THESIS

CHARACTERIZATION AND MOLECULAR MAPPING OF STRIPE RUST RESISTANCE IN A DENALI/HATCHER WINTER WHEAT DOUBLED HAPLOID POPULATION

Submitted By

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In partial fulfilment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2019

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ABSTRACT

CHARACTERIZATION AND MOLECULAR MAPPING OF STRIPE RUST RESISTANCE IN A DENALI/HATCHER WINTER WHEAT DOUBLED HAPLOID POPULATION

The majority of global wheat (*Triticum aestivum* L.) production is subject to infection by the stripe rust pathogen (*Puccinia striiformis* Westend. f. sp. *tritici* Erikss.). The evolution of new stripe rust races appears to be occurring more rapidly than in the past, causing significant economic loss through yield reduction and increased use of fungicides. A combination of all-stage resistance and high-temperature adult plant (HTAP) resistance in new cultivars may provide complete resistance or serve to reduce disease incidence, thus providing a greater overall level of protection. In addition, knowledge of the form of resistance present in a particular cultivar may help to minimize fungicide use with cultivars that show early-season infections prior to initiation of HTAP resistance. A doubled haploid population (n=210) developed from a cross between winter wheat cultivars 'Hatcher' (PI 638512) and 'Denali' (PI 664256) was developed and characterized for response to stripe rust during 2018 and 2019 at Fort Collins, CO and Rossville, KS. A high density genetic linkage map consisting of 4,441 single nucleotide polymorphism markers derived via genotyping by sequencing was used to identify markers for stripe rust resistance in this population. Four quantitative trait loci (QTL) for infection type (IT) and disease severity (DS) (*QYr.csu-1B, QYr.csu-3A, QYr.csu-3B,* and *QYr.csu-7B)* were found to contribute to stripe rust resistance. Among the resistance QTL, QYr.csu-1B and *QYr.csu-3A* were the most consistent for single environments and combined across environments and accounted for 9.6-16.3% and 10.1-14.4% of phenotypic variation, respectively. QYr.csu-3B showed a stronger effect than QYr.csu-7B and was detected in more than one environment.

Flanking markers for all the identified QTL, especially for QYr.csu-1B and QYr.csu-3A, will be useful to develop wheat cultivars with more effective and durable resistance to stripe rust.

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to my advisor, Dr. Scott Haley, for providing me with the opportunity to pursue graduate studies. His encouragement and support were invaluable throughout this project and his mentorship helped me develop personally and professionally. I am grateful to him. I would also like to thank my committee members, Dr. Patrick Byrne and Dr. Kirk Broders, for their advice and suggestions. I certainly enjoyed our extensive discussions and I learned a lot through our interactions. Their contributions are much appreciated.

This project was funded through funding provided by the Colorado Wheat Research Foundation (CWRF) and I am grateful for their generous support. I would like to thank Dr. Robert L. Bowden from USDA–ARS Manhattan KS and Drs. Xianming Chen and Meinan Wang from USDA-ARS Wheat Health, Genetics, and Quality Research, Pullman, WA for their help with the field and greenhouse testing.

Many thanks to friends and colleagues for their encouragement. I would like to thank my parents for their love and support and all the sacrifices they made to ensure I acquired proper education and a better future. Last but not least, special thanks to my dear wife, Manezha, as this would not have been possible without her.

DEDICATION

This is dedicated to the people of Afghanistan and the Afghan farmers who have suffered through a lot including severe crop disease and multiple droughts.

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CHAPTER I – LITERATURE REVIEW

Wheat and Wheat Production

Cereal crops have been highly important for humans, and have been the main food source for direct human consumption and livestock feed. The world's three staple cereal crops are maize (*Zea mays*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*). Wheat is ranked as the third most widely produced crop after maize (first) and rice (second), but it is second as a main food crop after rice given that a large part of maize production is used as animal feed. Because of its wide adaptation, a larger area in the world is covered by wheat than by any other food crop (FAOSTAT, 2013). Wheat is a unique crop in its growing range, as it can be grown in almost every temperature climate in the world. The crop can be grown at 67° north latitude in Russia to 45° south latitude in Argentina and from tropical to temperate conditions (Shewry, 2009). The reason behind the wide range of wheat's adaptability can be explained by its enormous and complex genome. The genetic composition of wheat allows for appropriate adaptation and acclimation to extreme conditions, including its ability to escape drought conditions, high temperatures in the summer, and survival from freezing temperatures in the winter (Snape et al., 2001).

One can say that the world's wheat production is perennial, and it is due to the fact that in every month of the year, wheat is harvested in some part of the world. Wheat production has been continually improved since its domestication. Farmers in the early world practiced selection by choosing favorable wheat heads, larger grains, average height and grains that are easier to thresh and grind. Significant improvements in wheat production have happened since the early 1800s that included the invention and use of chemical fertilizer and pesticides, improvements in milling technology, and the mechanization of the agricultural industries in the Europe and the U.S. (Mangelsdorf, 1953. Recent advances have remarkably accelerated wheat

production, most importantly, the majority of production increases have been driven by improving the yield per unit area rather than increases in the size of cultivation area.

Wheat was grown on 218 million ha in the world and the total production was close to 771 MMT in 2017. The United States alone produced around 6.2% (47 MMT) of the world total production and ranked as the fifth largest producer in the world. Other major wheat producer countries are the European Union (19.4%), China (17.4%), India (12.7%), and the Russian Federation (11.1%) (FAOSTAT, 2017).

Wheat origin and evolution

There is still a debate about the initial domestication of wheat (*Triticum spp.*), but a strong belief exists that common (hexaploid, 2n=6x=42) wheat was first domesticated around 10,500 years ago in southwest Asia (Barton and An, 2014). Before the domestication of common wheat, the wild diploid and tetraploid wheat species (*Aegilops* and *Triticum* species) had been grown by early farmers as the main source of food. Hexaploid wheat, with the genomic code of AABBDD, consists of three sub-genomes, namely A, B and D genome. Each genome was contributed from a distinct diploid species of the *Triticeae* tribe: *Triticum urartu* (AA),a species related to *Aegilops speltoides* (BB), and *Aegilops tauschii* (DD) (Marcussen et al., 2014).

Emmer wheat (*T. turgidum*; AABB), was the first genome hybridization in wheat that evolved few hundred thousand years ago from a hybridization between *Triticum urartu* and *Aegilops speltoides*. Emmer wheat is an ancestor to durum wheat (*T. turgidum* spp. *durum*) which is grown mostly for the purpose of pasta production. The modern hexaploid wheat is a subsequent result from hybridization between tetraploid emmer wheat and *Aegilops tauschii* (Huang et al., 2002; International Wheat Genome Sequencing Consortium, 2014). Common wheat has no hexaploid progenitor in nature and therefore it is considered to have been derived as a farming-associated accidental cross. Durum wheat and common wheat, the so-called ‘free-

threshing' wheats, are the latest domesticated species in *Triticum* genus. These two species became leading crops for human consumption after their domestication (Salamini et al., 2002).

The need to increase wheat production

Wheat is essential to human civilization and has played an important role in feeding a hungry world and ensuring global food security. The crop provides about 20% of the total dietary calories and protein of 4.5 billion people (FAO, 2012; Shiferaw et al., 2013). Further improvement in the yield potential of wheat in order to meet current and impending challenges, including rising consumption and the demand for grain for food as well as fuel, is still a need (Curtis and Halford, 2014).

The demand for increases in wheat production is clearly going to continue as the average projection for world population is to rapidly increase from the current level of seven billion to nine billion by 2050 and more than 10 billion by 2100 (United Nations world population prospects, 2015). While increases in wheat production in the past have been observed, gains at current levels are insufficient to meet the demands of population growth. Ensuring food security amidst the rapidly increasing population, together with the threats of the constantly changing climate and biotic (e.g., diseases, insects, weed infestations) and abiotic (e.g., high temperatures, drought, waterlogging, freezing) stresses have catalyzed efforts to improve wheat varieties through various breeding programs and initiatives. "The most direct solution to these problems will be to increase productivity on currently cultivated land through adoption of cultivars with improved genetic potential", says Reynolds et al. (2012). The new improved varieties are developed to be high yielding, resistant to biotic and abiotic stresses, and more adaptable to a wide range of environmental conditions than traditional or landrace varieties.

Wheat Stripe Rust

Wheat stripe rust (also known as "yellow rust") is the most important foliar fungal pathogen problem in the western United States. The disease has become increasingly important in the south-central states and the Great Plains in recent years (Chen et al., 2002; Hao et al., 2011). Stripe rust causes yield losses, affects the quality of grain and forage, and causes economic losses for producers that apply fungicides for stripe rust control. Crops damaged by stripe rust produce low vigor seeds and thus poor emergence after germination in the field (Chen, 2005).

Stripe rust is caused by a basidiomycete, which is an obligate biotrophic pathogen, meaning it only infects and grows on living plant tissues. Rusts and especially stripe rust have a complex life cycle compared to many other fungi. This pathogen is classified as a macrocyclic heteroecious fungus because the pathogen produces multiple types of spores and requires two hosts to complete its life cycle. The pathogen creates specialized structures called haustorium and mycelium that grow inter- and intra-cellularly within host plant tissues to obtain nutrients. The stripe rust urediniospore is the functional type of spore, which is produced on living plants and dispersed by the wind to other fields (Garnica et al., 2013; Chen et al., 2014).

Stripe rust pathogenicity and infection conditions

Stripe rust is capable of infecting many cultivated crops including wheat, rye (*Secale cereale*), barley (*Hordeum vulgare*) and triticale (*Triticosecale*), however, the most economically important form is the stripe rust of wheat that causes yield losses to commercial cultivars. Losses due to rust have caused concern since earliest recorded history. But greater damage by stripe rust epidemics can occur than in the past if multiple virulent races attack large-scale fields of genetically similar cultivars (Wellings, 2011). The green bridge between spring and winter wheat cultivation and volunteer cereals has been known as the primary facilitator for the stripe rust over-wintering and early inoculum build up. The recent discovery of the alternate host for stripe

rust, however, supports the hypothesis for the development of variability in pathogen's virulence due to possible sexual recombination on *Berberis spp*. (Jin et al., 2010; Park et al., 2015).

Among all types of rusts, stripe rust of wheat requires the lowest optimal temperature to infect the host plant. A conducive environment for stripe rust infection can be described as cooler nighttime temperatures (10 °C and lower) with higher humidity and days with the maximum temperature of ~20 °C, although nighttime temperature and humidity are more important than daytime temperature. Stripe rust requires a minimum of three hours of continuous free moisture or dew on the surface of the plant at dark and low temperature to germinate and initiate infection. Since both low temperature and dew formation generally occur during the night, it is believed that infection typically takes place at nighttime (Chen, 2005).

While the most favorable temperature for the stripe rust infection is between 10 and 15 °C (Sørensen et al., 2016), recent studies reported that stripe rust has caused severe losses to wheat under high temperatures. In China, the temperature sensitivity and tolerance of 126 stripe rust isolates was studied and it was found that isolates collected after 2010 were more tolerant to higher temperature and some temperature sensitive isolates died at 26 °C (Chen and Kang, 2017).

Stripe rust epidemics

The stripe rust pathogen is highly variable in virulence due to its ability to evolve, producing new races and changing in distribution and frequency of previously existing races (Wan et al., 2016). New races of the pathogen that are able to render previously resistant cultivars susceptible often cause stripe rust epidemics (Chen et al., 2010). The combination of a virulent pathogen and a conducive environment increases the possibilities of sexual recombination, mutation rates, long distance migration, and adaptation to different environments and makes this disease

an increasing problem worldwide (Ali et al., 2017; Brown and Hovmøller, 2002). For the last two decades, multiple stripe rust epidemics have been reported and it is believed that stripe rust has caused estimated annual losses of 5.5 MMT worldwide (Beddow et al., 2015). Several historical epidemics as well as new epidemics occurred in the U.S., Asia, Europe, Australia, and Africa.

Stripe rust incidence was reported for the first time in South Africa in 1996 (Boshoff et al., 2002) and Western Australia in 2002 (Wellings et al., 2003). In 2009, heavy stripe rust pressure was reported in North Africa and stripe rust has since occurred frequently in North and East Africa. In 2010, stripe rust epidemics caused economic losses in Central Asia and the Middle East, with severe losses in Tajikistan, Uzbekistan, Syria, and Lebanon (Ali et al., 2017). In Europe, stripe rust incidents were not very severe until 2011 when a new race known as '*Warrior*' emerged and spread over the European continent. The exotic Warrior race affected wheat and triticale in several European countries and overcame most of the race-specific wheat resistance genes in Europe (Hovmøller et al., 2016). The emergence of the Warrior race in Europe was shown to not be due to mutation nor sexual recombination within Europe, but to a sexually recombining population near the Himalayan region in Asia (Losert et al., 2017c). The widespread replacement of the pre-existing stripe rust population by new races in Europe highlights the complexity and capability of the stripe rust pathogen (Hovmøller et al., 2016). Recent destructive stripe rust epidemics in China occurred in 2002, which affected 6.6 million ha of wheat production and caused 1.3 MMT yield losses (Wan et al., 2004).

In the U.S., the first widespread occurrences of stripe rust due to a race change occurred in 2000, affecting a larger wheat growing area than ever in the past throughout the country. Despite efforts to control stripe rust using fungicides, the disease caused multimillion-dollar losses in the U.S. with Arkansas and California affected the most (Chen et al., 2002). Prior to 2000, only 59 stripe rust races were known in the U.S., and only four were reported east of the Rocky Mountains with very sporadic and localized incidence. For this reason, stripe rust

was known to be a disease primarily of wheat in the Pacific Northwest of the U.S. Since 2000, stripe rust has evolved rapidly in the U.S., as 40 new races were discovered between 2000 and 2004 and more than 20 states have reported stripe rust incidence east of the Rocky Mountains (Chen et al., 2002, Wan et al., 2004). Furthermore, a total of 41 stripe rust virulent races were characterized using new differential set with 18 *Yr* single-genes from 348 viable stripe rust isolates collected from 24 states during 2010 (Wan and Chen, 2014).

Stripe rust has been an annual problem throughout North America since the first widespread epidemic in 2000, and has caused significant yield losses in at least seven of the last 15 years (Hughes, 2016). The recent stripe rust epidemics occurred in the U.S. every year between 2010 to 2016, except 2014. The severity of the epidemics in 2010 and 2012 compared to 2011 were higher in the U.S. Great Plains. In 2012, stripe rust was reported in more than 25 states reaching from the west coast to the east coast and from Texas to North Dakota (Wan et al., 2016). Stripe rust infections in 2012 were more severe and widespread in the south-central U.S. and the Great Plains, particularly in Colorado, Kansas, Oklahoma, Mississippi, Nebraska, North Dakota and Minnesota (Wan et al., 2016). The epidemics in 2015, however, were more severe than any other year in the Great Plains because yield losses due to stripe rust were estimated up to 7.2% on spring wheat and up to 12.7% on winter wheat, not including fungicide applications costs (Kolmer et al., 2016). In particular, the epidemic of 2015 was recorded as the heaviest stripe rust outbreak in Colorado and Nebraska history (Kolmer et al., 2016; Lyon and Broders, 2017).

Wheat research collaborators monitor stripe rust through field surveys and disease nurseries throughout the U.S. The stripe rust epidemics in 2000, 2001, 2010, 2011, 2012, 2015 and 2016 alerted researchers to increase their efforts to breed for resistant cultivars throughout the U.S. The use of diverse sources of resistance genes and durable resistance in new cultivars

will be a good approach for preventing future widespread epidemics (Chen et al., 2002; Wan et al., 2016).

Stripe rust management

Stripe rust can be controlled or reduced through an integrated approach using genetic resistance, chemical applications, and cultural practices. The use of an integrated approach could be applicable in a situation where the available resistant cultivars are acceptable to the growers based on their quality and yielding properties, fungicides are affordable and effective, and cultural practices are appropriate. Despite the availability and effectiveness of the two other approaches, almost all researchers propose the use of genetic resistance as the most important. The use of fungicides comes as the second approach, and the use of cultural practices comes as the last approach because it is complex and less feasible than genetic and chemical approaches (Chen and Kang, 2017).

Chemical control may be considered the first option when there is a sudden threat due to appearance of new virulent races or when susceptible varieties are grown for some other reason. Fungicides have been used in the U.S. Pacific Northwest since 1981 and in Western Europe since the late 1970s and early 1980s to control stripe rust of wheat. It is still an important part of an integrated approach for controlling stripe rust in some countries. Advances in technology and science have helped researchers to discover new fungicides with different mode of actions and some of these fungicides are now available at a lower cost. The issues of cost and timeliness of chemical application still remain a hurdle in many parts of the world. Small-scale growers with lower yield and lower price for the grain cannot afford to pay for chemicals and machinery (Rathmell and Skidmore, 1982; Chen and Kang, 2017).

Cultural control of stripe rust consists of a wide range of practices, of which planting resistant cultivars is considered the most important. This approach is useful and can help reduce disease epidemics. For instance, the cropping system is considered an important issue in controlling stripe rust. Winter wheat and spring wheat growing near to one another in the same region provides a green bridge for disease transmission from one cropping season to another. This transmission provides an opportunity for building more inoculum in the next season, potentially leading to more frequent epidemics. As an example, in Washington State, spring wheat cultivation increased in 1999 from 10% to 25% of the total wheat cultivated area with winter wheat comprising the remaining 75%. The increases in spring wheat cultivation increased the green bridge and thus contributed to the occurrence of severe epidemics in the Pacific Northwest of the U.S. since 2000 (Chen, 2005; Chen et al., 2014; Chen and Kang, 2017).

Other cultural practices include, but are not limited to, control of volunteer plants, alteration or choosing an appropriate planting time, planting mixtures of crops or multiline cultivars, and avoiding excessive irrigation and fertilization. Many of these practices have effects on stripe rust as well as on the other components of farming and nature. For instance, changing the cropping system may be possible, but this may have downstream effects on other issues like food security, the local and national economy, and the environment.

The use of resistant cultivars is the most effective, economical, and environmentally-sound means for control of stripe rust, and in some situations is the only available option (Chen, 2005; Singh et al., 2016). Stripe rust genetic resistance studies date back to the early 1900s. Biffen (1905) was the first researcher who studied stripe rust resistance in wheat and applied Mendelian principles to plant disease resistance (Chen, 2005; Chen et al., 2014). Since then, a major discovery in the field of the genetics of host-parasite interactions was reported by H.H. Flor in 1955 from his work on flax rust (Wellings et al., 2012). Flor proposed the “gene-for-gene”

hypothesis, where he showed that the inheritance of resistance in the plant and the ability of the pathogen to infect the host is controlled by pairs of corresponding genes. This study paved the way for future studies and enabled researchers to better explain the genetic resistance mechanisms in plants and the type of pathogenicity (virulence/avirulence) of the pathogen (Wellings et al., 2012). Several other researchers also studied stripe rust of wheat but it was not until 1962 that the first stripe rust resistance gene was formally designated. Lupton and Macer (1962) studied the stripe rust resistance in the wheat variety “Chinese 166” and for the first time they introduced the *Yr* (for "yellow rust") nomenclature and designated the first stripe rust resistance gene as “*Yr1*” (Chen and Kang, 2017).

Recent studies with the help of molecular markers have led to more advancements in identification of stripe rust resistance genes. The improvement in principles and techniques of genetics and statistics toward identification of quantitative traits made it possible to discover quantitative trait loci (QTL) via biparental mapping and genome-wide association studies (GWAS) (Chen and Kang, 2017). Despite all the hard work of the breeding programs and the discovery of many resistance genes and QTLs, 88% of global wheat production is still susceptible to stripe rust, leading to annual losses of $1 billion worldwide (Schwessinger, 2017). Therefore, breeding for stripe rust resistance should be an essential part of a breeding program so breeders can deliver effective and durable stripe rust resistant cultivars to the farmers. Wellings et al. (2012) explains Johnson’s proposed term for durable resistance as “resistance that remains effective when deployed over extensive acreage and time, in an environment favorable for the disease”. Durable stripe rust resistance has been reported in European and CIMMYT wheat cultivars. The deployment of important seedling and adult-plant resistance genes in the same cultivar provides a durable resistance to the stripe rust of wheat in the field (Wellings et al., 2012; Chen and Kang, 2017).

Genetic factors conferring resistance to wheat stripe rust

Race-specific and non-race-specific resistance are two major types of resistance to stripe rust. All-stage resistance, which is also called seedling resistance, is generally race-specific and qualitatively inherited, whereas adult-plant resistance (APR) and high-temperature, adult-plant (HTAP) resistance are mostly non-race-specific, durable, and are often quantitatively inherited (Qayoum and Line, 1985; Chen and Line, 1995; Line and Chen, 1995; Line, 2002; Chen, 2005).

All-stage resistance (ASR)

All-stage resistance, also known as seedling resistance, is typically controlled by single genes. The term seedling resistance could be misinterpreted as the plant showing resistance only at the seedling stage, and therefore the term all-stage resistance is preferred. This resistance class also is referred to as race-specific resistance, gene-for-gene resistance and major-gene resistance. All-stage resistance can be detected at the seedling stage and it remains effective throughout plant development. All-stage resistance is typically race-specific and thus prone to rapid evolution of new virulent races (Chen, 2005; Hao et al., 2011). All-stage resistance against stripe rust is attractive for breeders due to the high level of resistance typically observed and the relative ease of incorporation of major (single) genes into improved cultivars (Chen, 2007). Numerous race-specific resistance genes conferring resistance to wheat stripe rust have been identified and incorporated into commercial cultivars. The emergence of new virulent races of stripe rust that overcome all-stage resistance and the changes in frequencies and distribution of virulent races has caused devastating epidemics in the U.S. and thus none of these genes remain effective against all U.S. and global stripe rust races (Ren et al., 2012). For instance, the epidemic in 2010 due to the shifts in the race broke down the long time effective resistance gene *Yr17* in the U.S. Great Plains (Yang et al., 2018). Changes in frequency of the virulent stripe rust races is another threatening issue for the race-specific resistance, since the frequency among the virulent races could change every year and could overcome multiple

resistances. A fluctuation between the two virulent stripe rust races *PSTv-11* and *PSTv-37* occurred in the U.S., with race *PSTv-37* as the most dominant race during the 2010 and 2012 epidemics and race *PSTv-11* as the most dominant race in 2011 (Wan and Chen, 2014; Wan et al., 2016).

Adult-plant resistance (APR)

In contrast to all-stage resistance, APR is expressed only in the adult-plant stage. APR is mostly effective at higher temperatures and is thus referred to as high-temperature adult-plant (HTAP) resistance (Chen, 2013). Unlike all-stage resistance, HTAP is typically non-race-specific and is considered to be more durable and often quantitatively inherited (Chen, 2005; Ren et al., 2012; Ellis et al., 2014). This type of resistance is often incomplete and is expressed gradually as the plant grows older and temperatures rise. Cultivars that carry only HTAP resistance are usually susceptible to most stripe rust races at the seedling stage, but as the plant ages and temperatures increase between 25-35 °C the plant becomes more resistant (Chen, 2005, 2013).

Multiple HTAP resistance genes or QTLs have been identified and incorporated in wheat cultivars in the U.S. Cultivars with HTAP resistance in the U.S. Pacific Northwest have shown resistance for over 50 years, confirming the durability of HTAP resistance. The effect of HTAP resistance may be seen and evaluated on the flag leaf of the adult-plant. The flag leaves of the plant are the major contributor to grain filling and grain yield and are thus very important when characterizing HTAP resistance to stripe rust. Cultivars are evaluated based on their flag leaf reaction to the stripe rust, and individuals with HTAP resistance always show more resistance on the flag leaves than lower leaves (Chen, 2005). Recently, more attention has been given to incorporating HTAP resistance in new cultivars together with all-stage resistance to provide a high level of durable resistance to stripe rust. All-stage resistance provides immunity to the plant when it is effective against the dominant race and when all-stage resistance is overcome, the HTAP resistance reduces the disease damage (Chen, 2005).

Plant Breeding and Marker-assisted Selection

Resistance breeding for quantitative traits

Plant breeding is a discipline that involves knowledge and skills of creation, selection, and combining of superior plant phenotypes in order to develop improved cultivars to meet the needs of growers and consumers. The primary goals of plant breeding for agricultural and horticultural crops have typically focused on higher yield, improved nutritional qualities, disease and insect pest resistance, and other traits of commercial value (Moose and Mumm, 2008).

Breeding for stripe rust resistance has most often focused on all-stage resistance in the past, as this type of resistance was in agreement with the Flor's gene-for-gene interaction hypothesis (Sorensen et al., 2016; Wan and Chen, 2014) and therefore many cultivars have been developed with this type of resistance. Plants with qualitative resistance typically show immunity or a hypersensitive reaction (HR) when attacked by an avirulent pathogen (Moody et al., 2003; Chen and Kang, 2017).

In contrast to all-stage resistance, adult-plant resistance typically shows a continuous phenotypic distribution among the progeny from a cross between a resistant and susceptible parent. This distribution does not conform to simple Mendelian segregation ratios as with qualitative traits controlled by alleles at a single locus. The main differences between qualitative and quantitative traits are as follow (Castro et al., 2003; Collard et al., 2005):

1- Qualitative traits have phenotypes that fall into easily recognizable categories whereas quantitative traits have a continuum of phenotypes.
2- Qualitative traits are controlled by only one or a few major genes whereas quantitative traits are controlled by several to many QTL.
3- Qualitative traits are studied via ratios and inheritance patterns whereas quantitative traits are studied via means and variances.

4- Qualitative traits are generally affected less by the environment whereas quantitative traits have a large component due to non-genetic effects. In fact quantitative traits are often influenced more by the environment than by the underlying QTL.

In summary, breeding for all-stage resistance follows classic genetic approaches, is more readily used in breeding programs, but often has shown less stability over time. On the other hand, breeding for adult-plant resistance tends to be more challenging, often requires a longer time to incorporate into commercial cultivars, but has generally shown a greater degree of stability over time.

Strategies for resistance breeding

There are many considerations important for improving the resistance in plants to biotic and abiotic stresses. Singh and Rajaram (2002) highlighted some of these factors in wheat breeding for biotic stresses. They emphasized that understanding the nature of the pest, availability, diversity and type of resistance, screening methodology and selection environment are the primary considerations that must be taken into account for effective resistance breeding.

In the past, different strategies have been proposed and applied by researchers and scientists to utilize host-plant resistance in breeding programs. Strategies that have been used for resistance breeding include: a) gene rotation; b) regional gene deployment; c) cultivar mixtures or blends; d) multiline cultivars; e) gene pyramiding; and f) combining different types of resistance. These strategies have been used by different programs subject to the availability of resources and technologies and a complete understanding of stripe rust resistance dynamics. While not all of these strategies are used today, they are still useful and have application in modern breeding programs (Chen, 2005; Chen and Kang, 2017). Among these, gene pyramiding and combining different types of resistance into one cultivar are the most useful strategies and breeders have reported using these two strategies in recent breeding activities.

Gene pyramiding may be used in wheat breeding programs to combine race-specific resistance genes into wheat cultivars. Breeding programs in the U.S. PNW have successfully controlled leaf rust (*P. triticina*) and stem rust (*P. graminis*) by pyramiding race-specific resistance genes in wheat cultivars (Chen and Kang, 2017). The spring wheat cultivar Patwin 515 (PI 666962) was developed with three race-specific stripe rust resistance genes (*Yr5, Yr15* and *Yr17*) and this variety is resistant to all current races. Chen and Kang (2017) suggest no firm rules for the number of resistance genes to be pyramided in a cultivar to provide an ideal level of resistance and durability. Wheeler and Diachun (1983) argue that if the gene-for-gene hypothesis is valid then four to five resistance genes pyramided into a cultivar can provide resistance "for up to centuries". Chen and Kang (2017) also suggest that for gene pyramiding to be effective, at least two or more effective genes conferring resistance to all races should be incorporated at the same time.

Combining different types of resistance is considered a more effective strategy. Combining both ASR with APR or HTAP resistance provides a high level of resistance in wheat cultivars. A good reason for incorporating both types of resistance in one cultivar is that ASR may provide complete resistance against the dominant virulent race when it is effective, and APR or HTAP resistance provides partial resistance if the ASR resistance is defeated by a new stripe rust race (Chen, 2005, 2013). The HTAP resistance is usually conferred by large effect genes or QTLs that explain a large portion of the phenotypic variation for resistance. Coram et al. (2010) and Chen et al. (2013), however, have suggested that durability of resistance is more a function of the resistance type than the effect size. For example, the winter wheat cultivar Stephens (CI 017596), developed in the U.S. PNW, carries both types of resistance and has remained resistant since its release in 1987 (Vazquez et al., 2012). Using conventional procedures, it is important to incorporate HTAP resistance first in a cultivar and then the ASR resistance, so to make sure that the ASR does not block the HTAP effect when making phenotypic selections. The use of marker assisted selection (MAS), however, makes it possible

to incorporate both resistance types at the same time when molecular markers for ASR and HTAP resistance are available (Chen and Kang, 2017).

The use of molecular markers in plant breeding

The use of molecular techniques has evolved in recent years and has significantly contributed to the development of improved cultivars of wheat and other crops. Developments in molecular marker technologies have helped researchers to explore the potential of improving varieties through improved understanding of the underlying genetic control of important traits. Molecular marker approaches have been integrated with plant breeding through the process of marker-assisted selection (Collard and Mackill, 2007).

Marker assisted selection has recently become a routine application in crop improvement programs. One of the main uses of molecular markers has been the construction of linkage maps for diverse crop species. Linkage maps have been used to identify chromosomal regions that carry genes controlling qualitative or quantitative traits (Collard et al., 2005). Apart from the use of DNA markers in the construction of linkage maps and MAS, they have abundant applications in plant breeding for assessment of genetic diversity within breeding germplasm and determining cultivar identity. The main advantages of the use of MAS in molecular breeding include: a) time saving from conducting complex and expensive field trials; b) selection of genotypes at any growth stage; c) elimination of unreliable phenotypic evaluation associated with field trials due to environmental effects; d) avoidance of transfer of undesirable genes, particularly resulting from introgression of genes from wild species; e) gene pyramiding; f) testing for specific traits where phenotypic evaluation is not feasible; and g) selection for traits with low heritability (Collard et al., 2005). Apart from all the advantages of DNA marker-assisted selection, Collard and Mackill (2007) and Boopathi (2013) have listed some main considerations that may affect the applicability of MAS:

Reliability: markers should be tightly linked to target loci. The use of flanking markers or intragenic markers will greatly increase the reliability of the markers to predict phenotype.

Level of polymorphism: ideally, the marker should be highly polymorphic in breeding material, especially in core breeding material.

Technical procedure: the level of simplicity and the time required for the technique are critical considerations. High-throughput, simple, and quick methods are highly desirable.

Cost: the marker assay must be cost-effective in order for MAS to be reasonable.

Transferability: markers developed for one population may not be transferable to other populations, either due to lack of marker polymorphism or the inconsistency of marker-trait association.

Ideal marker types for marker-assisted selection

Various marker systems have been developed and are applied to major crop species. These include Random Amplification Polymorphic DNAs (RAPDs), Restriction Fragment Length Polymorphisms (RFLPs), Amplified Fragment Length Polymorphism (AFLPs), Sequence Tagged Sites (STS), Simple Sequence Repeats (SSRs; also known as microsatellite markers), and Single Nucleotide Polymorphisms (SNPs). These marker systems have been used in a wide range of studies to evaluate differences in DNA sequence within and among species. These techniques may also help breeders to maximize the genetic diversity in a breeding program by incorporating desirable traits from related species into advanced or elite lines (ISAAA, 2006). Marker systems like RFLPs, AFLPs and SSRs have been the most widely used marker type for crop genetic studies in past decades. Recently, SNP markers have become popular as an alternative marker system for use in MAS. SNP markers, the most common form of genetic variation among individuals, are the most recently developed DNA marker technology. SNP markers are considered the preferred marker system for the study of complex

genetic traits through genome-wide association studies and genome-wide selection (He et al., 2014).

Single nucleotide polymorphisms have a wide range of application in marker-assisted breeding, including germplasm characterization, marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), linkage mapping, genome-wide association studies, genomic selection and linkage-based and linkage disequilibrium based quantitative trait loci (QTL) mapping (Semagn et al., 2013). The main advantages of SNPs as DNA-based markers are ease of data acquisition and management, abundance in the genome, high throughput platforms, flexibility, speed, and low cost per marker (Kanazin et al., 2002). Next-generation sequencing (NGS) is a remarkable technology that has led to major advances in whole genome sequencing. Among the several platforms of the NGS technology, genotyping-by-sequencing (GBS) (Elshire et al., 2011) approach has been developed to identify SNP markers across the genome. GBS is a suitable platform for genotyping in large scale plant breeding programs and it has been implemented successfully for GWAS, genomic selection, molecular marker discovery, and genetic linkage mapping (He et al., 2014).

Molecular Mapping of Resistance Genes/QTLs

Molecular mapping is an effective approach for identification of all-stage resistance genes and QTL for adult-plant HTAP resistance to stripe rust of wheat (Chen, 2013). In plant breeding, molecular markers are widely used in mapping studies to identify QTL associated with traits of interest. Genetic linkage analysis enables detection of genes or QTLs controlling target traits, which is based on the principle of genetic recombination during meiosis. This permits the construction of linkage maps composed of genetic markers for a specific population (Collard and Mackill, 2007). Linkage maps indicate the position and relative genetic distances between markers along chromosomes (Collard et al., 2005). A QTL is a genomic region associated with phenotypic variation of a trait. Usually, a QTL is linked to or contains gene(s) that control the

target trait. Statistical analysis to identify QTL may be performed using molecular mapping software such as R/qtl (Broman et al., 2003), QTL IciMapping (Meng et al., 2015), QTL Cartographer (Basten et al., 2004) and other programs. Other than the genome location, the variance explained by the markers (R^2), their significance (logarithm of the odds ratio, LOD), the additive genetic effect of the alleles, and the parental origin of the favorable alleles are also determined from a QTL analysis. The data generated from the QTL analysis can then be used to improve understanding of the overall genetic control of a trait and then one can decide which main-effect QTL should be used for a marker-assisted breeding approach. Many factors may affect the accuracy of a QTL mapping study such as population size, level of replication used to generate phenotypic data, and bias sampling can lead to a bias result in estimates of QTL (Byrne, 2005a, 2005b).

To date, around 80 permanently designated stripe rust resistant genes (*Yr*), 67 temporarily designated genes, and 327 QTL have been reported in different wheat cultivars from different breeding programs (Chen and Kang, 2017; Yuan et al., 2018). These numbers suggest that there is a very large number of genes for resistance to stripe rust available in wheat that may be used to develop cultivars with better stripe rust resistance. The identified genes and QTL have been mapped throughout all 21 wheat chromosomes except chromosome 5D, whereas, most of the previously identified QTL represent the same gene that have been introduced to different breeding programs through germplasm exchange. However, few of the identified resistance genes or QTL provides durable resistance, and even fewer have been cloned successfully and characterized with a clear gene structure (Rosewarne et al., 2013).

STUDY OBJECTIVES

Stripe rust disease in wheat has been an increasingly important problem in the U.S. The occurrence of frequent stripe rust epidemics during the past two decades, as well as recent

evidence of overwintering at more northern latitudes in North America, makes the disease a serious threat for hard winter wheat production in the Great Plains region (Lyon and Broders, 2017). The majority of the previously identified resistant genes and QTL for stripe rust have been characterized using older marker technologies that are not amenable to high throughput screening in applied breeding programs. Furthermore, the QTL information from one population or germplasm pool is usually not directly applicable to other breeding programs.

The increased prevalence and severity of stripe rust in Colorado winter wheat provides the primary justification for this study. The winter wheat cultivar Hatcher (PI 638512; Haley et al., 2005) has shown a moderate level of stripe rust resistance through three different stripe rust race shifts in the Great Plains (2001, 2010, 2012). The winter wheat cultivar Denali (PI 664256; Haley et al., 2012) was initially highly resistant to stripe rust but became highly susceptible with the race change that was detected in the Great Plains in 2012. The type of resistance of Hatcher and Denali was characterized in greenhouse studies and a double haploid mapping population developed with the two parents was subsequently used to genetically map the resistance using evaluations of stripe rust resistance in artificially inoculated field sites in Colorado and Kansas.

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CHAPTER II - CHARACTERIZATION AND MOLECULAR MAPPING OF STRIPE RUST RESISTANCE IN A DENALI/HATCHER WINTER WHEAT DOUBLED HAPLOID POPULATION

INTRODUCTION

Wheat stripe rust (Yellow rust), caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (*Pst*), is the most important foliar fungal pathogen of wheat in the western United States. The disease has become increasingly important in the south-central states and the Great Plains in recent years (Hao et al., 2011; Chen et al., 2002). Stripe rust causes yield losses, affects the quality of grain and forage, and may necessitate costly fungicide applications. Crops damaged by stripe rust produce low vigor seeds and thus poor emergence after germination in the field (Chen, 2005).

The stripe rust pathogen is highly variable in virulence due to the ability to evolve, produce new races, and change in distribution and frequency compared to previously existing races (Wan et al., 2016). New races of the pathogen that are able to render previously resistant cultivars susceptible often cause stripe rust epidemics (Chen et al., 2010). One of the most widespread occurrences of stripe rust due to a race change that occurred in 2000 and affected more than 20 states (Chen et al., 2002), causing multimillion-dollar losses in the United States despite extensive fungicide use (Chen et al., 2002). Recent epidemics of stripe rust occurred during 2010, 2011, 2012, 2015 and 2016. In 2012, stripe rust was reported in more than 25 states reaching from the west coast to the east coast and from Texas to North Dakota. Stripe rust infections have become more severe and widespread in the south-central U.S. and the Great Plains (Wan et al., 2016).

The use of resistant cultivars is the most effective, economical, and environmentally sound means to control stripe rust. All-stage resistance (ASR) and adult-plant resistance (APR) are the two general types of host-plant resistance that protect the plant from stripe rust. All-stage resistance, also known as seedling resistance, is effective against a specific race throughout the plant life cycle and tends to be inherited in a qualitative fashion. A major disadvantage of ASR is that often it is overcome by new virulent races and thus does not provide durable resistance. In contrast to ASR, APR is expressed only in the adult-plant stage. APR is mostly effective at higher temperatures and therefore is commonly referred to as high-temperature adult plant (HTAP) resistance. Unlike ASR, APR or HTAP is typically non-race-specific, is considered to be more durable, and is often quantitatively inherited (Chen, 2005; Ren et al., 2012; Ellis et al., 2014). This type of resistance is often incomplete and is expressed gradually as the plant ages and temperatures rise. Cultivars that carry only HTAP resistance are usually susceptible to almost all stripe rust races in the seedling stage, but as the plant ages and temperatures increase between 25-35 °C the plant becomes more resistant (Chen 2005, 2013).

Multiple HTAP resistance genes or quantitative trait loci (QTL) have been identified and incorporated in wheat cultivars in the U.S. Some cultivars with HTAP resistance in the U.S. Pacific Northwest have shown resistance for over 50 years, demonstrating the durability of HTAP resistance. The effect of HTAP resistance may be seen and evaluated on the flag leaf of the adult plant. The flag leaves of the plant are the most important contributor to grain filling and grain yield and are thus very important when characterizing HTAP resistance to stripe rust. Cultivars are evaluated based on their flag leaf reaction to stripe rust, and individuals with HTAP resistance typically show more resistance in the flag leaves than lower leaves (Chen, 2005). Recently, more attention has been given to developing new cultivars with HTAP resistance and to combining both HTAP and ASR resistance to provide a high level of durable resistance to stripe rust.

Adult-plant resistance to wheat stripe rust is conferred by the additive effects of multiple loci (Lu et al., 2009). Therefore, the observed resistance is due to the effect of several minor genes contributing to resistance. The location and effect of the responsible genes can be estimated through QTL mapping. Due to the increases in adoption of the HTAP resistance in breeding programs, several studies have investigated quantitative resistance to stripe rust using molecular marker approaches and have confirmed that this type of resistance is additively inherited (Singh et al., 2000; Lin and Chen, 2009). Single-nucleotide polymorphism (SNP) markers, the most common form of genetic variation among individuals, are the most recently developed DNA marker technology and are the preferred marker system for the study of complex genetic traits through genomic selection and linkage-based and linkage disequilibrium-based QTL mapping (Semagn et al., 2013).

'Hatcher' (PI 638512; Haley et al., 2005), a hard red winter (HRW) wheat cultivar, has shown a moderate level of resistance to stripe rust in the field since the original race change that appeared in 2001, while many other lines and cultivars previously showing resistance became susceptible in more recent epidemics in the Great Plains. Hatcher was crossed to another HRW wheat, 'Denali' (PI 664256; Haley et al., 2012), to establish a doubled haploid (DH) population of 210 individuals. These materials were used in this study to (i) characterize the parental lines for their reaction to multiple virulent races of *Pst* and assess them for the presence of HTAP resistance, and (ii) characterize the DH population, identify HTAP resistance QTL to stripe rust, and identify flanking markers surrounding those QTL to enable marker-assisted selection.

MATERIALS AND METHODS

Parent Characterization

Multiple race testing

The parental lines Hatcher and Denali and the susceptible check 'Ripper' (PI 644222; Haley et al., 2007) were evaluated in greenhouse studies to characterize their reactions to a group of virulent stripe rust races. Seedling tests conducted at the USDA-ARS Wheat Health, Genetics, and Quality Research Unit in Pullman, WA were done using six virulent stripe rust races (*PSTv-4*, *PSTv-14*, *PSTv-37*, *PSTv-40*, *PSTv-51* and *PSTv-198*). The plants were inoculated with the spores of each race at the two-leaf stage and tested under low temperature (4-20 °C diurnal temperature cycle changing from 4°C at 2:00 a.m. to 20 °C at 2:00 p.m.). Infection type (IT) were rated on the seedlings 16 to 18 days post inoculation following the scale described by Line and Qayoum (1992).

The IT scale from 0 to 9 is also explained by Chen and Kang (2017) as "IT 0 = no visible signs or symptom; 1 = necrotic and/or chlorotic flecks, no sporulation; 2 = necrotic and/or chlorotic blotches or stripes, no sporulation; 3 = necrotic and/or chlorotic blotches or stripes, trace sporulation; 4 = necrotic and/or chlorotic blotches or stripes, light sporulation; 5 = necrotic and/or chlorotic blotches or stripes, intermediate sporulation; 6 = necrotic and/or chlorotic blotches or stripes, moderate sporulation; 7 = necrotic and/or chlorotic blotches or stripes, abundant sporulation; 8 = chlorotic behind sporulation area, abundant sporulation; and 9 = No

necrosis or chlorosis, abundant sporulation". Generally, IT 0-3 is considered resistant, 4-6 is intermediate, and 7-9 is susceptible.

Four-way testing

To determine the type of resistance of the parental lines, a four-way test consisting of both seedling and adult-plant testing was done in the CSU Plant Growth Facilities in Fort Collins, CO. The four-way test is used to differentiate between ASR and APR resistance and separate the effects of plant growth stage and temperature to allow determination of the presence of HTAP resistance (Chen and Kang, 2017). Parental lines and the susceptible check Ripper were inoculated with virulent stripe rust isolates collected in Kansas during the stripe rust epidemics of 2010 and 2012 (provided by R.L. Bowden, USDA-ARS, Manhattan KS). The isolate from 2010 (designated PST2010) is virulent on wheat genotypes carrying the *Yr17* resistance gene and the isolate from 2012 (designated as PST2012) is virulent on the wheat cultivar Everest (PI 659807).

In the seedling test, seeds were planted in plastic pots of 6 cm x 6 cm and parental lines and the susceptible check were inoculated with the stripe rust spores at the two-leaf stage (12-14 days after planting) (Zadoks stage 11-13) as described by Chen and Kang (2017). Seeds for adult-plant tests were vernalized at 2 °C for 4-6 wk after germination. Vernalized seedlings were transplanted in plastic pots of 17 cm x 14 cm and adult plants were inoculated on the flag leaves when 50% of the plants had reached the heading stage (Zadoks stage 55-59) (Zadoks et al., 1974). Plants from both tests were placed in a dark dew chamber for 24 hours at 10 °C and 95% or higher relative humidity after inoculation. Plants were then moved to separate growth chambers at low (4-20 °C diurnal temperature cycle changing from 4 °C at 2:00 a.m. to 20 °C at 2:00 p.m.) and high (10-30 °C diurnal temperature cycle changing from 10 °C at 2:00 a.m. to 30 °C at 2:00 p.m.) temperatures (Chen and Line, 1995Chen, 2013; Chen and Kang, 2017).

Infection type of the seedling test were rated 16-18 days post inoculation and 18-22 days post inoculation for the adult-plant tests based on the 0 to 9 scale described previously.

QTL Mapping

Mapping population development

A doubled haploid (DH) mapping population between Hatcher and Denali was developed by Heartland Plant Innovations (Manhattan, KS) using the wheat x maize (*Zea mays* L.) hybridization method (Laurie and Bennett, 1988; Santra et al., 2017). Hatcher was selected from a cross with a complex pedigree (Yuma/PI 372129//TAM-200/3/4*Yuma/4/KS91H184/Vista) and released by CSU in 2004 (Haley et al., 2005). Hatcher has shown a consistent moderately resistant field reaction to stripe rust in Colorado since the original race change in the Great Plains that appeared in 2001. Denali was selected from the cross CO980829/TAM 111 and was released cooperatively by Colorado State University (CSU) and Kansas State University (KSU) in 2011 (Haley et al., 2012). Denali showed resistance to stripe rust prior to and during the stripe rust epidemic in 2010 but became susceptible to the new race outbreak in 2012. The cross was made in 2014 (with Denali as female and Hatcher as male), doubled haploid generation was done from the F_1 generation in 2015-2016, and seed of the doubled haploid plants was increased in the greenhouse at Fort Collins, CO in spring 2017.

Field evaluation

The DH population, its parents, and the susceptible check Ripper were evaluated in the field in 2018 and 2019 using the virulent stripe rust isolates PST2010 and PST2012 described above. Field environments included the Colorado State University Agricultural Research Development and Education Center (ARDEC) in Fort Collins, CO and the Kansas State University Kansas River Valley Experiment Field at Rossville, KS. The trials were drill seeded in 1-m long double-rows with three replications at Fort Collins and 1.4-m long single-rows with two replications at

Rossville in an augmented incomplete block design. Planting dates and stripe rust scoring dates varied between Rossville and Fort Collins due to the geographical differences between the two locations. The trials were sown on September 13, 2017 at Fort Collins and on October 18, 2017 at Rossville for the 2018 experiments and on September 21, 2018 at Fort Collins and on October 20, 2018 at Rossville for the 2019 experiments. At both locations, experiments were artificially inoculated with a mixture of spores of PST2010 and PST2012. To increase the chances of infection, Ripper (Fort Collins) or experimental line KS89180B (Rossville) were planted as susceptible spreaders within the trials.

Different methods were used to inoculate the experiments at Fort Collins and Rossville. For Fort Collins, Ripper plants were grown in the greenhouse, inoculated at the seedling stage, and transplanted throughout the field spreader rows in April (jointing stage) after stripe rust infection was observed. At Rossville, spreader rows were sprayed at the jointing stage with a suspension (1 ml liter^{-1}) of fresh urediniospores in Soltrol 170 isoparaffin oil (Chevron-Philips Chemical Co., Texas, U.S.). The suspension was applied using a battery-powered ultralow volume atomizer (Ulva+, Chinagros International Corporation Ltd., Zhejiang, China).

Due to unfavorable conditions for stripe rust infection in 2018, stripe rust infection type (IT) was rated (as described above) only once at Fort Collins at the soft dough stage (Zadoks stage 69 – 85) and twice at Rossville at the heading stage (Zadoks stage 55 – 60) and soft dough (Zadoks stage 69 – 85) stage (Zadoks et al., 1974). Disease severity (DS) was assessed once at the soft dough stage (Zadoks stage 69 – 85) at both locations, recorded based on a 0 to 100% scale representing the percentage leaf area covered by stripe rust; a DS score of 5% represents low severity, whereas, a DS score of 100% means highly severity. In 2019, both IT and DS were rated twice, once at the heading stage and again at the soft dough stage. Only one rating that was taken when the severity was at its highest, was used for analysis.

Genotyping and linkage map construction

Seeds of each entry were planted and grown at the CSU greenhouse facilities. Leaf tissue samples were collected from plants at the single leaf stage and genomic DNA was extracted in a 96-well format using King Fisher 96 magnetic bead Extraction Kits on the King Fisher Flex Purification system (ThermoFisher Scientific Inc., Waltham, MA, U.S.A). Libraries for genotyping by-sequencing (GBS) were constructed using a protocol modified from Poland et al. (2012). High-throughput sequencing and genotyping were done at the Roy J. Carver Biotechnology Center (University of Illinois, Urbana, IL). Single-nucleotide polymorphism calls were made using the TASSEL-GBSv1 pipeline (Glaubitz et al., 2014), which is a reference-based SNP calling procedure. The International Wheat Genome Sequencing Consortium (IWGSC) 'Chinese Spring' RefSeq (v1.0) was used as the reference genome for assigning SNP markers to chromosomes.

Genetic linkage map construction, marker filtering, and QTL detection procedures were performed in a number of steps in the desktop version of TASSEL 5.2 (Glaubitz et al., 2014) and R statistical software (R Core Team 2016). Prior to linkage map construction, SNPs were filtered by scoring heterozygous calls as missing data and removing SNPs with >20% missing data. SNPs with a minor allele frequency of 0.2 or greater were retained and the ABH-plugin option in TASSEL was used to convert SNPs from nucleotide-format to the parent-based format. Markers showing a high degree of segregation distortion (p-value < 0.01), duplicated markers, individuals with high genotypic errors (higher number of crossovers and double crossovers than the threshold), and linkage groups with five or fewer markers were removed using commands in *R/qtl* v1.42 package in R (Broman et al., 2003). After filtering and removing markers with high missing data, high segregation distortion, and individuals with high genotypic errors, the final linkage map was constructed with 202 DH lines and 4,441 high quality SNP markers. The *ASMap* package (Taylor and Butler, 2017) in R was used for map construction using the Kosambi mapping function.

Statistical analysis and QTL mapping

Best linear unbiased predictors (BLUPs) for IT and DS for each DH line (n=210) were obtained using the *lme4* package (Bates et al., 2015) in R. Data were analyzed using both single-environment and across-environment models with genotypes, and locations as random effects. Variance components for both IT and DS were estimated using a fully random model in *lme4* for each location separately to estimate entry-mean heritability (H^2) according to the following formula:

$$h^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_{ge}/e + \sigma^2_e/re)$$

where, σ^2_g is the variance component for genotypes, σ^2_{ge} is the variance components for genotype x environment interaction, σ^2_e is the error variance component, *r* is the number of replications, and *e* is the number of environments (Fehr, 1991). A rank correlation test using the 'Spearman' method was conducted to test the correlation association for IT and DS for within and among the environments in R. The QTL peak logarithm of odds ratio (LOD) scores were used to calculate the percentage of the phenotypic variation explained (PVE) by each QTL using the formula $PVE = 1 - 10^{((-2*LOD)/n)}$, where, n is the number of individuals and LOD is the LOD peak value calculated using the *scanone()* function in R/qtl. QTL were detected using the standard interval mapping and Expectation-Maximization (EM) algorithm method with a permutation test of = 2000 in R/qtl (Broman et al., 2003). QTL calls were made for each environment separately and also for across environments.

RESULTS

Greenhouse Stripe Rust Testing

The parents of the DH population, Denali and Hatcher, and the susceptible check Ripper showed highly susceptible infection types (IT) when inoculated with six virulent stripe rust races at the seedling stage under low temperature (4-20 °C) in the greenhouse. For each of the six races tested, an IT of 8 was observed for all three entries (Table 1).

In the four-way test, the parents and the susceptible check each showed susceptibility to both isolates under the low-temperature seedling-stage test, but differences among the entries were observed at the adult-plant stage under low temperature (Table 2). Denali showed an intermediate reaction to PST2010, with an IT=5, but was susceptible to PST2012 with an IT=8. Hatcher showed an intermediate reaction to both isolates with an IT=6 and Ripper was susceptible to both isolates, with an IT=8 (Table 2).

Under the high-temperature experiment, both Denali and Hatcher showed susceptible reactions to both isolates (IT=7 to 8) at the seedling stage, similar to the low-temperature experiment. At the adult-plant stage, Denali was resistant (IT=4) to isolate PST2010 and susceptible (IT=8) to isolate PST2012, whereas, Hatcher showed a moderately resistant reaction to both isolates IT=5. The susceptible check Riper had a susceptible reaction with an average IT=8 to both isolates (Table 2).

Field Stripe Rust Testing

In 2018, stripe rust infection was low at both field locations due to unfavorable temperature conditions for stripe rust development. Disease pressure was abnormally low and inconsistent across the field at Fort Collins as evidenced by a lack of stripe rust infection in the Ripper

spreader rows in various places throughout the field. The data for Fort Collins in 2018 were therefore not used for any further analyses. At Rossville in 2018, the parents showed similar infection types (Hatcher IT=4 and Denali IT=5) though Denali showed a greater disease severity than Hatcher (Hatcher DS=5 and Denali DS=20). Variation was observed for the DH population for infection types with a mean IT=3.7 (range 0 to 9). Disease severity ranged from 3 to 90% with a mean DS=12.5 (Table 3).

In 2019, stripe rust infection was very high at both locations, with the susceptible check Ripper showing IT=9 and DS=100 at Fort Collins and up to IT=8 and DS=90 at Rossville. Hatcher displayed a lower infection type and disease severity at Rossville (IT=6 and DS=15) compared to Denali (IT=7 and DS=25). Similarly, at Fort Collins, Hatcher had a lower infection type and disease severity (IT=5 and DS=30) compared with Denali (IT=8 and DS=70). While variation was observed among the DH lines at both locations, the overall IT average was relatively similar for the two locations (Fort Collins IT=5.6, Rossville IT=5.0). Across the DH population, a slightly higher disease severity was observed at Fort Collins (DS=40.7) compared with Rossville (DS=23.4). The histograms of the BLUP values shows that the DH population has transgressive phenotypic values for IT and DS compared to the phenotypic values observed in the parental lines. The extreme phenotypic values on the two ends of the distribution represent higher resistant and susceptible reactions compared to the parents, which is explained as positive and negative transgressive segregation beyond the parents (Figure 1). Furthermore, the average IT and DS values for the 10 most resistant and susceptible DH lines compared with the average values of the parental lines showed a transgressive segregation at all three environments (Table 3). The Spearman rank correlation test showed a high correlation between infection type and disease severity in the field. The correlations were highly significant within and among the environments except for the IT in 2018 at Rossville (Table 4).

Linkage Map and Marker Density

A total of 4,441 SNP markers were retained based on the filtering criteria and were used for linkage map construction (Figure 2). The 4,441 markers were distributed across 35 linkage groups and spanned a total length of 18486 cM, covering 20 of 21 wheat chromosomes (Figure 3). Due to the low marker coverage (less than 5 markers) in each linkage group on chromosome 4D, no markers were retained for this chromosome after filtering. The genetic linkage map produced an average marker spacing of 4.2 cM and a maximum spacing of 38.6 cM. Markers were unevenly distributed across the three genomes of wheat, with the A genome harboring 1,656 markers (37.3%), the B genome 2,339 markers (52.7%), and the D genome 446 markers (10.0%). Marker density was similar among the three genomes, with the A genome having the highest density (1 SNP per 3.9 cM) compared with the B genome (1 SNP per 4.2 cM) and D genome (1 SNP per 4.1 cM). In general, the genetic map was inflated due to higher genetic recombination frequencies than expected and the reported genetic distances are generally higher than usual.

QTL Analysis

Three major-effect (R^2 >10%) QTLs and one minor-effect (R^2 <10%) QTL were identified for resistance to stripe rust in the DH population. The major-effect QTL included chromosome 1BL (LOD = 7.79, R^2 = 16.3%), 3AL (LOD = 6.8, R^2 = 14.4%), and 3BS (LOD = 5.18, R^2 = 11.1%) while the minor-effect QTL was identified on chromosome 7BL (LOD = 3.43, R^2 = 7.5%) (Table 5). Of these four, the *QYr.csu-7B* QTL was identified only at Rossville in 2018, the *QYr.csu-3BS* QTL was identified only at Fort Collins in 2019, and the *QYr.csu-1BL* and *QYr.csu-3AL* QTLs were identified at both locations in 2019. Based on the combined analysis across locations, the *QYr.csu-1BL*, *QYr.csu-3AL*, and *QYr.csu-3BS* QTLs were each significant for 2019 (Figure 4). While *QYr.csu-1BL* and *QYr.csu-3AL* were significant for resistance based on both infection type and disease severity, *QYr.csu-3BS* and *QYr.csu-7B* were only significant for resistance

based on infection type. The resistance allele for the *QYr.csu-7BL* QTL at Rossville in 2018 was contributed by the Hatcher parent, whereas the resistance alleles for the *QYr.csu-1BL*, *QYr.csu-3AL*, and *QYr.csu3BS* QTLs were each contributed by the Denali parent (Figure 5).

DISCUSSION

Due to the rapid evolution of new races of the stripe rust pathogen, and its apparent increasing adaptation to warmer regions, stripe rust has become a prevalent problem in the United States over the last two decades (Chen, 2005; Markell and Milus, 2008). While the Great Plains region was not considered an overwintering area for the stripe rust pathogen, higher winter temperatures over the last 10-15 years, together with possible adaptation of stripe rust to milder winters, has increased the risk for stripe rust epidemics (Lyon and Broders, 2017).

In this study, both of the parents (Denali and Hatcher) were susceptible at the seedling stage under both low and high temperatures to stripe rust isolates (PST2010 and PST2012) that are virulent on *Yr17* and the resistance present in the winter wheat cultivar Everest (PI 659807). The parents also showed susceptibility to six virulent U.S. stripe rust races (*PSTv-4*, *PSTv-14*, *PSTv-37*, *PSTv-40*, *PSTv-51* and *PSTv-198*) when tested under low temperatures at the seedling stage. At the adult-plant stage, however, Hatcher showed an intermediate to resistant infection type under high temperatures when infected with both isolates. This result is in agreement with field observations of Hatcher showing a moderate level of resistance under multiple stripe rust epidemics since its release in 2004. Denali, on the other hand, showed moderate resistance under both temperatures with the PST2010 isolate but a susceptible reaction with the PST2012 isolate. This result for Denali is in close agreement with past field

observations that during the 2010 epidemic Denali was resistant but it became highly susceptible during and since the 2012 epidemic (Table 2).

In the field evaluations, disease pressure during 2018 was less severe, particularly at the Fort Collins location, most likely due to hot weather that suppressed the growth of stripe rust in susceptible entries. At Rossville KS in 2018, however, the DH population showed clear segregation for resistant and susceptible types (Table 3). In 2019, disease development was heavy and uniform at both locations and strong positive correlations were observed between IT and DS within and across environments except for IT 2018 (Table 4). The high positive correlations between the IT and DS within and between environments indicates that the stripe rust establishment was uniform at both locations and that the visual scoring was reliable.

In the QTL mapping, Denali contributed each of the resistant alleles for IT and DS with the exception of Rossville 2018 where Hatcher contributed the resistant allele for IT (Table 5). The genetic map was inflated due to a higher rate of crossovers and double crossovers observed in the DH population and QTL locations were detected at higher centimorgan (cM) distances compared to other QTL mapping studies reported in common wheat (Figure 3). Therefore, the following comparison of QTL with previously mapped genes or QTL is discussed based on the physical position of the peak QTL with respect to the IWGSC RefSeq v1.0 reference.

QYr.csu-1BL

QYr.csu-1BL was the second most consistently detected QTL after *QYr.csu-3AL* for IT and DS across environments. This QTL was detected on the long arm of chromosome 1B at 673.7 megabase pairs (Mbp) physical position in the IWGCS RegSeq v1.0 (Table 5). *QYr.csu-1BL* had the highest LOD score (7.8) among all detected QTL (Figure 4). *Qyr.csu-1BL* explained 9.6% of the total phenotypic variation for IT and 16.3% for DS at Fort Collins. It explained 6.9%

of the total phenotypic variation for IT at Rossville and 10% for IT and 14.5% for DS for the combined environments in 2019. The allele for resistance at *QYr.csu-1BL* was contributed by Denali.

Chromosome 1B is an enriched region for functional genes. Multiple genes for resistance to various pathogens have been mapped in this chromosome, including powdery mildew (*Blumeria graminis*), stem rust (*Puccinia graminis*), leaf rust (*Puccinia triticina*) and stripe rust (Cobo et al., 2018). More than 10 stripe rust resistance genes and 27 resistance QTL have been mapped to this chromosome, with two resistance genes (*Yr26*, and *Yr29/Lr46*) and 16 resistance QTL specifically located to the long arm of chromosome 1B (Chen and Kang, 2017; Rosewarne et al., 2006). Hou et al. (2015) identified loci for HTAP resistance on the long arm of chromosome 1B that explained 2.3% to 31.0% of the phenotypic variation for resistance. Lan et al. (2014) also identified a large effect APR QTL, explaining up to 43.6% of the phenotypic variation, on the long arm of chromosome 1B that was also closely linked to *Lr46/Yr29*. The stripe rust resistance gene *Yr29* is an adult plant resistance gene that is pleiotropic or co-located with the leaf rust resistance gene *Lr46* (Rosewarne et al., 2012). *Yr29* has since been regularly mapped to the long arm of chromosome 1BL between the flanking simple sequence repeat (SSR) markers '*wmc44*' and '*gwm140*', where these two markers correspond to a physical position between 662.2 and 684.9 Mb in the IWGSC RefSeq v1.0 (Cobo et al., 2018). Since, *QYr.csu-1BL* was mapped to the 673.7 Mb physical position of the IWGSC RefSeq v1.0, this position is within the previously mapped *Yr29* interval, therefore, these results suggest that *QYr.csu-1BL* may be allelic to *Yr29.* Fine mapping experiments may help to clarify possible relationships between *Yr29* and the resistance on chromosome 1BL found in Denali.

QYr.csu-3AL

Among the QTLs identified in this study, *QYr.csu-3AL* was the only QTL detected across years (for DS) and across environments in 2019 (for both IT and DS). This QTL was positioned on the

long arm of chromosome 3A at 624 Mb physical position in the IWGSC RefSeq v1.0 (Table 5), explaining 10% of the phenotypic variation for resistance for IT and 6.7% for DS at Rossville and 10% for IT and 14.4% for DS at Fort Collins Moreover. *QYr.csu-3AL* explained 11.7% of the phenotypic variation for IT and 13.8% for DS for combined environments analysis for the 2019 season. Previous studies have also reported stripe rust resistance QTL on chromosome 3A, mostly with minor phenotypic effects. For example, Zou et al. (2017) reported a minor effect (R^2=6.7–8.5) QTL on chromosome 3A. Lillemo et al. (2008) reported a minor effect QTL associated with stripe rust resistance that mapped on chromosome 3A, co-localized with a powdery mildew resistance QTL. Rosewarne et al. (2013) and Buerstmayer et al. (2014) also reported minor effect stripe rust resistance QTL on chromosome 3A in Avocet and Arina wheat cultivars, respectively. The stripe rust resistance gene *Yr76*, previously known as *Yr.Tye* (Xiang et al., 2016), is the only permanently named resistance gene that has been mapped on chromosome 3A so far and it is mapped on the short arm of this chromosome (Chen and Kang, 2017). *Yr76* confers an all-stage resistance reaction to race PSTv-37. Considering the chromosomal locations of *Yr76* and *QYr.csu-3AL*, *QYr.csu-3AL* is mapped on the opposite arm of the chromosome 3A than the *Yr76* and therefore, we do not propose if the *QYr.csu-3AL* is co-localized with the *Yr76*

QYr.csu-3BS

The *QYr.csu-3BS* was detected on the distal region of the short arm of chromosome 3B, mapped at 7.3 Mb physical position on IWGSC RefSeq v1.0 (Table 5). This QTL was identified only at Fort Collins in 2019, providing resistance through a reduced IT, with a LOD score of 5.18 (R^2=11.1%). With the analysis over environments for 2019, this QTL was detected with a lower level of significance (LOD=3.39, R^2=7.4%). Denali contributed the resistance allele for *QYr.csu-3BS*.

Chromosome 3B is considered the largest chromosome (Paux et al. 2008) in the wheat genome and contains at least four stripe rust resistance genes (*Yr4, Yr30, Yr57* and *Yr58*) and at least 30 stripe rust resistance QTLs (Q3B.1 – Q3B.30) (Chen and Kang 2017). The distal region of the short arm of chromosome 3B is well known due to the importance of the partial stripe rust resistance APR gene Yr30 (Spielmeyer et al., 2005) and it is pleiotropic responses with the stem rust resistance gene Sr2. Another important HTAP stripe rust resistance gene, designated as *Yr58* (Chhetri et al., 2016), has also been mapped on the short arm of chromosome 3B.

Basnet et al. (2014) described a QTL mapping study with a CIMMYT wheat line known as 'Quaiu 3', which shows an immune reaction to stripe rust in Mexico. A QTL for resistance in Quaiu 3 was mapped on the short arm of chromosome 3B, colocalized with *Yr30*, explaining up to 17% of the phenotypic variation observed. Similarly, Huo et al. (2011) mapped three significant stripe rust resistance QTL on the short arm of chromosome 3B in a soft red winter wheat recombinant inbred line population, explaining an average phenotypic variation of 10%. The chromosomal region where *QYr.csu-3BS* is located is close to the previously mapped adult plant resistance gene *Yr3*0 and the HTAP resistance gene *Yr58*. Further fine mapping experiments are needed to demonstrate if the *QYr.csu-3BS* and these other known genes are localized in the same region.

QYr.csu-7BL

QYr.csu-7BL was mapped on the long arm of chromosome 7B at the physical position of 723 Mb on the IWGSC RefSeq v1.0 (Table 5). This QTL was for infection type, and it was the only resistance QTL detected at Rossville in 2018. *QYr.csu-7BL* was significant with a LOD score = 3.43, explaining only 7% of the phenotypic variation. The resistance allele for this QTL was derived from Hatcher.

Chromosome 7B is an important chromosome that confers resistance to stripe rust in wheat. Several important stripe rust resistance genes or QTLs are located on the long arm of chromosome 7BL, including six resistance genes [*Yr6*, *Yr39 (HTAP)*, *Yr52 (HTAP)*, *Yr59 (HTAP)*, *Yr63* and *Yr67*] and 14 QTLs for resistance (Chen and Kang, 2017). All HTAP resistance genes within chromosome 7B are mapped on the long arm of this chromosome. Most of the QTL on this chromosome were reported as being small-effect QTLs, except the HTAP resistance gene *Yr39* (Lin and Chen, 2007) that explained 64.2% of the phenotypic variation in a QTL mapping study with the spring wheat cultivar Alpowa (Chen and Kang 2017). Vazquez et al. (2015) mapped a minor effect QTL (R^2=6.7%) on the long arm of chromosome 7B in a winter wheat population of 'Einstein' x 'Tubbs'. Ren et al. (2012a) and Rosewarne et al. (2012) mapped resistance QTL on the long arm of chromosome 7B that explained 14.7% and 5.4% of the phenotypic variation for resistance, respectively. The mapped region (7BL distal region) of *QYr.csu-7BL* and the effect size of this QTL are in agreement with several QTL studies, including Ren et al. (2012a) and Rosewarne et al. (2008). Additionally, three HTAP resistance genes also mapped in this region of chromosome 7BL. Although *QYr.csu-7BL*, *Yr39*, and *Yr52* are located on the same region of the chromosome, it is unclear if these represent the same genes or are alleles at the same locus. Future studies are needed to clarify any potential association between Yr39 and Yr52 and the QTL identified in this study.

CONCLUSIONS

In this study three major-effect QTLs and one small-effect QTL were identified in a winter wheat doubled haploid mapping population tested under field conditions at Fort Collins CO and Rossville KS. The large-effect QTL were identified on chromosome arms 1BL, 3AL, and 3BS and the small-effect QTL was identified on chromosome arm 7BL. While both of the parents contributed resistance QTL, the resistance QTL derived from Hatcher was detected in only one environment (Rossville 2018). Denali contributed a greater number of resistance QTL in this study, possibly due to the fact that the mixture of PST2010 and PST2012 isolates were used to infect the field. Since Denali had the lowest IT when infected with isolate PST2010 in the greenhouse, the results from the *QYr.csu-1BL*, *QYr.csu-3AL* and *QYr.csu-3BS* may suggest that these QTLs are more involved with resistance to isolate PST2010. In view of the Hatcher reactions in the field and under high temperature tests in the greenhouse, QYr.csu-7BL may at least partially explain the HTAP reaction observed in Hatcher. While the genetic map developed from the DH population was inflated to some extent, and the QTL were identified at larger genetic positions, at least three out of the four identified QTL were mapped to chromosomal regions previously reported as being highly associated with other stripe rust resistance genes or QTLs. As transgressive segregation was observed in the population, pyramiding these four QTL could reduce the infection type and disease severity in response to the virulent stripe rust isolates PST2010 and PST2012. Further fine mapping and QTL validation studies may be needed, however, to confirm the validity of this approach to further development of durable stripe rust resistance in new cultivars.

Table 1. Infection type of the parental cultivars Denali and Hatcher and the susceptible check Ripper inoculated with six virulent *Puccinia striiformis* f. sp. *tritici* races at the seedling stage in the greenhouse at low temperatures (4-20 °C).

	Race					
Cultivar	PSTv-4	PSTv-14	PSTv-37	PSTv-40	PSTv-51	PSTv-198
Denali (parent)	8[†]	8	8	8	8	8
Hatcher (parent)	8	8	8	8	8	8
Ripper (check)	8	8	8	8	8	8

[†] Infection type was recorded based on 0-9 scale. Generally IT 0-3 are considered resistant, 4-6 are considered intermediate, and 7-9 are considered susceptible.

Table 2. Infection type of the parental cultivars Denali and Hatcher and the susceptible check Ripper inoculated with two virulent isolates of *Puccinia striiformis* f. sp*. tritici* under the four-way test in the greenhouse.

	Low-temperature (4-20 °C) testing			
	Isolate PST2010		Isolate PST2012	
Cultivar	Seedling	Adult-Plant	Seedling	Adult-Plant
Denali (parent)	7	5	8	7
Hatcher (parent)	7	6	7	6
Ripper (check)	8	8	8	8

	High-temperature (10-30 °C) testing			
	Isolate PST2010		Isolate PST2012	
Cultivar	Seedling	Adult-Plant	Seedling	Adult-Plant
Denali (parent)	7	4	7	8
Hatcher (parent)	7	5	7	5
Ripper (check)	8	8	9	8

† Infection type was recorded based on 0-9 scale. Generally IT 0-3 are considered resistant, 4-6 are considered intermediate, and 7-9 are considered susceptible.

Table 3. Average Infection type and disease severity of the parent cultivars Denali and Hatcher and doubled haploid (DH) lines in each environment.

	Infection Type[†]	Disease Severity [‡]
	Rossville 2018	
All DHs	3.4	12.5
10 most Resistant DHs	1.0	1.0
10 most Susceptible DHs	8.4	60.0
Denali	3.5	8.0
Hatcher	3.0	5.0
	Fort Collins 2019	
All DH	5.6	40.7
10 most Resistant DHs	3.2	15.0
10 most Susceptible DHs	8.9	87.5
Denali	7.0	54.3
Hatcher	5.0	24.0
	Rossville 2019	
All DH	5.0	23.4
10 most Resistant DHs	3.5	3.0
10 most Susceptible DHs	8.1	82.5
Denali	5.7	18.0
Hatcher	5.0	9.0

[†] Infection type was recorded based on 0-9 scale. Generally IT 0-3 are considered resistant, 4-6 are considered intermediate, and 7-9 are considered susceptible.

[‡] Disease severity is recorded based on a 0-100% scale representing the percentage leaf area covered by stripe rust.

Table 4. Spearman rank correlation coefficients between measured variables for the Denali/Hatcher doubled haploid population at individual and across field environments.

	IT_19_Overall †	DS_19_Overall ǂ	IT_18_RV ¶	DS_18_RV §	IT_19_RV #	DS_19_RV ††	IT_19_FC ǂǂ	DS_19_FC §§
IT_19_Overall	1	0.89***	0.09^{NS}	0.76***	0.86***	0.76***	0.96***	0.80***
DS_19_Overall		1	0.05^{NS}	0.85***	0.77***	0.85***	0.85***	0.90***
IT_18_RV			1	0.02^{NS}	0.10^{NS}	0.02^{NS}	0.07^{NS}	0.07^{NS}
DS_18_RV				1	0.80***	1.00***	0.64***	0.53***
IT_19_RV					1	0.80***	0.69***	0.57***
DS_19_RV						1	0.64***	0.53***
It_19_FC							1	0.83***
DS_19_FC								1

† infection type for both locations during 2019.

ǂ disease severity for both locations during 2019.

¶ infection type Rossville 2018.

§ disease severity Rossville 2018.

infection type Rossville 2019.

†† disease severity Rossville 2019.

ǂǂ infection type Fort Collins 2019.

§§ disease severity Fort Collins 2019.

*** highly-significant.

NS non-significant

Table 5. Quantitative Trait Loci (QTL) detected using single and combined environments analysis from r/qtl and ASMap.

			Single Environment				Combined Environments 2019	
QTL‡	QTL ID	Trait#	IWGSC Ref Seq v1.0¶	Environments	R^2§	Additive Effectł	IWGSC Ref Seq v1.0¶	R^2§
			Mb		%		Mb	%
QYr.coh-1BL	1	IT	673.7	Fort Collins 2019	9.6	0.3	673.7	10
				Rossville 2019	6.9	0.2		
QYr.coh-1BL	1	DS	673.7	Fort Collins 2019	16.3	3.8	673.7	14.5
QYr.coh-3AL	2	IT	624.8	Fort Collins 2019	10	0.3	624.8	11.7
				Rossville 2019	10.1	0.2		
QYr.coh-3AL	2	DS	624.8	Fort Collins 2019	14.4	3.7	624.8	13.8
				Rossville 2019	6.7	3.8		
				Rossville 2018	6.7	3.7		
QYr.coh-3BS	3	IT	7.3	Fort Collins 2019	11.1	0.3	7.3	7.4
QYr.coh-7BL	4	IT	723.06	Rossville 2018	7.5	-0.4	--	--

‡ Only QTL with LOD = > 3. The QTL name includes trait, institute name and chromosome name.

DS, disease severity; It, Infection type.

¶ Mega base pair positions based on International Wheat Genome Sequencing Consortium (IWGSC) RegSeq v1.1.

§ The percentage of phenotypic variance explained by the significant QTL.

ł Average additive effect explained by each QTL based on the BLUP values for IT (0-9) and DS (%). Positive values indicates that Denali increased the resistance to stripe rust, negative values indicates that Hatcher increased the resistance to stripe rust

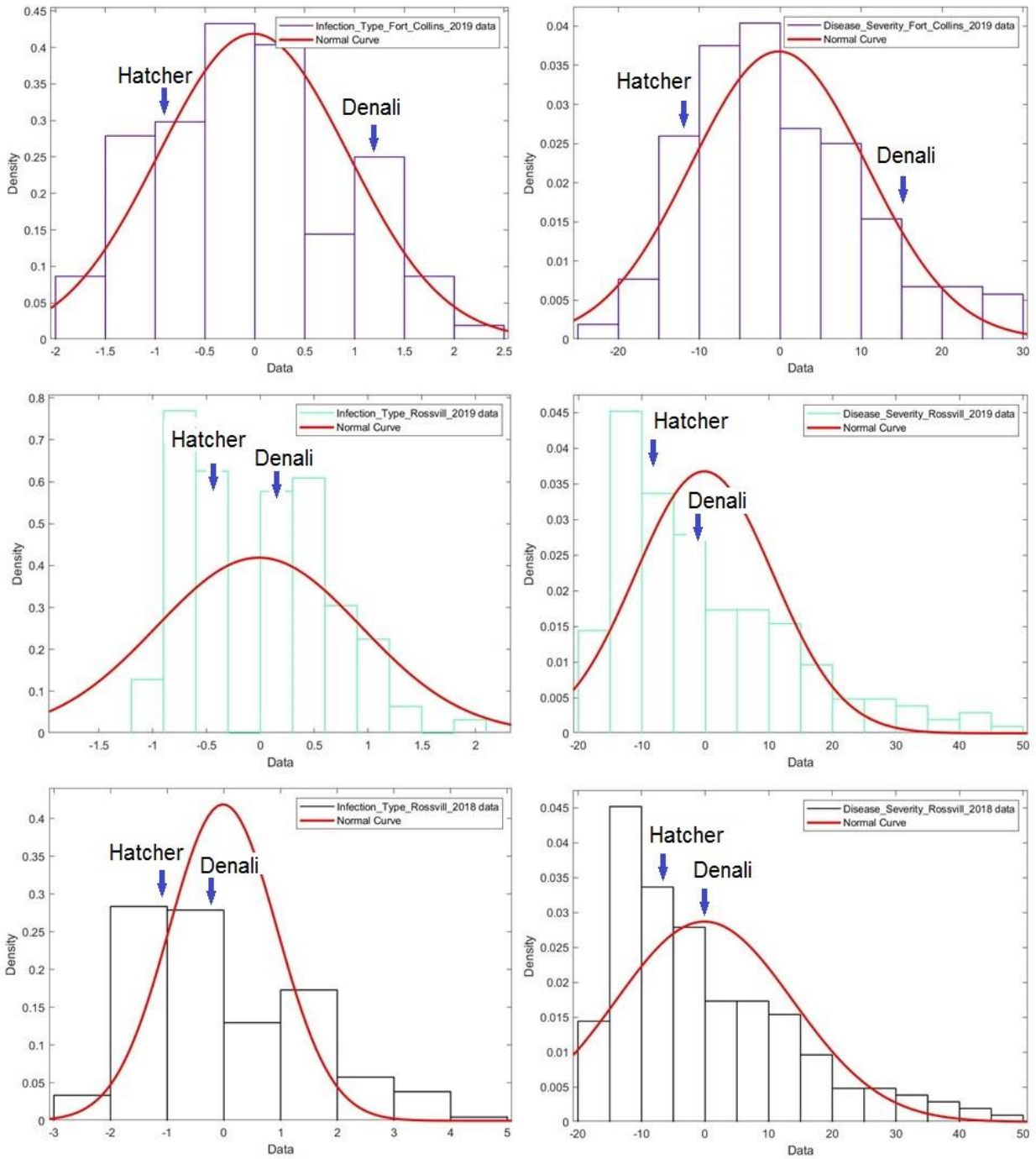

Figure 1. Histograms of the Best Linear Unbiased Predictor (BLUP) values of the DH population and the parent cultivars Hatcher and Denali for all environments.

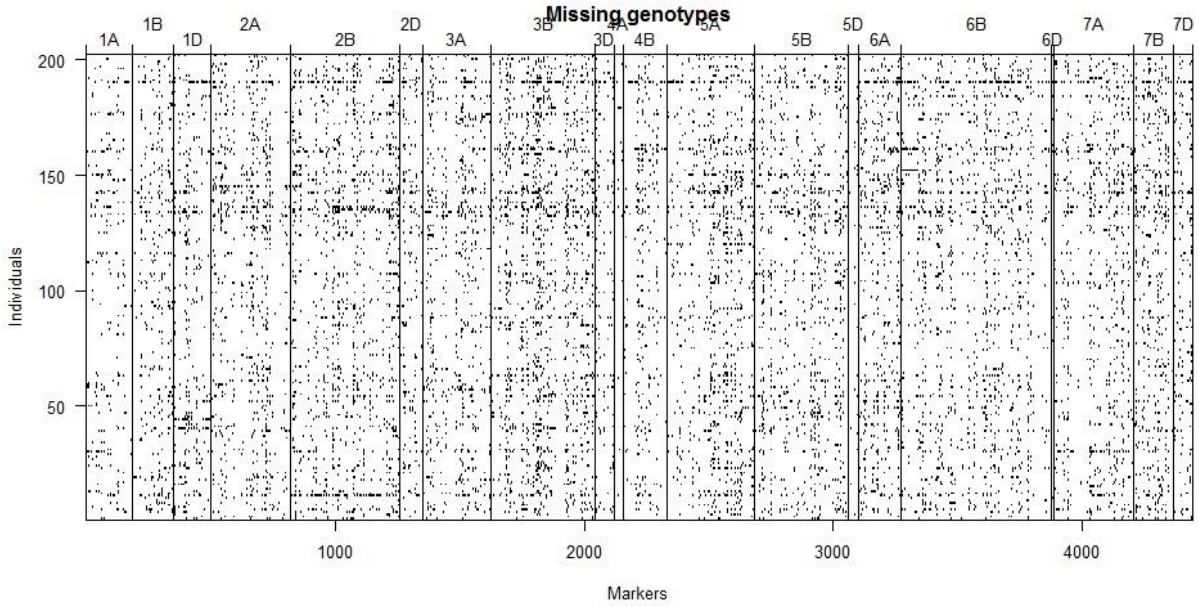

Figure 2. Pattern of missing marker data in the Denali/Hatcher doubled haploid population. Black pixels indicate missing genotypes. The X-axis represents the number of each marker and the Y-axis represents the number of each individual

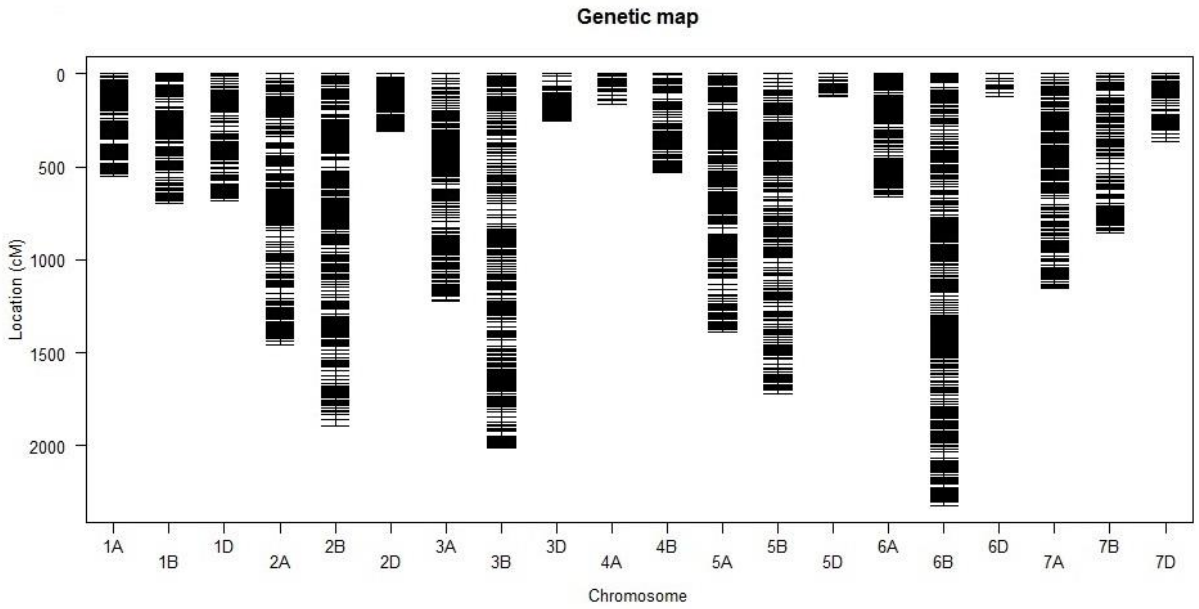

Figure 3. Estimated genetic map of the Denali/Hatcher doubled haploid population after assignment of linkage groups to the respective chromosome. The X-axis shows the name of each chromosome and the Y-axis represents the length of each chromosome in centiMorgans (cM).

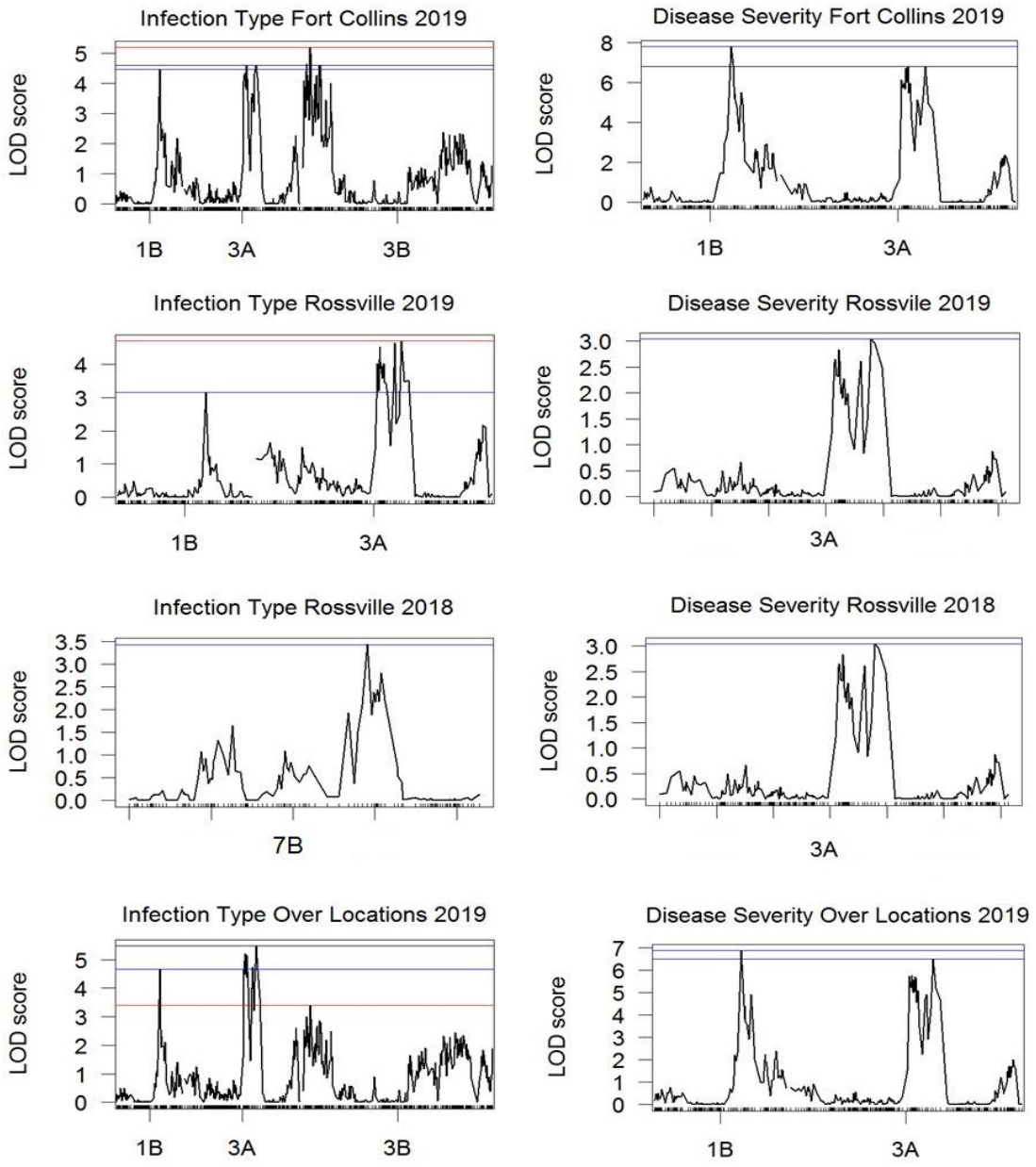

Figure 4. Significant quantitative trait loci (QTL) for infection type (IT) and disease severity (DS) identified in the Denali/Hatcher doubled haploid mapping population. Plots shows single and multiple QTL peaks for single environments and combined over environments. The X-axis represents the chromosomal location and the Y-axis represents the logarithm of odds (LOD) score for each QTL peak. The parallel black, blue and red lines to the X-axis are representing the peak logarithm of odds scores for each QTL.

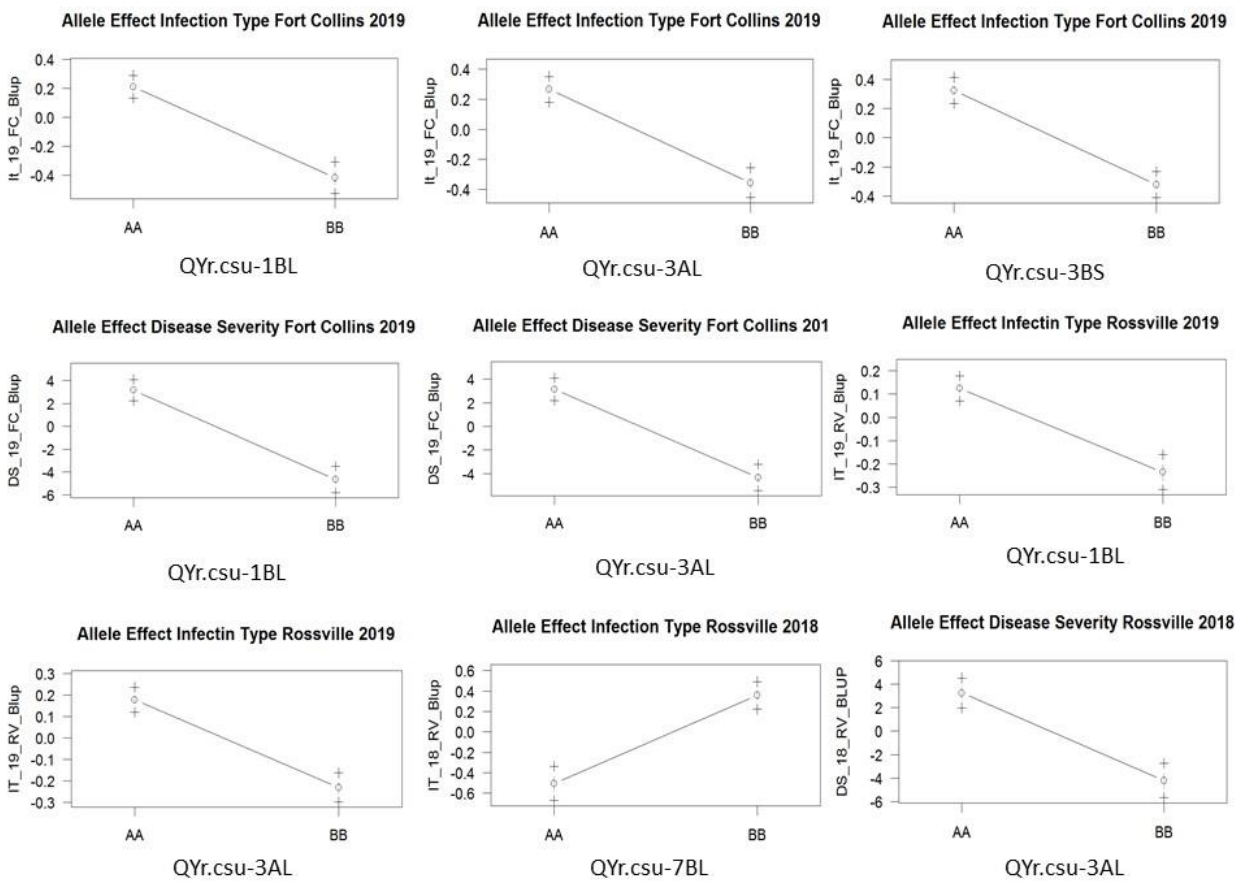

Figure 5. Parental lines contribution to resistance QTL for infection type (IT) and disease severity (DS). For each plot, the X-axis represents the genotype allelic effect of the parental lines (AA = Hatcher, BB = Denali), whereas the the Y-axis represents the phenotypes. SNP names on the X-axis represent the peak marker for that respective QTL. Error bars represent the standard error of the QTL effect.

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