PP			0.15	0.42	0.37 ⅂	-1.4*	-0.2 ⅂	0.4	9*	-53

⅂ Significant at P < 0.1; * Significant at *P < 0.01;* ** Significant at *P* = 0.001; *** Significant at *P* < 0.0001

Figure 3-1. Distribution based on grain yield (a) and protein content (b) of biparental Recombinant Inbred Lines population derived from a cross between 'Peace' and 'CDC Stanley' with the resistance alleles of *Lr34/Yr18* and/or *Lr37/Yr17* in 2017. Relative positions of 21 selected lines were indicated and five lines selected for the 2018 multi-site yield trial were highlighted in bold. Relative positions of four check cultivars Carberry (c), Glenn (g), Parata (p), and Splendor (s) along with two parents Peace (P') and CDC Stanley (S') were also represented.




PS027, PS045, PS047, PS101, PS102, PS117
PS025, PS034, PS076
PS022, PS122
PS015, PS113, PS135
PS035
PS064
PS087, PS088, PS104
PS168
PS130
C
P'
G
P
S'
S
Percent of population number (%)
Grain protein content (%)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
11
11.5
12
12.5
13
13.5
14
14.5
15
15.5
16
16.5
17
17.5
18
18.5
(b)

Chapter 4 General discussion and conclusions

4.1 Introduction

Hexaploid wheat (*Triticum aestivum* L.) serves as one of the most staple food sources in the world that not only provides predominant calories of carbohydrates in the human diet but also becomes the significant ingredients of animal feed. Canada is one of the largest exporter of wheat, with majority of wheat (~96%) produced in the three prairie provinces Alberta, Saskatchewan and Manitoba (McCallum and DePauw, 2008). Canada Western Red Spring (CWRS) wheat class that targets the premium market of high-volume bread loaf accounts for approximately 86% of total exported wheat (Canadian Grain Commission, 2018). The development of elite cultivar in Canada primarily focuses on (i) desirable agronomic traits including early maturity, relatively short plant stature, and high yield potential along with pre-harvest sprouting resistance; (ii) superior quality attributes including high grain protein content and dough strength; and (iii) durable fungal disease resistance possessed by major disease resistance loci (Prairie Grain Development Committee 2016).

Organic agriculture refers to a sustainable production system with reduced external inputs and no application of synthetic fertilizer, pesticide or genetically modified organisms (Bruinsma 2003). The benefits of organic management system not only support environmental stewardship but also protect the health of ecosystems, animals and humans in a long term (Mason and Spaner, 2006). However, crop yield reductions are commonly associated with organic production in comparison with conventional production, possibly due to soil nutrient deficiencies and higher weed pressure (Degenhardt et al., 2005). In Canada, wheat cultivars exhibiting improved competitive ability including increased plant height and early maturity could effectively suppress aboveground weeds biomass and enhance grain yield potential in organic management system (Reid et al., 2009).

Pre-harvest sprouting (PHS) refers to the in-spike germination of kernels at physiological maturity in response to relatively high humidity prior to harvest. As a major downgrading

parameter, the occurrence of PHS often leads to great economic loss from substantial reduction of grain yield, end-use quality and possibly the seed variability for planting in the next year (Derera 1989). Sprouted grains are low in kernel weight and the bread loaves made from the flour of germinated grain exhibits reduced functional quality, represented as having sticky crumb, dark colored crust, large air bubble holes in the bread and they are hard to slice (Singh et al., 2010). Therefore, breeding for an elite wheat cultivar with PHS resistance is of necessity in order to save approximately 100 million annual loss for grain producers in western Canada particularly under PHS favorable weather conditions (Clarke et al., 2005). However, the quantitative inheritance of PHS resistance plus the high susceptibility of genetic expression to environmental effects most likely hinder the phenotypic selection process. Marker assisted selection based on the genetic information of identified quantitative trait loci that are associated with PHS resistance would improve the efficiency and reliability of conventional breeding methodologies (DePauw et al., 2012).

Wheat stem rust (*Puccinia graminis* f. sp. *tritici*), leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*) are three major rust diseases in Canada (McCallum et al., 2007). Under pathogen favorable conditions, stem rust is able to cause total crop loss within a relatively short period due to significant water loss from infected lesions (McCallum et al., 2007). Leaf rust has been more prevalent in western Canada with various degrees of disease severity of the disease epidemics each year, which could result in tremendous economic losses. In addition to fungicide application to control the diseases, growing modern cultivars that carry durable effective resistance genes is an effective strategy to protect yield loss from disease pressure. The deployment of multiple effective resistance genes in adapted germplasm could potentially improve the resistance durability against common pathogen races (REX consortium 2016).

Molecular markers have been commonly adopted in specific developing populations, due to their relatively low cost, co-dominant inheritance, precision of selecting specific traits of interest and ability to speed up the process of cultivar development in comparison with conventional breeding process (H. S. Randhawa et al., 2013b). Single nucleotide polymorphism (SNP) for

example has emerged to be the powerful marker system to detect specific chromosomal regions that are associated with economically important traits exhibiting complex inheritance. The discovery of major genes and quantitative trait loci characterized by linkage-based analysis and association mapping therefore provides optimistic insight of the prospects of molecular assisted selection in crop improvement (Mulualem and Bekeko, 2016).

The goal of this thesis was to explore the potential improvement of disease resistance to leaf and stripe rusts from pooling *Lr34/Yr18* and *Lr37/Yr17* and to understand the genetic control of agronomic traits in a Recombinant Inbred Lines (RILs) population derived from two Canadian western hard red spring wheat cultivars. The specific objectives were: 1) to assess the field performance of Canadian hard spring wheat under both conventional and organic systems and to uncover the QTLs for phenotypic traits in two management systems; 2) to validate the presence of a major QTL (*Qphs.usask-4A*) for pre-harvest sprouting resistance and to evaluate the effect of this QTL on phenotypic traits of breeding interest; and 3) to evaluate the effects of *Lr34/Yr18* and *Lr37/Yr17* on disease resistance and phenotypic traits of breeding importance and to select superior genotypes for multi-environment yield trial.

4.2 Contribution to Knowledge

The contribution of the first study is the detection of 30 QTLs for phenotypic traits in conventional management system that explained 0.5 – 23.3% of phenotypic variance and 20 QTLs in organic management system that explained 1.3 – 25.9% of phenotypic variance in the 'Peace' × 'CDC Stanley' Canadian western red spring wheat RIL population. Three coincidental QTLs shared common confidence intervals that are associated with multiple traits. QTLs on chromosome 2D, 4B, 6B, 6A and 6D for plant height, protein content, grain yield, one QTL for thousand kernel weight (TKW), falling number, lodging resistance expressed consistency across management systems. All agronomic traits in the RIL population exhibited transgressive segregation. Only days to heading and maturity, grain yield, TKW, test weight and grain protein content showed high broad-sense heritability across management systems. Strong correlations were observed between

days to heading and maturity, between days to heading and plant height, between days to heading and grain protein content, between plant height and grain protein content under conventional management. Correlation between heading time and days to maturity and test weight, between plant height and grain yield and test weight, between test weight and TKW were identified in organically managed systems. In addition, the major QTL *Qphs.usask-4A* associated with pre-harvest spouting resistance was only identified in 'CDC Stanley'. RILs carrying this QTL expressed significant falling number, test weight and TKW.

The second study was to investigate the differences in disease responses and phenotypic traits of 'Peace' × 'CDC Stanley' RIL population with respect to the resistance allelic combination of the presence/absence of *Lr34/Yr18* and/or *Lr37/Yr17*. Genotypes with resistance *Lr34/Yr18* allele were shorter with lower SDS sedimentation volume and took longer to flower and to reach physiological maturity. They also exhibited lower TKW, test weight and grain yield. RILs carrying *Lr37/Yr17* resistance allele were taller with greater SDS sedimentation. Lines with or without *Lr37/Yr17* did not differ in days to heading and maturity, grain yield, test weight and falling number. Genotypes with both *Lr34/Yr18* and *Lr37/Yr17* resistance alleles exhibited significantly greater leaf and stripe rust resistance. Lines with either *Lr34/Yr18* or *Lr37/Yr17* represented significant greater stripe rust resistance. Yet the two genes seemed to have little impact on bunt infection. Twenty-one lines from the RIL population with high yield combined with the presence of *Lr34/Yr18* and/or *Lr37/Yr17* resistance allele(s) were grown in replicated yield trials over three locations. We attempted to select superior lines with higher grain yield and protein content, desirable plant height and improved disease resistance conferred by *Lr34/Yr18* and/or *Lr37/Yr17*. However, we failed to select one elite genotype as a much larger population size is required for the combined selection of multiple quantitatively inherited traits.

4.3 General discussion

One hundred sixty-eight (168) recombinant inbred lines of 'Peace' × 'CDC Stanley' population generally matured six days earlier with on average 19 % less grain yield and 12 % less

grain protein content and lower dough strength in the organically managed land. Lower days to grain maturity with lower protein concentration was also reported in malting barley cultivars under limited nitrogen supply (O'Donovan et al., 2011). A negative relationship between heading time and plant height in our population was possibly due to the absence of polymorphism at *Ppd-D1* gene in both parents. Negative correlation between protein content and days to heading in conventional management system was aligned with previous studies, explaining that 80 % of grain protein content was ascribed to nitrogen assimilation from post-anthesis plant biomass (Austin et al., 1977; Sylvester-Bradley and Kindred, 2009). On the other hand, early heading lines producing lower protein content in organic land can be explained by the nitrogen source for protein content accumulation under nitrogen limited conditions that were derived from plant biomass prior to heading or leaf senescence (Osman et al., 2012). Only six QTLs out of 30 QTLs in conventional management system were consistent in organic management system indicating the substantial effect of management on the expression of most QTLs identified in the present study. Major effect QTLs that are management specific have potential to be used in cultivar development adapted to specific management systems. Marker assisted selection for coincidental QTLs could be advantageous for incorporation of multiple target traits with minimal negative effect of linkage drag. However, the stable coincidental QTL on 2D was located in considerably larger chromosomal segments. A larger population containing sufficient recombinants therefore is required to break the tight linkage of possible loci that are associated with corresponding traits of breeding importance. We also validated a major PHS resistance QTL (*Qphs.usask-4A*) with three molecular markers DuPw004, Barc170 and wms650 in 'Peace × CDC Stanley" population. Lines carrying this QTL headed significantly earlier possibly due to genetic linkage of these two traits, which was also reported in rice (Lin et al., 1998). The various components of PHS resistance cannot be only reflected by falling number, as this test serves as a quantification of α-amylase activities, but by more direct measurement of the liquefaction of a sample (Johansson, 2002). Although all three diagnostic markers are associated with the PHS resistance QTL, the physical

position of barc170 and wms650 were considerably apart from each other in spite of the short genetic distance between these two markers.

The presence of both *Lr34/Yr18* and *Lr37/Yr17* resistance alleles conferred enhanced resistance against rusts in our mapping population. However, lines with *Lr34/Yr18* expressed reduced plant height, SDS sedimentation and more importantly yield penalty, possibly due to genetic linkage or pleiotropic effects of co-expression of leaf tip necrosis on flag leaf and rust resistance (Mishra et al., 2005). The necrotic area on canopy leads to lower photosynthetic ability that ultimately impacts grain yield potential. On the other hand, no negative effect on grain yield and quality was associated with the presence of *Lr37/Yr17* resistance allele. Five lines out of 21 lines exhibited acceptable agronomic performance and quality attributes with improved disease resistance. However, whole-scale dependence on markers in marker assisted selection (MAS) program will likely eliminate desirable genotypes. An effective large population size is more likely to maintain the effectiveness of MAS for desirable traits to avoid random genetic drift (Wang et al., 2016). Previous study suggested that a single seed descent or doubled haploid derived population should at least have more than 500 lines for a MAS for one target marker in integration with multiple target traits that express quantitative inheritance (Chen et al., 2016).

4.4 Future research

i. The population size should be expanded to potentially pool *Lr34/Yr18* and *Lr37/Yr17* resistance genes with multiple traits of economic importance that express quantitative inheritance.

ii. Genetic dissection of the coincident genomic region on 2D where the stable coincidental QTL for multiple phenotypic traits was located in both management systems; a large population size with sufficient recombinants therefore is required to break the tight linkage of loci attributing to corresponding traits of breeding interests.

iii. Standardized methods including artificial sprouting of intact spikes, germination tests, and artificial weathering trials in addition to falling number as an indirect measure

should be used to examine the effect of pre-harvest sprouting resistance QTL *Qphs.usask-4A*.

iv. Future QTL mapping analysis in 'Peace' × 'CDC Stanley' RIL population could be employed to determine major effect QTLs that are associated with disease resistance of leaf rust, stem rust, stripe rust, common bunt and tan spot, with an emphasis on uncovering disease resistance QTLs against *Ug99* race of stem rust pathogen, as previous study has reported a disease resistance locus *SrCad* on 'Peace'.

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