# Genetic Mapping for Stripe Rust Resistance in Canadian Spring Wheat Populations

By

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## Abstract

Stripe rust, caused by *Puccinia striiformis* Westend. f.sp. *tritici* Erikss. (*Pst*), is one of the most devastating diseases of wheat (*Triticum aestivum* L.) globally. Exploring and utilising new sources of resistance is essential for breeding resistant wheat cultivars. The objective of this thesis was to identify QTL associated with stripe rust resistance in two hexaploid spring wheat populations. In the first study, a doubled haploid (DH) population (n=291) derived from the cross AAC Innova/AAC Proclaim was evaluated to dissect the genetics of resistance in cv. AAC Innova. This population was evaluated for stripe rust severity at the adult plant stage in disease nurseries at Creston, British Columbia (in 2016 and 2020) and Lethbridge, Alberta (in 2016, 2017 and 2020), and genotyped using wheat 90K SNP (single nucleotide polymorphism) assay. A high-density genetic map was constructed using 7,112 SNP markers with an average marker interval of 2.3 cM. Quantitative trait loci (QTL) mapping identified one major (*QYr.lrdc-2A*) and ten minor effect (*QYr.lrdc-2B.1*, *QYr.lrdc-2B.2, QYr.lrdc-2B.3, QYr.lrdc-2B.4, QYr.lrdc-2D, QYr.lrdc-3B, QYr.lrdc-5A, QYr.lrdc-5B, QYr.lrdc-5D* and *QYr.lrdc-7D*) loci. The *QYr.lrdc-2A* was consistently effective against *Pst* races across all environments and explained up to 33.0% of the phenotypic variation. Other QTLs were either relatively less consistent or were environment specific. AAC Innova contributed stripe rust resistance alleles for most of the QTLs except for *QYr.lrdc-2D, QYr.lrdc-5A and QYr.lrdc-7D*.

In the second study, a recombinant inbred line (RIL) population of 252 individuals was developed from the cross AAC Cameron/P2711. This population was evaluated for stripe rust severity at the adult plant stage at the nurseries in Creston, BC (in 2018, 2019 and 2020) and Lethbridge, AB (in 2018 and 2020), and was genotyped using the wheat 90K Infinium

iSelect SNP assay. A high-density genetic map of 8,914 SNP markers was constructed covering all wheat chromosomes. Four resistance alleles were contributed by the resistant parent P2711 and three from the partially resistant parent AAC Cameron. *QYr.lrdc-2A.1*, corresponding to the *Yr17* gene, was the most stable QTL and was detected in four out of five environments, whereas *QYr.lrdc-2B* was the second most stable QTL. These two QTL along with *QYr.lrdc-5A* showed a significant reduction in stripe rust severity when present together. Except for *QYr.lrdc-2A.1* and *QYr.lrdc-2B*, all other QTL were location specific. The stable QTLs and their closely associated markers identified in these studies could be utilized in marker assisted selection for stripe rust resistant cultivar development.

## Preface

A version of second chapter of this thesis has been accepted by *Canadian Journal of Plant Pathology* titled as "Mapping quantitative trait loci associated with stripe rust resistance from Canadian wheat cultivar AAC Innova" and authored by Momna Farzand, Raman Dhariwal, Colin W. Hiebert, Dean Spaner and Harpinder Randhawa. The AAC Innova/AAC Proclaim DH mapping population was developed at Agriculture and Agri-Food Canada (AAFC), Lethbridge before I joined the study in 2019. The phenotypic data was collected by Dean Spaner, Harpinder Randhawa and Raman Dhariwal in 2016 and 2017. Raman Dhariwal extracted and shipped the DNA samples to AAFC, Morden, where samples were genotyped using the wheat 90K Infinium iSelect SNP assay. I was responsible for collecting phenotypic data in 2020, curating phenotypic and genotypic data, statistical analysis of phenotypic data, QTL analysis, interpretation of results and writing manuscript. Raman Dhariwal provided extensive support in QTL analysis and manuscript editing. Dean Spaner, Harpinder Randhawa and Colin W. Hiebert assisted with manuscript editing and interpretations of results.

A version of third chapter of this thesis has been submitted to the journal *Crop Science* titled as "QTL mapping for adult plant resistance to stripe rust in the AAC Cameron/P2711 spring wheat population" and authored by Momna Farzand, Raman Dhariwal, Colin W. Hiebert, Dean Spaner and Harpinder Randhawa. The AAC Cameron/P2711 RIL spring wheat mapping population was developed at AAFC, Lethbridge, before I joined the study in 2019. I was responsible for collecting phenotypic data in 2019 and 2020, extracting and shipping the DNA samples, curating phenotypic and genotypic data, statistical analysis of phenotypic data, QTL analysis, interpretation of results and writing manuscript. Raman Dhariwal, Dean

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# Chapter 1 Literature review

## 1.1 Wheat

Wheat is a widely cultivated crop with a record estimated production of 766 million tonnes (Mt) in 2019 globally (FAOSTAT, 2020). It is one of the world's most important staple crops providing significant amount of daily calories and 20% of the daily protein requirements for human (Shewry & Hey, 2015). It is also a major field crop in Canada in terms of acreage and annual production; approximately 9.7 Mha of wheat was harvested in 2019 in Canada with a record production of about 32.3 Mt (FAOSTAT, 2020). The types of wheat produced in Canadian prairie regions are: hexaploid spring or winter wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L.). Hexaploid spring wheat is predominant type of wheat grown in prairie provinces Alberta, Manitoba, and Saskatchewan (McCallum & DePauw, 2008). These provinces produce more than 95% of the spring wheat (Canadian Wheat, 2019).

Hexaploid wheat ($2n = 6x = 42$, AABBDD) is derived from independent hybridizations between tetraploid emmer wheat (*Triticum dicoccoides*; $2n = 4x = 28$, AABB) and diploid grass (*Aegilops tauschii*; $2n = 2x = 14$, DD) (Matsuoka, 2011). Western Canadian spring wheat is presently classified into ten market classes with different quality parameters such as kernel hardness, grain protein content, gluten strength and kernel color (Canadian Grain Commission, 2020). Three major wheat classes grown in western Canada are Canada Western Red Spring (CWRS), Canada Western Amber Durum (CWAD) and Canada Prairie Spring Red (CPSR).

CWRS wheat accounts for more than 60% of annual wheat production in western Canada (Canadian Wheat, 2019). It is well known for its wide adaptability, high protein

content, and good flour milling and baking characteristics (McCallum & DePauw, 2008). CWRS is often blended with low quality wheat to improve overall baking quality. CWRS has three milling grades and can be used to make noodles, pasta, hearth, and flat bread (Canadian Grain Commission, 2018).

The history of CWRS wheat grown in Canadian prairies date back to the early 1800's. However, it failed to grow periodically due to poor weather conditions and pest problems. The spring cultivar Red Fife was introduced in 1870s in western Canada, which successfully replaced previous poorly adapted cultivars because of its excellent end use quality (Campbell & Shebeski, 1986). Agronomic drawbacks of Red Fife included, late maturity and susceptibility to lodging, stem rust and shattering (McCallum & DePauw, 2008; Newman, 1928). Subsequently, Red Fife was replaced by Marquis (developed in 1909), which matured 3-4 days earlier than Red Fife and was less susceptible to lodging, stem rust and shattering (McCallum & DePauw, 2008; Morrison, 1960). However, Marquis suffered from frost damage and was subsequently replaced by Garnet, Red Bobs, and Park due to their certain improved agronomic traits. Thatcher was dominant from 1939 to 1968 due to its excellent end use quality and high resistance to stem rust (McCallum & DePauw, 2008) was used as a progenitor in the parentage of subsequent CWRS cultivars Manitou, Neepawa, Katepwa, Columbus, CDC Teal and AC Barrie (McCallum & DePauw, 2008).

Canada is the main exporter of durum wheat (Canadian Wheat, 2019) which has been grown in Canadian prairies since the introduction of Mindum and Carleton, two leading CWAD cultivars, in 1940s from United States of America (USA) (McCallum & DePauw, 2008). The CWAD wheat is very popular for its recognized bright yellow color, high protein content and semolina yield. Currently, CWAD has four milling grades and used for making high-quality pasta and couscous (Canadian Grain Commission, 2018). Additionally, flour

milled from high protein CWAD is used in the production of artisan, flat breads, and hearth breads (Canadian Wheat, 2019).

Stewart 63 was the first CWAD cultivar to be bred in Canada and released in 1963. Hercules, registered in 1969, was better than Stewart in terms of color and gluten strength (Dexter, 2008). Wascana (registered in 1971) and Wakooma (registered in 1973) had better end use quality and agronomic characteristics than previous cultivars and were dominant from 1970s to 1980s (McCallum & DePauw, 2008). Subsequent CWAD leading cultivars are: Kyle, AC Avonlea and AC Navigator and AC Strongfield (Dexter, 2008; McCallum & DePauw, 2008).

CPSR is the third most common wheat produced in western Canada. It is well known for its medium protein content, kernel hardness, and dough strength (Canadian Grain Commission, 2018; McCallum & DePauw, 2008). It has two milling grades and used to make hearth bread, pan bread, steamed bread, crackers, and Asian noodles (Canadian Wheat, 2019) The CPSR class has lower protein content than CWRS but for producers, lower price of grains due to lower protein content is compensated by higher yield (McCallum & DePauw, 2008).

CPSR wheat was introduced in 1985 and its breeding objectives primarily focused on improving end use quality and genetic resistance to common bunt and loose smut. HY320 is an important progenitor in the parentage of many of CPSR wheat cultivars.

**1.2 Breeding objectives**

Wheat productivity is threatened by several biotic (caused by bacteria, fungi, insects, and viruses) and abiotic stresses (nutrient deficiencies, poor soil structure, variable temperature, frost, and drought) globally (Afzal et al., 2015). Important breeding objectives for western Canadian wheat classes include higher grain yield, early maturity, good straw

strength, intermediate height, end use quality, and resistance to various biotic and abiotic stresses (McCallum & DePauw, 2008). New cultivars to be registered in western Canada must have good levels of resistance to stripe rust, leaf rust, stem rust, Fusarium head blight and common bunt (http://www.pgdc.ca/). Among the insect pest, orange wheat blossom midge and stem sawfly can cause most economic damage in western Canada. Modern breeding strategies are needed to deal with the continuous evolution of these pathogens. Deployment of resistant cultivars is the most economical and environment friendly approach to limit the damage caused by these diseases (Chen, 2005).

### 1.2.1 Stripe rust

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici (Pst)*, is the most threatening disease of wheat and categorized as 'priority one' diseases for western Canadian breeding programs. The pathogen is highly aggressive and destructive on wheat and quickly evolves into new virulent races to overcome the existing resistance genes (Chen, 2005). Generally, mutation, somatic hybridization, and sexual recombination are three main factors responsible for this pathogen variation (Little & Manner, 1967; Park & Wellings, 2012; Lei et al., 2017). Severe stripe rust epidemics have occurred in 2005, 2006, 2011 and 2016 in western Canada (Aboukhaddour et al., 2020). The disease is common in western Canada and become a serious concern on both spring and winter wheat grown in western Canada especially under irrigated areas which provide proliferative conditions to the pathogen infection (Su et al., 2003).

The primary source of stripe rust in western Canada is its spores entering via wind currents from the infected fields of Pacific Northwest of United States (Su et al., 2003) but the prevalence of stripe rust increases due to the occurrence of new high temperature-adapted exotic races (Milus et al., 2009) and early winter snow cover which enables the overwintering of the pathogen on winter wheat in southern Canadian prairies (Brar & Kutcher, 2016). Stripe

rust has also caused serious epidemics in the other important Canadian wheat classes including Canada Western Red Winter (CWRW), CWRS and CPSR (Puchalski & Gaudet, 2010).

#### 1.2.1.1 Life cycle

The life cycle of *Pst* include five different spore stages, which occur on two botanically distinct plant hosts (Figure 1-1). Wheat is considered as primary host while some species of genera *Berberis* and *Mahonia* are known to be secondary hosts (Jin et al., 2010; Zhao et al., 2016). In its asexual cycle, dikaryotic urediniospores (N+N′) infect wheat several times during a wheat growing season. Thick walled, dikaryotic teliospores (N+N′) are produced usually at the end of the wheat growing season when weather is hot. In its sexual cycle, teliospores undergo karyogamy (NN′) during spring and germinate into haploid basidia (the site of meiosis).

Four binucleated double-haploid basidiospores (2*N) are produced from each basidium, which infect the alternate host, barberry (*Berberis spp.*), and then develop into haploid pycnia. Pycniospores (N) produced from pycnia, cross fertilize with receptive hypha of pycnia and give rise to a dikaryon with two unfused nuclei in each cell. The hyphae penetrate through the barberry leaves and form dikaryotic aecia on the abaxial side of leaves. Aeciospores (N+N′) dispersed from aecia germinate into dikaryotic uredinia, which produce urediniospores (N+N′) to reinfect wheat and complete the cycle (Hovmøller et al., 2011).

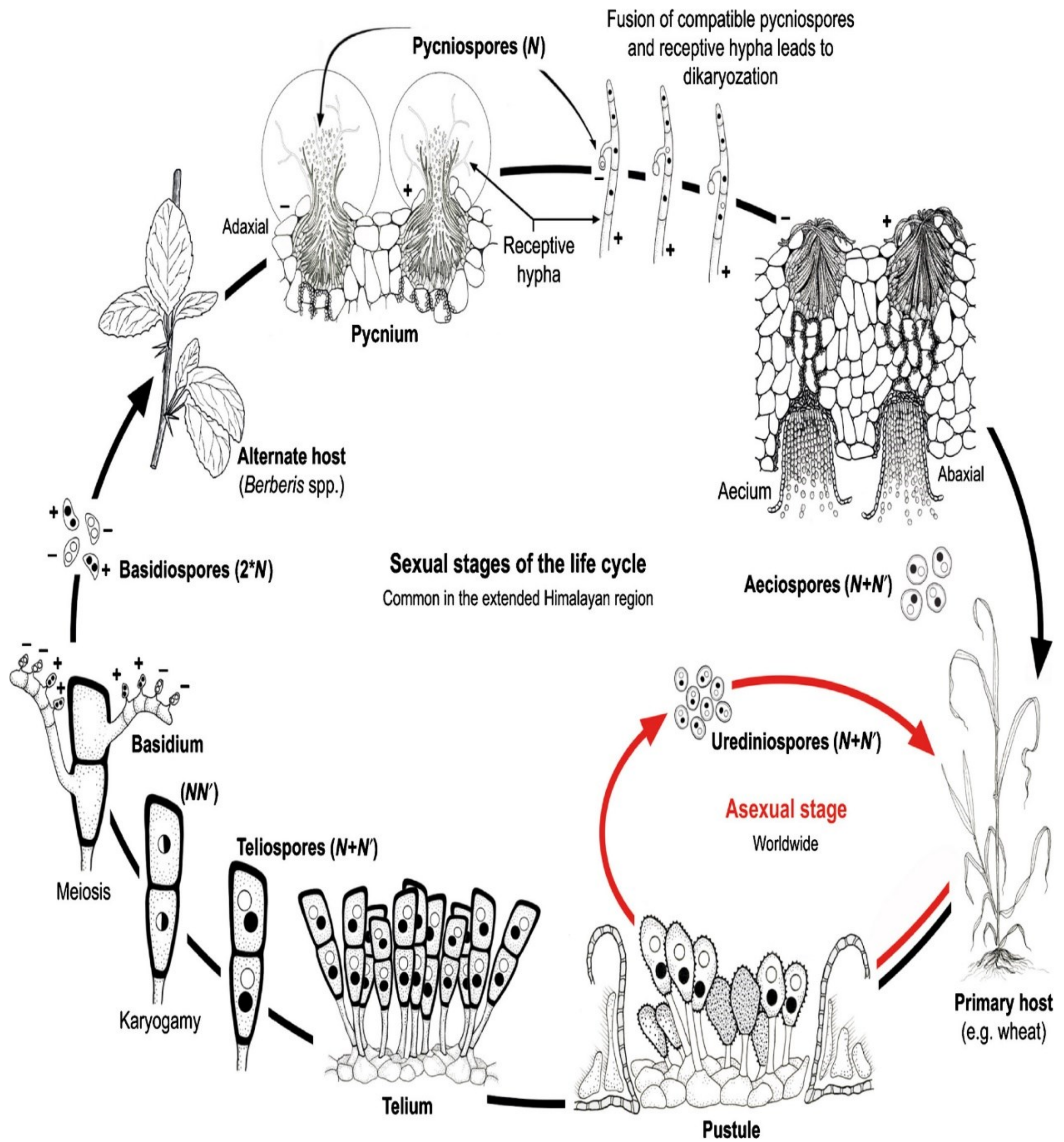


**Figure 1-1.** Life cycle of *Puccinia striformis* f. sp. *tritici*. (Adapted from Schwessinger, 2017)

#### 1.2.1.2 Genetic resistance

The genetic resistance to stripe rust could be characterized into seedling or all stage resistance (ASR) and adult plant resistance (APR) based on their effectives at different plant growth stages (Wang & Chen, 2017). The ASR is generally race specific and provides high level of resistance against a particular race at all plant growth stages (Chen, 2005). This type of resistance is mostly controlled by a single or few highly resistance genes, which are qualitatively inherited and confer hypersensitive reaction to combat a specific race. However, resistance provided by a single major gene could be easily defeated by the evolution of new virulent pathogen races (Chen, 2013; Liu et al., 2019). Whereas APR is not effective at seedling stage but gradually expresses at post seedling stages.

APR is generally quantitively inherited and confers partial but durable resistance against different pathogen races (Chen, 2005; 2013). The APR is mostly non race specific (Wang & Chen, 2017) but race specificity has also been reported for some APR genes (McIntosh et al., 1995). High-temperature adult-plant resistance (HTAP) is a type of APR, which shows resistance at later plant development stages with the rise in temperature (Chen, 2013). A single APR gene is generally not sufficient to provide resistance under high disease pressure. Therefore, a combination of 3-5 APR is generally recommended to achieve maximum level of resistance (Singh et al., 2000).

So far, several stripe rust (*Yr*) resistance genes have been reported in hexaploid wheat and its wild relatives (Wang & Chen 2017; Gessese et al., 2019; Pakeerathan et al., 2019; Li et al., 2020). Moreover, many multiple resistance genes including slow-rusting *Lr46/Yr29* on chromosome 1BL(William et al., 2003), *Lr34/Yr18* gene on 7DS (Lagudah et al., 2009), *Lr67/Yr46* on 4DL (Herrera-Foessel et al., 2014), *Yr17/Lr37/Sr38* cluster on 2AS (Milus et al., 2015) and *Sr2/Yr30* on 3BS (Randhawa et al., 2018) have been reported to confer partial,

but durable resistance to multiple diseases for a long time. Of these, *Lr34/Yr18* gene has been widely deployed in breeding programs across Canada and globally (Randhawa et al., 2012; Randhawa et al., 2013).

Stripe rust resistance genes *Yr5*, *Yr15* and *YrSP* could be useful in breeding since most of the stripe rust races prevalent in North America are avirulent to these genes (Brar & Kutcher, 2016; Ghanbarnia et al., 2021). Although, no perfect marker exists for *Yr5* but a combination of two KASP assays reliably detects carriers of *Yr5* (Naruoka et al., 2016).

In addition to above mentioned genes, many QTL conferring resistance to stripe rust have been reported (Rosewarne et al., 2013; Wang & Chen, 2017). Stripe rust resistance QTLs have also been studied in several Canadian cultivars. Singh et al. (2014) reported QTLs on chromosomes 2A, 2B, 3A, 3A, 4B, 5B, 7A and 7B in the AC Cadillac/ Carberry DH population. Bokore et al. (2017) detected many QTL conferring resistance to stripe rust on chromosomes 1B, 2A, 2B, 2D, 3B, 3D, 4A, 4B, 5B, 6A, 7A and 7D in five DH populations (Carberry/AC Cadillac, Carberry/Vesper, Vesper/Lillian, Vesper/Stettler and Stettler/Red Fife).

Three QTL on chromosomes 3A, 4A and 5B were detected in the Attila/CDC Go RIL population (Zou et al., 2017a). Furthermore, Bemister et al. (2019) identified two QTLs on chromosomes 4B and 3A in a RIL population derived from cross between Canadian spring wheat cultivars Peace and Carberry.

### 1.2.2 Stem rust

Stem rust (caused by *Puccinia graminis* f. sp. *tritici* [*Pgt*]) was reported in western Canada in 1916, which resulted in an estimated yield loss of 43% (Johnson, 1961). The virulence in *Pgt* population prevailing in North America has been quite variable up to the mid of the 20th century since 71 races were discovered between 1919 and 1955 (Johnson &

Green, 1957). Globally, stem rust has become a serious concern with the emergence of a highly virulent *Pgt* race TTKSK (*Ug99*) (Pretorius et al., 2000; Jin et al., 2008). This race was designated after the discovery of virulence to stem rust resistance gene *Sr31* in wheat nurseries in Uganda in 1999 (Pretorius et al., 2000). About 90 % of the wheat cultivars grown worldwide (Singh et al., 2011) and 78 % of the spring wheat cultivars in Canada are susceptible to race TTKSK (Fetch et al., 2012). So far, many stem rust resistance genes/alleles have been identified and numbered (Mclntosh et al., 2014; Chen et al., 2018; Toth et al., 2018).

However, many of stem rust resistance genes derived from *Triticum* and related species are race specific (Toth et al., 2018). To date, five *Ug99* stem rust resistance genes including, *Sr22*, *Sr33, Sr35*, *Sr45* and *Sr50* have also been cloned successfully (Saintenac et al., 2013; Periyannan et al., 2014; Mago et al., 2015; Steuernagel et al., 2016). Of these, *Sr22*, *Sr35* and *Sr50* confer resistance to multiple pathogenic races (Steuernagel et al., 2016). *SrCad* located on short arm of chromosome 6D is a well studied gene in Canadian wheat cultivars, which imparts resistance against *Ug99* races TTKST and TTKSK (Hiebert et al., 2011). Stem rust resistance QTLs have been identified on chromosome 2B, 3B, 4B, 5A, 5B, 6B, 7B and 7D in the AC Cadillac/Carberry Canadian DH population (Singh et al., 2013).

**1.2.3 Leaf Rust**

*Puccinia triticina*, a causal agent of wheat leaf rust, is one the largest biotic threat to global wheat production (Bolton et al., 2008). In Canada, breeding for leaf rust resistance started in mid of 20th century after detecting virulence in wheat cultivar Thatcher (McCallum & DePauw, 2008). Currently, several permanently designated *Lr* genes have been reported in wheat (Aktar-Uz-Zaman et al., 2017; Qureshi et al., 2018). The most common *Lr* genes in

Canadian wheat germplasm are: *Lr14a*, *Lr16*, *Lr21*, *Lr22a*, *Lr34*, *Lr37*, and *LrCen* (Toth et al., 2018).

Among these, *Lr21* (Huang et al., 2003), *Lr22a* (Thind et al., 2017) and *Lr34* (Krattinger et al., 2009) have also been cloned successfully. *Lr34*, race-specific APR gene, has been effective in many countries for more than 50 years (Krattinger et al., 2009). Several QTLs associated with leaf rust resistance have been reported in Canadian spring wheat populations. Singh et al. (2014) mapped three QTLs on chromosomes 2A, 2B and 4B in the AC Cadillac/Carberry DH population. Zou et al. (2017a) studied two QTLs on chromosome 2D and one on chromosome 3A in the Attila/CDC Go RIL population. Two QTLs on chromosomes 4A and 3D were identified in the Peace/Carberry RIL population (Bemister et al., 2019). In a Recent study, Bokore et al. (2020) used five DH populations (Carberry/AC Cadillac, Carberry/Vesper, Vesper/Lillian, Vesper/Stettler and Stettler/Red Fife) and reported QTLs on chromosomes 1A, 1B, 1D, 2A, 2B, 2D, 4A, 4B, 5A, 6A, 6B, 7A, 7B and 7D.

**1.2.4 Fusarium head blight**

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a major limitation to wheat growing in temperate regions of the world (Dexter et al., 1996). High humidity and frequent rainfall are two main factors favoring the crop infection at flowering or early seed development stage (McMullen et al., 1997). The earliest record of FHB in Canada was found in 1884 followed by several minor to major epidemics so far (MacInnes & Fogelman, 1923; Zhu et al., 2019). The greatest concern with FHB-infected wheat is the accumulation of the secondary metabolites such as deoxynivalenol (DON), which constitutes a threat to the safety of human food and livestock feed (Pestka, 2010).

Chinese landrace Sumai 3 and Brazilian wheat cultivar Frontana are the most common exotic sources of FHB resistance utilized in wheat breeding in North America (Pandeya et al., 1996; Bai et al., 2018). Furthermore, a new Spring wheat line 00Ar134-1 derived from *Elymus repens* (quack grass) was introduced as a new source of FHB resistance in Canadian wheat (Brar & Hucl, 2016). Five CWRS wheat cultivars: Carberry, Cardale, AAC Brandon, AAC Elie and CDC Morris VR, with Sumai 3 in their pedigrees are being widely grown in FHB hot spot regions in Canada (Zhu et al., 2019). Although, several FHB resistance QTLs have been identified but only a limited number of these QTLs are successfully utilized in breeding programs worldwide (Buerstmayr et al., 2009; Buerstmayr et al., 2020). Among them, the strongest and best-validated QTLs include: *Fhb1*, *Fhb2*, *Fhb4,* and *Qfhs.ifa-5A* (Buerstmayr et al., 2020). Two FHB resistance QTLs, *Fhb1* and *QFhb.mgb-2A*, have also been cloned (Rawat et al., 2016; Gadaleta et al., 2019).

Sumai 3 derived *Fhb1, Fhb2,* and *Fhb5* genes have been successfully inbred into Canadian hard red spring wheat cultivars, CDC Go and CDC Alsask (Brar et al., 2019). Recently, Dhariwal et al. (2020) identified two major QTLs on chromosome 2D which together explained 34.2% of the phenotypic variance in FHB resistant wheat cultivar AAC Tenacious.

### 1.2.5 Common Bunt

Common bunt of wheat, caused by *Tilletia tritici* and *T. laevis*, can cause extensive grain yield and quality losses by replacing healthy grains with stinky bunt balls (Goates, 1996). In North America, Common bunt was the most destructive disease between 1800s and 1930s. Later on, it was significantly controlled using fungicides and genetically resistant cultivars (Aboukhaddour et al., 2020). But it is a typical problem in organic wheat production, where chemical seed treatments are not allowed (Matanguihan et al., 2011). To date, over 15

common bunt race specific resistance genes including, *Bt1-Bt15* and *Btp*, have been catalogued . Of these, *Bt10* on the short arm of 6D (Menzies et al., 2006), is widely utilized in wheat breeding programs in Canada and all over the world because it is highly effective to combat all known common bunt races around the globe (Gaudet et al., 1993; Demeke et al., 1996; Gaudet et al., 2007; Singh et al., 2016).

QTLs associated with common bunt resistance have been mapped in some Canadian cultivars. For example, QTLs on chromosomes 1B and 7A were mapped in the RL4452/AC Domain DH population (Fofana et al., 2008). Singh et al. (2016) studied QTLs on chromosomes 1B, 4B, 4D, 6D and 7D in the Carberry/AC Cadillac DH population. Recently, Bokore et al. (2019) reported QTLs on chromosomes 1D, 2A, 3D, 5A and 7A in the Lillian/Vesper DH population.

### 1.2.6 Orange wheat blossom midge

Orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), is among the most devastating insect pests of spring wheat in western Canada (Lamb et al., 1999). The female adult midge lays eggs on newly emerging wheat spikes. Once the larva hatches, the larva feeds upon young grains which results in yield and quality reduction (Thomas et al., 2005). There are two major sources of midge resistance, antibiotic resistance (antibiosis) and oviposition deterrence (antixenosis). To date, *Sm1* (located on wheat chromosome 2BS) is the only well-defined antibiosis gene for midge resistance (Thambugala et al., 2021). Cultivars carrying *Sm1* are developed as varietal blends of 90% midge resistant and 10% midge susceptible refuge that decrease the selection pressure on the wheat midge (Toth et al., 2018). Recently, a major effect locus for oviposition deterrence was reported on chromosome 1A in the Canadian breeding line BW278 (Thambugala et al., 2021).

### 1.2.7 Wheat stem sawfly

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a major pest of wheat in western Canada (Beres et al., 2011). The female adult sawflies lay eggs on stems, after hatching larvae begin feeding inside the stem and destroy parenchyma tissue and vascular bundles of the plant, which causes reduction in the photosynthetic activity (Macedo et al., 2005). It also causes lodging when the larvae move downwards to the base of the plant and cut a ring around the stem at ground level (Beres et al., 2017). The development of solid-stemmed cultivars can reduce the disease associated risks. A single major stem solidness locus has been reported on wheat chromosome 3BL that contributed 76% of the total variation in the Rampart/Jerry RIL population (Cook et al., 2004).

### 1.2.6 Early maturity

Early maturity in crop is a very important breeding objective, in a region where growing season is very short (95-125d) and days are long (> 14), such as in western Canada (Kamran et al., 2013a). Early maturity in Canadian wheat is a key feature to minimize the risk associated with frost damage which can badly effect grain yield and quality (Iqbal et al., 2006). It may also reduce pre harvest sprouting that is very common in cold and damp conditions (Hucl & Matus-Cádiz, 2002). The wide adaptability of wheat to diverse environments mainly depends on synchronization between flowering and maturation timing and local environmental conditions (Chen et al., 2015). Flowering time in wheat is governed by three major groups of genes: vernalization (*Vrn*), photoperiod (*Ppd*) and earliness per se (*eps*) genes (Distelfeld et al., 2009). The *Vrn* and *Ppd* genes are more important to regulate flowering and maturity timing than *eps* genes (Chen et al., 2015).

*Vrn* group of genes (*Vrn A1, VrnB1, VrnD1* and *VrnD5)* regulates the growth habit and classifies cereals into spring and winter type (Distelfeld et al., 2009). Spring wheat carry

one or more dominant allele at one of *Vrn* loci while winter wheat carry recessive alleles (except dominant allele at *Vrn-2*) at all these loci (Iqbal et al., 2007). Winter wheat demands continuous chilling treatment (vernalization) to initiate flowering. Spring wheat does not require vernalization to initiate reproductive phase, but some cultivars show positive response to vernalization by flowering early (Jedel et al., 1986; Iqbal et al., 2006).

*Vrn-A1* is epistatic to other dominant *Vrn-B1, Vrn-D1,* and *Vrn-D5* genes and different combination of these genes produces different phenotypes (Pugsley, 1972). Iqbal et al. (2007) reported that spring wheat genotypes carrying two dominant allele (*Vrn* A1 and *Vrn* B1) were both early maturing and high yielding while genotypes carrying *Vrn-D5* alone or in combination with other dominant alleles were late maturing. Similarly, Kamran et al. (2013b) reported that 74% of the high yielding soft white spring wheat lines posses *Vrn-B1* gene alone or in combination with other dominant *Vrn* genes.

Photoperiod genes are the second most important after vernalization genes in determining flowering time. *Ppd* group of genes confers day length sensitivity and characterizes cereals into photoperiod sensitive or insensitive (Distelfeld et al., 2009). Photoperiod insensitive cultivars possess dominant alleles at *Ppd* loci while photoperiod sensitive cultivars carry recessive alleles at these loci (Pugsley, 1966). A photoperiod insensitive cultivar flowers independently of day length while photoperiod sensitive cultivar flowers only when day length increases and fulfills its photoperiod requirement (Bentley et al., 2011). Three most important photoperiod insensitive genes include *Ppd-A1, Ppd-B1* and *Ppd-D1* on chromosomes 2A, 2B and 2D, respectively (Worland et al., 1998). *Ppd-D1a* is predominant in Canadian wheat germplasm as 72 % of the genotypes were reported to carry this locus (Kamran et al., 2013b). Photoperiod insensitivity results from

the deletion mutations in the coding regions, which respond to environmental signals (Beales et al., 2007).

Earliness per se genes are quantitatively inherited and tend to have relatively smaller effect on controlling flowering time (Yasuda & Shimoyama,1965; Appendino & Slafer, 2003). *Eps* genes have been reported to fine-tune flowering time in mega environments independent of *Ppd* and *Vrn* genes (Lewis et al., 2008; Griffiths et al., 2009). The effect of *Eps* genes have been found on the ear emergence time (Miura & Worland, 1994) and different stages of early reproductive and vegetative growth (Lewis et al., 2008). QTLs associated with earliness per se have been studied on chromosomes 1B, 2B, 3A , 3B, 4A, 4D, 5A, 5B, 6A and 6B, 6D, 7A, 7B and 7D (Kamran et al., 2014; Lombardo et al., 2019).

QTLs associated with flowering and maturity have also been studied in some Canadian spring wheat populations. For example, QTLs on chromosomes 1B, 2D, 3B, 4A, 4D, 5A, 5B, 6B and 7A have been mapped in the Cutler/AC Barrie RIL population (Kamran et al., 2013; Perez-Lara et al., 2016). Similarly, Zou et al. (2017b) reported one flowering QTLs on chromosome 5A and two maturity QTLs on chromosomes 4B and 5A in the Attila/CDC Go RIL population.

**1.2.7 Plant height**

Plant height is positively correlated with lodging and thus effects grain yield and quality (Navabi et al., 2006). There was a tremendous increase in wheat and rice yields during the Green Revolution when semi dwarf genes were introduced into the plants (Hedden, 2003). Jamal and Ali (2008) reported that reduction in plant height was associated with the improvement of grain yield in wheat. To date, 25 major dwarfing genes including *Rht1*-*Rht25*, have been characterized in wheat (Mclntosh et al., 2015; Tian et al., 2017; Mo et al., 2018). Of these, *Rht-B1b*, *Rht-D1b*, *Rht-B1c* and *Rht-D1c* (on chromosomes 4BS, 4DS, 4BS

and 4DS, respectively) have been cloned (Peng et al., 1999; Pearce et al., 2011; Wu et al., 2011). Three dwarfing genes, *Rht-B1*, *Rht-D1* and *Rht8* (on chromosomes 4BS, 4DS and 2DL, respectively) have been utilized in wheat breeding programs extensively (Tian et al., 2017). *Rht-B1* and *Rht-D1* are insensitive to gibberellic acid (GA) while *Rht8* is responsive to GA (Gale et al., 1985).

QTLs associated with plant height have been reported in many Canadian and other wheat cultivars around the globe (Griffiths et al., 2012). Four QTLs were mapped on chromosomes 4B, 4D, 5D and 7B, which accounted for 4.1 to 29.2% of the phenotypic variance in the AC Karma/87E03-S2B1 DH population (Huang et al., 2006). Perez-Lara et al. (2016) reported a QTL adjacent to *Rht-D1* gene on chromosome 4D, which explained up to 37.8% of the phenotypic variance in the Cutler/AC Barrie RIL population, and Singh et al. (2016) mapped QTLs on chromosomes 4B and 6D explaining 10.7 to 62.3% of the phenotypic variance in the Carberry/AC Cadillac DH population. Similarly, Zou et al. (2017b) reported QTLs on chromosomes 2D, 4B, 5A and 6B, which explained 3.2 to 23.9% of the phenotypic variance in the Attila/CDC Go RIL population. Furthermore, Cabral et al. (2018) mapped QTLs on chromosomes 4B and 4D, which explained 6.2 to 58.6% of the phenotypic variance in the RL4452/AC Domain DH population. Recently, Dhariwal et al. (2020) mapped QTLs on chromosomes 1B, 2D, 4A, 4B, 5A, 5D and 7D, which explained 2.0 to 53.0 % of the phenotypic variance in the AAC Innova/AAC Tenacious DH population.

**1.3 Molecular markers**

The choice of markers for genotyping depends on several factors such as, reproducibility, level of polymorphism, codominance in expression, quantity and quality of available DNA, technical handling, and cost (Jiang, 2013; Miah et al., 2013). Botstein et al. (1980) first used restriction fragment length polymorphisms (RFLP) molecular markers to

produce a molecular map of the human genome. However, RFLP was no longer frequently utilized in genotyping due to certain limiting factors such as requirements for high quantity DNA (5–20 µg), and laborious handling (Grover & Sharma, 2016). RFLP was replaced by several alternative polymerase chain reaction (PCR) based markers such as, random amplified polymorphic DNA (RAPD; Williams et al., 1990), amplified fragment length polymorphism (AFLP; Vos et al., 1995), and simple sequence repeats (SSR; Gupta et al., 2002).

Since last two decade, SSR have been favorable markers for research and breeding due to their high polymorphism, high accuracy, locus specificity, uniform distribution, codominance, and reproducibility (Jiang, 2013). Diversity array technology (DArT) marker system has also caught attention of researchers due to its low cost and typing hundred of polymorphic loci simultaneously (Schouten et al., 2012). However, this system is not suitable for genotyping early generation populations due to dominant nature of DArT markers, which cannot distinguish between homozygous dominant and heterozygous individuals.

Single nucleotide polymorphisms (SNPs) are the most preferred markers for genotyping due to their high genome coverage. High-throughput SNP genotyping can be done using different GBS pipelines available following next generation sequencing of plant samples or using different SNP arrays available such as wheat 9K Infinium iSelect SNP assay (Cavanagh et al., 2013) and 90K Infinium iSelect SNP assay (Wang et al., 2014) from Illumina and 35K Axiom® wheat HD Genotyping Array (Breeders' Array; Allen et al., 2017), 660K Axiom® wheat HD Genotyping Array (Cui et al., 2017) and 820K Axiom® wheat HD Genotyping Array (Winfield et al., 2016) from Affymetrix.

Low-throughput SNP genotyping methods are also available. There are several useful techniques for these and simplest one includes the allele-specific PCR (AS-PCR) that is

designed to detect two different SNP alleles running in two separate reactions (Bundock et al., 2006; Toth et al., 2018). A modification of this genotyping method is Kompetitive Allele Specific PCR (KASP), which requires KASP master mix and detects two different SNP alleles running in a single reaction. The reaction results in fluorescent signals(Semagn et al., 2014). Recently, semi-thermal asymmetric reverse PCR is developed that is almost similar to KASP but does not require KASP master mix (Long et al., 2017).

**1.4 Quantitative trait locus mapping**

Most of the economically important traits in plant breeding are quantitatively inherited, meaning the genetic variation in such traits are controlled by the cumulative action of multiple genes and their interaction with the environment (Sham et al., 2002). Quantitative trait locus (QTL) mapping is a highly practical approach to uncover the chromosomal regions controlling such complex traits in plants and identify their closely linked markers (Collard et al., 2005). Sax (1923) was the pioneer to propose the idea of QTL mapping, which was further extended in several independent studies by Rasmusson (1935), Thoday (1961) and Law (1967). Latest QTL mapping techniques are based on these above-mentioned studies. Linkage based QTL mapping requires: (i) developing a large biparental mapping population for phenotyping the desirable traits, (ii) genotyping the population with suitable marker system, and (iii) constructing linkage map and mapping of QTLs (Collard et al., 2005).

Mapping populations such as doubled haploids (DHs), near isogenic lines (NILs), and recombinant inbred lines (RILs) are most preferred for linkage-based QTL analysis since they are homozygous and can be used for phenotyping experiments in multiple environments. Different statistical methods are available for linkage-based QTL analysis. Single marker analysis (SMA) is simplest one, which uses t test, analysis of variance (ANOVA) and linear regression methods (Collard et al., 2005). The major drawback of this method is the

recombination between marker and target loci, which mislead about the effect of QTL (Tanksley, 1993). Interval mapping methods include several methods such as simple interval mapping (SIM; Lander & Botstein, 1989), composite interval mapping (CIM; Zeng, 1994), inclusive composite interval mapping (ICIM; Li et al., 2007), multiple interval mapping (MIM; Hackett et al., 2001), and Bayesian interval mapping (BIM; Satagopan et al., 1996) which overcome the misleading effect of QTL by the recombination between marker and target loci and detect multiple QTLs simultaneously.

## 1.5 Conclusions

Major goals of wheat breeding programs in western Canada are to develop cultivars with good agronomic performance, suitable end use quality and resistance to priority one diseases. In the ever-evolving dynamic population, due to population shifts and/or mutations, and appearance of new more aggressive strains of pathogens, wheat cultivars can be rendered susceptible to one or more diseases. Hence, there is continuous need to identify and incorporate new source of resistance to these diseases. With the advancements in low-cost molecular techniques in combination with phenotypic selection and high throughput genetic mapping protocol, linkage-based QTL mapping has enabled breeders to trace chromosomal regions with important loci that could be potentially used as distinguishing markers for marker assisted breeding (Collard et al., 2005). Although QTL mapping facilitated by molecular markers gave us a better understanding about genetic architecture of quantitative traits, but scientist still need to learn more about its biological bases because QTL mapping information is highly statistical (Bernardo, 2016).

## 1.6 Thesis objectives and null hypotheses

1. Map QTLs associated with stripe rust resistance in a DH population derived from the cross between spring wheat cultivars AAC Innova and AAC Proclaim.
2. Map QTLs associated with stripe rust resistance in a RIL population derived from the cross between spring wheat cultivar AAC Cameron and germplasm line P2711.
3. Identify molecular markers linked to stripe rust resistance loci in both populations.

The objectives were tested under the following null hypotheses:

1. No stripe rust resistance QTL could be mapped in AAC Innova/AAC Proclaim DH population.
2. No stripe rust resistance QTL could be mapped in AAC Cameron/P2711 RIL population.

## Chapter 2 Mapping quantitative trait loci associated with stripe rust resistance from Canadian wheat cultivar AAC Innova[1]

### 2.1 Introduction

Wheat (*Triticum aestivum* L.) is a widely cultivated crop globally. In 2019, wheat occupied 216 million hectares (Mha) of arable land with an approximate production of 766 million tonnes (Mt) globally (FAOSTAT, 2020). Canada is one of the top wheat producing nations in the world in terms of acreage and annual production (FAOSTAT, 2020). In 2019, approximately 9.7 Mha of hexaploid wheat was harvested, with a record estimated production of 32.3 Mt in Canada (FAOSTAT, 2020). Fungal diseases constitute a major threat to wheat production around the globe. Stripe rust (also known as yellow rust) caused by *Puccinia striiformis* (*Pst*), is one of the most devastating diseases of wheat (Chen, 2005). It can cause yield losses up to 70 to 100%, depending upon the nature of inoculum, prevailing climatic conditions and genetics of the cultivar grown (Chen, 2005; Wellings, 2011). In western Canada, stripe rust epidemics have been reported in 2005, 2006, 2011 and 2016 (Aboukhaddour et al., 2020).

The disease is widespread in western Canada due to the inoculum carried by wind from the Pacific Northwest and the Great Plains of North America, where epidemics occur frequently (Brar & Kutcher, 2016). In addition, the recent expansion in the range of disease can be attributed to evolution of new high temperature adapted races (Milus et al. 2009) and overwintering of the pathogen on winter wheat (Brar & Kutcher, 2016). The management of disease through fungicide application is costly and imposes adverse effects on the

[1] A version of this chapter with the same title has been accepted by the *Canadian journal of Plant Pathology* on August 31, 2021; authored by Farzand et al.

environment. The most cost effective, sustainable, and environmentally friendly approach is growing resistant wheat cultivars.

Resistance to stripe rust can be categorized into two types: (i) all stage resistance (ASR) and (ii) adult plant resistance (APR) (Chen, 2013). ASR is widely used in wheat breeding programs because it is easy to transfer to new cultivars as it inherits qualitatively and is conferred by major genes. It provides complete resistance against a specific stripe rust race throughout all plant growth stages. Although ASR is more attractive, if a single major gene protects a large wheat growing area, it can easily be defeated by the evolution of new virulent races (Lin & Chen, 2007; Saharan & Tiwari, 2011). Race non-specific APR provides more durable resistance, however, it is usually ineffective in the seedling stage but gradually manifests at later plant growth stages (Chen, 2013). Since APR is generally controlled by minor genes, which confer partial or incomplete resistance to a broad range of races (Chen, 2013; Brown, 2015), a combination of 4-5 minor genes provides a sufficient level of protection over the longer term (Singh et al., 2000; Singh et al., 2005).

To date, 83 yellow rust (*Yr*) resistance genes have been catalogued from hexaploid wheat and its wild relatives (Wang & Chen, 2017; Gessese et al., 2019; Pakeerathan et al., 2019; Li et al., 2020). In addition, dozens of temporarily designated genes and QTLs have also been reported in literature (Rosewarne et al., 2013; Wang & Chen, 2017). Most *Yr* genes are race-specific and ineffective against current *Pst* races (Aktar-Uz-Zaman et al., 2017). Therefore, the significance of identification and characterization of new genes, especially APR genes, is increasingly emphasized in breeding programs (Line, 2002) and a few APR genes controlling resistance to stripe rust such as *Yr18* (Bossolini et al., 2006; Krattinger et al., 2009), *Yr36* (Fu et al., 2009) *Yr39* (Lin & Chen, 2007) and *Yr46* (Moore et al., 2015) have been cloned.

Among the cloned genes, *Yr18* is widely used in different breeding programs in Canada and around the globe because it is pleiotropic and confers resistance to leaf rust, stem rust and powdery mildew (Randhawa et al., 2012; Randhawa et al., 2013). However, cultivars with only one *Yr* gene may not provide an adequate level of resistance under high disease pressure (Zhang et al., 2019). A combination of several APR genes with one or more ASR gene is required to develop durable and adequate resistance against rapidly evolving races of stripe rust (Ellis et al., 2014; Liu et al., 2018).

Recent advancements in single nucleotide polymorphism (SNP) array-based platforms have revolutionised the genetic mapping in wheat (Cavanagh et al., 2013; Wang et al., 2014; Winfield et al., 2016; Allen at al., 2017; Cui et al., 2017). Gene associated SNPs, high-throughput genotype calling and low cost per data point make array-based genotyping platforms ideally suitable for construction of high-resolution genetic maps and identifying markers closely linked to desirable traits (Wang et al., 2014; Dhariwal et al., 2018; Dhariwal et al., 2020).

AAC Innova is a stripe rust resistant cultivar from the Canada Western General Purpose (CWGP) spring wheat class developed by Agriculture and Agri-Food Canada (AAFC), Lethbridge, AB (Randhawa et al., 2015a). The objective of this study was to identify and locate QTL controlling stripe rust resistance at adult plant stage in AAC Innova.

## 2.2 Materials and Methods

### 2.2.1 Plant material

A doubled haploid (DH) mapping population consisting of 291 lines was generated from the cross of susceptible cultivar AAC Proclaim with resistant cultivar AAC Innova

following the wheat-maize hybridization method (Sadasivaiah et al., 1999) at AAFC Lethbridge, AB. AAC Innova is a stripe rust resistant semi-dwarf spring wheat cultivar, which was derived from the cross AC Andrew/N9195. The male parental line N9195 was of Chinese origin and believed to carry stripe rust resistance, but had an unknown pedigree (Randhawa et al., 2015a). AAC Proclaim is also a spring wheat cultivar and is susceptible to prevalent races of *Pst* in western Canada (Randhawa et al., 2015b).

A set of six differential genotypes (Avocet +*Yr1,* Avocet +*Yr5,* Avocet +*Yr7,* Avocet +*Yr10* Avocet +*Yr31* and Avocet +*Yr32*) was also screened to determine the effectiveness of these resistance genes against the prevalent races of the stripe rust pathogen. Highly susceptible cultivars (AC Barrie, AC Crystal, and Morocco) were planted in the field for disease spreading and to evaluate levels of infection.

### 2.2.2 Disease evaluation

Parents, DH lines and stripe rust differential genotypes were evaluated for stripe rust severity in disease nurseries at Creston, BC in 2016 and 2020, as well as at Lethbridge, AB in 2016, 2017 and 2020. The experimental sites, Creston, BC (49°09'N, 116°51'W), and Lethbridge, AB (49°41'N, 112°49'W), are situated in proximity to the Pacific Northwest (PNW) of the United States, which is a hot spot region for stripe rust occurrence in North America (Chen, 2005) and provides natural inoculum in western Canada through wind trajectories. The experiment was planted in a randomized complete block design without replication in 2016 and 2017 and two replicates in 2020 at both sites. Each line was planted as one-meter-long rows with the space of 25 cm between them to facilitate disease evaluation. Approximately 60 seeds were planted in individual rows. Spread rows, consisting of a

mixture of stripe rust susceptible cultivars, were planted as border rows to create sufficient levels of infection.

While Creston, BC disease nurseries were solely exposed to natural infection of stripe rust, a urediniospores mixture of unknown stripe rust races collected in the previous years from disease hot-spot field site Lethbridge was used to inoculate in disease nurseries in Lethbridge, AB. Stripe rust severity at adult plant stage was recorded visually as 0 to 100% (0 = immune and 100% = completely susceptible) based on a modified Cobb scale (Peterson, 1948).

### 2.2.3 Plant DNA extraction and Genotyping

Two seeds from each DH line and two parents were sown in 72-cell trays containing professional growing mix (Sun Go Horticulture Canada Ltd., Seba Beach, AB) and grown in the greenhouse. The day and night greenhouse growth conditions were 22°C/18°C temperature and 16/8 hours photoperiod. Approximately 100 mg fresh leaf tissues from each DH line and parents were collected at the three-leaf growth stage. The tissue samples were frozen immediately in liquid nitrogen in 2-mL nuclease-free eppendorf tubes, each containing 3 stainless steel beads. Leaf tissues were ground using TissueLyser II (QIAGEN, Germany). The ground tissue samples were stored at -80°C before DNA extraction. Genomic DNA was extracted from the ground tissues using the DNeasy 96 Plant Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's instructions. The DNA pellets were dissolved and stored in 100 µL of 1x TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0).

DNA quality and integrity were determined using 1.5% agarose gel electrophoresis. DNA concentration was quantified using Quant-iT$^{TM}$ dsDNA Assay Kit (Thermo Fisher Scientific Inc, Bartlasville, OK, USA) following the manufacturer's instructions. Stock DNA

solutions were diluted to 50 ng/μL for marker genotyping. A random selection of 190 DH and two parents were genotyped with the wheat 90K Infinium iSelect SNP assay (Wang et al., 2014) following the manufacturers protocols (Illumina Inc., San Diego, USA).

Genome Studio 2.0 (Illumina, San Diego, USA) software was used to filter high quality SNPs using default expression features described by Dhariwal and Randhawa (2021; in press) (i) AA Freq: !=1, (ii) AB Freq: !=1, (iii) BB Freq: !=1, (iv) Minor Allele Freq: >0.03, and (v) Call Freq: >0.50. Additional filtering was done by removing monomorphic markers, markers with > 10% missing values and markers significantly distorted from the expected segregation ratio (1:1) using Chi-squared ($\chi^2$) test. Only high quality, polymorphic markers that fit into the expected segregation ratio (1:1) were used to construct a linkage map.

### 2.2.4 Phenotypic data analysis

Analysis of variance (ANOVA) was carried out using the agricolae (version 1.2-4) package of the R (version 4.0.3) software (R Core Team, 2013). For the ANOVA model, parents, DH lines and check cultivars were treated as fixed effects, while environments were treated as random effects. A histogram was generated to show the distribution of disease severity data in DH population across all five environments using Microsoft Excel (2016).

### 2.2.5 Linkage map construction

Linkage groups were constructed by a two-step process following Dhariwal and Randhawa (2021; in press). In the first step, draft linkage groups were created using MSTmap software version 2.0 (Wu et al., 2008). The following parameters were used to calculate the order of linkage groups: (i) kosambi map function (Kosambi, 1944), (ii) cut-off *p* value of $1^{-10}$ (iii) no mapping size threshold of 1 (iv) no mapping distance threshold of 15.0 cM, (vi) no

mapping missing threshold of 0.1 and (v) maximum likelihood (ML) function (Jansen et al., 2001). Each linkage group was assigned to a particular wheat chromosome using a previously published consensus map (Wang et al., 2014). In the second step, draft linkage groups were refined by recalculating marker order and distance within the linkage groups using MapDisto version 2.0 software (Heffelfinger et al., 2017).

The following parameters were used for computation: (i) kosambi map function (Kosambi, 1944), (ii) minimum logarithm of odd (LOD min) threshold of 3.0, (ii) maximum recombination frequency (*r* max) threshold of 0.3 and (iv) minimum missing data threshold of 10%. Two or more linkage groups created for the same chromosome were attempted to merge into a single linkage group using less stringent cut off values (*r* max > 0.3). "AutoCheckInversions" and "AutoRipple" commands were used to create the best order of markers.

**2.2.6 QTL analysis**

QTL analysis was undertaken for each environment separately and across the environments using pooled data. Composite interval mapping (CIM) was carried out with Windows QTL Cartographer version 2.5 software (Wang et al., 2005), using a forward-backward regression method with the window size set at 1 cM and walk speed of 1.0 cM. A thousand permutation tests (Doerge & Churchill, 1996) were performed to determine significant logarithm of odd (LOD) thresholds. QTLs were also reported when reaching an arbitrary significance level of LOD 2.5 or above to detect QTLs with large additive effect and phenotypic variation explained following the method described by Yi et al. (2018). QTL confidence intervals were determined using LOD peak above the calculated permutation

threshold. Final chromosome map charts were generated using MapChart 2.32 software (Voorrips, 2002).

### 2.2.7 Physical position of QTLs

Physical positions of all QTLs identified in DH population were obtained using Blast searches of the surrounding probe/primer sequences of each QTL against the International Wheat Genome Sequencing Consortium (IWGSC) RefSeq v.1.0 (Appels et al., 2018) available at https://wheat-urgi.versailles.inra.fr/Tools/BLAST.

## 2.3 Results

### 2.3.1 Phenotypic observations

ANOVA of stripe rust severity for the AAC Innova/AAC Proclaim population revealed highly significant effects ($p < 0.0001$) for genotype, environment, and genotype x environment interaction (Table 2-1). The disease severity value for the resistant parent AAC Innova ranged from 0% in Lethbridge 2017 and 2020 to 10% in Creston. Disease severity for the susceptible parent AAC Proclaim ranged from 40% in Creston 2020 to 90% in Lethbridge 2020 (Table 2-2). The mean value of stripe rust severity in the DH population ranged from 14.7% in Lethbridge 2016 to 29.7% in Creston 2020 (Table 2-2). The median value of stripe rust severity in the DH population ranged from 1.0% in Lethbridge 2020 to 20.0% in Creston 2020 (Table 2-2). Histogram showed skewed distribution towards greater resistance across all five environments (Figure 2-1).

### 2.3.2 Linkage map

In total, 7,369 high quality polymorphic markers (approximately 9.0% of the total 81587 SNPs) were used for constructing the linkage map. Of the markers, 7,112 out of 7,369

markers were mapped to 29 linkage groups belonging to all 21 wheat chromosomes (Table 2-2). While single linkage groups were observed for almost two thirds of the chromosomes, two linkage groups were observed each for chromosome 1A, 3A, 5A, 6A, 7A, 5D, 6D and 7D (Figure 2-2; Table 2-3).

The total map length of all linkage groups was 3,074 cM with an average distance of 2.3 cM between markers. The map lengths for the sub genomes A, B, and D were 1,077, 1,357 and 640 cM, with a density of 2.3, 2.8 and 1.4 markers per cM, respectively. The highest number of markers were mapped to wheat homoeologous group 2 chromosome (# of markers = 1,354 and density = 2.2 markers/cM) and the lowest number of markers were mapped on homoeologous group 5 (# of markers = 752 and density = 1.3 markers/cM), respectively (Table 2-3).

Considering wheat sub-genomes, the highest number of markers were mapped on sub-genome B (# of markers = 3,733 and density = 2.8 markers/cM) followed by sub-genome A (# of markers = 2,493 and density = 2.3 markers/cM) and sub-genome D (# of markers = 886 and density = 1.4 markers/cM), respectively. Since many markers co-segregated (mapped at the same position), the number of linkage bins spanned across all 21 chromosomes were 1,381 (Table 2-3).

### 2.3.3 QTLs for stripe rust resistance

A total of 11 stripe rust resistance loci (*QYr.lrdc-2A, QYr.lrdc-2B.1, QYr.lrdc-2B.2, QYr.lrdc-2B.3, QYr.lrdc-2B.4, QYr.lrdc-2D, QYr.lrdc-3B, QYr.lrdc-5A, QYr.lrdc-5B, QYr.lrdc-5D* and *QYr.lrdc*-7D), were identified in this study (Figure 2-2; Table 2-4). These QTLs were mapped to chromosomes 2A, 2B, 2D, 3B, 5A, 5B, 5D and 7D, respectively (Figure 2-2 and Table 2-4). Amongst these, *QYr.lrdc-2A* was the only major effect QTL

detected in all environments. *QYr.lrdc-2A* was identified within the 218.1-240.6 cM interval with the LOD score ranged from 8.02 in Lethbridge 2016 to 20.28 in Lethbridge 2017 and 21.25 in the combined analysis using the pooled data (Figure 2-3 and Table 2-4). The proportion of phenotypic variation explained by this QTL varied from 14% in Lethbridge 2016 to 29% in Lethbridge 2019 and 33% using the pooled data. The resistant parent AAC Innova was the source of the resistant allele for this major QTL (Table 2-4).

The second most stable QTL, *QYr.lrdc-5A*, was detected in four out of five environments and accounted for 5% to 7% of the phenotypic variation in individual environments and 7% in the combined analysis using the pooled data. The *QYr.lrdc-5A* was mapped within the 57.1-65.7 cM interval with a LOD score ranging from 3.07 in Lethbridge 2016 to 5.61 in Lethbridge 2017 and 5.99 in the combined analysis using the pooled data. The resistant allele at *QYr.lrdc-5A* was derived from AAC Innova (Table 2-4). The *QYr.lrdc-7D* was identified in two environments, with a LOD score of 5.0 and 6.0, and explained phenotypic variation up to 5 and 6%, respectively. AAC Proclaim contributed resistance alleles at *QYr.lrdc-5A* and *QYr.lrdc*-7D (Table 2-4).

Remaining QTLs were detected in one environment, with or without pooled data. However, chromosome 2B harboured four QTLs namely *QYr.lrdc-2B.1, QYr.lrdc-2B.2, QYr.lrdc-2B.3* and *QYr.lrdc-2B.4*, between 103.9 and 189.3 cM, with the possibility that they represent a single locus. These loci explained 5%, 7%, 6% and 8% of the phenotypic variation, respectively. AAC Innova contributed stripe rust alleles at all the four loci (Table 2-4).

Similar to the 2B resistance loci, *QYr.lrdc-2D, QYr.lrdc-3B, QYr.lrdc-5B* and *QYr.lrdc-5D* were also detected in a single environment and each explained less than 10% of the phenotypic variation (Table 2-4). AAC Innova contributed stripe rust alleles at *QYr.lrdc-*

*3B, QYr.lrdc-5B* and *QYr.lrdc-5D*, while AAC Proclaim contributed at *QYr.lrdc-2D* (Table 2-4).

The three most stable QTLs (*QYr.lrdc-2A*, *QYr.lrdc-5A* and *QYr.lrdc-7D*) were assessed individually and in combination for their effect on stripe rust severity in the DH population. It was observed that in combinations, these QTLs contributed to maximum reductions in stripe rust severity (Figure 2-4). *QYr.lrdc-2A* was the only QTL sufficient to reduce stripe rust severity individually, while *QYr.lrdc-5A* and *QYr.lrdc-7D* were only significantly effective in combination with *QYr.lrdc-2A* (Figure 2-4).

### 2.4 Discussion

The linkage map generated in this study consisted of a total of 7,112 SNP markers representing 1,381 unique markers or linkage bins and mapped across 29 linkage groups with a total map length of 3,074 cM. This map is comparable to some recently published wheat SNP linkage maps (Case et al., 2014; Bokore et al., 2017; Bemister et al., 2019), and provides enough coverage to dissect the stripe rust resistance variation present in this population.

Stripe rust resistance in the DH population AAC Innova/AAC Proclaim was conditioned by a combination of one major QTL detected across all environments and few minor loci detected either in specific environment or in few environments. Our results are in agreement with the Avocet/Pastor population results, where 13 QTLs with different size effects were found to reduce stripe rust severity (Rosewarne et al., 2012) and the Druchamp/Michigan Amber population, where stripe rust resistance was controlled by 11 QTLs with various degrees of effectiveness (Hou et al., 2015). Vazquez et al. (2015) also reported similar results from the Tubbs/NSA-98-0995 population, where eight stripe rust resistance QTLs were identified in unique environments.

It may be recalled that the *QYr.lrdc-2A* was the most significant QTL identified in this study, and it was detected across environments as well as in pooled data. The disease pressure was high in all trials planted at Creston, BC and Lethbridge, AB, providing circumstances where any plant might have very little chance to escape disease. Despite of high disease pressure, the *QYr.lrdc-2AL* was effective in all trial environments, suggesting its practical value in a breeding program. This QTL was mapped on the long arm of chromosome 2A, within the 218.2-240.6 cM interval, and flanked by SNP markers *BS00067284_51* and *RAC875_c10408_90*. (Figure 2-3). The proportion of phenotypic variation (14-33%) explained by *QYr.lrdc-2A* across a considerable diversity of environments indicates that it could be the source of a major stripe rust resistance gene in AAC Innova.

To date, five stripe rust resistance genes including, characterized designated genes *Yr1* (Bansal et al., 2009) and *Yr32* (Eriksen et al., 2004), and temporarily designated genes *Yrxy2* (Zhou et al., 2011) *YrJ22* (Chen et al., 2016) and *YrH9017* (Lu et al., 2019) have been reported on the long arm of chromosome 2A. In addition, major QTLs *QYrns.orz-2AL* (Vazquez et al., 2015)*, QYr.spa-2A* (Bokore et al., 2017)*,* and *QYr.cau-2AL* (Wang et al., 2019) conferring resistance to stripe rust have also been reported on the long arm of chromosome 2A.

We observed that, differential genotype Avocet +*Yr1* was highly rust resistant while differential genotype Avocet +*Yr32* was highly susceptible (Table 2-2) across environments in this study, which indicates that *QYr.lrdc-2A* could be *Yr1* but not *Yr32*. However, the physical map position (717-765 Mb) of *QYr.lrdc-2A* (Table 2-4) only overlapped with *YrJ22* (760-771 Mb) and *QYr.cau-2AL* (761-772 Mb)*,* when compared to the flanking marker sequences of all these reported genes and QTLs with IWGSC RefSeq v1.0. The *YrJ22* and

*QYr.cau-2AL* were reported in Chinese wheat cultivar Jimai 22 and landrace Hong Qimai, respectively. Male parental line N9195 of resistant parent AAC Innova is also Chinese in origin, suggesting that *QYr.lrdc-2A* comes from the Chinese wheat germplasm. We also validated our results by comparing marker positions on the consensus genetic map produced by Wang et al. (2014) and Wen et al. (2017), where SNP marker *Kukri_c365_345* closely associated with *QYr.cau-2AL* was mapped approximately 3 cM and 5 cM away from *QYr.lrdc-2A*'s flanking marker *RAC875_c10408_90,* respectively, suggesting that these two QTLs might relate to the same stripe rust resistance gene.

*QYr.lrdc-2B.1*, *QYr.lrdc-2B.2*, *QYr.lrdc-2B.3* and *QYr.lrdc-2B.4* were mapped on the long arm of chromosome 2B, each except *QYr.lrdc-2B.4* identified in a single environment and explained less than 10% of phenotypic variation (Table 2-4). The long arm of wheat chromosome 2B is rich with many stripe rust resistance genes such as *Yr5, Yr7, Yr43, Yr44, Yr53* and *Yr72* (Wang & Chen, 2017). All our identified QTLs on chromosome 2B were derived from the resistant parent AAC Innova, indicating that they might relate to one of these single genes. However, considering the phenotypic variation, all of our 2B loci seem different from these already reported genes on the long arm of chromosome 2B, which have been reported providing greater resistance than observed in this study. Furthermore, we observed that differential genotype Avocet + *Yr5* was highly resistant across environments (Table 2-2) in this study but *Yr5* being an alien derived gene is not present in the pedigree of AAC Innova, confirming that *Yr5* could not be the source of resistance for all of 2B loci identified in this study. There are several reported minor QTLs contributing to stripe rust resistance on chromosome 2B (Lan et al.,2010; Singh et al., 2014; Hou et al., 2015; Bokore et al., 2017). Based on consensus genetic maps (Wang et al., 2014; Maccaferri et al., 2015), flanking markers of QTLs reported in these studies are positioned far away (>10-100 cM)

from the flanking markers of our QTLs, suggesting that our reported QTLs are likely new and different from those previously reported on the long arm of chromosome 2B.

*QYr.lrdc-5A* mapped on chromosome 5A was the second most stable QTL in our study, identified in four out of five environments and accounted for 5-7% of phenotypic variation (Table 2-4). The QTL interval was flanked by SNP markers *CAP7_c4064_162* and *Kukri_c16087_281.* There are several studies supporting the presence of minor effect QTLs on chromosome 5A (Rosewarne et al., 2012; Buerstmayr et al., 2014; Lan et al., 2014; Hou et al., 2015). There is a possibility that *QYr.lrdc-5A* and *QYrdr.wgp.5AL* identified by Hou et al. (2015) could be the same, based on consensus genetic maps produced by Maccaferri et al. (2015) and Wang et al. (2014); SNP marker *wsnp_Ex_c20899_30011827* closely associated with *QYrdr.wgp.5AL* is positioned 2 cM and 3 cM away from our flanking maker *Kukri_c16087_281,* respectively. *QYrdr.wgp.5AL* explained 2.27-17.22% phenotypic variation which is also comparable to *QYr.lrdc-5A.*

*QYr.lrdc-7D* derived from susceptible parent AAC proclaim was identified in two individual environments (Lethbridge 2016 and Creston 2020), where it accounted for 5 and 7% of the phenotypic variation, respectively (Table 2-4). Minor effect QTLs on chromosome 7D have only been detected in a few studies (Basnet et al., 2014b). In contrast, several studies reported the presence of the *Lr34/Yr18* gene on the short arm of chromosome 7D (Lu et al., 2009; Yuan et al., 2018). Randhawa et al. (2012) reported that most of the Canadian wheat cultivars carry the *Lr34*/*Yr18* gene. However, the phenotypic effect of *QYr.lrdc-7D* is not typical of the *Lr34/Yr18* because it exhibited only minor (5-6%) phenotypic variation and derived from susceptible parent AAC proclaim, which is not supposed to carry the *Lr34/Yr18* gene.

*QYr.lrdc-2D,* contributed by susceptible parent AAC Proclaim was identified only in the Lethbridge 2020 environment and pooled data, where it contributed 4% of the phenotypic variation and was closely associated with the SNP marker *wsnp_JD_rep_c63957_40798083* (Table 2-4). There are several previous studies reporting stripe rust resistance QTLs on chromosome 2D (Lu et al., 2009; Agenbag et al., 2012; Lan et al., 2014; Ren et al., 2015; Ren et al., 2017). Most of these QTLs were present on the short arm of chromosome 2D like *QYr.lrdc-2D*. But, regardless of their mapping on the same chromosome arm, the physical position (21-22 Mb) of *QYr.lrdc-2D* (Table 2-4) does not overlap with any of the QTLs on IWGSC RefSeq v1.0, suggesting it might be different from these QTLs.

*QYr.lrdc-3B*, mapped on chromosome 3B, was only identified in Lethbridge 2020 and accounted for 5% of the phenotypic variation (Table 2-4). The QTL was flanked by *SNPs BS00064177_51* and *BobWhite_c25670_425.* Minor effect QTLs conferring resistance to stripe rust have been reported on chromosome 3B in many studies (Agenbag et al., 2012; Lan et al., 2014; Singh et al., 2014; Bokore et al., 2017; Yuan et al., 2018). Among these studies, *QYr.lrdc-3B* showed similarity with *QYr.spa-3B* reported in Canadian wheat cultivars AC Cadillac and Red Fife (Bokore et al., 2017). According to consensus genetic maps produced by Maccaferri et al. (2015) and Wang et al. (2014), SNP marker *Tdurum_contig79629_538* closely associated with *QYr.spa-3B* is just 2 and 3 cM away from *QYr.lrdc-3B*'s flanking marker *BobWhite_c25670_425,* respectively. Phenotypic variation (2.6-8.7%) explained by *QYr.spa-3B* is also comparable to *QYr.lrdc-3B.* Since both QTLs are derived from Canadian spring wheat cultivars, we can conclude that these two QTLs could be the same gene.

*QYr.lrdc-5B* was mapped on the long arm of chromosome 5B and identified in Lethbridge 2017. This QTL was closely associated with SNPs *RAC875_c19099_308* and explained 7% of the phenotypic variation (Table 2-4). There are multiple minor effect QTLs

conferring resistance to stripe rust on chromosome 5B (Lu et al., 2009; Hao et al., 2011; Agenbag et al., 2012; Singh et al.,2014; Hou et al., 2015). Our findings suggest that *QYr.caas-5BL.1* identified by Lu et al. (2009) on the long arm of chromosome 5B and *QYr.lrdc-5B* might relate to the same gene because on the consensus genetic map (Maccaferri et al., 2015), flanking simple sequence repeat (SSR) marker *Xwmc415* of *QYr.caas-5BL.1* was less than 1 cM away from *QYr.lrdc-5B*'s closely associated SNP marker *RAC875_c19099_308*. Phenotypic variation (3.6-5.1%) explained by *QYr.caas-5BL.1* is similar to that of *QYr.lrdc-5B* (Table 2-4).

*QYr.lrdc-5D* on chromosome 5D was identified in Lethbridge 2017 and explained only 3% of the phenotypic variation. This QTL was closely associated with the SNP marker *Excalibur_c50584_358* (Table 2-4). Several minor effect QTLs conferring resistance to stripe rust have been previously reported on chromosome 5D (Imtiaz et al., 2004; Case et al., 2014; Ren et al., 2015; Vazquez et al., 2015). However, the physical position (549-550 Mb) of *QYr.lrdc*-5D (Table 2-4) does not overlap with any of the QTLs reported in these studies.

The detailed comparison of QTLs detected in this study with the previously reported genes/QTLs might not be straightforward due to several limiting factors such as the platform used for genotyping, the population size, recombination frequency, genotype x environment interactions and methods used for linkage map construction and QTL analysis (Bemister et al., 2019). However, regardless of proving novelty or similarity of these identified QTLs, they could be valuable for diversifying the sources of genetic resistance in breeding programs. This is particularly the case for the major QTL *QYr.lrdc-2A*, which could be used to incorporate into commercial cultivars and combine with the other genes using the associated SNP markers identified in this study.

## 2.5 Conclusions

Our findings suggest that the genetic architecture of the AAC Innova/AAC Proclaim population was modulated by a major and ten minor QTLs. Four of these QTLs were located on chromosome 2B and one on each of chromosome 2A, 2D, 5A, 3D, 5A, 5D and 7D; although most of them (except QTL on chromosome 2A) had small phenotypic effects and were not consistently identified across the environments, they could be valuable for diversifying the sources of genetic resistance in breeding program. *QYr.lrdc-2A* derived from the resistant parent was particularly interesting, as it explained a high proportion of the phenotypic variation (up to 33%) and was consistently identified in all environments. From the viewpoint of practical breeding, *QYr.lrdc-2A* could be a valuable source of resistance to incorporate into commercial cultivars and combine with the other genes for stripe rust resistance.

## 2.6 Tables and Figures

**Table 2-1.** ANOVA table for stripe rust severity of AAC Innova/AAC Proclaim doubled haploid spring wheat mapping population.

| **Source** | **df** | **Sum Sq** | **Mean Sq** | **F value** | **Pr (>F)** |
|---|---|---|---|---|---|
| Genotype | 292 | 1114076 | 3815.3 | 25.149 | < 2.2e-16 *** |
| Environment | 4 | 43949 | 10987.2 | 72.423 | < 2.2e-16 *** |
| Genotype x Environment | 1168 | 410386 | 351.4 | 2.316 | < 2.2e-16 *** |
| Error | 151.709 | | | | |

Note: df: degrees of freedom; significance codes: 0 '***'.

**Table 2-2.** Summary of stripe rust severity of parents, differential genotypes and AAC Innova/AAC Proclaim doubled haploid spring wheat mapping population.

| Env | Parental lines | | Stripe rust differential genotypes | | | | | | Population | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **AAC Proclaim** | **AAC Innova** | **Avocet +*Yr1*** | **Avocet +*Yr5*** | **Avocet +*Yr7*** | **Avocet +*Yr10*** | **Avocet +*Yr31*** | **Avocet +*Yr32*** | **Min** | **Max** | **Mean** | **Median** |
| LET 2016 | 65.0 | 1.0 | 1.0 | 1.0 | 100.0 | 35.0 | 95.0 | 85.0 | 1.0 | 75.0 | 14.7 | 5.0 |
| CRE 2016 | 85.0 | 1.0 | 1.0 | 1.0 | 100.0 | 25.0 | 95.0 | 85.0 | 1.0 | 85.0 | 21.6 | 5.0 |
| LET 2017 | 85.0 | 0.0 | 1.0 | 1.0 | 85.0 | 35.0 | 85.0 | 75.0 | 0.0 | 100.0 | 25.0 | 0.0 |
| LET 2020 | 90.0 | 0.0 | 1.0 | 1.0 | 95.0 | 65.0 | 95.0 | - | 0.0 | 100.0 | 21.1 | 1.0 |
| CRE 2020 | 40.0 | 10.0 | - | - | 90.0 | 20.0 | 80.0 | - | 0.0 | 90.0 | 29.7 | 20.0 |

Note: CRE: Creston; LET: Lethbridge; -: missing value.

**Table 2-3.** Summary of SNP markers used for linkage mapping using the AAC Innova/AAC Proclaim doubled haploid spring wheat mapping population.

| Chr | Map Length (cM) | Number of Linkage bins | Number of Markers | Markers per cM | Linkage Bins per cM |
|---|---|---|---|---|---|
| 1A.1 | 93 | 67 | 479 | 5.2 | 0.7 |
| 1A.2 | 7 | 5 | 13 | 1.9 | 0.7 |
| 2A | 241 | 95 | 458 | 1.9 | 0.4 |
| 3A.1 | 18 | 14 | 106 | 6 | 0.8 |
| 3A.2 | 73 | 23 | 59 | 0.8 | 0.3 |
| 4A | 181 | 95 | 474 | 2.6 | 0.5 |
| 5A.1 | 131 | 78 | 256 | 1.9 | 0.6 |
| 5A.2 | 66 | 27 | 91 | 1.4 | 0.4 |
| 6A.1 | 77 | 47 | 254 | 3.3 | 0.6 |
| 6A.2 | 39 | 28 | 89 | 2.3 | 0.7 |
| 7A.1 | 119 | 35 | 115 | 1 | 0.3 |
| 7A.2 | 32 | 26 | 99 | 3.1 | 0.8 |
| **A Genome Total** | **1077** | **540** | **2493** | **2.3** | **0.5** |
| % | 35 | 39.1 | 35.1 | 35.4 | 38.5 |
| 1B | 115 | 66 | 619 | 5.4 | 0.6 |
| 2B | 233 | 114 | 629 | 2.7 | 0.5 |
| 3B | 212 | 108 | 694 | 3.3 | 0.5 |
| 4B | 158 | 68 | 386 | 2.4 | 0.4 |
| 5B | 243 | 75 | 234 | 1 | 0.3 |
| 6B | 185 | 92 | 545 | 3 | 0.5 |
| 7B | 211 | 144 | 626 | 3 | 0.7 |

| | | | | | |
|---|---|---|---|---|---|
| **B Genome Total** | **1357** | **667** | **3733** | **2.8** | **0.5** |
| % | 44 | 48.3 | 52.5 | 43.1 | 38.5 |
| 1D | 79 | 26 | 95 | 1.2 | 0.3 |
| 2D | 137 | 32 | 267 | 1.9 | 0.2 |
| 3D | 11 | 9 | 116 | 11 | 0.9 |
| 4D | 25 | 6 | 12 | 0.5 | 0.2 |
| 5D.1 | 118 | 16 | 36 | 0.3 | 0.1 |
| 5D.2 | 36 | 22 | 135 | 3.7 | 0.6 |
| 6D.1 | 27 | 10 | 74 | 2.7 | 0.4 |
| 6D.2 | 88 | 15 | 37 | 0.4 | 0.2 |
| 7D.1 | 35 | 18 | 70 | 2 | 0.5 |
| 7D.2 | 83 | 20 | 44 | 0.5 | 0.2 |
| **D Genome Total** | **640** | **174** | **886** | **1.4** | **0.3** |
| **%** | **21.0** | **12.6** | **12.4** | **21.5** | **23.0** |
| **A+B+D** | | | | | |
| 1 | 294 | 164 | 1206 | 4.1 | 0.6 |
| 2 | 611 | 241 | 1354 | 2.2 | 0.4 |
| 3 | 314 | 154 | 975 | 3.1 | 0.5 |
| 4 | 364 | 169 | 872 | 2.4 | 0.5 |
| 5 | 594 | 218 | 752 | 1.3 | 0.4 |
| 6 | 416 | 192 | 999 | 2.4 | 0.5 |
| 7 | 481 | 243 | 954 | 2 | 0.5 |
| **Total** | **3074** | **1381** | **7112** | **2.3** | **0.4** |

Note: Chr: chromosome; cM: centimorgan.

**Table 2-4.** Details of quantitative trait loci identified for stripe rust (*Yr*) resistance on different wheat chromosomes in AAC Innova/AAC Proclaim doubled haploid spring wheat mapping population.

| QTL | Chr | Env | Position (cM) | Confidence Interval (cM) | LOD | Additive Effect | %$R^2$ | Closet Marker | Physical Position (Mb) | Donor Parent |
|---|---|---|---|---|---|---|---|---|---|---|
| *QYr.lrdc-2A* | 2A | LET16 | 238.40 | 237.8 - 240.6 | 8.02 | 12.60 | 14.00 | *Excalibur_c10461_520* | 717-765 | I |
| | | CRE16 | 234.20 | 218.2 - 239.9 | 13.04 | 15.10 | 25.00 | *Excalibur_c10461_520* | | I |
| | | LET17 | 240.10 | 237.6 - 240.6 | 20.28 | 19.40 | 29.00 | *Excalibur_c10461_520* | | I |
| | | LET20 | 238.10 | 218.2 - 239.9 | 12.15 | 16.10 | 22.00 | *Excalibur_c10461_520* | | I |
| | | CRE20 | 237.60 | 218.4 - 239.9 | 14.51 | 14.80 | 28.00 | *Excalibur_c10461_520* | | I |
| | | Combined | 238.40 | 218.1 - 239.9 | 21.25 | 14.90 | 33.00 | *Excalibur_c10461_520* | | I |
| *QYr.lrdc-2B.1* | 2B | LET20 | 108.80 | 103.9 - 112.8 | 3.41 | 7.78 | 5.00 | *CAP12_rep_c3980_87* | 115-174 | I |
| *QYr.lrdc-2B.2* | 2B | LET17 | 145.40 | 139.3 - 149.3 | 5.73 | 9.48 | 7.00 | *Tdurum_contig10219_706* | 694-744 | I |
| *QYr.lrdc-2B.3* | 2B | CRE20 | 168.90 | 163.3 - 172.5 | 3.90 | 7.44 | 6.00 | *RAC875_c65882_668* | 750-763 | I |
| *QYr.lrdc-2B.4* | 2B | CRE16 | 184.20 | 182.6 - 189.3 | 5.80 | 8.53 | 8.00 | *BS00074091_51* | 766-779 | I |
| | | Combined | 184.20 | 182.6 - 189.3 | 5.92 | 6.74 | 7.00 | *BS00074091_51* | | I |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *QYr.lrdc-2D* | 2D | LET20 | 6.30 | 3.0 - 7.6[a] | 2.82 | -6.87 | 4.00 | *wsnp_JD_rep_c63957_40798083* | 21-22 | P |
| | | Combined | 6.30 | 4.3 - 8.4 | 3.70 | -5.43 | 4.00 | *wsnp_JD_rep_c63957_40798083* | | P |
| *QYr.lrdc-3B* | 3B | LET20 | 26.30 | 25.3 - 27.4[a] | 3.27 | 7.21 | 5.00 | *RAC875_c36432_197* | 16-18 | I |
| *QYr.lrdc-5A* | 5A | LET16 | 60.80 | 57.1 - 60.9[a] | 3.07 | -5.23 | 5.00 | *Tdurum_contig17216_310* | 700-702 | P |
| | | CRE16 | 64.60 | 63.6 - 65.7 | 4.09 | -7.36 | 6.00 | *Tdurum_contig17216_310* | | P |
| | | LET17 | 65.40 | 64.6 - 65.7 | 5.61 | -9.63 | 7.00 | *Tdurum_contig17216_310* | | P |
| | | LET20 | 64.70 | 63.6 - 65.7 | 4.81 | -8.96 | 7.00 | *Tdurum_contig17216_310* | | P |
| | | Combined | 64.70 | 63.6 - 65.7 | 5.99 | -6.88 | 7.00 | *Tdurum_contig17216_310* | | P |
| *QYr.lrdc-5B* | 5B | LET17 | 88.40 | 87.2 - 88.7[a] | 2.61 | 14.22 | 3.00 | *RAC875_c19099_308* | 519-534 | I |
| *QYr.lrdc-5D* | 5D | LET17 | 5.40 | 5.4 - 5.4[a] | 2.51 | 6.01 | 3.00 | *Excalibur_c50584_358* | 549-550 | I |
| *QYr.lrdc-7D* | 7D | LET16 | 0.10 | 0.0 - 0.8[a] | 2.68 | -4.84 | 5.00 | *wsnp_Ra_c6894_11980338* | 55-59 | P |
| | | CRE20 | 0.00 | 0.0 - 1.2 | 4.41 | -7.55 | 6.00 | *wsnp_Ra_c6894_11980338* | | P |

Note: QTL: quantitative trait loci; Chr: chromosome; Env: environment; cM: centimorgan; LOD: logarithm of the odds score; $R^2$: phenotypic variance; Mb: mega base pairs; I: resistant parent AAC Innova; P: susceptible parent AAC Proclaim; LET16, LET17 and LET20: Lethbridge field trials in 2016, 2017 and 2020, respectively; CRE16 and CRE20: Creston field trials in 2016 and 2020, respectively; LOD score thresholds were 3.2, 3.4, 3.2, 3.3, 3.3 and 3.2 at Lethbridge 2016, Lethbridge 2017, Lethbridge 2020, Creston 2016, Creston 2020 and for the pooled data, respectively; [a] QTL interval determined using an

arbitrary LOD threshold of 2.5; - additive effect: resistance alleles are contributed by the parent AAC Proclaim; + additive effect: resistance alleles are contributed by the parent AAC Innova.

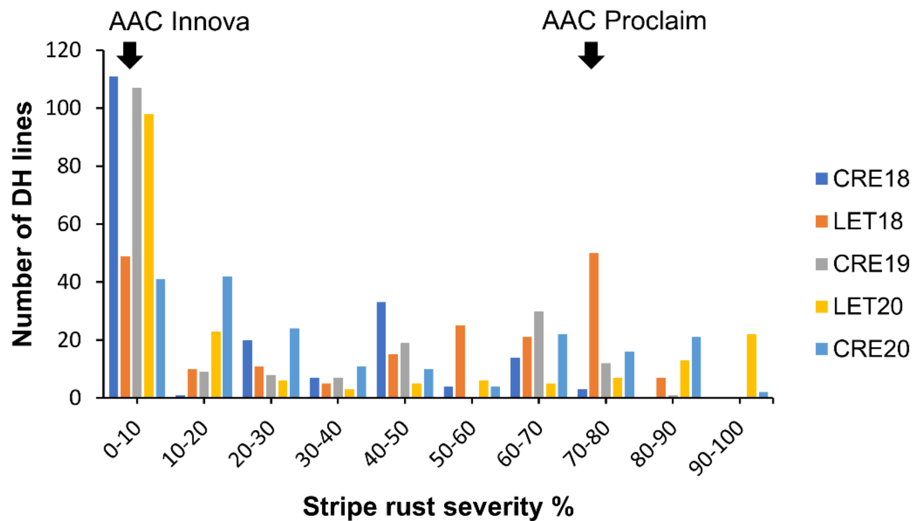


**Figure 2-1.** Frequency distributions of stripe rust severity at the adult plant stage in AAC Innova/AAC Proclaim doubled haploid spring wheat mapping population in each of the five trial environments. Black arrows indicate the mean percentage disease severity of the parental lines, AAC Innova and AAC Proclaim. The trial locations and years are indicated on the right side of graph. LET16, LET17 and LET20: Lethbridge field trials in 2016, 2017 and 2020, respectively; CRE16 and CRE20: Creston field trials in 2016 and 2020, respectively.

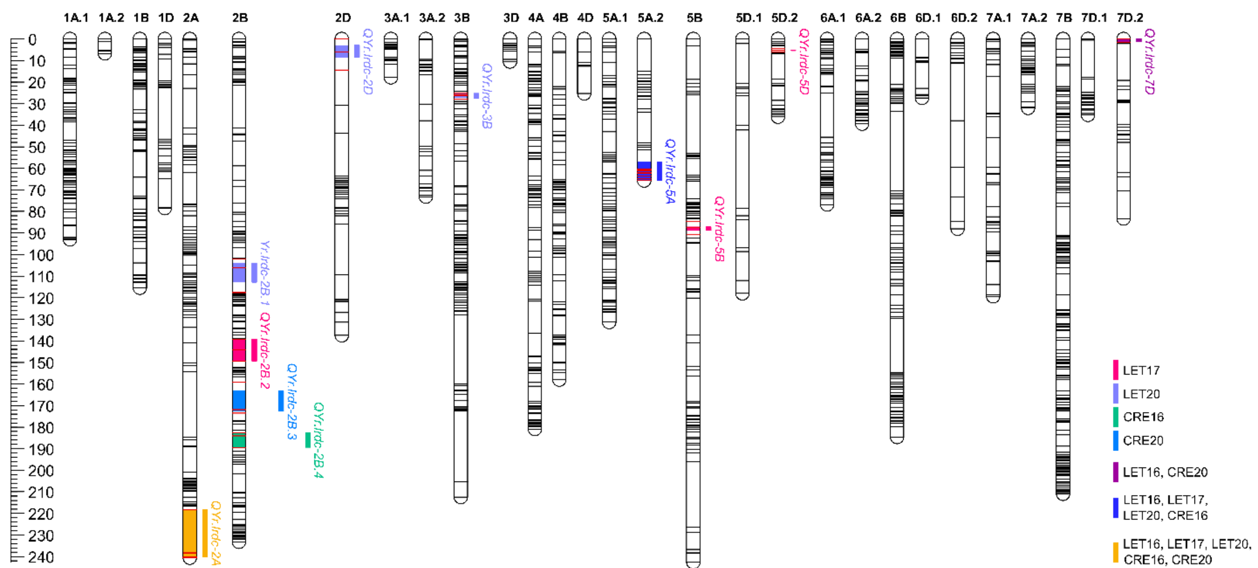


**Figure 2-2.** AAC Innova/AAC Proclaim doubled haploid spring wheat mapping population linkage map. The twenty-nine linkage groups (LGs) are labeled as 1A.1 to 7D.2 according to their relatedness to homoeologous group chromosomes 1 - 7 and three genomes A, B and D of hexaploid spring wheat. A scale ruler for marker positions (in cM) are shown on the left side of the chart. Marker loci are shown as horizontal lines of either black or red colour on LGs. Red colour lines for marker loci represent linked markers of quantitative trait loci (QTL) located in the respective chromosome segment, while black colour represent loci outside QTL regions. Coloured lines represent QTL intervals. Different colours of LG blocks/segments and QTL bars represent different individual or group of years for QTLs detected as shown in the legend on the right-lower corner of the chart.

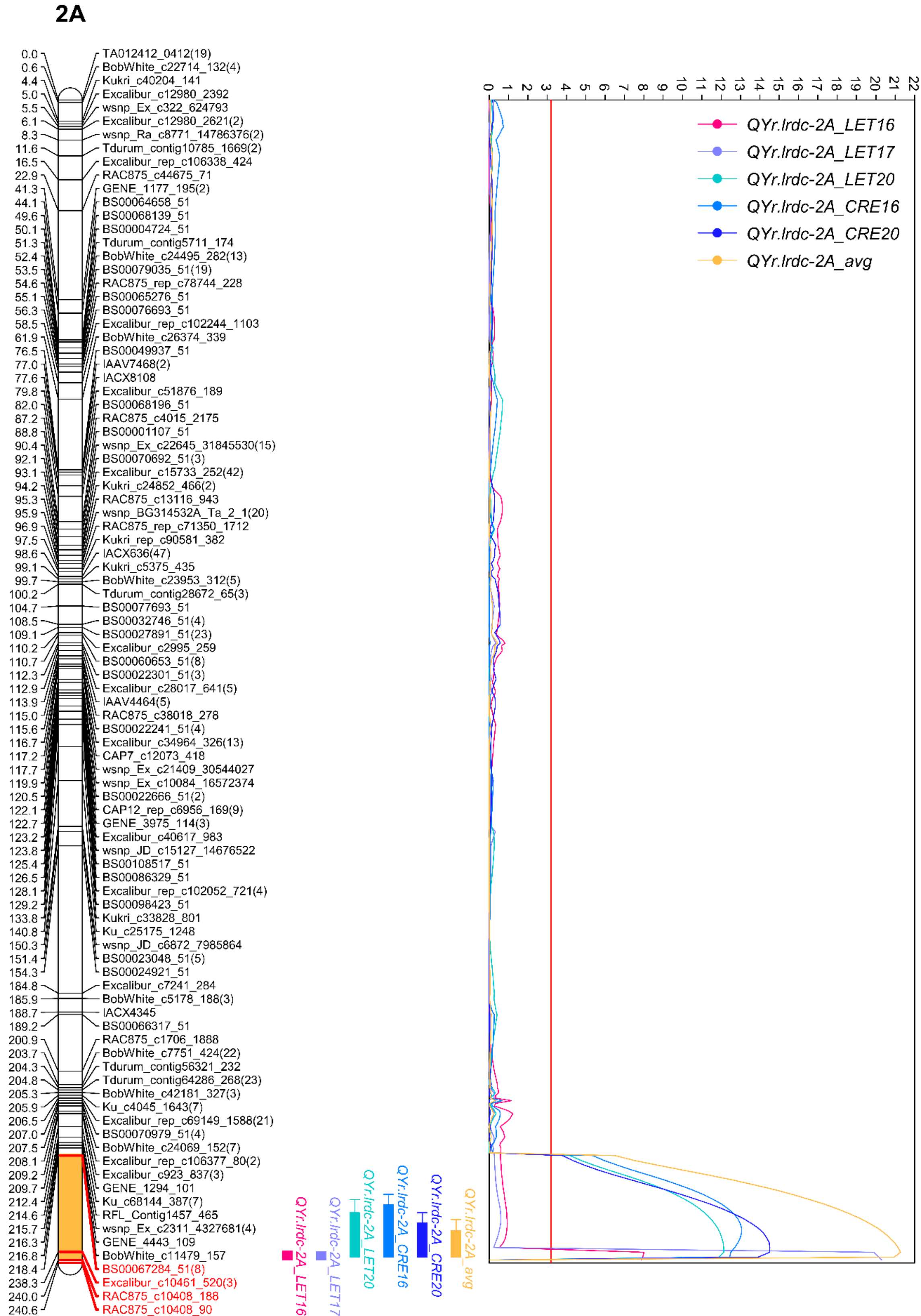


**Figure 2-3.** The linkage map of chromosome 2A along with the logarithm of odds (LOD) contours

for major stripe rust resistance quantitative trait locus (QTL) *QYr.lrdc-2A* detected in each of the five trial environments and pooled data. Left panel: linkage map of chromosome 2A with the QTL *QYr.lrdc-2A* highlighted on the chromosome in orange and the delimiting markers indicated in red. Positions (cM) of markers are shown to the left of the linkage group, and the marker names are shown on the right. Right panel: LOD curve of major stripe rust resistance QTL *QYr.lrdc-2A* detected using composite interval mapping in each of the five trial environments and pooled data. The vertical straight line represents the single LOD significance threshold of 3.2 for pooled data. LET16, LET17 and LET20: Lethbridge field trials in 2016, 2017 and 2020, respectively; CRE16 and CRE20: Creston field trials in 2016 and 2020, respectively.

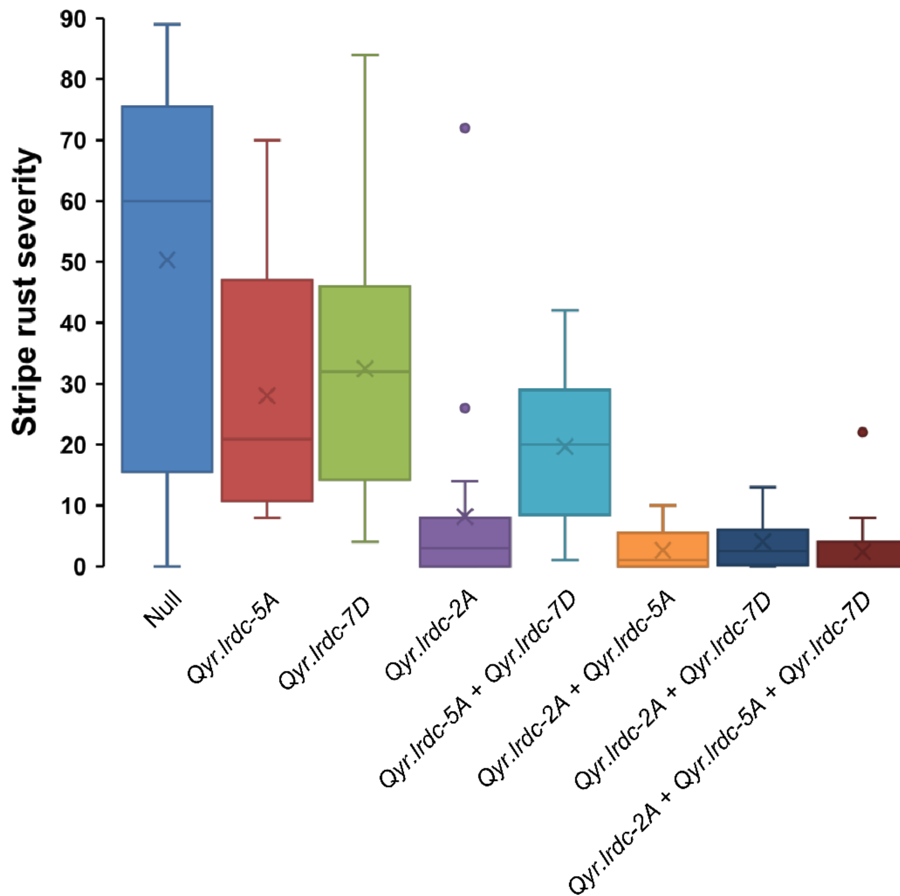


**Figure 2-4.** Boxplot distributions of AAC Innova/AAC Proclaim doubled haploid spring wheat mapping population. Effects of single QTLs (*QYr.lrdc-2A*, *QYr.lrdc-5A* and *QYr.lrdc-7D*) and their combinations on average stripe rust severity is depicted alongside susceptible (Null) alleles at all three loci using pooled phenotypic data (average of all environments). Quartiles and medians are represented by boxes and continuous lines, respectively. Whiskers extend to the farthest points that are not outliers, whilst outliers are shown as black dots.

# Chapter 3 QTL mapping for adult plant resistance to stripe rust in the AAC Cameron/P2711 spring wheat population[2]

## 3.1 Introduction

Stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici Erikss*. (*Pst*), is one of the major biotic threats to wheat production globally. It can cause complete yield loss in wheat, depending upon the nature of inoculum, specific climate conditions and the genetics of the wheat grown (Chen, 2005; Wellings, 2011). Severe stripe rust epidemics have occurred in 2005, 2006, 2011 and 2016 in western Canada (Aboukhaddour et al., 2020). The disease is widespread in western Canada due to inoculum carried by winds from the Pacific Northwest and the Great Plains of North America, where epidemics occur frequently (Brar & Kutcher, 2016). The evolution of new high-temperature-adapted races (Milus et al., 2009) and the overwintering of the pathogen on winter wheat (Brar & Kutcher, 2016) has increased disease incidence in western Canada. Although fungicides can provide effective disease control, this increases production costs and environmental degradation. Breeding resistant cultivars minimizes the risks associated with fungicides and represents the most cost effective and ecologically sustainable solution to control diseases (Line, 2002).

Resistance to stripe rust can generally be characterized as seedling or all-stage resistance (ASR) and adult plant resistance (APR) (Chen, 2005). Seedling or all-stage resistance is effective at the seedling stage and remains effective throughout the growth stages (Chen, 2013). This type of resistance is usually race specific and generally more easily overcome by the evolution of new pathogen races (Chen, 2005; Chen, 2013). Due to race specificity, many ASR genes, including

[2] A version of this chapter with the same title has been submitted to the *Crop Science* on July 25, 2021; authored by Farzand et al.

*Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr27*, *Yr31*, *Yr43* and *Yr44* have been rendered ineffective in combating the predominant *Pst* races in Canada (Brar et al., 2018; Ghanbarnia et al., 2021). Only a limited number of ASR genes, such as *Yr1*, *Yr5* and *Yr15* are currently effective in Canada (Brar et al., 2018; Ghanbarnia et al., 2021).

In contrast, adult plant resistance (APR) is mostly race-nonspecific, even though race specificity has also been reported for some APR genes (Wang & Chen, 2017). The APR genes conferring race non-specificity provide partial but durable resistance against different *Pst* races (Chen, 2013). Therefore, APR genes, which are less likely to be overcome by new virulent races, have been deployed by many wheat breeding programs. For example, the APR gene *Yr18* has been widely deployed in breeding programs across Canada and globally (Randhawa et al., 2012; Randhawa et al., 2013) and is linked to resistance to leaf rust (*Lr34*), stem rust (*Sr57*) and powdery mildew (*Pm18*) due to its pleiotropic effect (Lagudah et al., 2009). Plants with APR genes are generally susceptible at the seedling stage but gradually express their resistance as adult plants (Chen, 2013). Since the level of resistance provided by the incorporation of a single moderate effect APR gene is generally not adequate under extreme epidemics (Chen, 2013; Chen, 2014), a combination of 3-5 moderately effective APR genes is recommended (Singh et al., 2000).

To date, several stripe rust (*Yr*) resistance genes have been reported in hexaploid wheat and its wild relatives (Wang & Chen 2017; Gessese et al., 2019; Pakeerathan et al., 2019; Li et al., 2020). Many temporarily designated genes and QTLs have also been documented (Rosewarne et al., 2013; Wang & Chen, 2017). Mapping molecular markers linked to resistance genes is a key step for efficient gene pyramiding into breeding material (Chen et al., 2016). Several types of marker systems have been employed, including restriction fragment length polymorphism (RFLP; Devos et al., 1992; Chen et al., 1994), amplified fragment length polymorphism (AFLP; Shao et

al., 2001; Li et al., 2009), simple sequence repeats (SSR; Ren, Li, et al., 2012; Xiang et al., 2016) and Diversity Arrays Technology (DArT; Prins et al., 2011; Vazquez et al., 2015). Recent advances in single nucleotide polymorphism (SNP) array-based platforms have revolutionized the field of genetic mapping to dissect the chromosomal region associated with desirable traits (Cavanagh et al., 2013; Wang et al., 2014; Winfield et al., 2016; Allen at al., 2017; Cui et al., 2017).

The experimental wheat germplasm line P2711 exhibited high levels of stripe rust resistance in the field. AAC Cameron (Fox et al., 2016) is a hard red spring wheat cultivar which is moderately susceptible to stripe rust races under field conditions. The objective of this study was to identify and locate QTL controlling adult stripe rust resistance in the AAC Cameron/P2711 recombinant inbred line (RIL) mapping population.

## 3.2 Materials and Method

### 3.2.1 Plant material

In this study, a mapping population comprising of 252 RILs was developed from a cross between wheat cultivar AAC Cameron (Fox et al., 2016) and germplasm line P2711. AAC Cameron is a hard red spring wheat cultivar (Fox et al., 2016) that is moderately susceptible to the current prevalent *Pst* races in western Canada; whereas P2711 is an experimental wheat germplasm line that is highly resistant to these races. The RILs were produced by the single seed descent (SSD) method, where individual spikes were picked in each generation and advanced to $F_7$ generation. Seeds of $F_{7:8}$ generation were then multiplied and used for all genotyping and phenotyping filed experiments in this study. Differential genotypes Avocet +*Yr1,* Avocet +*Yr17,* Avocet +*Yr27,* Avocet +*Yr31* and Avocet +*Yr32* were also screened to determine the effectiveness of the resistance genes against prevalent races of stripes rust.

### 3.2.2 Disease evaluation

Parents, RILs and stripe rust differential genotypes were screened for stripe rust severity in disease nurseries at Creston, BC in 2018, 2019 and 2020, as well as at Lethbridge, AB in 2018 and 2020. The experimental sites, Creston, BC (49°09'N, 116°51'W) and Lethbridge, AB (49°41'N, 112°49'W), are situated in proximity to the Pacific Northwest (PNW) of the United States, which is a hot spot region for stripe rust occurrence in North America (Chen, 2005). Infected fields of the PNW provide natural inoculum in western Canada through wind trajectories. This experiment was planted in a randomized complete block design without replication in 2018 and 2019 and two replicates in 2020 at both sites. Each line was planted as a one-meter-long row with a space of 25 cm between them to facilitate disease evaluation. Approximately 60 seeds were planted in each row. Spreaders rows, consisting of a mixture of stripe rust susceptible wheat cultivars (AC Barrie, AC Crystal, and Morocco), were planted as border rows for disease spreading and also to evaluate the level of infection.

A representative collection of stripe rust spores from the disease hot-spot fields of the previous year were used to inoculate disease nurseries in Lethbridge, AB. Artificial inoculation was not done in Creston, BC disease nurseries since natural infection has always been sufficient. Stripe rust severity at adult plant stage was recorded visually as 0 to 100% (0 = immune and 100% = completely susceptible) based on the modified Cobb scale (Peterson 1948).

### 3.2.3 Plant DNA extraction and Genotyping

Genomic DNA was extracted from fresh leaf tissues of each RIL and parents using the same procedure as described in 2.2.3 section of this thesis. A random selection of 190 RIL and two parents, representing two 96-well plates, were genotyped with the wheat 90K Infinium iSelect SNP assay (Wang et al., 2014) following the manufacturers instructions.

Genome Studio 2.0 (Illumina Inc., San Diego, USA) software was used to filter high quality SNPs using the following expression features filters as described by Dhariwal and Randhawa (2021; in press): (i) AA Freq: !=1, (ii) AB Freq: !=1, (iii) BB Freq: !=1, (iv) Minor Allele Freq: >0.03, and (v) Call Freq: >0.50. Additional filtering was done by removing monomorphic markers, markers with > 10% missing genotyping calls, and markers significantly distorted from the expected segregation ratio (1:1) using Chi-square ($\chi^2$) test. Only high quality, polymorphic markers that fit into expected segregation ratio (1:1) were used to construct the linkage map. The RIL population was also genotyped with PCR marker VENTRIUP/LN2, specific for the 2NS from *Aegilops ventricosa* carrying the *Yr17* gene using the forward VENTRIUP (5′-AGGGGCTACTGACCAAGGCT-3′) and reverse LN2 (5′-TGCAGCTACAGCAGTATGTACACAAAA-3′) primers (Helguera et al., 2003). PCR products were then analyzed by electrophoresis in 3.5% agarose gel containing 1.5% SYBR Safe DNA Gel Stain (QiaGen Toronto, Canada) .

**3.2.4 Phenotypic data analysis**

Analysis of variance (ANOVA) was carried out using the agricolae (version 1.2-4) package of R (version 4.0.3) (R Core Team, 2013). For the ANOVA model, parents and RILs were treated as fixed effects, while environments were treated as random effects. The broad sense heritability was estimated across environments based on formula: $H^2 = \sigma^2 g / \sigma^2 p$ where $\sigma^2 g$ = genetic variance and $\sigma^2 p$ = phenotypic variance. The genetic variance ($\sigma^2 g$) was calculated from $(\sigma^2 L - \sigma^2 E)/r$, where $\sigma^2 L$ = mean variance of the RILs, $\sigma^2 E$ = error variance and r = number of replications (Hou et al., 2015). Histograms were generated to show the distribution of disease severity data in RIL population across all five environments individually and using pooled data in Microsoft Excel (2016).

### 3.2.5 Linkage map construction

Linkage groups were constructed by a two-step process following Dhariwal and Randhawa (2021; in press). In the first step, draft linkage groups were created using MSTmap software version 2.0 (Wu et al., 2008). The following parameters were used to calculate order of linkage groups: (i) kosambi map function (Kosambi, 1944), (ii) cut-off $p$ value of $1^{-10}$, (iii) no mapping size threshold of 2, (iv) no mapping distance threshold of 15.0 cM, (vi) no mapping missing threshold of 0.25 and (v) maximum likelihood (ML) function (Jansen et al. 2001). Each linkage group was assigned to a particular wheat chromosome based on common markers between the previously published consensus map (Wang et al., 2014). In the second step, draft linkage groups of each chromosome were refined by recalculating marker order and distance within the linkage groups as described by Dhariwal et al. (2018; 2020) using MapDisto version 2.0 software (Heffelfinger et al., 2017).

Briefly, the following parameters were used for recalculation of the linkage groups: (i) kosambi map function (Kosambi, 1944), (ii) minimum logarithm of odd (LOD min) threshold of 3.0, (ii) maximum recombination frequency ($r$ max) threshold of 0.3 and (iv) maximum missing data threshold of 10%. Two or more linkage groups created for the same chromosome were merged into a single linkage group using less stringent cut off values ($r$ max > 0.3). “AutoCheckInversions” and “AutoRipple” commands were used to create the best order of markers.

### 3.2.6 QTL analysis

QTL analysis was undertaken for each environment separately and across the environments using pooled data. Composite interval mapping (CIM) was carried out with Windows QTL Cartographer version 2.5 software (Wang et al., 2005), using a forward-backward regression

method with both window size and walk speed set at 1.0 cM. A thousand permutation tests (Doerge & Churchill, 1996) were performed to determine significant logarithm of odd (LOD) thresholds. We also report QTLs (2.5 < LOD < threshold) when identified either in single environment analysis or combined analysis, reaching the significance level following the method described by Yi et al. (2018). QTL confidence intervals were determined using LOD peak above the calculated threshold or LOD 2.5. Final chromosome map charts were generated using MapChart 2.32 software (Voorrips, 2002).

### 3.2.7 Physical position of QTLs

Physical positions of all QTLs identified from RIL population in this study were obtained through the BLAST search of probe/primer sequences of the flanking marker of each QTL against the International Wheat Genome Sequencing Consortium (IWGSC) RefSeq v.1.0 (Appels et al., 2018) available at https://wheat-urgi.versailles.inra.fr/Tools/BLAST.

## 3.3 Results

### 3.3.1 Phenotypic observations

The ANOVA of stripe rust severity for the AAC Cameron/P2711 population revealed highly significant effects ($p < 0.0001$) for genotype, environment, and genotype x environment interaction (Table 3-1). The estimated broad-sense heritability ($H^2$) was 0.74 (Table 3-1). The disease severity value for P2711 ranged from 0% in Lethbridge 2018 to 10% in Creston 2020. Disease severity for AAC Cameron ranged from 15% in Creston 2018 to 80% in Lethbridge 2018 (Table 3-2). The mean value of stripe rust severity in the RIL population ranged from 14.3% in Creston 2018 to 48.6% in Lethbridge 2018 (Table 3-2). The median value of stripe rust severity in the RIL population ranged from 1.0% in Creston 2018 to 60.0% in Lethbridge 2018 (Table 3-2). Histograms showed skewed distribution towards greater resistance in Creston 2018, 2019 and 2020

and Lethbridge 2020 environments (Figure 3-1A, 1C, 1D and 1E), while skewed towards greater susceptibility in Lethbridge 2018 environment (Figure 3-1B).

### 3.3.2 Linkage map

In total, 9,312 high quality SNP markers showing polymorphism between two parents were used for the construction of genetic map. A total of 8,914 out of 9,312 SNPs were incorporated into 29 linkage groups covering all 21 wheat chromosomes (Table 3-3). Single linkage group were observed for almost three-fourth of the chromosomes, two linkage group for each of chromosome 1D, 3D and 7D, and three linkage groups for each of chromosome 5D and 6D, respectively (Table 3-3). The total map length for all linkage groups was 4075.8 cM with an average distance of 2.2 between markers. The total map lengths of linkage groups for genomes A, B, and D were 1666.4, 1567.2 and 842.3, respectively (Table 3-3). Since 5,795 SNPs were redundant (co-segregated or mapped at the same position), the number of linkage bins was 3,119 (Table 3-3).

The highest number of linkage bins were assigned to homoeologous group 7 chromosome (# of linkage bins = 564 and density = 0.9 linkage bins/cM) and the lowest number of markers were assigned to homoeologous group 4 chromosome (# of linkage bins 168 and density = 0.4 linkage bins/cM) (Table 3-3). Considering wheat genomes, the highest number of linkage bins were mapped on genome B (# of linkage bins = 1498 and density = 1.0 linkage bins/cM) followed by A (# of linkage bins = 1262 and density = 0.8 linkage bins/cM) and D (# of linkage bins = 359 and density = 0.4 linkage bins/cM), in that order (Table 3-3).

### 3.3.3 QTLs for stripe rust resistance

A total of seven stripe rust resistance QTLs including *QYr.lrdc-1A.1*, *QYr.lrdc-1A.2*, *QYr.lrdc-2A.1*, *QYr.lrdc-2A.2*, *QYr.lrdc-2B*, *QYr.lrdc-3B* and *QYr.lrdc-5A* were identified in this study (Table 3-4). These QTLs were mapped on chromosomes 1A, 2A, 2B, 3B and 5A,

respectively (Figure 3-2). *QYr.lrdc-2A.1* was the most stable QTL identified in this study. It was detected across four environments/two locations as well as in pooled data and accounted for 7.4 to 12.6 % of phenotypic variance in individual environments and 8.6% in combined analysis. This QTL was identified within 0.0-7.8 cM interval with the LOD score ranged from 3.9 in Lethbridge 2020 to 6.5 in Creston and Lethbridge 2018 and 5.2 in combined analysis. Resistant parent P2711 was the source of the resistant allele for *QYr.lrdc-2A.1* (Table 3-4).

*QYr.lrdc-2B* was the second most stable QTL identified in three out of five environments/ two locations in addition to pooled data and explained 8.1 to 12.1 % of the phenotypic variance in individual environments and 14.0% in combined analysis. This QTL was identified within 91.2-104.5 cM with LOD score ranging from 4.3 in Lethbridge 2020 to 7.1 in Lethbridge 2018 and 8.0 in combined analysis. The resistant alleles of *QYr.lrdc-2B* were derived from parent AAC Cameron (Table 3-4).

*QYr.lrdc-5A* was detected all three years in Creston. This QTL was mapped within 166.9-182.7 cM interval with the LOD score ranged from 2.8 in Creston 2018 to 11.7 in Creston 2020 and 5.7 in combined analysis using pooled data. The proportion of phenotypic variance explained by this QTL ranged from 5.2% in Creston 2018 to 20.0% in Creston 2020 and 8.4% in combined analysis. This QTL was derived from parent P2711 (Table 3-4).

Other QTLs (*QYr.lrdc-1A.1, QYr.lrdc-1A.2, QYr.lrdc-2A.2* and *QYr.lrdc-3B),* except *QYr.lrdc-1A.2,* were identified in unique environments and each explained less than 10% of phenotypic variance. P2711 contributed stripe rust alleles at *QYr.lrdc-2A.2 and QYr.lrdc-3B,* while AAC Cameron at *QYr.lrdc-1A.1* and *QYr.lrdc-1A.2* (Table 3-4). The three most stable QTLs (*QYr.lrdc-2A.1*, *QYr.lrdc-2B* and *QYr.lrdc-5A*) were also assessed individually and in combination for their effect on stripe rust severity in the RIL population. It was observed that none of the QTLs

was sufficient to reduce stripe rust severity alone but contributed to maximum reductions in stripe rust in combinations (Figure 3-3; Table 3-5).

### 3.4 Discussion

The genetic map of AAC Cameron/P2711 consisted of 8,914 SNP markers representing 3,119 unique SNPs or linkage bins across 29 linkage groups and a total map length of 4,075.8 cM (Table 3-3). The map length, number of unique markers and linkage groups of our genetic map are in agreement with the genetic maps of Brundage/Coda (Case et al., 2014), Avocet/Kundan (Ren et al., 2017), AvS/Madsen (Liu et al., 2018), Humai 15 × Mingxian 169 (Yuan et al., 2018) and Fuyu 3/Zhengzhou 5389 (Gebrewahid et al., 2020). The high density AAC Cameron/P2711 linkage map covering all wheat chromosomes provides enough coverage to dissect the genetic variation of stripe rust resistance present in this population.

Resistance in AAC Cameron/P2711 was modulated by a combination of seven stripe rust resistance loci of varying effectiveness. Our results are in agreement with the Capo/Arina and Capo/Furore RIL populations, where resistance was conditioned by five minor and major stripe rust resistance loci (Buerstmayr et al., 2014) and Avocet-*YrA*/Francolin#1 RIL population, where six minor and major stripe rust resistance loci were found to control genetic resistance (Lan et al., 2014). Liu et al. (2018), also reported similar results for an Avocet S/Madsen RIL population, where five stripe rust resistance QTLs with different level of effectiveness were found to be associated with resistance.

*QYr.lrdc-2A.1* mapped on the short arm of chromosome 2A was identified across four environments/two locations as well as in pooled data. This QTL was derived from parent P2711 and explained up to 12.6% of the phenotypic variance (Table 3-4). There are three reported genes including *Yr17*, *Yr56* and *Yr69*, which could be a source of stripe rust resistance on the 2AS

chromosome arm (Wang & Chen, 2017). Furthermore, QTLs on chromosome 2AS have been identified in wheat cultivars Renan (Dedryver et al., 2009), Pioneer 26R61 (Hao et al., 2011), Apache (Paillard et al., 2012), Cappelle-Desprez (Agenbag et al., 2012), Stephens (Vazquez et al., 2012) Madsen (Liu et al., 2018) and Fuyu 3 (Gebrewahid et al., 2020). According to Dedryver et al. (2009), Paillard et al. (2012) and Liu et al. (2018), QTLs identified on chromosome 2AS in cv. Renan, cv. Apache and cv. Madsen, respectively, correspond to the *Yr17* gene. Tests with the PCR marker VENTRIUP/LN2 (Helguera et al., 2003), specific for the 2NS/2AS from *Aegilops ventricosa* carrying *Yr17* gene confirmed that *Yr17* is present in parent P2711 and all RILs carrying *QYr.lrdc-2A.1* (data not shown). Therefore, we can postulate that *QYr.lrdc-2A.1* also corresponds to the *Yr17* gene.

Furthermore, we observed that differential genotype Avocet+*Yr17* was moderately susceptible across environments in this study (Table 3-2). RILs carrying only *QYr.lrdc-2A.1* were also moderately susceptible across environments (Table 3-5) indicating that *QYr.lrdc-2A.1* and *Yr17* are same. Interestingly, while *QYr.lrdc-2A.1* was the most stable QTL identified in this study, use of it with other QTLs can provide maximum reduction in disease severity (Figure 3-3; Table 3-5).

*QYr.lrdc-2B* mapped on the short arm of chromosome 2B was identified in three out of five environments as well as in pooled data. This QTL was derived from AAC Cameron and explained up to 14.0% of the phenotypic variance (Table 3-4). To date, three permanently designated stripe rust resistance genes *Yr27*, *Yr31* and *Yr41* have been reported on the short arm of chromosome 2B (Wang & Chen, 2017). QTLs on chromosome 2BS have also been reported in some wheat cultivars, lines and landraces including, Luke (Guo et al., 2008), Louise (Carter et al., 2009), Renan (Dedryver et al., 2009), Pingyuan 50 (Lan et al., 2010), Kariega (Prins et al., 2011)

, Stephens (Vazquez et al., 2012), Francolin#1 (Lan et al., 2014) and Fuyu 3 (Gebrewahid et al., 2020).

We observed that differential genotypes Avocet+*Yr27* and Acovet+*Yr31* were highly susceptible across environments in this study (Table 3-2), suggesting that *QYr.lrdc-2B* could not belong to either *Yr27* or *Yr31*. Moreover, the physical map position (165.6-304.2 Mb) of *Yr41* does not coincide with *QYr.lrdc-2B* (47.4-68.2 Mb), indicating that it might also be different from this gene. According to an integrated genetic map (Maccaferri et al., 2015), flanking SSR marker *Xbarc200* of *QYr.sgi-2B.1* reported in cv. Kariega are just < 4 cM away from *QYr.lrdc-2B*'s closely associated SNP marker *Tdurum_contig54704_176.* Furthermore, physical map position (47.6 Mb) of *QYr.sgi-2B.1* also overlap with *QYr.lrdc-2B* (47.4-68.2 Mb), confirming that both QTLs belong to the same gene. However, the phenotypic effect of *QYr.sgi-2B.1* (45.7 %) was stronger than *QYr.lrdc-2B* (14.0%), indicating difference in virulence composition among the pathogen populations at both locations and the number and strength of other resistance genes/QTLs in the populations being studied. RILs carrying *QYr.lrdc-2B* alone were not completely resistant across all environments (Table 3-5). However, this QTL showed significant additive effect with two other important QTLs (*QYr.lrdc-2A.1* and *QYr.lrdc-5A*) identified in this study among all environments.

*QYr.lrdc-5A* mapped on the long arm of chromosome 5A in this study was only significant in the Creston location. This QTL was derived from male parent P2711 and explained up to 20.0% of the phenotypic variance (Table 3-4). So far, one designated gene *Yr34* (synonym *Yr48*) has been reported on the long arm of chromosome 5A (Bariana et al., 2006; Qureshi et al., 2018; Chen et al., 2021).

In addition, some stripe rust resistance QTLs have been mapped on chromosome 5AL in wheat such as, Opata 85 (Boukhatem et al., 2002), Pingyuan 50 (Lan et al., 2010), Pastor

(Rosewarne et al., 2012), Shanghai 3/Catbird (Ren, He, et al., 2012), Avocet (Lan et al., 2014), SW 8588 (Zhang et al., 2019) , and Fuyu 3 (Gebrewahid et al., 2020) and its related species *T. boeoticum* (Chhuneja et al., 2008). Based on the genetic map produced by Maccaferri et al. (2015), *QYr.lrdc-5A* is > *10-38 cM* away from these QTLs/*Yr34* gene indicating that it could differ from all of them. Further validation is provided by physical map position (593.3-611.6 Mb) of *QYr.lrdc-5A*, which also does not overlap with any of these QTLs/*Yr34* gene suggesting that *QYr.lrdc-5A* could be a novel stripe rust resistance QTL in P2711. However, this QTL only expressed in Creston environments postulating that it is race specific. There is a possibility that some virulent races might have evolved to overcome *QYr.lrdc-5A's* resistance in Lethbridge. Moreover, this QTL was not sufficient for reduction of stripe rust severity alone but in combination with other QTLs among all environments (Figure 3-3; Table 3-5). RILs carrying *QYr.lrdc-5A* in combination with other QTLs were almost resistant (Figure 3-3; Table 3-5), indicating that *QYr.lrdc-5A* needs to be combine with other QTLs to achieve maximum level of resistance.

*QYr.lrdc-1A.1* and *QYr.lrdc-1A.2* were identified on the long arm of chromosome 1A, where they explained up to 7.2% and 6.0% of the phenotypic variance, respectively (Table 4). *QYr.lrdc-1A.2* was more stable than *QYr.lrdc-1A.1* since it was identified in two environments as well as in pooled data, but both were detected only at the Creston location. Minor QTLs on chromosomal arm 1AL have been mapped in wheat cultivars Naxos (Ren et al., 2012), Pastor (Rosewarne et al., 2012), Stephen (Vazquez et al., 2012), TAM 112 (Basnet et al., 2014a) and Stettler (Bokore et al., 2017). Based on the genetic maps produced by Wang et al., (2014) and Maccaferri et al. (2015), SNP marker *Excalibur_rep_c110054_341* closely associated with *QYr.spa-1A* identified in cv. Stettler is <2 cM and < 1cM away from *QYr.lrdc-1A.2*'s flanking SNP marker *BobWhite_c44164_151*, respectively. Moreover, physical map position (560.5-572.0

Mb) of *QYr.lrdc-1A.2* also overlaps with *QYr.spa-1A* (551.5 Mb), suggesting that both QTLs are controlled by the same gene. Since both AAC Cameron and Stettler are Canadian spring wheat cultivars, they might have common ancestry. *QYr.spa-1A* explained 7.6% of the phenotypic variance in cv. Stettler, which is also in agreement with *QYr.lrdc-1A.2* explaining 6.0% of the phenotypic variance.

*QYr.lrdc-2A.2* mapped on the long arm of chromosome 2A was identified only in Creston 2020, where it explained 6.2% of the phenotypic variance (Table 3-4). The long arm of chromosome 2A has always been a major source of stripe rust resistance (Vazquez et al., 2015; Bokore et al., 2017; Wang et al., 2019). Furthermore, minor effect QTL on chromosome 2AL have been reported in wheat cultivars Recital (Dedryver et al., 2009), Taldor (Paillard et al., 2012), Kundan (Ren et al., 2017), Chuanmai 55 (Yang et al., 2019) and PI 197734 (Liu et al., 2020). According to genetic maps produced by Wang et al., (2014) and Maccaferri et al., (2015), flanking SNP marker *wsnp_Ex_rep_c102538_87682273* of *QYrPI197734.wgp-2A* identified in cv. PI 197734 is < 1 cM and < 3 cM from *QYr.lrdc-2A.2*'s flanking SNP marker *Ra_c42714_1137*, respectively. Moreover, Physical map position of *QYr.lrdc-2A.2* (597.5-675.0 Mb) also overlaps with *QYrPI197734.wgp-2A* (580.3-709.7 Mb) indicating that both QTLs are derived from the same gene. Liu et al., (2020) attributed 7.34-11.36% of the phenotypic variance to *QYrPI197734.wgp-2A,* which is also comparable to *QYr.lrdc-2A.2* explaining 6.2% of the phenotypic variance.

*QYr.lrdc-3B* was identified in Creston 2019 only, where it explained 6.2% of the phenotypic variance (Table 3-4). Minor effect QTLs on chromosome 3B have been detected in some wheat cultivars, lines and landraces including Francolin#1 (Lan et al., 2014), AC Cadillac (Singh et al., 2014), AC Cadillac and Red Fife (Bokore et al., 2017), and Humai 15 (Yuan et al., 2018). Based on integrated genetic map (Maccaferri et al., 2015), SSR marker *Xbarc147* closely

associated with *QYr.spa-3B.1* identified in AC Cadillac (Singh et al., 2014), is almost 3.5 cM away from flanking SNP marker *BS00087757_51* of *QYr.lrdc-3B*. The physical map position of *QYr.spa-3B.1* (1.9-7.1) also overlaps with *QYr.lrdc-3B* (6.3-13.8) showing that the source of resistance for both QTLs is the same. *QYr.spa-3B.1* explained 4.0 to 9.4% of the phenotypic variance in AC Cadillac which is also consistent with *QYr.lrdc-3B* exhibiting 6.0% of the phenotypic variance.

In summary, we identified seven stripe rust resistance QTLs in this study. All identified QTLs had a moderate effect on stripe rust severity when used individually but showed larger effects when deployed with other QTLs identified in this study. Results suggest that these QTLs need to be pyramided to achieve a high level of resistance. Singh et al. (2000) also highlighted the importance of combining 3-5 minor to intermediate effect genes which are not effective individually, to achieve a high level of resistance. QTLs *QYr.lrdc-2A.1*, *QYr.lrdc-2B* and *QYr.lrdc-5A* may be manipulated within a breeding program using the flanking SNP markers reported in this study.

**3.5 Conclusions**

Our finding suggests that resistance in AAC Cameron/P2711 was conditioned by seven stripe rust resistance QTLs with different levels of effectiveness. These include, two on chromosome 1A and 2A and one on each of chromosome 2B, 3B and 5A. The resistant parent P2711 contributed alleles for most of these QTLs except the QTLs on chromosome 1A and 2B, which were derived from the moderately susceptible parent AAC Cameron. Among these, *QYr.lrdc.2A.1*, *QYr.lrdc.2B* and *QYr.lrdc.5A* were most consistent and showed a significant reduction in disease severity when employed together. Except *QYr.lrdc.5A*, the remaining QTLs identified had chromosome locations reported previously in literature. *QYr.lrdc-5A* could be a

novel source of resistance in P2711 but requires further study for validation. From the viewpoint of practical breeding, these three selected QTLs could be pyramid into commercial cultivars with other stripe rust resistance genes to achieve maximum level of resistance.

## 3.6 Tables and Figures

**Table 3-1.** ANOVA table for stripe rust severity of AAC Cameron/P2711 recombinant inbred line spring wheat mapping population.

| **Source** | **df** | **Sum Sq** | **Mean Sq** | **F value** | **Pr (>F)** |
|---|---|---|---|---|---|
| Genotype | 253 | 877595 | 3469 | 15.06 | < 2.2e-16 *** |
| Environment | 4 | 216739 | 54185 | 235.23 | < 2.2e-16 *** |
| Genotype*Environment | 1012 | 463110 | 458 | 1.99 | < 2.2e-16 *** |
| **Error** = 230.34, **CV%** = 45.51, $\mathbf{H}^2$ = 0.74 | | | | | |

Note: df: degrees of freedom; $H^2$: heritability; CV: coefficient of variance; Significance codes: 0 '***'.

**Table 3-2.** Summary of stripe rust severity of parents, differential genotypes and AAC Cameron/P2711 recombinant inbred line spring wheat mapping population.

| Env | Parental lines | | | | | | | Population | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **AAC Cameron** | **P2711** | **Avocet *+Yr1*** | **Avocet *+Yr5*** | **Avocet *+Yr17*** | **Avocet *+Yr27*** | **Avocet *+Yr31*** | **Min** | **Max** | **Mean** | **Median** |
| CRE2018 | 15.0 | 1.0 | 1.0 | 1.0 | 1.0 | 85.0 | 100.0 | 1.0 | 90.0 | 14.3 | 1.0 |
| LET2018 | 80.0 | 0.0 | 1.0 | 1.0 | 45.0 | 75.0 | 85.0 | 0.0 | 90.0 | 48.6 | 60.0 |
| CRE2019 | 65.0 | 1.0 | 1.0 | 1.0 | 65.0 | 100.0 | 100.0 | 1.0 | 85.0 | 25.2 | 5.0 |
| CRE2020 | 55.0 | 10.0 | - | - | 90.0 | 90.0 | 80.0 | 0.0 | 100.0 | 42.0 | 40.0 |
| LET2020 | 75.0 | 1.0 | 1.0 | 1.0 | 45.0 | 90.0 | 95.0 | 0.0 | 100.0 | 30.4 | 15.0 |

Note: Env: Environment; CRE18, CRE19 and CRE20: Creston field trials in 2018, 2019 and 2020, respectively; LET18 and LET20: Lethbridge field trials in 2018 and 2020, respectively -: missing data.

**Table 3-3.** Summary of SNP markers used in the AAC Cameron/P2711 recombinant inbred line spring wheat mapping population.

| Chromosome | Map Length (cM) | No. of Linkage Bins | No. of Markers | Markers/cM | Linkage Bins/cM |
|---|---|---|---|---|---|
| 1A | 197.8 | 186 | 572 | 2.9 | 0.9 |
| 2A | 182.0 | 133 | 348 | 1.9 | 0.7 |
| 3A | 251.3 | 170 | 440 | 1.8 | 0.7 |
| 4A | 241.2 | 101 | 163 | 0.7 | 0.4 |
| 5A | 274.2 | 149 | 314 | 1.1 | 0.5 |
| 6A | 185.0 | 179 | 648 | 3.5 | 1.0 |
| 7A | 334.9 | 344 | 895 | 2.7 | 1.0 |
| **A-Genome** | **1666.4** | **1262** | **3380** | **2.0** | **0.8** |
| **%** | **40.9** | **40.5** | **37.9** | **32.8** | **36.4** |
| 1B | 213.9 | 249 | 834 | 3.9 | 1.2 |
| 2B | 281.4 | 274 | 923 | 3.3 | 1.0 |
| 3B | 261.1 | 192 | 363 | 1.4 | 0.7 |
| 4B | 134.2 | 56 | 134 | 1.0 | 0.4 |
| 5B | 259.9 | 274 | 868 | 3.3 | 1.1 |
| 6B | 250.6 | 275 | 806 | 3.2 | 1.1 |
| 7B.1 | 146.4 | 170 | 443 | 3.0 | 1.2 |
| 7B.2 | 19.6 | 8 | 33 | 1.7 | 0.4 |
| **B-Genome** | **1567.2** | **1498** | **4404** | **2.8** | **1.0** |
| **%** | **38.4** | **48** | **49.4** | **45.9** | **45.4** |
| 1D.1 | 112.3 | 75 | 234 | 2.1 | 0.7 |
| 1D.2 | 44.6 | 12 | 14 | 0.3 | 0.3 |
| 2D | 126.0 | 35 | 64 | 0.5 | 0.3 |
| 3D.1 | 3.4 | 12 | 73 | 21.7 | 3.6 |

| | | | | | |
|---|---|---|---|---|---|
| 3D.2 | 63.2 | 62 | 363 | 5.7 | 1.0 |
| 4D | 72.8 | 11 | 18 | 0.2 | 0.2 |
| 5D.1 | 83.2 | 8 | 11 | 0.1 | 0.1 |
| 5D.2 | 56.1 | 40 | 148 | 2.6 | 0.7 |
| 5D.3 | 34.1 | 7 | 8 | 0.2 | 0.2 |
| 6D.1 | 25.3 | 14 | 31 | 1.2 | 0.6 |
| 6D.2 | 22.6 | 29 | 66 | 2.9 | 1.3 |
| 6D.3 | 62.4 | 12 | 13 | 0.2 | 0.2 |
| 7D.1 | 15.8 | 21 | 56 | 3.5 | 1.3 |
| 7D.2 | 120.5 | 21 | 31 | 0.3 | 0.2 |
| **D-Genome** | **842.3** | **359** | **1130** | **1.3** | **0.4** |
| **%** | **20.7** | **11.5** | **12.7** | **21.3** | **18.2** |
| **A+B+D** | | | | | |
| **1** | 568.6 | 522 | 1654 | 2.9 | 0.9 |
| **2** | 589.4 | 442 | 1335 | 2.3 | 0.7 |
| **3** | 579.0 | 436 | 1239 | 2.1 | 0.8 |
| **4** | 448.2 | 168 | 315 | 0.7 | 0.4 |
| **5** | 707.5 | 478 | 1349 | 1.9 | 0.7 |
| **6** | 546.0 | 509 | 1564 | 2.9 | 0.9 |
| **7** | 637.2 | 564 | 1458 | 2.3 | 0.9 |
| **Total** | **4075.8** | **3119** | **8914** | **2.2** | **0.8** |

Note: cM: centimorgan

**Table 3-4.** Details of quantitative trait loci (QTL) identified for stripe rust (*Yr*) resistance on different wheat chromosomes in AAC Cameron/P2711 recombinant inbred line spring wheat mapping population.

| QTL | LG | Env | Position (cM) | Confidence Interval (cM) | LOD | Additive Effect | %R2 | Closet Marker | Physical Position (Mb) | Donor Parent |
|---|---|---|---|---|---|---|---|---|---|---|
| *QYr.lrdc-1A.1* | 1A | CRE20 | 63.6 | 62.9-64.3 | 4.5 | -7.94 | 7.2 | *Tdurum_contig47183_205* | 41.9-47.2 | C |
| *QYr.lrdc-1A.2* | | CRE19 | 153.8 | 153.4-154.6[a] | 3.3 | -6.18 | 4.9 | *Kukri_c52420_112* | 560.5-572.0 | C |
| | | CRE20 | 153.8 | 153.2-154.6[a] | 3.2 | -6.44 | 4.8 | *Kukri_c52420_112* | | |
| | | Combined | 153.8 | 153.4-153.7[a] | 3.7 | -5.72 | 6.0 | *Kukri_c52420_112* | | |
| *QYr.lrdc-2A.1* | 2A | CRE18 | 0.9 | 0.0-7.3 | 6.5 | 8.57 | 12.6 | *BS00010087_51* | 31.1-36.9 | P |
| | | LET18 | 0.0 | 0.0-7.8 | 6.5 | 10.93 | 10.9 | *GENE-0137_1660* | | |
| | | CRE19 | 0.0 | 0.0-6.7 | 5.8 | 8.40 | 8.9 | *GENE-0137_1660* | | |
| | | LET20 | 0.0 | 0.0-4.6 | 3.9 | 9.83 | 7.4 | *GENE-0137_1660* | | |
| | | Combined | 0.0 | 0.0-5.0 | 5.2 | 7.03 | 8.6 | *GENE-0137_1660* | | |
| *QYr.lrdc-2A.2* | | CRE20 | 69.2 | 68.4-77.2 | 4.1 | 7.35 | 6.2 | *BS00022301_51* | 597.5-675.0 | P |
| *QYr.lrdc-2B* | 2B | LET18 | 94.8 | 91.8-98.6 | 7.1 | -11.27 | 12.1 | *Tdurum_contig54704_176* | 47.4-68.2 | C |
| | | CRE19 | 94.8 | 91.8-98.6 | 6.0 | -8.60 | 9.4 | *Tdurum_contig54704_176* | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | LET20 | 103.4 | 102.5-104.5 | 4.3 | -10.31 | 8.1 | *BobWhite_c31129_60* | | |
| | | Combined | 94.8 | 91.2-98.0 | 8.0 | -8.80 | 14.0 | *Tdurum_contig54704_176* | | |
| *QYr.lrdc-3B* | 3B | CRE19 | 20.1 | 19.2-20.3 | 4.0 | 6.90 | 6.0 | *RFL_Contig4531_1195* | 6.3-13.8 | P |
| *QYr.lrdc-5A* | 5A | CRE18 | 169.6 | 168.6-171.8[a] | 2.8 | 5.45 | 5.2 | *RFL_Contig316_572* | 593.3-611.6 | P |
| | | CRE19 | 179.3 | 177.5-182.7[a] | 3.5 | 6.51 | 5.3 | *IAAV108* | | |
| | | CRE20 | 169.1 | 167.7-170.3 | 11.7 | 13.26 | 20.0 | *wsnp_Ex_rep_c109532_92292121* | | |
| | | Combined | 169.6 | 166.9-176.1 | 5.7 | 7.06 | 8.4 | *RFL_Contig316_572* | | |

Note: QTL: quantitative trait loci; Chr: chromosome; Env: environment; cM: centimorgan; LOD: logarithm of the odds score; $R^2$: phenotypic variance; Mb: mega base pairs; C: moderately susceptible parent AAC Cameron; P: resistant parent P2711; CRE18, CRE19 and CRE20: Creston field trials in 2018, 2019 and 2020, respectively; LET18 and LET20: Lethbridge field trials in 2018 and 2020, respectively; LOD thresholds were 3.6, 3.8, 3.8, 3.7, 3.7, 3.6 at CRE18, LET18, CRE19, LET20, CRE20 and combined analysis, respectively ; [a] Intervals determined at LOD score 2.5; - additive effect: resistance alleles are contributed by the parent AAC Cameron; + additive effect: resistance alleles are contributed by the parent P2711.

**Table 3-5.** Mean stripe rust severities of AAC Cameron/P2711 recombinant inbred line population carrying different QTL combinations.

| QTL | CRE18 | LET18 | CRE19 | LET20 | CRE20 | Mean | SEM |
|---|---|---|---|---|---|---|---|
| *Null* | 46.5 | 66.7 | 52.0 | 57.1 | 72.9 | 57.6 | 3.9 |
| *QYr.lrdc-2A.1* | 18.0 | 56.3 | 31.9 | 38.1 | 53.8 | 38.8 | 6.8 |
| *QYr.lrdc-2B* | 29.3 | 59.4 | 35.7 | 47.5 | 53.6 | 40.8 | 5.7 |
| *QYr.lrdc-5A* | 26.9 | 68.8 | 38.9 | 42.8 | 45.6 | 43.0 | 5.8 |
| *QYr.lrdc-2A.1+ QYr.lrdc-2B* | 12.4 | 37.9 | 7.7 | 12.1 | 41.4 | 21.7 | 5.8 |
| *QYr.lrdc-2A.1+ QYr.lrdc-5A* | 19.3 | 56.3 | 27.0 | 38.8 | 25.0 | 30.6 | 5.4 |
| *QYr.lrdc-2B+ QYr.lrdc-5A* | 19.3 | 35.0 | 22.4 | 24.0 | 34.0 | 25.3 | 2.7 |
| *QYr.lrdc-2A.1+ QYr.lrdc-2B+QYr.lrdc-5A* | 5.9 | 7.2 | 1.0 | 2.5 | 15.6 | 6.5 | 2.2 |

Note: QTL: quantitative trait loci; SEM: standard error of the mean; CRE18, CRE19 and CRE20: Creston field trials in 2018, 2019 and 2020, respectively; LET18 and LET20: Lethbridge field trials in 2018 and 2020, respectively.

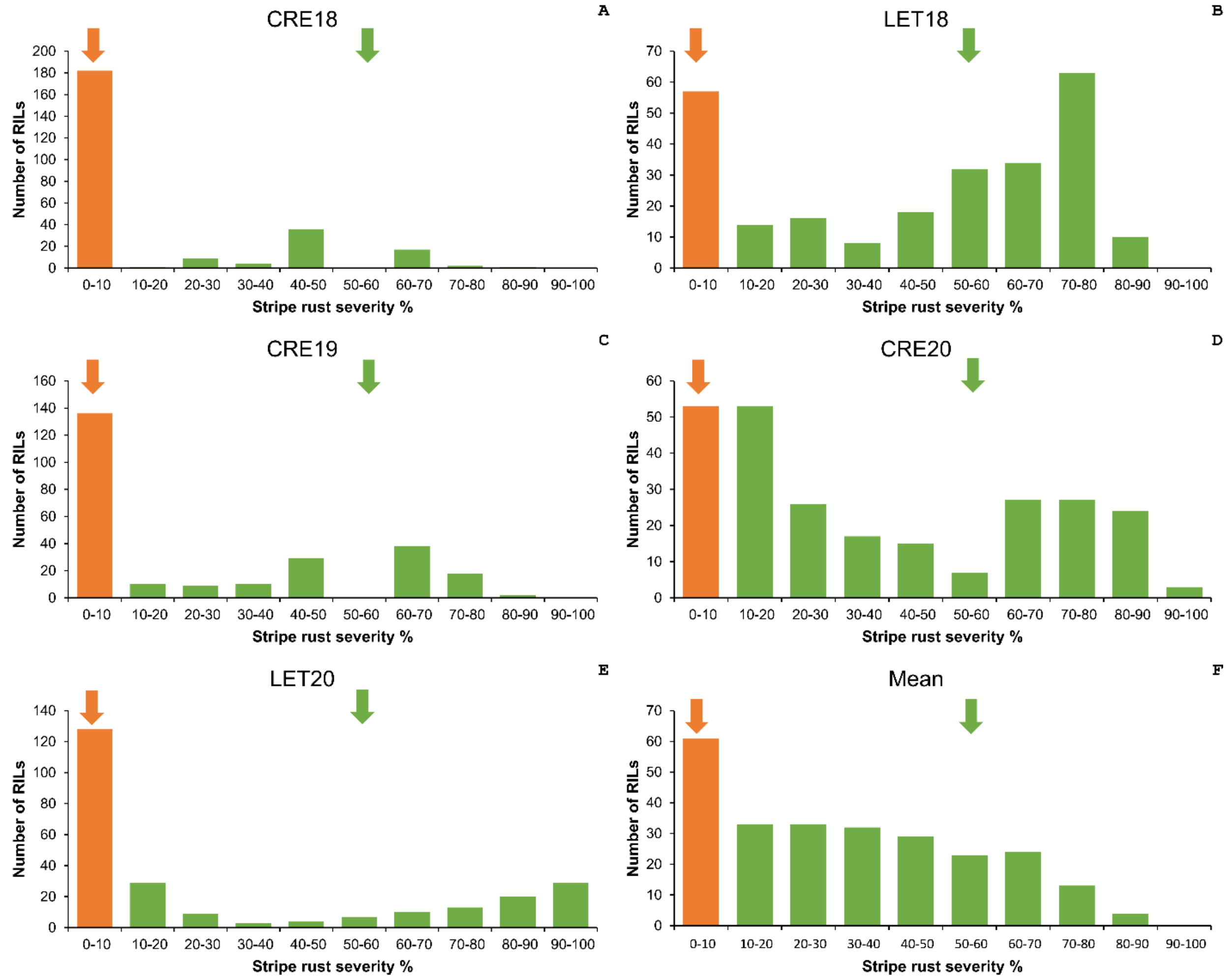


**Figure 3-1.** Frequency distribution of stripe rust severity at the adult plant stage among recombinant inbred lines of AAC Cameron/ P2711 mapping population in trials at Creston 2018 (A), Lethbridge 2018 (B), Creston 2019 (C), Creston 2020 (D) and Lethbridge 2020 Environments (E) and Mean (using average data of all environments) (F). Arrows indicate the mean percentage disease severity of parents, with orange arrow representing resistant parent P2711 and green arrow representing moderately susceptible parent AAC Cameron.

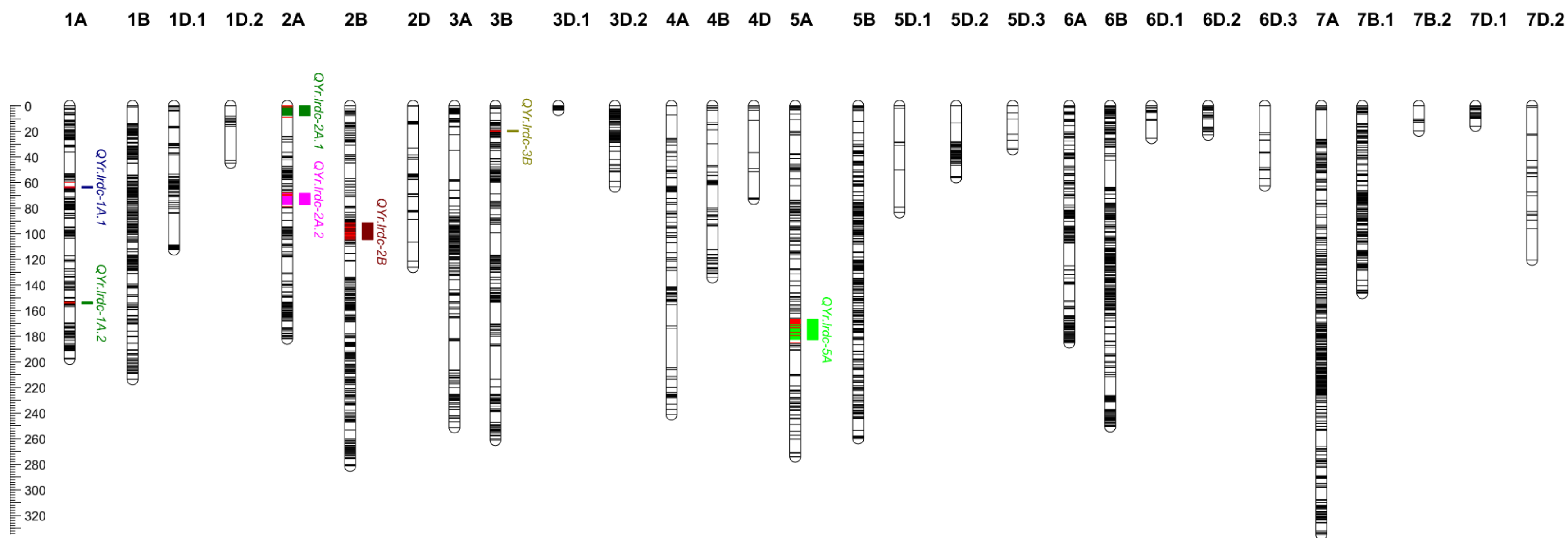


**Figure 3-2.** AAC Cameron/ P2711 recombinant inbred line spring wheat mapping population genetic map. The 29 linkage groups (LGs) are labelled as 1A.1 to 7D.2 according to their relatedness to homoeologous group chromosomes 1-7 and three genomes A, B and D of hexaploid spring wheat. A scale ruler for marker positions (cM) are shown on the left side of chart. Marker loci are shown as horizontal lines of either black or red color on LGs. Red color lines for marker loci represent linked markers of quantitative trait loci (QTL) located in respective chromosome segment, while black color lines represent loci outside of QTL regions. Colored LG blocks represent QTL intervals.

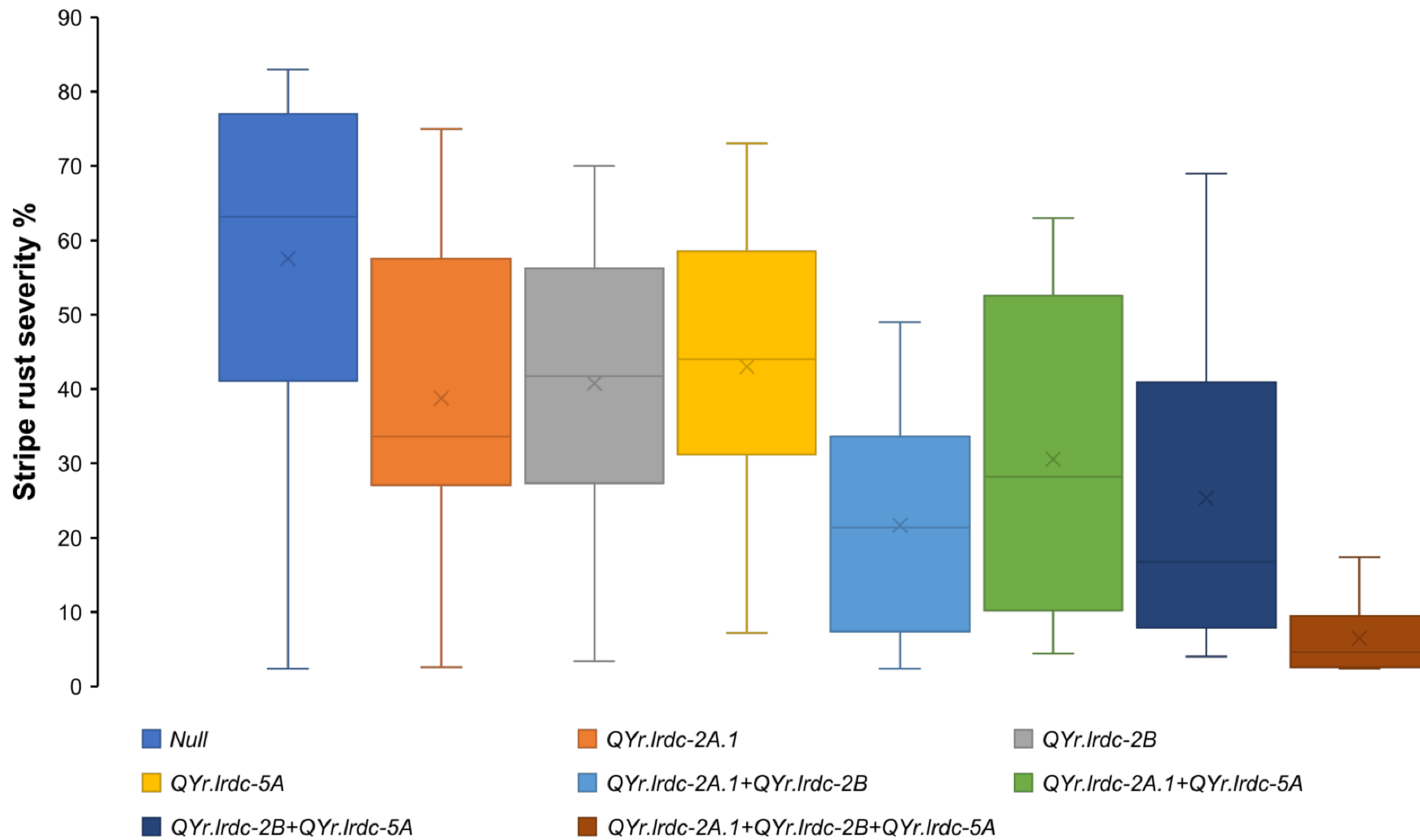


**Figure 3-3.** Boxplot distributions of AAC Cameron/P2711recombinant inbred line mapping population. Effects of single QTL (*QYr.lrdc-2A.1*, *QYr.lrdc-2B* and *QYr.lrdc-5A*) and their combinations on stripe rust severity using pooled phenotypic data (average of

all environments). Quartiles and medians are shown by boxes and continuous lines, respectively. Whiskers extend to the farthest points that are not outliers.

# Chapter 4 General discussions and conclusions

## 4.1 Introduction

Wheat is one the major cereal crops in Canada in terms of area under cultivation and annual yield. Global wheat production was 766 million tonnes (Mt) in 2019, of which Canada contributed approximately about 32.3 (Mt) and was among the top 5 wheat producing countries globally (FAOSTAT, 2020). The Prairie Provinces comprising Alberta, Saskatchewan, and Manitoba produced more than 95% of the spring wheat in Canada (Canadian Wheat, 2019). However, Canadian wheat production faces many challenges, including several destructive fungal pathogens such as stripe rust, stem rust, leaf rust, fusarium head blight and common bunt (Aboukhaddour et al., 2020). Thus, new cultivars to be registered in western Canada are required to possess moderate to high level of resistance to these diseases (http://www.pgdc.ca/).

Stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici Erikss*. (*Pst*), is an emerging threat to wheat production in western Canada, where stripe rust spores enter via wind currents from infected fields of Pacific Northwest, United States (Su et al., 2003). The prevalence of stripe rust has also increased due to the evolution of new high temperature-adapted races (Milus et al., 2009) and overwintering of the pathogen on winter wheat in southern Canadian prairies (Brar & Kutcher, 2016). Application of fungicides can control the disease to some extent but increases production costs and can lead to environmental degradation. The development of resistant wheat cultivars is one of the best strategies to mitigate disease associated risks (Line, 2002).

Mapping of resistance genes with the aid of molecular markers is an important step for identifying and pyramiding appropriate genes into breeding material (Chen et al., 2016). Several types of marker systems, including Restriction Rragment Length Polymorphism (RFLP; Waldron

et al., 1999), Amplified Fragment Length Polymorphism (AFLP; Guo et al., 2003), Simple Sequence Repeats (SSR; Ren et al., 2012), and Diversity Array Technology (DArT; Vazquez et al., 2015) have been utilized to uncover the genomic regions associated with disease resistance in wheat. At present, Single Nucleotide Polymorphisms (SNPs) are preferred due to their high throughput analysis potential, low genotyping error, codominance, and high genome coverage (Cavanagh et al., 2013; Wang et al., 2014).

Linkage-based QTL mapping is a valuable approach to dissect chromosomal regions associated with resistance and identify a subset of markers linked to these regions (Collard & Mackill, 2008). Mapping populations, including doubled haploid (DH), near isogenic line (NIL), and recombinant inbred line (RIL) are highly suitable for linkage-based QTL analysis since they are homozygous and can be used for phenotyping experiments in multiple environments.

The goal of this thesis was to explore and understand the genetic basis of stripe rust resistance in a doubled haploid (DH) population derived from the cross between spring wheat cultivars AAC Innova and AAC Proclaim, and a recombinant inbred line (RIL) population derived from the cross between spring wheat cultivars AAC Cameron and germplasm line P2711. The specific objectives were 1) to identify and locate QTLs associated with stripe rust resistance in the DH population AAC Innova/AAC Proclaim; 2) to identify and locate QTLs associated with stripe rust resistance in the RIL population AAC Cameron/P2711.

## 4.2 Contribution to knowledge

In the first study, we identified 11 QTLs associated with stripe rust in the AAC Innova/AAC Proclaim DH population tested in disease nurseries at Creston, British Columbia (in 2016 and 2020) and Lethbridge, Alberta (in 2016, 2017 and 2020). Four of these QTLs were located on chromosome 2B and one on each of chromosome 2A, 2D, 5A, 3D, 5A, 5D and 7D.

AAC Innova contributed stripe rust resistance alleles for QTLs identified on chromosome 2A, 2B, 3D and 5D while AAC proclaim contributed resistance alleles for QTLs mapped on chromosome 2D, 5A and 7D. *QYr.lrdc-2A* identified on the long arm of chromosome 2A was the only major effect and most stable QTL in this study, which explained up to 33% of the phenotypic variance and was identified in all five environments.

*QYr.lrdc-5A* mapped on chromosome 5A was the second most stable QTL in this study which was identified in four out of five environments and accounted for 5-7% of the phenotypic variance. *QYr.lrdc-7D* mapped on chromosome 7D was the third most stable QTL in this study, identified in two individual environments, where it accounted for 5 and 7% of the phenotypic variance, respectively. Other QTLs were inconsistent or environment specific and explained less than 10% of the phenotypic variance. DH lines carrying *QYr.lrdc-2A* alone and three most stable QTLs (*QYr.lrdc-2A*, *QYr.lrdc-5A* and *QYr.lrdc-7D*) in combination were resistant.

In the second study, we identified a total of seven stripe rust resistance QTLs in the AAC Cameron/P2711 RIL population evaluated for stripe rust severity at the adult plant stage in nurseries at Creston, British Columbia (in 2018, 2019 and 2020) and Lethbridge, Alberta (in 2018 and 2020). Two QTLs were mapped on each of chromosome 1A and 2A and one on each of chromosome 2B, 3B and 5A. Resistance at QTLs *QYr.lrdc-2A.1*, *QYr.lrdc-2A.2*, *QYr.lrdc-3B* and *QYr.lrdc-5A* was provided by the male parent P2711, while those at *QYr.lrdc-1A.1*, *QYr.lrdc-1A.2* and *QYr.lrdc-2B* came from the female parent AAC Cameron.

*QYr.lrdc-2A.1* mapped on the short arm of chromosome 2A was the most stable QTL in this study, identified across four environments as well as in pooled data and explained up to 12.6% of the phenotypic variance. Our finding suggests that *QYr.lrdc-2A.1* corresponds to *Yr17* gene. *QYr.lrdc-2B* mapped on the short arm of chromosome 2B was the second most stable QTL in this

study, identified in three out of five environments as well as in pooled data, and explained up to 14.0% of the phenotypic variance. *QYr.lrdc-5A* mapped on the long arm of chromosome 5A was identified in three environments (only in Creston location) in addition to the pooled data where it explained up to 20.0% of the phenotypic variance. Other QTLs were either location specific or identified in unique environments and explained less than 10% of the phenotypic variance. None of the most stable QTLs *QYr.lrdc-2A.1*, *QYr.lrdc-2B* and *QYr.lrdc-5A* identified in this study was sufficient for the reduction in stripe rust severity alone, but only in combination with other QTLs.

**4.3 General discussion**

In the first study, stripe rust resistance in the AAC Innova/AAC Proclaim DH population was found to be modulated by a combination of one major QTL detected across all environments and few minor QTLs detected either in a specific environment or in a few environments. *QYr.lrdc-2A* was the most significant QTL identified in this study since it was detected across all environments and explained up to 33% of the phenotypic variance. Our findings indicated that *QYr.lrdc-2A* belongs to *YrJ22* gene reported in Chinese wheat cultivar Jimai 22 (Chen et al., 2016). The physical map position (717-765 Mb) of *QYr.lrdc-2A* overlapped with *YrJ22* (760-771 Mb), when compared to the flanking marker sequences of all these reported genes and QTLs using IWGSC RefSeq v1.0. The male parental line N9195 of resistant parent AAC Innova is also Chinese in origin, further suggesting that *QYr.lrdc-2A* comes from the Chinese wheat germplasm.

*QYr.lrdc-5A* and *QYr.lrdc-7D* derived from the susceptible parent AAC Proclaim were the second and third most stable QTLs in this study, respectively. Previous research has mapped a minor stripe rust QTL on chromosome 5A (Hou et al., 2015), which was positioned 2-3 cM away from *QYr.lrdc-5A* (Maccaferri et al., 2015; Wang et al., 2014), suggesting that source of resistance for both QTLs could be similar. *QYr.lrdc-2A* was the only QTL in this study that was responsible

for the reduction in stripe severity alone while *QYr.lrdc-5A* and *QYr.lrdc-7D* were only effective in combination with *QYr.lrdc-2A*. Results of this study suggests that major effect QTL *QYr.lrdc-2A* could be a valuable source of resistance to incorporate into commercial cultivars and combined with other genes for developing stripe rust resistant cultivars.

In the second study, resistance in the AAC Cameron/P2711 RIL population was found to be controlled by seven moderate effect stripe rust resistance loci. *QYr.lrdc.2A.1*, *QYr.lrdc.2B* and *QYr.lrdc.5A* were the most consistent QTLs identified in this study. Our findings indicated that *QYr.lrdc-2A.1* derived from parent P2711 corresponds to *Yr17* gene. Tests with the PCR marker VENTRIUP/LN2 (Helguera et al., 2003) specific for 2NS/2AS from *Aegilops ventricosa* carrying *Yr17* gene confirmed that *Yr17* gene was present in parent P2711 and RILs carrying *QYr.lrdc-2A.1*. A previous study (Prins et al., 2011) had identified a QTL (*QYr.sgi-2B.1*) on the short arm of chromosome 2A whose physical map position (47.6 Mb) overlaps with *QYr.lrdc-2B* (47.4-68.2 Mb), indicating that both QTLs might be derived from the same gene.

*QYr.lrdc.5A* identified on the long arm of chromosome 5A was mapped > *10-38 cM* away from previously reported QTLs/*Yr34* gene on the same chromosome (Maccaferri et al., 2015; Wang et al., 2014). Furthermore, its physical map position (593.3-611.6 Mb) also does not overlap with any of these QTLs/ *Yr34* gene suggesting that it could be potentially a novel source of stripe rust resistance in the resistant parent P2711. However, none of these most stable QTLs (*QYr.lrdc.2A.1*, *QYr.lrdc.2B* and *QYr.lrdc.5A*) was sufficient for reduction in stripe rust severity alone, but rather in combination with other QTL/genes.

We observed that RILs carrying these three most stable and moderate effect QTLs (*QYr.lrdc.2A.1*, *QYr.lrdc.2B* and *QYr.lrdc.5A*) in combination contributed to an experimentally maximum reduction in stripe rust severity, suggesting that these QTLs need to be pyramided to

achieve maximum level of resistance. Singh et al. (2000) also highlighted the importance of combining 3-5 minor to intermediate effect genes, which are not effective individually, to achieve a high level of resistance. The major effect QTL (*QYr.lrdc.2A*) identified in the first study and three moderate effect QTLs (*QYr.lrdc-2A.1*, *QYr.lrdc-2B* and *QYr.lrdc-5A*) identified in the second study could be manipulated (combined) within a breeding program using the flanking SNP markers reported in these studies. Combining the QTLs we report here into adapted germplasm should serve to increase stripe rust resistance in Canadian spring wheat.

### 4.4 Future research

i. Potential QTL by environment interactions could be explored to understand the stability of QTLs.
ii. KASP markers might be developed for *QYr.lrdc-2A* identified in the first study and *QYr.lrdc-2B* and *QYr.lrdc-5A* identified in the second study, respectively.
iii. KASP markers could be validated in a wide set of germplasm before deployment into marker assisted selection.
iv. Fine Mapping could be done to narrow down the QTL region and find more effective markers.
v. Allelism test might be conducted to confirm the uniqueness of QTLs from the previously identified genes.

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