IDENTIFICATION OF STEM RUST RESISTANCE QUANTITATIVE TRAIT LOCI IN

DURUM WHEAT POPULATIONS

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Kennedy Lauren Lund

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Program:
Genomics and Bioinformatics

April 2022

Fargo, North Dakota

North Dakota State University
Graduate School

Title

IDENTIFICATION OF STEM RUST RESISTANCE QUANTITATIVE TRAIT LOCI IN DURUM WHEAT POPULATION

By

Kennedy Lauren Lund

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota

State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Upinder Gill

Co-Chair

Dr. Jason Fiedler

Co-Chair

Dr. Zhaohui Liu

Approved:

04/12/2022	Phil McClean
Date	Department Chair

ABSTRACT

Stem rust (*Puccinia graminis* f. sp. *tritici*) negatively impacts durum wheat production worldwide. Resistance loci from four resistant landrace durum wheat lines were identified in biparental F_5 recombinant inbred line (RIL) populations after crossing with the susceptible line 'Rusty'. The populations were tested with foreign race of stem rust from Eastern Africa and Europe (JRCQC, TRTTF, TTRTF, and TTKSK) and local races from United States Upper Midwest (MCCF and RKQQ), followed by genotyping and linkage map construction to identify stem rust resistant quantitative trait loci (QTLs). At least one stem rust resistance QTL was identified in each population with a total of twelve QTLs identified overall. Seven of the identified QTL regions validated previously published stem rust resistance genes and the other five identified potentially novel stem rust resistance genes. Various resistance mechanisms were determined from QTL regions that provide stem rust resistance to the four durum wheat RIL populations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

xi

CHAPTER 1. INTRODUCTION

Wheat is a staple cereal crop grown around the world that contains many important vitamins and minerals. It is used to make bread, pasta, pastries, semolina, and many other foods. Globally, China is the largest wheat consumer with Europe, India, and Russia following behind (indexmundi.com 2021). The breeding objectives of wheat, like most crops, allow for expression of desired traits such as yield, cooking qualities, disease resistance, kernel size, and plant height. Research of desired traits has resulted in the identification of numerous genes that impact the desired traits, as well as continued research proving new insights. With global population continuing to rise, food demands keep increasing, and crop producers and scientists are faced with finding solutions to increase yield and production while arable land decreases. Increasing yields of crops would allow for increased production, but there is more to production volume than just the yield.

Diseases and pests impact yield, resulting in losses, and lead to epidemics when they are not effectively controlled by disease/pest management strategies. While many diseases and pests threaten worldwide agricultural production, this study focuses on how stem rust threatens durum wheat production and how to combat this issue. Stem rust can attack any portion of the wheat plant that is above soil level, impacting plant vigor and grain quality. Identification of stem rust resistance genes within durum wheat would allow for locations that experience severe stem rust infections to fight back against those races of stem rust and limit yield loss. This study aimed to 1) Identify stem rust resistant durum wheat lines to use for crossing, 2) Evaluate the progeny of crosses from parent durum wheat lines that have been identified to have high resistance to various races of stem rust, and 3) Perform a genetic analysis to determine the locations of

1

quantitative trait loci that contribute to stem rust resistance and cross reference those locations

with previously discovered stem rust resistance genes.

CHAPTER 2. LITERATURE REVIEW

Durum Wheat Domestication and Importance

Several times throughout history cereal grasses have diverged from one another into separate species. Wheat and rice shared a common ancestor roughly 40-54 million years ago and more recently, wheat and brachypodium shared a common ancestor roughly 32-39 million years ago ("Evolution of Wheat" cerealsdb.uk.net). Wheat and barley diverged from one another roughly 3-4 million years ago ("Evolution of Wheat" cerealsdb.uk.net). Durum wheat (*Triticum turgidum* subsp. *durum*) is a species of wheat derived from emmer wheat and accounts for a small fraction of total wheat production around the world. One of the first domestication events to take place in a cereal was the domestication of wild emmer wheat with the transition to a non-shattering spike (Özkan et al. 2010). Approximately 12,000-10,000 years ago, a second domestication event occurred where domesticated emmer wheat transitioned to durum wheat with a non-hulled grain (Sall et al. 2019). Durum wheat and common wheat genetically diverged from one another approximately 8,000 to 10,000 years ago with the hybridization of *Aegilops tauschii* and the addition of the D-sub genome (Mastrangelo and Cattivelli 2021).

When durum wheat was derived from domesticated emmer wheat, several desirable traits were selected for, such as easy threshability from loss of tough glumes, reduced spike shattering, reduced number of tillers, increased erect growth, and reduced dormancy for seeds (Gioia et al. 2015). Durum wheat and common wheat are closely related; however, durum wheat is a tetraploid (AABB), and common wheat is a hexaploid (AABBDD). Durum wheat is used to make semolina, which is the main ingredient in pasta and couscous, and common wheat is known for making bread, cookies, and cakes (Mastrangelo and Cattivelli 2021).

3

Wheat Production in the World and the United States

Worldwide wheat production was led by China in 2021 with the European Union, India, Russia, United States, and Canada following in production ("World Wheat Production, Consumption, and Stocks," www.usda.gov). Durum wheat only represents approximately 5% of global wheat production. Figure 2.1 shows the proportion of common wheat (winter and spring wheat) to durum wheat planted in the United States since 2000. Durum wheat only accounts for 2-5% of the United States production of wheat and is predominately grown in North Dakota (19,680,000 bushels harvested in 2021) and Montana (10,160,000 bushels harvested in 2021) ("Wheat Sector at a Glance" 2022; Small Grains 2021 Summary). Idaho, California, and Arizona are the other states where durum wheat was harvested in the United States with 539,000, 2,200,000, and 4,680,000 bushels harvested in 2021, respectively (Small Grains 2021 Summary).

U.S. wheat production by class, 2000/01–2021/22
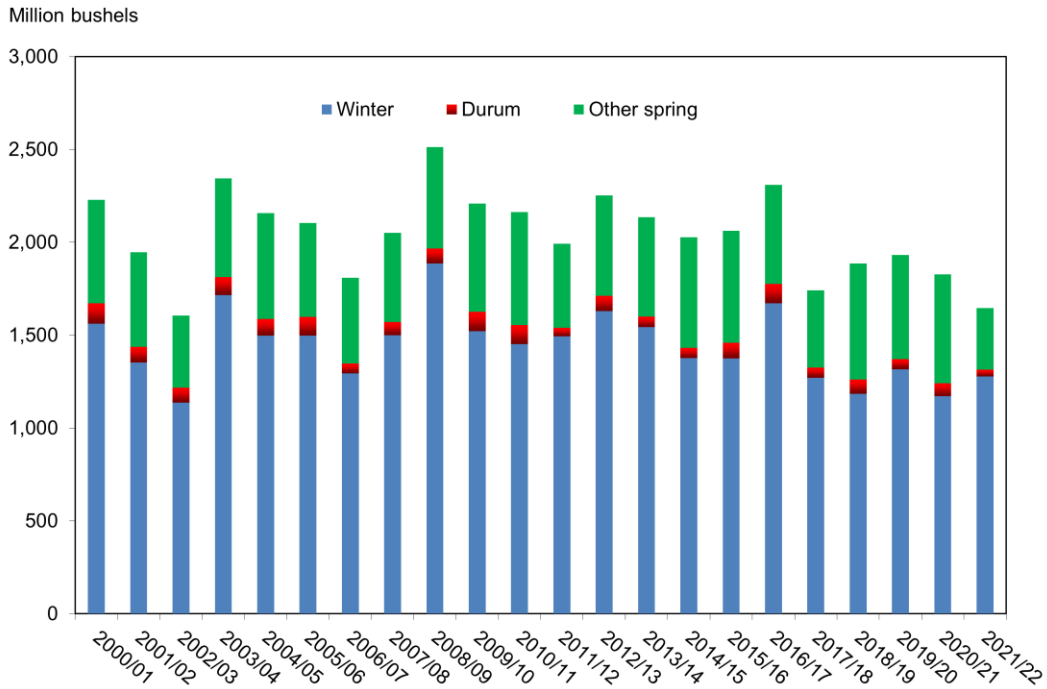
Figure 2.1. U.S. wheat production by class from 2000/01-2021/22.
Note: From "Wheat Sector at a Glance" 2022.

Durum wheat thrives in an environment that provides cool summer nights with long warm days and adequate rainfall during the growing season ("Durum Production", ndwheat.com). With limited areas producing durum wheat, the constantly increasing global demand of agricultural production, and evolving abiotic and biotic stress factors, developing cultivars that can provide increased disease resistance and yield is essential to meet current and future demands. A few abiotic factors that impact durum wheat include frost and drought. Frost, drought, and heat shock impact yield by damaging floral organs and developing grains with the severity dependent upon when the stress occurs during the growing season (Beres et al. 2020).

Biotic stress factors that influence yield and grain quality include weeds, diseases, and insects. Insects and weeds interfere and compete with durum plants to cause yield loss that can range from 5% to more than 80% (Beres et al. 2020). Fungal diseases have the greatest impact on durum wheat production with numerous diseases impacting yield and quality. Fusarium head blight (FHB) is caused by *Fusarium graminearum* and results in seed shrinkage and poor quality (Schmale and Bergstrom 2003). Crown rot, caused by *Fusarium pseudograminearum*, infects seedlings, and causes them to prematurely die or result in heads with no grain or shriveled grain if the plant survives (Kazan and Gardiner 2018). Tan spot results in lesions on the leaf blades and red smudge disease on seeds and is caused by *Pyrenophora tritici-repentis* (Wegulo 2011). Septoria leaf blotch is similar to tan spot as it results in chlorotic spots on the leaves of plants and can decrease yield by 35-50%, caused by *Mycosphaerella graminicola* (Ponomarenko et al. 2011). Like the previously listed fungal diseases, stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), results in significant damage to wheat and poses a major threat to global wheat production.

Stem Rust History and Life Cycle

Stem rust was first described by Italian scientists Fontana and Tozzetti in 1767 with

Puccinia graminis receiving its name in 1797 by Persoon (Schumann and Leonard 2000). Even

before being described by Fontana and Tozzetti, stem rust existed in Israel dating as far back as

1300 B.C. in the Fertile Crescent where wheat and barberry originated (Schumann and Leonard

2000). Although unknown at the time, European farmers identified a connection between

barberry, wheat, and stem rust and implemented laws prohibiting the placement of barberry near

fields of wheat in 1660 (Schumann and Leonard 2000). The connection between barberry, wheat,

and stem rust was made by Anton de Bary in the 1800s when he discovered that stem rust

requires an alternate host (barberry) to complete its life cycle. Stem rust sexual cycle starts with

the production of teliospores in a telium towards the end of the growing season, and this is how

Puccinia graminis overwinters on wheat or other grass hosts. Once spring arrives, karyogamy

and meiosis take place within the teliospore to produce haploid basidiospores that will go on to

infect the alternate host barberry (Figure 2.2). Basidiospores produce a haploid mycelium, which

will then create pycnia that creates hyphae and pyciniospores. Pyciniospores are cross-fertilized

through the help of insects and rain and aecium begins forming following fertilization. The

aecium grows for a few days until it releases the aeciospores that will then infect wheat or other

hosts of *P. graminis* and become urediniospores. Urediniospores are the asexual stage of stem

rust. Under favorable conditions, urediniospores produce a new generation every 7-14 days and

can reinfect the same plant or neighboring plants several times leading to an epidemic.
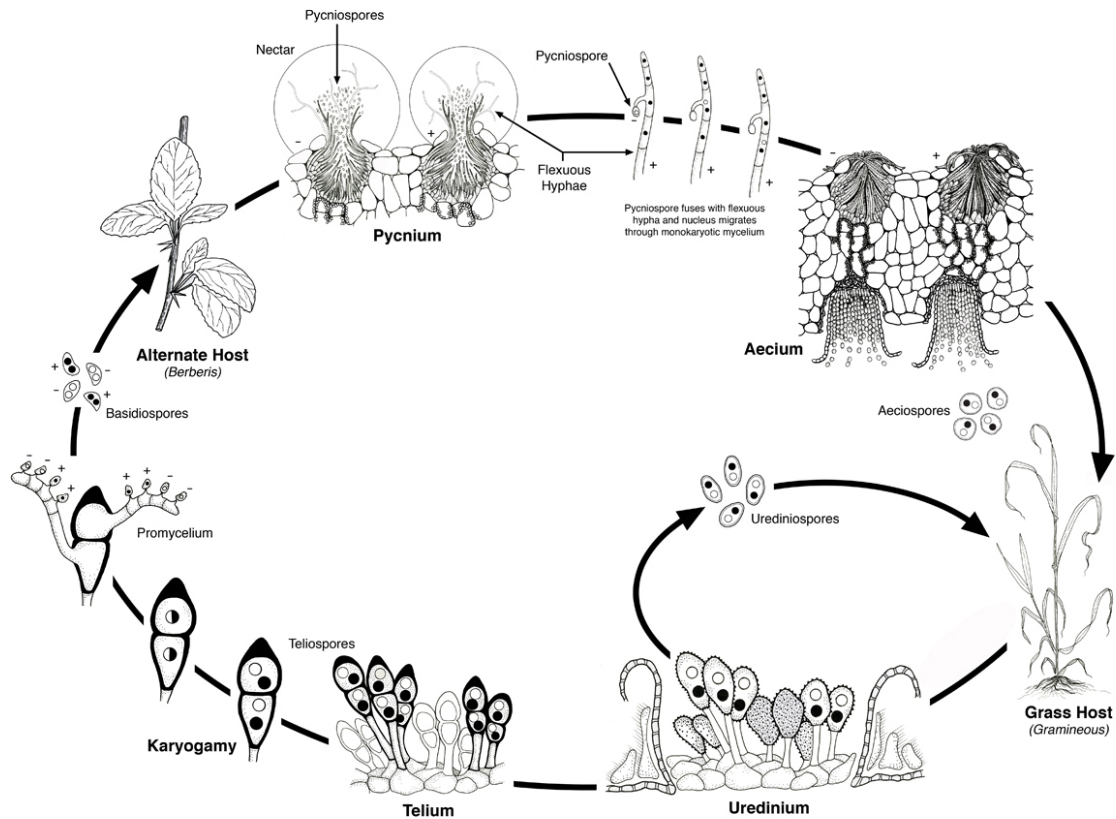
Figure 2.2. Lifecycle of *Puccinia graminis.*
Note: From https://www.ars.usda.gov; accessed March 23, 2022.

Stem rust pathogens commonly evolve in the presence of selective pressure. Sexual recombination is the main source of genetic variation and recombination, and commonly thought to be only possible on barberry. However, a recent article has suggested that a concerning foreign race may have emerged because of somatic hybridization on the main host (Li et al, 2019). The only other main source for genetic variation is random mutations in virulence genes.

Stem Rust Races

From the beginning of wheat production, stem rust races have existed and evolved to overcome resistance efforts. TTKSK stem rust, also known as Ug99, was first detected in 1998 in Uganda (Singh et al. 2015). TTKSK has been determined to be virulent to *Sr5, Sr6, Sr7b,*

Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr21, Sr30, Sr31, Sr38, and *SrMcN* and

avirulent to *Sr22, Sr24, Sr35, Sr36,* and *SrTmp* (Rouse and Jin 2011; Chao et el. 2017). Since the

discovery of Ug99, 15 variants have been confirmed residing in 14 different countries

("Pathotype Tracker – Where is Ug99?", rusttracker.cimmyt.org). TTKSF is a variant of TTKSK

first identified in 2000 in South Africa with avirulence to *Sr31*, also avirulent to *Sr31* are TTKSP

(identified in 2007, virulent to *Sr24*) and TTKSF+ (identified in 2012, virulent to *Sr9h*)

("Pathotype Tracker – Where is Ug99?", rusttracker.cimmyt.org). Other variants of TTKSK that

have been identified include TTHTT, TTKTT+, TTHST, PTKTK, TTHSK, TTKTK, TTKTT,

PTKST, TTTSK, TTKST, and most recently PTKSK ("Pathotype Tracker – Where is Ug99?",

rusttracker.cimmyt.org). PTKSK was first identified in South Africa in 2017 and is virulent to

Sr5, Sr6, Sr7b, Sr8a, Sr8b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr30, Sr31, Sr38, and

SrMcN and avirulent to *Sr24, Sr27, Sr36,* and *SrTmp* (Terefe et el. 2019).

  While the Ug99 races were adapting, other races that unrelated to the Ug99 lineage were

also evolving and infecting plants around the world. First, race TRTTF, which is a virulent race

found in Yemen, is virulent to *Sr5, Sr6, Sr7b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr13,*

Sr21, Sr30, Sr36, Sr38, SrMcN, and *SrTmp* and avirulent to *Sr8a, Sr22, Sr24, Sr31,* and *Sr35* (Yu

et al. 2017; Rouse and Jin 2011; Singh et al. 2015; Chao et al. 2017). TRTTF is virulent to

effective resistance genes to TTKSK including *Sr36* and *SrTmp* (Singh et al. 2015). TRTTF and

JRCQC both poses virulence to the most recurring resistance genes/alleles *Sr13b* and *Sr9e*

(Olivera et al. 2012; Megerssa et al. 2020). JRCQC stem rust originated from Ethiopia and is a

virulent race that most of the global durum wheat germplasm is susceptible to, and additionally

virulent to *Sr6, Sr9a, Sr9d, Sr9e, Sr9g, Sr11, Sr13, Sr17,* and *Sr21* (Chao et al. 2017; Letta et al.

2014; Olivera et al. 2012). JRCQC is avirulent to *Sr5, Sr7b, Sr8a, Sr9b, Sr10, Sr24, Sr30, Sr31, Sr36,* and *Sr38* (Chao et al. 2017).

Race TTRTF was detected in Sicily, Italy in 2016 and was responsible for a stem rust epidemic in 2016 (Hovmøller et al. 2018). TTRTF was also discovered in Ethiopia through a stem rust survey during the main growing season in 2017 (Tesfaye et al. 2019). TTRTF was determined to be virulent to *Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr 13b, Sr17, Sr21, Sr35, Sr36, Sr37, Sr38, SrMcN,* and *SrTmp* and avirulent to *Sr24, Sr30,* and *Sr31* (Olivera et al. 2019; Tesfaye et al. 2019). In the United States, foreign and Ug99 races aren't present, however, it's only a matter of time before they become entrenched and threaten domestic wheat production.

In addition to foreign races of stem rust threatening wheat production in the United States, there are local races MCCF, QCCJ, and RKQQ that pose a more imminent threat to wheat production. Race MCCF is virulent to *Sr5, Sr7b, Sr9g, Sr10, Sr17, Sr35, SrMcN,* and *SrTmp* and is avirulent to *Sr6, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr11, Sr21, Sr22, Sr24, Sr30, Sr31, Sr36,* and *Sr38* (Rouse and Jin 2011; Rouse et al. 2011). QCCJ was first identified in the United States in 1898 and in Minnesota in 1989 and is virulent to *Sr5, Sr9d, Sr9g, Sr10, Sr15, Sr16,* and *Sr17* and avirulent to *Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9e, Sr11, Sr13, SrTmp,* and *SrMcN* (Murray et al. 2010; Roelfs et al. 1991; Roelfs et al. 1997). RKQQ is virulent to *Sr5, Sr6, Sr7b, Sr8a, Sr9b, Sr9d, Sr9g, Sr21, Sr36,* and *SrMcN* and is avirulent to *Sr9e, Sr10, Sr11, Sr17, Sr24, Sr30, Sr31, Sr38,* and *SrTmp* (Rouse et al. 2011). Notably, QCCJ is avirulent to *SrMcN* whereas the other local races MCCF and RKQQ are virulent to *SrMcN.*

Controlling Stem Rust in Wheat

Since the discovery of barberry as an alternate host within the stem rust life cycle, extreme barberry eradication measures have taken place including the federal Barberry Eradication Program implemented in the United States in 1918. The Barberry Eradication Program lasted until 1980, when the program switched from being a federal program to state programs. Through the federal program over 500 million bushes were destroyed in the United States (Peterson 2003). Even with the barberry eradication program, stem rust still poses a threat to wheat production due to continental spread. Stem rust urediniospores are able to survive the winter on wheat and other grasses in areas that remain above 10°C year-round (Figure 2.3). By the year 2100, the projected areas where stem rust spores are able to survive the winter will be increased (Figure 2.3).
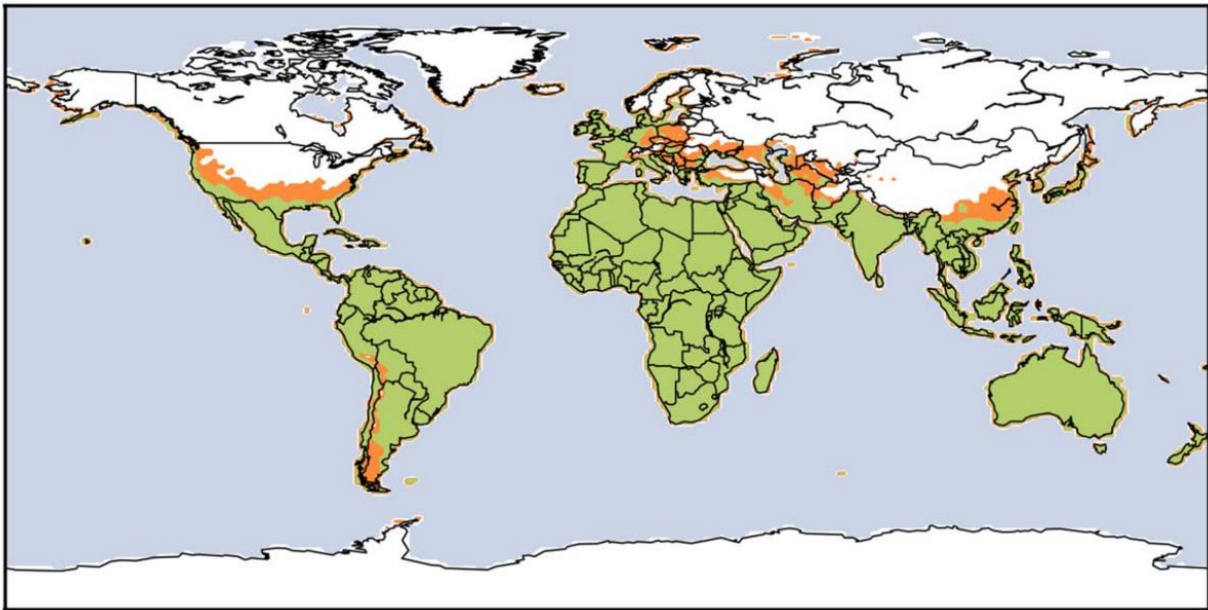
Figure 2.3. Areas where stem rust spores are able to survive the winter currently as shown in green and projected to be able to survive the winter by 2100 as shown in orange.
Note: Image taken from Prank et al. (2019).

Even though stem rust spores are not able to survive the winters everywhere in the world, nearly the entire world is still susceptible to stem rust infection due to the ability of stem rust urediniospores to utilize atmospheric transportation to relocate to areas where spores are not able to survive the winter and where susceptible cultivars that are grown. Figure 2.4 shows a simulation that highlights the distance a stem rust spore may travel within one day and within three days (Prank et al. 2019). After roughly 35 hours of sunlight over a three-day period the germination percentage of spores is reduced to less than 0.1%, but it doesn't take many to start an infection cycle in a new place. Southern spring winds could transport stem rust spores that are able to overwinter in Mexico to North Dakota and Canada within one day (Prank et al. 2019; Schumann and Leonard 2000).
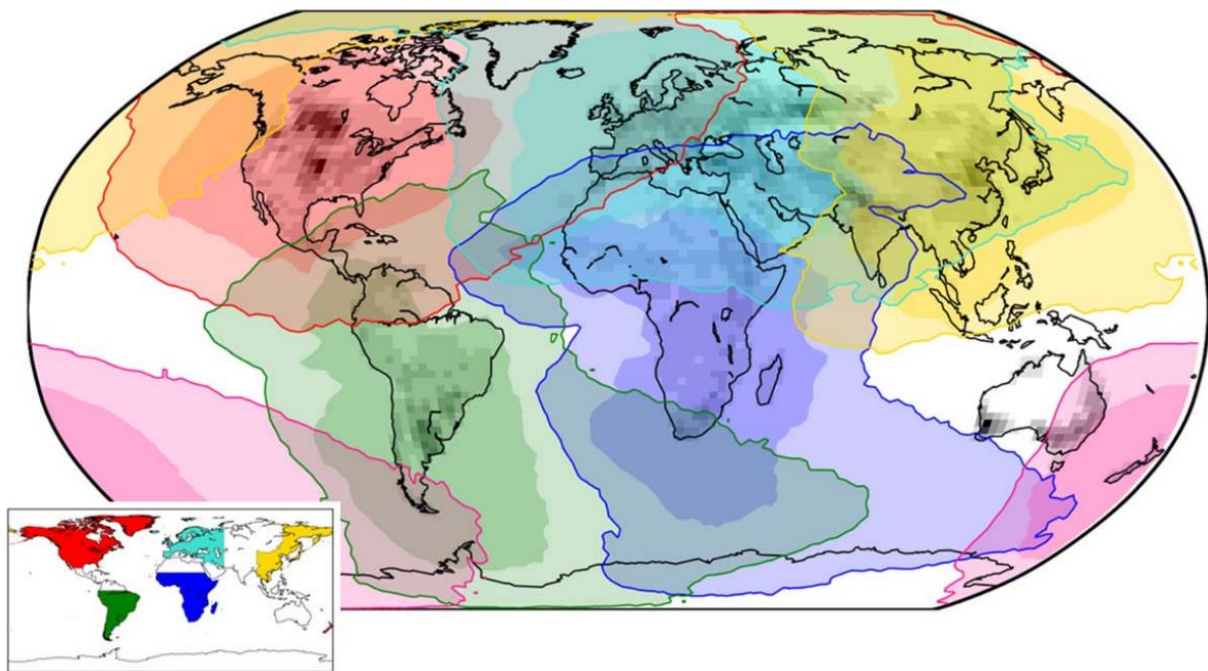
Figure 2.4. Projections of how far a stem rust spore can travel in one day (darker shades) and three days (lighter shades).
Note: Image taken from Prank et al. (2019).

To assist in combating stem rust, there are several control measures that growers can take. A way to limit disease development and reduce disease severity is the timely application of chemical, specifically a fungicide containing a triazole is one of the most effective chemicals (Murray et al. 2010). Another preventative measure to reduce the likelihood of an epidemic occurring is planting of rust resistant cultivars. The utilization of rust resistant cultivars is the most effective, lease expensive, and environmentally friendly approach to combat stem rust disease (Murray et al. 2010; Gyawali et al. 2017). Cultivar mixtures and multiline cultivars, a practice developed by Normal Borlaug, is a practice where several cultivars with different race-specific rust resistances are planted within a field to slow the potential spread of stem rust infection across it (Murray et al. 2010). Resistant cultivars may have different mechanisms of resistant including seedling, or adult plant resistance. Gene combinations or gene pyramiding is the practice of deploying more than one resistance genes and or/mechanisms to provide a longer lasting, more durable resistance to stem rust. Another way to limit disease development and reduce disease severity is the timely application of chemical, specifically a fungicide containing a triazole is one of the most effective chemicals (Murray et al. 2010).

Four important themes were identified for stem rust research by Murray et al. (2010). First, the breeding for host resistance in wheat and barley by incorporating known resistance genes in germplasms and identification of new all stage resistance sources that can provide effective and long-lasting resistance. Second, the identification of new stem rust races by developing detection methods and deploying cultivars to be used as biological indicators to track the effectiveness of known stem rust resistance genes. Third, the continued in-depth research of stem rust spore survival and development to assist in understanding and predicting emerging races of stem rust in the United States and races from foreign countries. And the fourth, the

identification of fungicides and studying the timely application and technologies for the most effectiveness.

Types of Resistance

There are two different types of rust resistance in plants: all stage resistance or adult-plant resistance. All stage resistance is also known as seedling resistance and is effective at all stages of plant growth, typically resulting in hypersensitive reactions (Gyawali et al. 2017). Adult-plant resistance is a form of resistance that is only effective once the plant reaches the adult stage.

All stage resistance screening can be performed in a greenhouse at seedling stage and numerous lines can be tested at once with a single race of stem rust (Letta et al. 2014). When testing with a single race of stem rust at a time, linkage mapping and QTL identification is race specific. In comparison, adult-plant resistance requires screening of adult plants in a field setting under natural or artificial inoculations with multiple rust race and the identified resistance QTL are generally non-race-specific (Letta et al. 2014). Adult-plant resistance is more durable and long-lasting resistance than all-stage resistance that is typically race specific and can be overcome by stem rust pathogens (Farrakh et al. 2018). All stage resistance heavily relies upon a single gene or a few genes to provide resistance, whereas adult-plant resistance is dependent upon many minor genes providing resistance to stem rust.

Published Stem Rust Resistance Genes

Stem rust resistance genes have been discovered for several decades with *Sr31* being a well-known resistance gene first deployed to combat stem rust in Kenya in 1982 (Fetch 2021). However, by 1998 virulence to *Sr31* was reported in Uganda and this race of rust became known as *Pgt-Ug99* (TTKSK) (Fetch 2021; Schumann and Leonard 2000). In addition to *Sr31*, there are nearly 70 other known stem rust resistance genes/alleles to help combat stem rust (ars.usda.gov;

13

Megerssa et al. 2020). Despite identification of numerous stem rust genes and alleles, a single stem rust gene is not universally effective against all races of stem rust and the majority of identified resistance genes are race specific (Mergerssa et al. 2020).

Stem rust resistance gene *Sr2* is an adult plant resistance gene located on chromosome 3BS that is closely associated with leaf rust resistance gene *Lr27* (Hare and McIntosh, 1979). *Sr6* is located on chromosome 2D and shows close linkage with *Lr2* and *Lr15* (globalrust.org). *Sr6* used to be effective when first used in cultivars Eureka and Selkirk, however, most stem rust races are now virulent to this gene (Luig, 1983). *Sr7a* is located on chromosome 4A and has greater resistance capabilities when paired with *Sr12* (Singh and McIntosh 1986). Several different alleles of *Sr9* have been identified including *Sr9a* (Green et al. 1960)*, Sr9b* (Green et al. 1960)*, Sr9c* (eventually changed to *Sr36*) (globalrust.org)*, Sr9d* (globalrust.org)*, Sr9e* (McIntosh and Luig, 1973)*, Sr9f* (Loegering 1975)*,* and *Sr9g* (McIntosh and Luig 1973) all located on chromosome 2B. And the list goes on and on for resistance genes and resistance alleles that have been identified. Importantly, the number of identified resistance genes does not correlate with the number of resistance genes that are effective today as many have become virulent to stem rust races, thus highlighting the continued importance of identification of stem rust resistance genes.

In addition to stem rust races becoming virulent to known resistance genes, suppression of the genes can occur which does not allow resistance genes to provide resistance despite being present. With the removal of the D genome of hexaploid wheat, several resistance genes became activated to virulent races of stem rust (Hiebert et al. 2020). Further studies confirmed the presence of *SuSr-D1,* a dominant gene located on chromosome 7DL that suppresses stem rust resistance in hexaploid wheat (Hiebert et al. 2020). Understanding how suppression genes are

14

able to essentially block resistance genes effects may help uncover more stem rust resistance genes within germplasms that have not been discovered.

Genetic Mapping of Stem Rust Resistance Genes/Loci

The need for new stem rust resistance is important as stem rust races can overcome currently deployed genes. Stem rust resistance genes have many ways of being identified through genetic mapping methods to highlight quantitative trail loci (QTL) that affect genetic variation. Genetic mapping can be performed by linkage mapping with biparental populations or association mapping with multiparent populations (Xu et al. 2017). Linkage mapping involves a defined population that is genotyped with molecular markers to determine groupings of the markers and order of the markers within linkage groups that correspond to physical chromosomes. Linkage groups are formed based on the recombination frequency and commonly described in terms of mapping units (centimorgans (cM)) distance. Marker locations and phenotype data are analyzed to identify cosegregation within the population (Myles et al. 2009). Biparental populations include F_2, backcrosses (BC), double haploids (DH), recombinant inbred lines (RIL), and near-isogenic lines (NIL), and each population type has its strengths and weaknesses as shown in Table 2.1 from Xu et al. (2017). Linkage mapping with biparental populations has limited QTL resolution (10 to 20 cM) capabilities due to the typically low number of recombination events that take place within the population development and the limited number of markers used to determine the location of those recombination events (Xu et al. 2017; Myles et al 2009).

Table 2.1. Commonly used biparental populations with their strengths and weaknesses (Xu et al. 2017).

Population	Strengths	Weaknesses
F_2	Rapid construction, estimation of both additive and dominant effects	Lower power, limited recombination, temporary nature
Backcross (BC)	Utility for introgressing specific genes	Impossibility of estimation of dominant effects, time requirement, temporary nature
Double haploid (DH)	Rapid construction, immortality, easy replication	Limited recombination, expense, impossibility of estimation of dominant effects
Recombinant inbred lines (RIL)	Abundance of recombination, immortality, easy replication	Impossibility of estimation of dominant effects, time requirement

In contrast to biparental populations, association mapping utilizes the history of

recombination events throughout many generations of evolution to give rise to high resolution

QTL regions (Myles et al. 2009). Association mapping combines the genotype data with

phenotype data of unrelated individuals to determine any correlations. Using association

mapping with multiparent populations allows for greater QTL resolution than linkage mapping

alone. Nested association mapping (NAM) and multiparent advanced generation intercrosses

(MAGIC) are two ways to map multiparent populations (Xu et al. 2017). Association mapping is

very reliant on the strength of linkage disequilibrium (LD) between genotyped markers and the

functional variant to map QTL regions and can generate false positives from genotype-phenotype

covariance (Myles et al. 2009). Correcting for false positives can be done by using the Q matrix,

which is the identification of sub-populations and the proportion of each line's variation that was

derived from a particular sub-population (Myles et al. 2009). Solely using the Q matrix to correct

population structure is sometimes not enough and requires a marker-generated kinship matrix (K

matrix) to control for relatedness of individuals (Xu et al. 2017; Myles et al. 2009). The combination of the Q matrix and K matrix is determined to be the most powerful approach to correct false positives (Bradbury et al. 2007).

References

Beres, B. L., Rahmani, E., Clarke, J. M., Grassini, P., Pozniak, C. J., Geddes, C. M., Porker, K. D., May, W. E., & Ransom, J. K. (2020). A systematic review of durum wheat: Enhancing production systems by exploring genotype, environment, and management (G × E × m) synergies. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.568657

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). Tassel: Software for association mapping of complex traits in diverse samples. *Bioinformatics*, *23*(19), 2633–2635. https://doi.org/10.1093/bioinformatics/btm308

Durum Production. ND Wheat Commission. (n.d.). Retrieved March 23, 2022, from https://www.ndwheat.com/buyers/NorthDakotaWheatClasses/Durum/#:~:text=Durum%20Production&text=Durum%20thrives%20in%20a%20climate,commonly%20associated%20with%20wet%20conditions

Evolution of Wheat. WheatBP NEW. (n.d.). Retrieved April 10, 2022, from https://www.cerealsdb.uk.net/cerealgenomics/WheatBP/Documents/DOC_Evolution.php

Farrakh, S., Wang, M., & Chen, X. (2018). Pathogenesis-related protein genes involved in race-specific all-stage resistance and non-race specific high-temperature adult-plant resistance to *Puccinia striiformis* f. sp. *tritici* in wheat. *Journal of Integrative Agriculture*, *17*(11), 2478–2491. https://doi.org/10.1016/s2095-3119(17)61853-7

Fetch, T. G., Park, R. F., Pretorius, Z. A., & Depauw, R. M. (2021). Stem rust: Its history in

    Kenya and research to combat a global wheat threat. *Canadian Journal of Plant*

    Pathology, *43*(sup2). https://doi.org/10.1080/07060661.2021.1902860

Gioia, T., Nagel, K. A., Beleggia, R., Fragasso, M., Ficco, D. B., Pieruschka, R., De Vita, P.,

    Fiorani, F., & Papa, R. (2015). Impact of domestication on the phenotypic architecture of

    durum wheat under contrasting nitrogen fertilization. *Journal of Experimental Botany*,

    66(18), 5519–5530. https://doi.org/10.1093/jxb/erv289

Green, G. J., Knott, D. R., Watson, I. A., & Pugsley, A. T. (1960). Seedling reactions to stem

    rust of lines of marquis wheat with substituted genes for rust resistance. *Canadian*

    Journal of Plant Science, *40*(3), 524–538. https://doi.org/10.4141/cjps60-069

Gyawali, S., Verma, R. P., Kumar, S., Bhardwaj, S. C., Gangwar, O. P., Selvakumar, R.,

    Shekhawat, P. S., Rehman, S., & Sharma-Poudyal, D. (2017). Seedling and adult-plant

    stage resistance of a world collection of barley genotypes to stripe rust. *Journal of*

    Phytopathology, *166*(1), 18–27. https://doi.org/10.1111/jph.12655

Hare, R. A., & McIntosh, R. A. (1979). Genetic and cytogenetic studies of durable adult-plant

    resistances in 'Hope' and related cultivars to wheat rusts. *Zeitschrift Fur*

    Pflanzenzuchtung, *83*, 350–367.

Henderson, C. R. (1975). Best linear unbiased estimation and prediction under a selection model.

    Biometrics, *31*(2), 423. https://doi.org/10.2307/2529430

Hiebert, C. W., Moscou, M. J., Hewitt, T., Steuernagel, B., Hernández-Pinzón, I, Green, P., Pujol, V., Zhang, P., Rouse, M. N., Jin, Y., McIntosh, R. A., Upadhyaya, N., Zhang, J., Bhavani, S., Vrána, J., Karafiátová, M., Huang, L., Fetch, T., Doležel, J., … Spielmeyer, W. (2020). Stem rust resistance in wheat is suppressed by a subunit of the Mediator Complex. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-14937-2

Hovmøller, M. S., Hansen, J. G., Justesen, A. F., Thach, T., Rodriguez-Algab, J., Hodson, D., & Randazzo, B. (2018). Epidemics of yellow and stem rust in southern Italy 2016-2017. *BRGI*. Retrieved March 23, 2022, from https://globalrust.org/content/epidemics-yellow-and-stem-rust-southern-italy-2016-2017

Kazan, K., & Gardiner, D. M. (2018). Fusarium crown rot caused by *Fusarium pseudograminearumin* cereal crops: Recent progress and future prospects. *Molecular Plant Pathology*, *19*(7), 1547–1562. https://doi.org/10.1111/mpp.12639

Letta, T., Olivera, P., Maccaferri, M., Jin, Y., Ammar, K., Badebo, A., Salvi, S., Noli, E., Crossa, J., & Tuberosa, R. (2014). Association mapping reveals novel stem rust resistance loci in durum wheat at the seedling stage. *The Plant Genome*, *7*(1). https://doi.org/10.3835/plantgenome2013.08.0026

Li, F., Upadhyaya, N. M., Sperschneider, J., Matny, O., Nguyen-Phuc, H., Mago, R., Raley, C., Miller, M. E., Silverstein, K. A., Henningsen, E., Hirsch, C. D., Visser, B., Pretorius, Z. A., Steffenson, B. J., Schwessinger, B., Dodds, P. N., & Figueroa, M. (2019). Emergence of the Ug99 lineage of the wheat stem rust pathogen through somatic hybridization. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-12927-7

Loegering, W. Q. (1975). An allele for low reaction to *Puccinia graminis tritici* in Chinese spring wheat. *Phytopathology*, *65*(8), 925. https://doi.org/10.1094/phyto-65-925

Luig, N. H. (1983). A survey of virulence genes in wheat stem rust, *Puccinia graminis* f. sp. *tritici*. *Advances in Plant Breeding*, *11*, 1-198.

Mastrangelo, A. M., & Cattivelli, L. (2021). What makes bread and durum wheat different? *Trends in Plant Science*, *26*(7), 677–684. https://doi.org/10.1016/j.tplants.2021.01.004

McIntosh, R. A., & Luig, N. H. (1973). Recombination between genes for reaction to P. graminis at or near the *Sr9* locus. *'Proceedings of the Fourth International Wheat Genetics Symposium*, 425–432.

McIntosh, R. A., Wellings, C. R., Park, R. F., & Jeans, K. (1995). Wheat rusts: An atlas of resistance genes. *CSIRO Publications*, Melbourne, 208. https://doi.org/10.1007/978-94-011-0083-0

Megerssa, S. H., Ammar, K., Acevedo, M., Brown-Guedira, G., Ward, B., Degete, A. G., Randhawa, M. S., & Sorrells, M. E. (2020). Multiple-race stem rust resistance loci identified in durum wheat using genome-wide association mapping. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.598509

Murray, T., Milus, G., DeWolf, E., Dill-Macky, R., Steffenson, B., Wegulo, S., Bergstrom, G., Sorrells, M., McMullen, M., Paul, P., Hunger, R., Mundt, C., Isard, S., Stein, J., Baker, H., Bulluck, R., Divan, C., Engle, J., Hebbar, P., … Szabo, L. (2010). Recovery plan for STEM rust of wheat. *ARS-USDA*. Retrieved from https://www.ars.usda.gov/ARSUserFiles/00000000/opmp/WheatStemRustUg99100331.pdf

Myles, S., Peiffer, J., Brown, P. J., Ersoz, E. S., Zhang, Z., Costich, D. E., & Buckler, E. S. (2009). Association mapping: Critical Considerations shift from genotyping to experimental design. *The Plant Cell*, *21*(8), 2194–2202. https://doi.org/10.1105/tpc.109.068437

Olivera, P. D., Jin, Y., Rouse, M., Badebo, A., Fetch, T., Singh, R. P., & Yahyaoui, A. (2012). Races of *Puccinia graminis* f. sp. *tritici* with combined virulence to *Sr13* and *Sr9e* in a field stem rust screening nursery in Ethiopia. *Plant Disease*, *96*(5), 623–628. https://doi.org/10.1094/pdis-09-11-0793

Olivera, P. D., Sikharulidze, Z., Dumbadze, R., Szabo, L. J., Newcomb, M., Natsarishvili, K., Rouse, M. N., Luster, D. G., & Jin, Y. (2019). Presence of a sexual population of *Puccinia graminis* f. sp. *tritici* in Georgia provides a hotspot for genotypic and phenotypic diversity. *Phytopathology, 109*(12), 2152–2160. https://doi.org/10.1094/phyto-06-19-0186-r

Özkan, H., Willcox, G., Graner, A., Salamini, F., & Kilian, B. (2010). Geographic Distribution and domestication of wild emmer wheat (*Triticum dicoccoides*). *Genetic Resources and Crop Evolution*, *58*(1), 11–53. https://doi.org/10.1007/s10722-010-9581-5

Pathotype Tracker – Where is Ug99? Rusttracker.org. (2021, September). Retrieved March 23, 2022, from https://rusttracker.cimmyt.org/?page_id=22

Peterson, P. D. (2003). *The common barberry: The past and present situation in Minnesota and the risk of Wheat Stem Rust epidemics* (dissertation).

Ponomarenko, A., Goodwin, S. B., & Kema, G. H. J. (2011). Septoria tritici blotch (STB) of wheat. *Plant Health Instructor*. https://doi.org/10.1094/phi-i-2011-0407-01

Prank, M., Kenaley, S. C., Bergstrom, G. C., Acevedo, M., & Mahowald, N. M. (2019). Climate change impacts the spread potential of wheat stem rust, a significant crop disease. *Environmental Research Letters*, *14*(12), 124053. https://doi.org/10.1088/1748-9326/ab57de

Roelfs, A. P. (1989). Races of *Puccinia graminis* in the United States and Mexico during 1987. *Plant Disease*, *73*(5), 385. https://doi.org/10.1094/pd-73-0385

Roelfs, A. P., McCallum, B., McVey, D. V., & Groth, J. V. (1997). Comparison of virulence and isozyme phenotypes of PGT-QCCJ and Great Plains races of *Puccinia graminis* f. sp. *tritici*. *Phytopathology*, *87*(9), 910–914. https://doi.org/10.1094/phyto.1997.87.9.910

Schmale III, D. G., & Bergstrom, G. C. (2003). Fusarium head blight (FHB) or SCAB. *APSnet Feature Articles*. https://doi.org/10.1094/phi-i-2003-0612-01

Schumann, G. L., & Leonard, K. J. (2000). Stem rust of wheat (Black Rust). *The Plant Health Instructor*. https://doi.org/10.1094/phi-i-2000-0721-01

Singh, R. P., & Mcintosh, R. A. (1986). Genetics of resistance to *Puccinia graminis tritici* and *Puccinia recondita tritici* in Kenya plume wheat. *Euphytica*, *35*(1), 245–256. https://doi.org/10.1007/bf00028563

Singh, R. P., Hodson, D. P., Jin, Y., Lagudah, E. S., Ayliffe, M. A., Bhavani, S., Rouse, M. N., Pretorius, Z. A., Szabo, L. J., Huerta-Espino, J., Basnet, B. R., Lan, C., & Hovmøller, M. S. (2015). Emergence and spread of new races of wheat stem rust fungus: Continued threat to food security and prospects of genetic control. *Phytopathology*, *105*(7), 872–884. https://doi.org/10.1094/phyto-01-15-0030-fi

Small Grains 2021 Summary. United States Department of Agriculture National Agricultural

      Statistics Service. (2021, September 30). Retrieved March 23, 2022, from

      https://www.nass.usda.gov/Publications/Todays_Reports/reports/smgr0921.pdf

Terefe, T., Pretorius, Z. A., Visser, B., & Boshoff, W. H. (2019). First report of *Puccinia*

      graminis f. sp. *tritici* race PTKSK, a variant of Wheat Stem Rust Race Ug99, in South

      Africa. *Plant Disease*, *103*(6), 1421–1421. https://doi.org/10.1094/pdis-11-18-1911-pdn

Tesfaye, T., Chala, A., Shikur, E., Hodson, D., & Szabo, L. J. (2020). First report of TTRTF race

      of wheat stem rust, *Puccinia graminis* f. sp. *tritici*, in Ethiopia. *Plant Disease*, *104*(1),

      293–293. https://doi.org/10.1094/pdis-07-19-1390-pdn

Wegulo, S. N. (2011). Tan spot of cereals. *The Plant Health Instructor*.

      https://doi.org/10.1094/PHI-I-2011-0426-01

Wheat sector at a glance. USDA ERS - Wheat Sector at a Glance. (2022, February 2). Retrieved

      March 23, 2022, from https://www.ers.usda.gov/topics/crops/wheat/wheat-sector-at-a-

      glance/

Xu, Y., Li, P., Yang, Z., & Xu, C. (2017). Genetic mapping of quantitative trait loci in crops. *The*

      Crop Journal, *5*(2), 175–184. https://doi.org/10.1016/j.cj.2016.06.003

CHAPTER 3. EVALUATION OF DURUM WHEAT POPULATIONS FOR RESISTANCE TO FOREIGN AND LOCAL RACES OF STEM RUST

Abstract

Stem rust (*Puccinia graminis* f. sp. *tritici*) is a severe pathogen that affects wheat production worldwide. Stem rust resistance genes give wheat the ability to combat stem rust pathogens. Five parental lines (Rusty, PI192711, PI383416, PI520392, and PI636501) were screened for their resistance to local races of stem rust RKQQ, HKHJ, QCCJ, and MCCF to supplement preliminary data of evaluation with foreign races BCCBC, TTTTF, TTKSK, TRTTF, and JRCQC. Four durum wheat populations were developed and screened for stem rust resistance to local races RKQQ and MCCF as well as foreign races JRCQC, TRTTF, TTRTF, and TTKSK. Resistant to susceptible ratios indicative of one (1:1), two (1:3/3:1), and three (1:7/7:1) gene interactions were observed that provide stem rust resistance in these populations, as well as distorted segregations ratios that require more research to understand the interactions that are taking place. Two very distorted segregation ratios resulting in near entire population susceptibility despite resistant parent lines having high levels of resistance, may be indicative of a suppression gene somewhere within the population.

Introduction

Durum wheat, *Triticum turgidum* subsp. *durum*, plays an essential role in providing key ingredients to make pasta, semolina, couscous, and many other dishes. Durum's ancestors were first grown in the Fertile Crescent as early as 1300 B.C. (Schumann and Leonard 2000). Durum was selected over time and is derived from domesticated emmer wheat (*Triticum turgidum* subsp. *dicoccum* (Schrank ex Schübl) Thell.), and domesticated emmer wheat is derived from wild emmer wheat (*Triticum turgidum* subsp. *dicoccoides* (Körn. Ex Asxh. & Graebn.)) (Sall et

24

al. 2019). Desired traits such as yield, plant height, days to flowering, disease resistance, and drought tolerance can be selected for with breeding. Breeding for stem rust resistance is one aspect of durum breeding to assist in reducing the threat of stem rust disease infection.

Stem rust, caused by the fungus *Puccinia graminis* f. sp. *tritici*, threatens durum wheat production worldwide and the search for stem rust resistance genes is an important objective for the scientific community working on cereal diseases. When a field or area is infected with stem rust, yield losses can be as high as 90% with severe infections (Prank et al. 2019). Year-to-year infection severity differs based on the weather conditions, prevalent stem rust races, and varieties being grown. As new stem rust genes are identified and deployed, stem rust races evolve to combat the resistance genes. The stem rust sexual life cycle requires the alternate host barberry to complete the cycle. Even with massive efforts to eliminate barberry, stem rust is still able to infect durum wheat all over the world. Stem rust urediniospores have the capabilities to travel from one continent to another through atmospheric transportation as well as move locally to nearby fields with the wind (Prank et al. 2019). Stem rust spores can also be translocated from one area to another through transportation of infected plant material that has spores on it as well as contaminated farm equipment and clothing.

No region or area is completely safe from the stem rust pathogens and to maintain reliable durum wheat production for food security and sustainability, stem rust resistance genes must be identified to combat the different races of stem rust. This study focuses on four durum wheat populations and six races of stem rust (two local Upper Midwestern United States races and four foreign East African and European races) with the aim of understanding the mechanisms that provide resistance to stem rust within the four populations that were tested.

Materials and Methods

Preliminary Identification of Durum Lines Resistant to Stem Rust

Selection of resistant parent lines was based off the work of Chao et al. (2017). Chao et al. (2017) performed stem rust testing on 497 durum wheat cultivars obtained from USDA-ARS NSGC in Aberdeen, ID. Seedling testing was performed with stem rust races BCCBC, TTTTF, TTKSK, TRTTF, and JRCQC and adult plant testing with a bulk mixture of races MCCFC, QFCSC, QTHJC, RCRSC, RKQQC, and TPMKC. Seedling testing was done at the Cereal Disease Laboratory in St. Paul, MN in 2016. Adult plant resistance was also tested in this study with field tests occurring in St. Paul, MN (April-July, 2012-2014) and Debre Zeit, Ethiopia (June 2014-October 2014). Adult plant resistance in St. Paul was evaluated with a mixture of MCCFC, QFCSC, QTHJC, RCRSC, RKQQC, and TPMKC. In Ethiopia the plants were inoculated with a mixture of JRCQC and TTKSK stem rust urediniospores. Infection type scores were assigned to seedlings following the 0-4 scale as described by Stakman et al. (1962) and adult plants were scored following the modified Cobb Scale (Peterson et al. 1948). From the seedling stem rust resistance testing by Chao et al (2017), four lines were chosen to be used for recombinant inbred line population development: PI192711, PI383416, PI520392, and PI636501.

PI192711 originated in Gotland, Sweden and was submitted to the National Small Grains Collection in 1950 with an unknown improvement status. The other three lines are breeding lines originating from Herault, France (PI383416), Federal District, Mexico (PI520392) and North Dakota, United States (PI636501) (Chao et al. 2017).

Development of Recombinant Inbred Line Populations

Rusty, a well-known stem rust susceptible durum wheat line (Klindworth et al. 2006), was crossed with the selected lines from the previous study by Chao et al. (2017). Four

populations were developed from the following crosses: Rusty x PI192711, Rusty x PI383416, Rusty x PI520392, and Rusty x PI636501. The initial crossing took place in 2017 at the USDA-ARS Edward T. Schafer Agricultural Research Center in Fargo, North Dakota. 189 recombinant inbred lines (RILs) were developed from each cross. Populations had been advanced to F_5 RILs through single-seed descent.

Phenotyping of the Parent Lines in the Greenhouse with Upper Midwestern United States Races of Stem Rust

Rusty, PI192711, PI383416, PI520392, and PI636501 were tested against local races of stem rust RKQQ, HKHJ, QCCJ, and MCCF at the North Dakota Agricultural Experiment Station Greenhouse Complex in Fargo, North Dakota during the fall of 2020 and into the spring of 2021. Parent lines were planted in three replicates with three seeds planted per replicate and grown in the greenhouse room at 19-22°C with a 16 hour photoperiod. Standard seedling stem rust inoculation procedures were followed. Briefly, frozen stem rust spores were heat shocked in a 45°C water bath for 15 minutes. A solution of stem rust urediniospores and Soltrol-170 oil was prepared at $1x10^6$ spores/ml in a gelatin capsule. Inoculum was applied to plants at second leaf stage (approximately 12 to 14 days after planting) using a vacuum pump and spray nozzle at roughly 2 psi. Plants were allowed to dry for 15 minutes before being placed in a misting chamber, where the plants remained for 16-20 hours receiving mist for 20 seconds every four minutes with no light and maintained at 20-22°C. After the misting chamber, plants were allowed to dry for 2 hours with the lights on before being placed back into the greenhouse room at 19-22°C with a 16 hour photoperiod.

Fourteen days after inoculation, seedlings were evaluated for infection types (ITs) based on the 0-4 Stakman scale (Stakman et al. 1962). ITs are described by Stakman et al. (1962) as:

'0' = immune, no uredia nor other indications of infections, '0;' = nearly immune, no uredia but hypersensitive flecks present, '1' = very resistant, uredia minute; surrounded by distinct necrotic areas, '2' = moderate resistant, uredia small to medium; usually in green islands surrounded by a decidedly chlorotic or necrotic border, '3' = moderately susceptible, uredia medium in size; coalescence infrequent; no necrosis, but chlorotic areas may be present, especially under unfavorable growing conditions, and '4' = very susceptible, uredia large, and often coalescing; no necrosis, but chlorosis may be present under unfavorable growing conditions. Plus (+) and minus (-) signs were added to scores that had slightly larger or smaller sized uredia than normally associated with a particular score. When a combination of scores can be observed, the most prevalent score is listed first followed by additional scores. Scores ranging from 0-2 were considered resistant and scores 3-4 were considered susceptible. All 0-4 scores were converted to linear 0-9 scores as described by Zhang et al. (2014) and as shown in Table 3.1. Linear scores of 0-6 are considered resistance and 7-9 susceptible. Resistant to susceptible segregation ratios were calculated based on the total number of resistant lines and total number of susceptible lines observed within a population. Chi-square goodness of fit tests were performed to determine if the segregation ratios fit the expected ratios. Expected ratios were 1:1 (single gene provides resistance), 1:3/3:1 (two genes provide resistance), or 1:7/7:1 (three genes provide resistance).

Table 3.1. The conversion of stem rust infection type scores from the 0-4 Stakman scale score assigned to plant infection severity to a linear 0-9 scale for use in genotyping.

Stakman Scale	Linear Scale
0	0
;	0
1-	1
1	2
1+	3
2-	4
2	5
2+	6
3-	7
3	8
3+	9
4	9

Phenotyping of the RIL Populations in the Greenhouse with Upper Midwestern United States Races of Stem Rust

During the fall of 2021, 186 Rusty/PI192711 F_5 RILs, 165 Rusty/PI383416 F_5 RILs, 147 Rusty/PI520392 F_5 RILs, and 143 Rusty/PI636501 F_5 RILs, as well as the parent lines, were evaluated for seedling resistance to RKQQ and MCCF at the North Dakota Agricultural Experiment Station Greenhouse Complex in Fargo, North Dakota. Two replicates of three seeds for each line were planted into 50 cell flats and grown in the greenhouse at 19-22°C with a 16-hour photoperiod. The inoculum preparation and inoculation procedure were performed the same as above for testing the parent lines. Seedlings were scored fourteen days after inoculation following the Stakman scale as previously described and converted to a linear scale as described by Zhang et al. (2014).

Phenotyping of the Parent Lines and RIL Populations in the Greenhouse with East African and European Races of Stem Rust

Seedling population testing was performed with various foreign stem rust races in a Biosafety level 3 greenhouse at the Cereals Disease Laboratory in St. Paul, Minnesota during the spring of 2021. 120 Rusty/PI192711 F_4 RILs, 124 Rusty/PI383416 F_4 RILs, and 118 Rusty/PI520392 F_4 RILs, as well as the parent lines, were tested against JRCQC (isolate 09ETH08-3), TRTTF (isolate 06YEM34-1), and TTRTF (isolate 14GEO189-1). Additionally, Rusty/PI192711 RILs and corresponding parent lines were also tested against TTKSK (isolate 04KEN156/04). Seedling infection-type assays were performed as outlined by Hundie et al. (2019). Briefly, five seeds per line per replicate were planted into peat pots filled with vermiculite. After planting, pots were placed in the greenhouse at 19-22°C with a 16-hour photoperiod. Once the seedlings reached the second leaf stage (roughly 10 days after planting), they were inoculated with isolates from single pustules that were stored at -80°C. Stem rust spores were heat shocked in a water bath at 45°C for 15 minutes. Fourteen mg of stem rust spores were mixed with 0.75 ml of Soltrol-170 mineral oil in a gelatin capsule. The spore suspension was applied to seedlings using a custom rust inoculator (St. Paul Machine Shop, University of Minnesota) and air pump (30 kPa). Inoculum was allowed to dry for 15 minutes and then plants were placed into misting chambers at 22°C for 16 hours with no light and mist for 2 minutes every 15 minutes by humidifiers. Then, 400-W high-pressure sodium vapor lights were run for 2 hours above the plants before returning the plants to a greenhouse at 19-22°C with a 16-hour photoperiod. Fourteen days following inoculation seedlings were evaluated for ITs based on the Stakman scale, as described above. ITs scores were converted from Stakman to linearized scores as described by Zhang et al. (2014).

Results

Preliminary Identification of Durum Lines Resistant to Stem Rust

Chao et al. (2017) observed the greatest overall resistance from seedling testing and adult

plant field testing to race BCCBC (56.1% resistance). Three lines were also identified with very

high levels of resistance to race JRCQC. Two of the three lines with high resistance to JRCQC

were PI383416 and PI636501, and these lines also exhibited moderate resistance to BCCBC.

Overall, all four durum cultivars were very resistant to BCCBC and JRCQC with moderate

resistance to TTTTF and TRTTF (Table 3.2). PI192711 was the only line with resistance to race

TTKSK, while the other three lines were susceptible.

Table 3.2. The ITs scores based on the Stakman scale for parent lines when inoculated with foreign races of rust BCCBC, TTTTF, TTKSK, TRTTF, and JRCQC as reported by Chao et al. (2017). Seedlings were tested at CDL in St. Paul, MN in 2016.

Line	BCCBC	TTTTF	TTKSK	TRTTF	JRCQC
PI192711	0;	2+3	0;	;13	;1
PI383416	;1	122+	3+	2+	0;
PI520392	;	0;	3	1;	;1+
PI636501	0;	-22	3+	0;	0

Phenotyping of the Parent Lines with Upper Midwestern United States, East African and European Races of Stem Rust

Parent lines were tested with the local races of stem rust prevalent in the wheat growing

regions of North Dakota prior to populations being tested. Parent lines PI192711, PI383416,

PI520392, and PI636501 exhibited high levels of resistance to the local races of stem rust

MCCF, RKQQ, QCCJ, and HKHJ (Table 3.3) Parent lines tested with foreign races occurred at

the same time as population testing. Only PI192711 and the Rusty/PI192711 population were

tested with race TTKSK. PI636501 and population Rusty/PI636501 were only tested with the

local races of rust and no foreign races. Line PI192711 was resistant to all eight races it was

tested with (Table 3.3). PI383416 was resistant to all local races as well as JRCQC, moderately resistant to TRTTF, and susceptible to TTRTF (Table 3.3). PI520392 was also resistant to all local races and JRCQC and was moderately resistant to TRTTF and TTRTF (Table 3.3). PI636501 was not tested with the foreign races but was resistant to the local races. Rusty, the susceptible parent, was susceptible to all eight races of stem rust.

Table 3.3. ITs scores for parent lines when tested with four Upper Midwestern United States local races of stem rust and four East African and European foreign races of stem rust. Local races of stem rust were tested at the AES greenhouse on NDSU's campus in 2020 and foreign races of rust were tested at CDL in St. Paul, MN in 2021.

Line	MCCF	RKQQ	QCCJ	HKHJ	JRCQC	TRTTF	TTRTF	TTKSK
PI192711	;	;	1;	;1	;12	;1	;12	0;
PI383416	;1	;	1;	;	13-	22-	3-1	/
PI520392	;	1;	;	1;	1;	2-	2	/
PI636501	;1	1;	1	1;	/	/	/	/
Rusty	4	3	3	4	3+	3+	3+	3+

Note: '/' Parent line not tested with race of rust.

Phenotyping of the RIL Populations with MCCF Stem Rust

The RIL populations were phenotyped with local stem rust race MCCF at the AES greenhouse on NDSU's campus in 2021 following parent testing. Table 3.4 below shows the total number of resistant and susceptible lines of the populations with MCCF stem rust. The parent lines were tested again with the rest of the population. Converted linear scores for the parent lines to race MCCF are as follows: PI192711 scored 1 and 2, PI383416 scored 3 and 4, PI520392 scored 2 and 4, and PI636501 scored 4 and 4. Rusty inoculated with MCCF yielded linear scores of 7, 7, 7, and 8.

Table 3.4. Total number of resistant and susceptible lines for four durum populations when tested with local stem rust race MCCF with Chi Squared p-values for expected segregation ratios.

Population	Resistant Lines	Susceptible Lines	Total Lines	Chi Square p-values				
				1:1	1:3	3:1	1:7	7:1
Rusty/PI192711	76	113	189	0.007	NS	NS	NS	NS
Rusty/PI383416	160	8	168	NS	NS	NS	NS	NS
Rusty/PI520392	146	4	150	NS	NS	NS	NS	NS
Rusty/PI636501	138	8	146	NS	NS	NS	NS	0.010

Note: "NS" Not significant (p-value<0.001).

The Rusty/PI192711 population was the most susceptible population when tested with MCCF stem rust with 40% resistance and 60% susceptibility (Table 3.4). Rusty/PI383416, Rusty/PI520392, and Rusty/PI636501 were all very resistant to MCCF with 95%, 97%, and 95% resistance observed to MCCF. None of the populations fit any of the expected segregation ratios as evidenced by Chi Squared p-values (p>0.05) (Table 3.4). The scoring distributions are shown in Figure 3.1 for each population when tested with MCCF, and the parent line scores are indicated in orange.

Figure 3.1. Distribution of linear scores throughout populations when tested with MCCF stem rust.
Note: Parent line scores indicated in orange and red dashed lines shows the cutoff between resistant lines and susceptible lines.

Phenotyping of the RIL Populations with RKQQ Stem Rust

Population testing with local race of stem rust RKQQ was done at the AES greenhouse on NDSU's campus following parent testing in 2021. Table 3.5 below shows the number of resistant and susceptible lines of the populations with RKQQ stem rust. Parent lines were tested again with the populations. The linear scores of the parent lines when tested with the population with RKQQ stem rust were scores of 9, 9, 9, and 9 for susceptible parent Rusty, 0 and 0 for resistant parent PI192711, 3 and 4 for resistant parent PI383416, 4 and 7 for resistant parent PI520392, and 5 and 7 for resistant parent PI636501.

Table 3.5. Total number of resistant and susceptible lines for four durum populations when tested with local stem rust race RKQQ with Chi Squared p-values for expected segregation ratios.

Population	Resistant Lines	Susceptible Lines	Total Lines	Chi Square p-values				
				1:1	1:3	3:1	1:7	7:1
Rusty/PI192711	20	169	189	NS	NS	NS	0.425	NS
Rusty/PI383416	117	50	167	NS	NS	0.140	NS	NS
Rusty/PI520392	103	45	148	NS	NS	0.129	NS	NS
Rusty/PI636501	70	68	138	0.865	NS	NS	NS	0.010

Note: "NS" Not significant (p-value<0.001).

The Rusty/PI192711 population did follow expected segregation with 10% resistance and 90% susceptibility observed ($\chi^2_{1:7}$ p=0.425). The Rusty/PI383416 and Rusty/PI520392 populations also did fit expected ratios with 70% of the lines being resistant and 30% of the line being susceptible in both populations to stem rust race RKQQ (Rusty/PI383416 $\chi^2_{3:1}$ p=0.140; Rusty/PI520392 $\chi^2_{3:1}$ p=0.129). Rusty/PI636501 fit the expected 1:1 ratio of resistant to susceptible with 51% resistance and 49% susceptibility ($\chi^2_{1:1}$ p=0.865). Figure 3.2 below shows the distribution of scores among the populations with the parent lines being indicated in orange.

Figure 3.2. Distribution of linear scores throughout populations when tested with RKQQ stem rust.
Note: Parent line scores indicated in orange and red dashed lines shows the cutoff between resistant lines and susceptible lines.

Phenotyping of the RIL Populations with JRCQC Stem Rust

The parents were tested in addition to the F4 RILs at CDL in St. Paul, MN with stem rust race JRCQC. The Rusty/PI636501 population was not tested with JRCQC. Table 3.6 below shows the total number of resistant lines and susceptible lines for each population when tested with JRCQC stem rust. Rusty's linear scores for JRCQC stem rust were 9 and 9. PI192711, PI383416, and PI520392 scored 5 and 2, 4 and 4, and 4 and 1 with JRCQC stem rust, respectively.

36

Table 3.6. Total number of resistant and susceptible lines for four durum populations when tested with foreign stem rust race JRCQC with Chi Squared p-values for expected segregation ratios.

Population	Resistant Lines	Susceptible Lines	Total Lines	Chi Square p-values				
				1:1	1:3	3:1	1:7	7:1
Rusty/PI192711	1	119	120	NS	NS	NS	NS	NS
Rusty/PI383416	60	64	124	0.719	NS	NS	NS	NS
Rusty/PI520392	66	52	118	0.197	NS	NS	NS	NS

Note: "NS" Not significant (p-value<0.001).

Rusty/PI192711 was very susceptible with only a single line observed being resistant and all other 119 lines being susceptible. Rusty/PI383416 fit the 1:1 expected ratio with 60 lines being resistant and 64 lines being susceptible ($\chi^2_{1:1}$ p=0.719). Rusty/PI520392 also fit the expected 1:1 ratio with 66 lines being resistant and 52 lines being susceptible ($\chi^2_{1:1}$ p=0.197). Scoring distributions for the three populations tested with JRCQC stem rust can be observed in Figure 3.3, below.
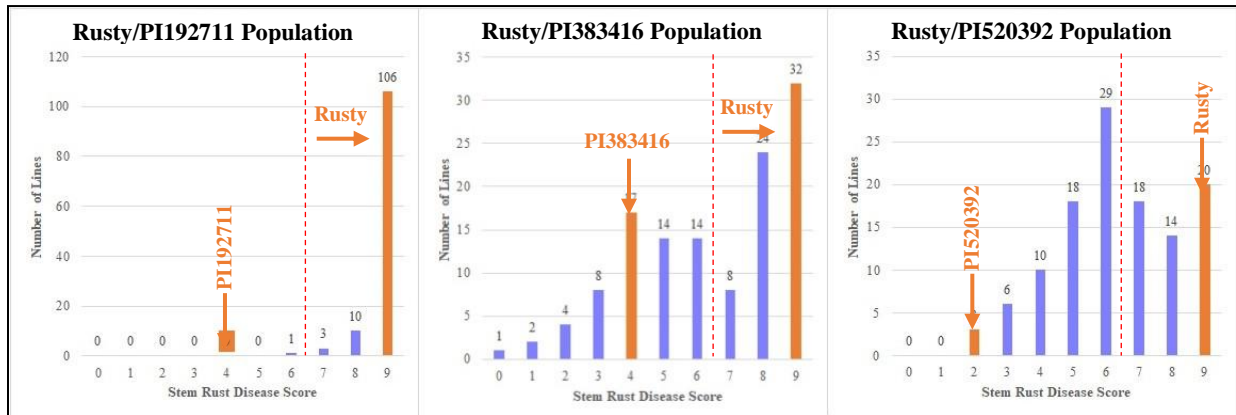
Figure 3.3. Distribution of linear scores throughout populations when tested with JRCQC stem rust.
Note: Parent line scores indicated in orange and red dashed lines shows the cutoff between resistant lines and susceptible lines.

Phenotyping of the RIL Populations with TRTTF Stem Rust

F_4 RILs, as well as the parent lines, were tested with foreign race of stem rust TRTTF at CDL in St. Paul, MN in 2021. The Rusty/PI636501 population was not tested with TRTTF stem

rust. Table 3.7 below shows the total number of lines considered resistant (linear scores 0-6) and

susceptible (linear scores 7-9) of the populations tested with TRTTF stem rust. Linear scores for

parent lines Rusty, PI192711, PI383416, and PI636501 were 9 and 9, 2 and 3, 5 and 5, and 4 and

5, respectively.

Table 3.7. Total number of resistant and susceptible lines for four durum populations when tested with foreign stem rust race TRTTF with Chi Squared p-values for expected segregation ratios.

Population	Resistant Lines	Susceptible Lines	Total Lines	Chi Square p-values				
				1:1	1:3	3:1	1:7	7:1
Rusty/PI192711	25	95	120	NS	0.292	NS	0.006	NS
Rusty/PI383416	69	55	124	0.209	NS	NS	NS	NS
Rusty/PI520392	99	17	118	NS	NS	NS	NS	0.237

Note: "NS" Not significant (p-value<0.001).

Rusty/PI192711 fit the expected 1:3 ratio with 25 resistant lines and 95 susceptible lines

(χ^2 $_{1:3}$ p=0.292). Rusty/PI383416 fit the expected 1:1 ratio with 69 resistant lines and 55

susceptible lines (χ^2 $_{1:1}$ p=0.209). Lastly, Rusty/PI520392 fit the expected 7:1 ratio with 99

showing resistance and 17 lines showing susceptibility to race TRTTF (χ^2 $_{7:1}$ p=0.237). The

distribution of scores for populations Rusty/PI192711, Rusty/PI383416, and Rusty/PI520392 can
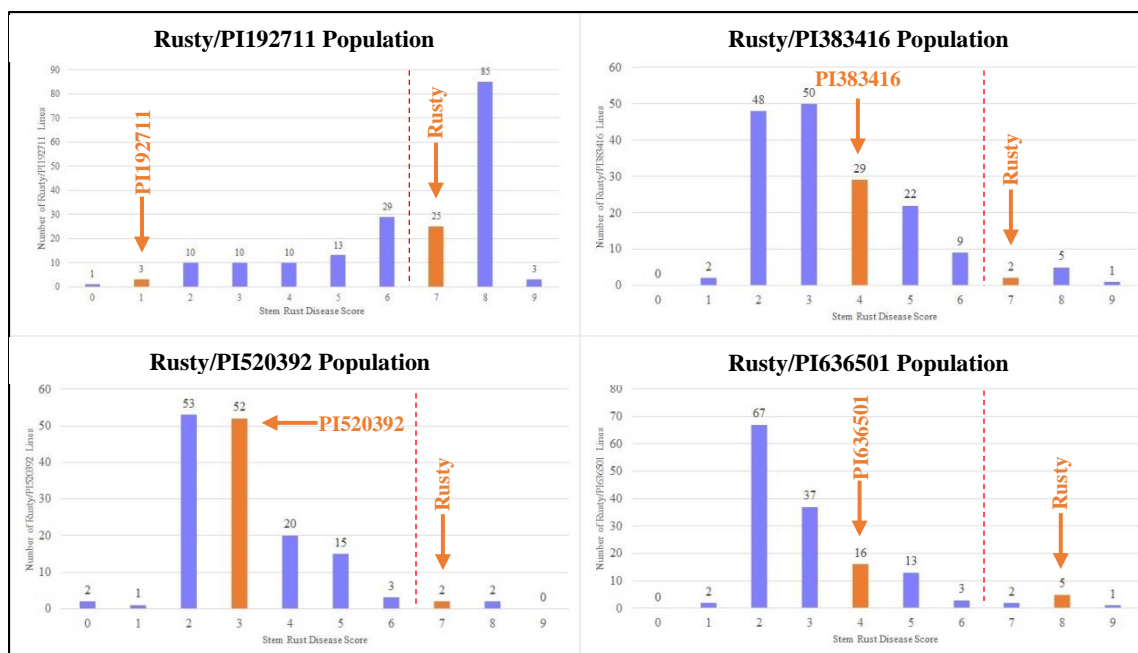
be viewed in Figure 3.4, below.
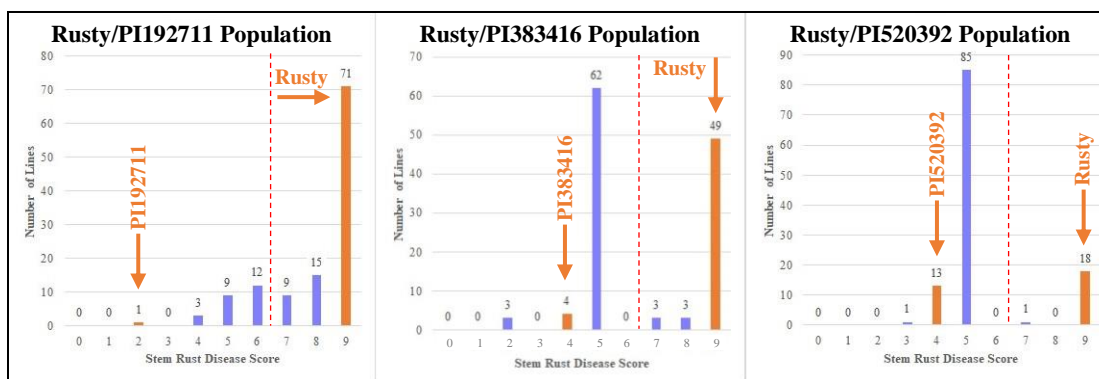
Figure 3.4. Distribution of linear scores throughout populations when tested with TRTTF stem rust.
Note: Parent line scores indicated in orange and red dashed lines shows the cutoff between resistant lines and susceptible lines.

Phenotyping of the RIL Populations with TTRTF Stem Rust

The Rusty/PI192711, Rusty/PI383416, and Rusty/PI520392 populations, as well as their parent lines, were tested with TTRTF stem rust at CDL in St. Paul, MN in 2021. The Rusty/PI636501 population was not tested with TTRTF stem rust. Table 3.8 below shows the number of resistant lines and number of susceptible lines observed for each population when tested with TTRTF stem rust.

Table 3.8. Total number of resistant and susceptible lines for four durum populations when tested with foreign stem rust race TTRTF with Chi Squared p-values for expected segregation ratios.

Population	Resistant Lines	Susceptible Lines	Total Lines	Chi Square p-values				
				1:1	1:3	3:1	1:7	7:1
Rusty/PI192711	9	111	120	NS	NS	NS	0.098	NS
Rusty/PI383416	4	120	124	NS	NS	NS	0.002	NS
Rusty/PI520392	62	56	118	0.581	NS	NS	NS	NS

Note: "NS" Not significant (p-value<0.001).

When tested with TTRTF stem rust, Rusty scored 9, PI192711 scored 2, PI383416 scored 5, and PI520392 scored 5. Rusty/PI192711 was very susceptible to TTRTF but did fit an expected ratio of 1:7 with 9 resistant lines and 111 susceptible lines ($\chi^2_{1:7}$ p=0.098).

39

Rusty/PI383416 RILs were very susceptible to TTRTF with only 4 lines being resistant to

TTRTF and did not fit any of the expected ratios. Rusty/PI520392 fit the expected 1:1 ratio with

62 resistant lines and 56 susceptible lines being observed ($\chi^2_{1:1}$ p=0.581). Scoring distribution for

the three populations and parent line scores in orange to race TTRTF can be seen in Figure 3.5,
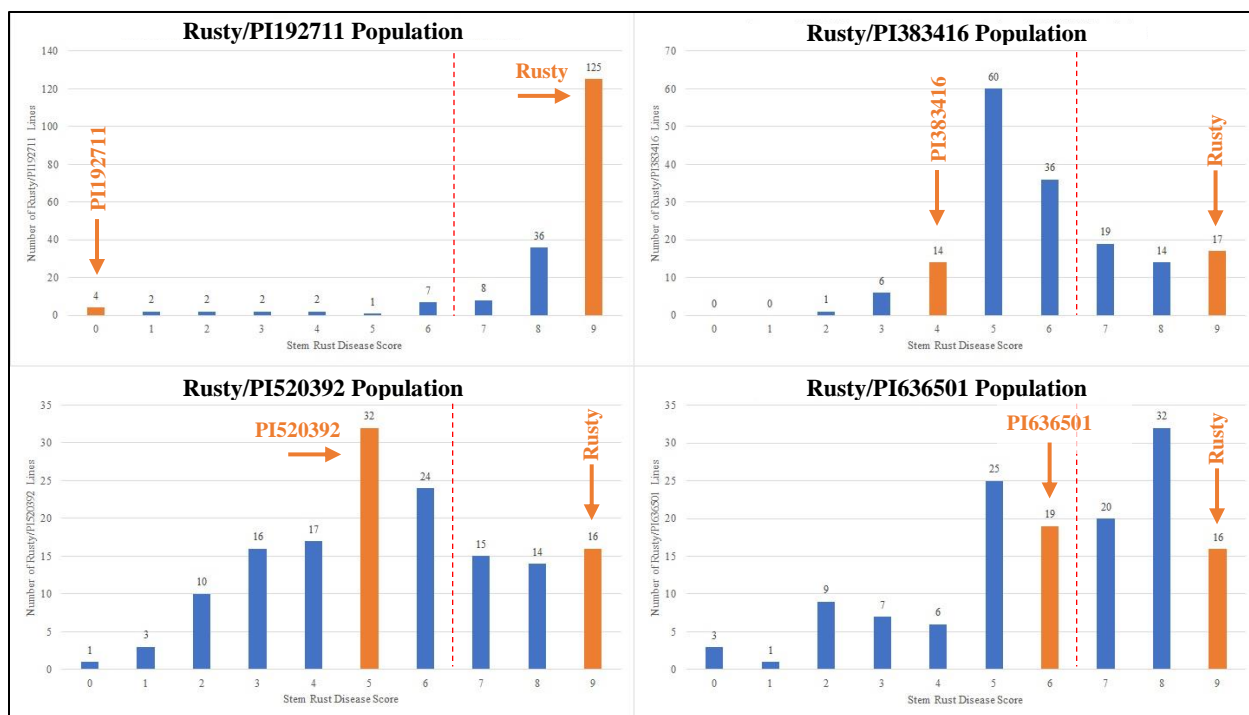
below.
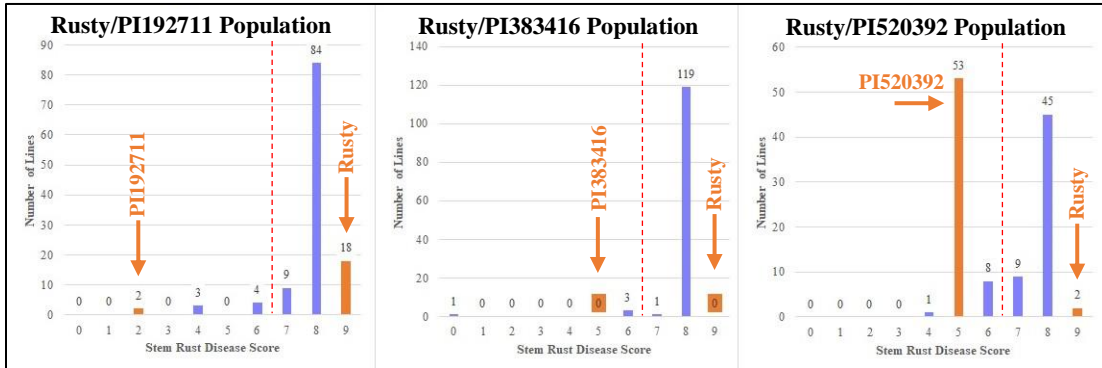
Figure 3.5. Distribution of linear scores throughout populations when tested with TTRTF stem
rust.
Note: Parent line scores indicated in orange and red dashed lines shows the cutoff between
resistant lines and susceptible lines.

Phenotyping of a RIL Population with TTKSK Stem Rust

Only population Rusty/PI192711 was tested with stem rust race TTKSK at CDL in St.

Paul in 2021. Table 3.9 below shows the resistant line total and susceptible line total for

Rusty/PI192711 with TTKSK stem rust testing. The linear scores for Rusty when tested with

TTKSK stem rust were 9 and 9 and PI192711 scored 0 and 0. The Rusty/PI12711 population fit

the expected ratio 1:3 with 27 lines being resistant and 93 lines being susceptible ($\chi^2_{1:3}$ p=0.527).

The scoring distribution of the lines can be viewed in Figure 3.6 below, with the parent line

scores being represented in orange.

Table 3.9. Total number of resistant and susceptible lines for four durum populations when tested with foreign stem rust race TTKSK with Chi Squared p-values for expected segregation ratios.

Population	Resistant Lines	Susceptible Lines	Total Lines	Chi Square p-values				
				1:1	1:3	3:1	1:7	7:1
Rusty/PI192711	27	93	120	NS	0.527	NS	0.001	NS

Note: "NS" Not significant (p-value<0.001).
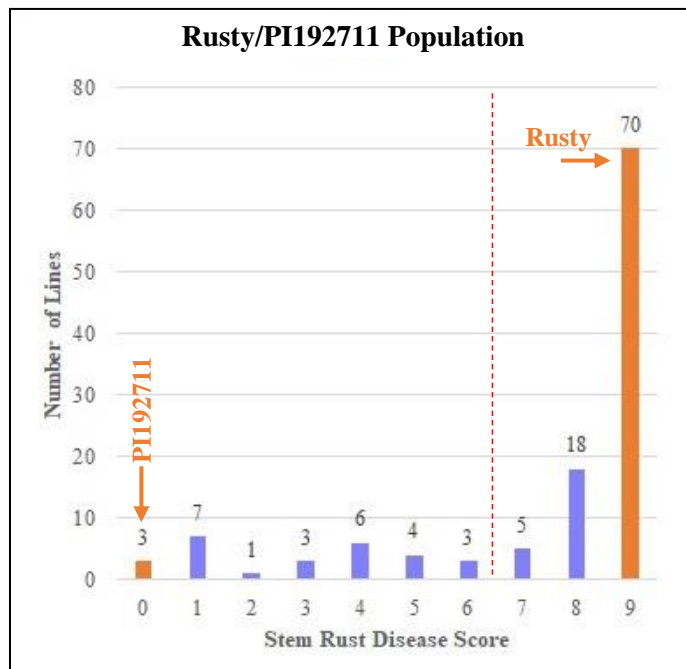
Figure 3.6. Distribution of linear scores of population Rusty/PI192711 when tested with TTKSK stem rust.
Note: Parent line scores indicated in orange and red dashed lines shows the cutoff between resistant lines and susceptible lines.

Discussion

Identification of stem rust resistant durum wheat lines is essential for global food security and sustainability. Within those resistant lines may be a novel stem rust gene or a previously discovered gene that provides resistance to the plant. The identification of a new stem rust resistance genes opens the door for further research to fine map the QTL and/or introgress the gene into adapted germplasm for release. This study first focused on determining the stem rust

resistance of parent lines PI192711, PI383416, PI520392, and PI636501 to local races of rust

RKQQ, HKHJ, QCCJ, and MCCF to supplement the preliminary data of the durum lines having

resistance to East African and European races of rust, including Ug99. When parent lines Rusty,

PI192711, PI383416, PI520392, and PI636501 were inoculated with local races of rust RKQQ,

HKHJ, QCCJ, and MCCF there were various levels of resistance observed. All four parent PI

lines were very resistant to race MCCF with all primary scores showing a flecking reaction ('';'')

with a few secondary scores of '1'. Resistance to race RKQQ among all the parent lines was

relatively strong as well. HKHJ and QCCJ testing had all parents considered resistant (ITs of 0-2

considered resistant, 3-4 susceptible), however, more moderate resistance scores when compared

to RKQQ and MCCF scores. The resistant parent lines also exhibited high levels of resistance

when tested with foreign races from the preliminary data from Chao et al. (2017). In that study,

the lines were tested with BCCBC, TTTTF, TTKSK, TRTTF, and JRCQC. PI192711 exhibited

high resistance to BCCBC ('0;'), TTKSK ('0;'), TRTTF (';13'), and JRCQC (';1'). PI192711

had moderate resistance to TTTTF with a score of '2+3'. PI383416 had similar results with

BCCBC (';1') and JRCQC ('0;'), however was moderately resistant to TTTTF ('122+') and

TRTTF ('2+') and susceptible to TTKSK ('3+'). PI520392 had some of the highest levels of

resistance overall to the foreign races with scores of ';' (BCCBC), '0;' (TTTTF), '1;' (TRTTF),

and ';1+' (JRCQC) but was considered susceptible to TTKSK ('3'). Finally, PI636501 was very

resistant to BCCBC ('0;'), TRTTF ('0;'), and JRCQC ('0'). PI636501 was moderately resistant

to TTTTF ('2-2') and susceptible to TTKSK ('3+').

Due to the overall high resistance of the resistant parent lines to the Upper Midwestern

United States, East African and European races of stem rust, the second part of this study was to

evaluate resistance of the F_4 and F_5 RILs with the stem rust races the parent lines were screened

with. Progeny were binned into 'Resistant' and 'Susceptible' reaction scores and segregation ratios were determined to provide evidence for the number of resistance genes present in the population. Chi square goodness of fit tests were performed to determine the significance of observed segregation ratios compared to the expected segregation ratios of one gene, two genes, or three genes providing resistance.

The Rusty/PI192711 population was tested with two local races of rust from the Upper Midwest United States as well as four foreign races of rust from Eastern Africa and Europe. With PI192711 exhibiting high resistance to all races of rust tested, high levels of resistance were expected in the population. However, Rusty/PI192711 was more susceptible than resistant to all races of rust. Distorted segregations from the expected segregations were observed from the population when tested with local races MCCF and foreign race JRCQC (Table 3.10). The expected 1:3 ratio indicative of two genes jointly providing resistance was observed with foreign races TRTTF and TTKSK and the expected 1:7 ratio indicating three genes are all required to provide resistance was observed when testing with local race RKQQ and foreign race TTRTF (Table 3.10). The distorted segregation ratios observed in with MCCF and JRCQC cannot be explained by one, two, or three genes providing resistance and require additional research to determine the mechanism of resistance within the population. With only one line being resistant to JRCQC, despite PI192711 exhibiting a high level of resistance to JRCQC, there may be a suppression gene that is hindering resistance to JRCQC.

Table 3.10. Resistant to susceptible ratios for all four populations tested with local Upper Midwestern United States races of stem rust MCCF and RKQQ as well as foreign races of stem rust from Eastern Africa and Europe including JRCQC, TRTTF, TTRTF, and TTKSK.

Population	MCCF	RKQQ	JRCQC	TRTTF	TTRTF	TTKSK
Rusty/PI192711	40%:60%	10%:90%[4]	1%:99%	21%:79%[2]	8%:92%[4]	23%:77%[2]
Rusty/PI383416	95%:5%	70%:30%[3]	48%:52%[1]	56%:44%[1]	3%:97%	-
Rusty/PI520392	97%:3%	70%:30%[3]	56%:44%[1]	84%:16%[5]	53%:47%[1]	-
Rusty/PI636501	94%:6%	51%:49%[1]	-	-	-	-

Note: '-' Population was not tested with that race of stem rust. [1] Fit the expected 1:1 ratio. [2] Fit the expected 1:3 ratio. [3] Fit the expected 3:1 ratio. [4] Fit the expected 1:7 ratio. [5] Fit the expected 7:1 ratio.

The Rusty/PI383416 population was tested with the two Upper Midwest United States races of rust and three of the East African and European races of stem rust. PI383416 exhibited high resistance to the local races of rust and foreign race JRCQC, moderate resistance to foreign race TRTTF, and was susceptible to foreign race TTRTF. Overall, Rusty/PI383416 displayed more resistance than susceptibility to all races except TTRTF. The population only expressed 3% resistance to race TTRTF and was otherwise susceptible (Table 3.10). On the contrary, Rusty/PI383416 expressed high levels of resistance to race MCCF with 95% resistance being observed (Table 3.10). When tested with race RKQQ, a 3:1 resistant to susceptible ratio was observed indicating two genes were independently providing resistance (Table 3.10). The expected 1:1 ratio was observed with foreign races JRCQC and TRTTF for the Rusty/PI383416 population signaling a single gene is providing resistance (Table 3.10). Like Rusty/PI192711 and JRCQC stem rust, when Rusty/PI383416 was tested with foreign stem rust race TTRTF only 3% resistance was observed and when PI383416 was tested high levels of resistance were observed. There may be a suppression gene that is hindering the resistance that PI383416 should be contributing to the population.

Rusty/PI520392 was also tested with two local Upper Midwestern United States stem rust races as well as three of the foreign races of stem rust from Eastern Africa and Europe. PI520392 was very resistant to the local races and foreign race JRCQC, as well as moderately resistant to foreign races TRTTF and TTRTF. Rusty/PI383416 was more resistant than susceptible to all races and only one race tested did not result in an expected segregation ratio. The population was very resistant to local race MCCF but did not follow one of the expected ratios (Table 3.10). Expected segregations ratios were observed with races JRCQC, TRTTF, and TTRTF. JRCQC and TTRTF segregated into 1:1 resistant to susceptible ratios indicating a single gene is able to provide resistance (Table 3.10). A 7:1 resistant to susceptible ratio was observed when tested with race TRTTF inferring three genes are independently capable of providing resistance (Table 3.10).

The Rusty/PI636501 population was only tested with the Upper Midwestern United States local races of rust. PI636501 was very resistant to both local races MCCF and RKQQ that the population was tested with. To emulate the Rusty/PI383416 and Rusty/PI520392 populations, Rusty/PI636501 was very resistant to local race MCCF with 94% resistance observed (Table 3.10). Unlike any other population, Rusty/PI636501 yielded a 1:1 ratio of resistant to susceptible with race RKQQ indicating a single gene is providing resistance (Table 3.10).

Overall, the expected segregation ratio resistant to susceptible of 1:1 was only met a few times with the populations tested against the local and foreign races of rust (Rusty/PI383416 + JRCQC; Rusty/PI383416 + TRTTF; Rusty/PI520392 + JRCQC; Rusty/PI520392 + TTRTF; Rusty/PI636501 + RKQQ). Two population and rust combinations resulted in the expected 1:3 resistant to susceptible ratio indicating two genes jointly provide resistance (Rusty/PI192711 +

TRTTF; Rusty/PI192711 + TTKSK). Two combinations resulted in the expected 3:1 ratio where two genes are able to independently provide resistance (Rusty/PI383416 + RKQQ; Rusty/PI520392 + RKQQ). Two populations and rust combinations resulted in a 1:7 segregation ratio indicating three genes are required for resistance (Rusty/PI192711 + RKQQ; Rusty/PI192711 + TTRTF) while only one combination yielded a 7:1 ratio where three genes are able to independently provide resistance (Rusty/PI520392 + TRTTF). The remaining six population and rust combinations resulted in distorted segregations that did not fit the expected ratios. Rusty/PI192711 when tested with JRCQC and Rusty/PI383416 when tested with TTRTF resulted in very distorted segregation ratios with only 1% and 3% resistance observed, potentially indicating a suppressor gene is responsible for the lack of resistance observed within these population to the given races of rust or there is a much more complex mechanism occurring.

In this study we evaluated four biparental populations with six different stem rust races, to local to the Upper Midwest United States and four foreign races from Eastern Africa and Europe. We identified each of the expected segregation ratios from the canonical 1:1 ratio indicating a single dominant resistance gene to the 1:3 ratio that requires two resistance genes to provide resistance to the 1:7 resistant to susceptible ratio that indicates the requirement of three genes to provide resistance. Multi-gene models for resistance to stem rust are scientifically interesting, but complicated to investigate and are not desirable for breeding as it is difficult to maintain the gene combinations during introgression and selection to an elite cultivar. In some cases, we have identified two independent genes that were passed from the resistance parent line to the progeny. It is possible that segregation ratios could vary slightly due to error while phenotyping the plants and assigning ITs scores, however, severe distortion to segregation ratios

are likely real (Coulton et al. 2020). Since only inbred lines were tested in this study, follow-up efforts should include the screening of segregating families to determine if genes display dominance in the heterozygous state.

Conclusion

While some expected 1:1 segregation ratios were observed, most of the population and stem rust race combinations evaluated did not yield ratios indicative of single gene resistance mechanisms. Further research into the location of the resistance genes within the populations and how the genes are interacting with one another will reveal important information about multiple gene interactions to provide higher levels of resistance to stem rust as well as if there are suppression genes hindering resistance potential. Crossing of Rusty with progeny lines that exhibit high resistance will help separate independent different genes into separate populations for higher-resolution mapping studies.

References

Chao, S., Rouse, M. N., Acevedo, M., Szabo-Hever, A., Bockelman, H., Bonman, J. M., Elias, E., Klindworth, D., & Xu, S. (2017). Evaluation of genetic diversity and host resistance to stem rust in USDA NSGC durum wheat accessions. *The Plant Genome*, *10*(2). https://doi.org/10.3835/plantgenome2016.07.0071

Coulton, A., Przewieslik-Allen, A. M., Burridge, A. J., Shaw, D. S., Edwards, K. J., & Barker, G. L. (2020). Segregation distortion: Utilizing simulated genotyping data to evaluate statistical methods. *PLOS ONE*, *15*(2). https://doi.org/10.1371/journal.pone.0228951

Hundie, B., Girma, B., Tadesse, Z., Edae, E., Olivera, P., Abera, E. H., Bulbula, W. D., Abeyo, B., Badebo, A., Cisar, G., Brown-Guedira, G., Gale, S., Jin, Y., & Rouse, M. N. (2019). Characterization of Ethiopian wheat germplasm for resistance to four *Puccinia graminis* f. sp. *tritici* races facilitated by single-race nurseries. *Plant Disease*, *103*(9), 2359–2366. https://doi.org/10.1094/pdis-07-18-1243-re

Peterson, R. F., Campbell, A. B., & Hannah, A. E. (1948). A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian Journal of Research*, *26c* (5), 496–500. https://doi.org/10.1139/cjr48c-033

Prank, M., Kenaley, S. C., Bergstrom, G. C., Acevedo, M., & Mahowald, N. M. (2019). Climate change impacts the spread potential of wheat stem rust, a significant crop disease. *Environmental Research Letters*, *14*(12), 124053. https://doi.org/10.1088/1748-9326/ab57de

Sall, A. T., Chiari, T., Legesse, W., Seid-Ahmed, K., Ortiz, R., van Ginkel, M., & Bassi, F. M. (2019). Durum wheat (*Triticum durum desf.*): Origin, cultivation, and potential expansion in Sub-Saharan Africa. *Agronomy*, *9*(5), 263. https://doi.org/10.3390/agronomy9050263

Stakman, E. C., Stewart, D. M., & Loegering, W. G. (1962). *Identification of physiologic races of Puccinia graminis var. tritici*. U.S. Dept. of Agriculture Revised.

Schumann, G. L., & Leonard, K. J. (2000). Stem rust of wheat (Black Rust). *The Plant Health Instructor*. https://doi.org/10.1094/phi-i-2000-0721-01

Zhang, D., Bowden, R. L., Yu, J., Carver, B. F., & Bai, G. (2014). Association analysis of STEM Rust Resistance in U.S. winter wheat. *PLOS ONE*, 9(7). https://doi.org/10.1371/journal.pone.0103747

CHAPTER 4. GENETIC MAPPING OF STEM RUST RESISTANT QTL IN DURUM WHEAT POPULATIONS

Abstract

Rusty, a stem rust susceptible line, was crossed with stem rust resistant lines PI192711, PI383416, PI520392, and PI636501 to develop F_5 RILs that were used for stem rust testing. QTL mapping within the four populations gave rise to confirmation of five previously known stem rust resistance genes and identification of six potentially novel stem rust genes. The confirmed genes were *Sr28* on chromosome 2B, *Sr12* on chromosome 3B, *Sr7/SrND643* on chromosome 4A, and *Sr22* on chromosome 7A. Three separate QTLs confirmed the presence and location of *Sr9*,* a potentially novel allele of *Sr9* on chromosome 2B. The other potentially novel genes were identified on chromosome 4A, two on 5B, 6A, and 6B. Several multi-gene models were discovered to provide resistance with some single genes identified that provide resistance. Several complex interactions that are not understood very well were also found.

Introduction

Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) affects durum wheat production worldwide. Identification of stem rust resistance genes allows for the breeding of improved resistant lines and increases the understanding of underlying resistance mechanisms. There are several known stem rust resistance genes that are utilized in breeding programs to provide resistance including *Sr13*, *Sr22, Sr25, Sr33,* and *Sr50* (Kosgey et al. 2021). Stem rust continuously evolves to generate virulent isolates to previously discovered resistance genes highlighting the need for continued research to identify new genes that can provide resistance in the future. A single resistance gene is also not universally effective towards all races of rust again highlighting the need to identify new genes.

Table 4.1. Virulence and avirulence of evaluated foreign races and local races of rust to known stem rust resistance genes.

Pgt Race	Avirulent	Virulent
JRCQC	*5 7 8a 9b 10 24 30 31 36 38*	*6 9(a, d, e, g) 11 13 17 21*
TRTTF	*8a 22 24 31 35*	*5 6 7b 9(a, b, d, e, g) 10 11 13 21 30 36 38 McN Tmp*
TTRTF	*24 30 31*	*5 6 7b 8a 9(a, b, d, e, g) 10 11 13b 17 21 35 36 37 38 McN Tmp*
TTKSK	*22 24 35 36 Tmp*	*5 6 7b 8a 9(a, b, d, e, g) 10 11 17 21 30 31 38 McN*
MCCF	*6 8a 9(a, b, d, e) 11 21 22 24 30 31 36 38*	*5 7b 9g 10 17 35 McN Tmp*
RKQQ	*9e 10 11 17 24 30 31 38 Tmp*	*5 6 7b 8a 9(b, d, g) 21 36 McN*

Table 4.1 shows the avirulence and virulence to stem rust resistance genes of foreign races of stem rust JRCQC, TRTTF, TTRTF, TTKSK and local races of rust MCCF and RKQQ. As shown in the table, there are numerous known genes with various avirulence and virulence. *Sr36* and *SrTmp* are both avirulent to TTKSK and provide resistance, however, those same two genes are virulent to race TRTTF. JRCQC, TRTTF, and TTRTF are virulent to the well-known resistance gene *Sr13*. Some resistance genes work well against some races of rust but are not effective against another race. This study focuses on identifying stem rust resistant gene QTLs within four durum wheat populations.

Materials and Methods

Plant Materials and Phenotyping Data

Four durum wheat RIL populations at the F_5 generation were developed from the following crosses: Rusty x PI192711, Rusty x PI383416, Rusty x PI520392, and Rusty x PI636501. The parent lines and populations were inoculated and scored with foreign East African and European races of stem rust JRCQC, TRTTF, TTRTF, and TTKSK as well as local Upper Midwestern races of stem rust MCCF and RKQQ. Foreign races of stem rust were tested at the Cereals Disease Laboratory in St. Paul, Minnesota and local races of stem rust were tested at the North Dakota Agricultural Experiment Station Greenhouse Complex in Fargo, North

Dakota. The infection type (ITs) scores were assigned to the plants as described by Stakman et al. (1964) and then were converted to a linear 0-9 scale as described by Zhang et al. (2014) for use in QTL analysis. The phenotype data used for QTL analysis was previously described in Chapter 3.

DNA Extraction

Tissue was harvested from the seedlings of the F_5 plants prior to inoculation and placed in 96-deep well plates. Leaves were dried and ground for 4 minutes at 1500 strokes using a Genogrinder (SPEX Sample Prep). DNA was extracted using a modified SDS extraction method (modified June 2007 from Pallotta et al. 2003) on a Matrix PlateMate Plus (Thermo Fisher, Waltham, MA, United States). DNA was quantified using a NanoDrop 1000 spectrometer (Thermo Fisher, Waltham, MA, United States) and then normalized to 12 ng/ul.

Genotyping of the Populations

Genotyping was performed at the USDA-ARS Small Grains Genotyping Lab in Fargo, North Dakota. Genotyping data of the F_5 plants was obtained with the iSelect 90k SNP assay that contains 81,587 SNPs and was developed by Wang et al. (2014). The data from the 90K SNP assay was uploaded into Illumina's GenomeStudio v. 2.0 software. Genotype clustering and calling were manually inspected, and the data exported from the software. Any heterozygote calls were converted to missing and only SNPs that were polymorphic between the parents for a given population were further evaluated.

QTL Mapping and Identification

The genotype calls from GenomeStudio were utilized to develop linkage groups in JoinMap 5 (Stam 1993). SNPs polymorphic for the parents were filtered so that markers with significant segregation distortion (p<0.01) were removed from analysis. Groups of markers were

generally separated by logarithm of the odds (LOD) of 6, but in some case, higher LODs up to 12 were required to break linkage groups in the same genome group (e.g., 2A and 2B). Ordering of the groups proceeded through a two-step approach to decrease computation time. First, maximum likelihood algorithm maps were initially created, and this order was input as a starting order for the regression mapping algorithm to determine genetic distances. The data from regression mapping, as well as F_5 population phenotype scoring for rusts RKQQ and MCCF and F_4 population phenotype scoring for rusts TTKSK, JRCQC, TRTTF, and TTRTF, were loaded into QGene v. 4.4.0 (Joehanes and Nelson 2008) to identify QTLs in the four populations. Composite interval mapping (CIM) was used to identify preliminary QTLs based on LOD > 3. Identified QTL LOD values were determined by running 1000 permutation tests to determine $\alpha_{0.01}$ and $\alpha_{0.05}$ values. The flanking sequence of the SNP markers were BLAST aligned wheat reference Chinese Spring v2.1 to facilitate connection to published results and postulated genes (Zhu et al. 2021).

GWAS Analysis

In addition to QTL mapping, a Genome-Wide Association Study (GWAS) was performed as an orthologous method to confirm significance of markers within QTL intervals. The SNP-based GWAS analysis was performed using TASSEL v.5 as a mixed linear model that combines the Q method with the kinship (K) method (Bradbury et al., 2007). The first five principal components were used as covariates to capture population structure (Q) in association analysis. The K matrix was created in TASSEL using 50,714 SNPs from all four populations and six races of stem rust tested. The significant SNP markers identified from the GWAS analysis were compared to the significant SNP markers uncovered using QGene.

Results

Genetic Linkage Map Construction

In the Rusty/PI192711 population, 4,866 polymorphic SNP markers were combined into 14 linkage groups that corresponded to the 14 chromosomes (Table 4.2). The groups spanned a total distance of 2,200 cM with an average marker density of 2.4 markers/cM.

Table 4.2. Summary of linkage groups for Rusty/PI192711 population.

Chromosome	Number of Markers	Genetic Distance (cM)	Marker Density
1A	355	176	2.0
1B	482	106	4.5
2A	205	116	1.9
2B	489	172	2.8
3A	266	191	1.4
3B	488	199	2.5
4A	343	221	1.7
4B	270	132	2.0
5A	145	138	1.1
5B	439	80	5.5
6A	383	180	2.1
6B	145	117	1.2
7A	384	232	1.8
7B	472	138	3.4
Total	4866	2200	2.4

A total of 6,630 SNP markers were used to generate linkage groups for Rusty/PI383416 population. 16 linkage groups were identified that corresponded to the 14 chromosomes of durum with chromosome 3A yielding three very small linkage groups (Table 4.3). The total genetic distance for the linkage groups was 1,247 cM and an average marker density was 5.4 markers/cM.

Table 4.3. Summary of linkage groups for Rusty/PI383416 population.

Chromosome	Number of Markers	Genetic Distance (cM)	Marker Density
1A	517	139	3.7
1B	1059	73	14.4
2A	439	157	2.8
2B	794	102	7.8
3A.1	28	16	1.8
3A.2	23	10	2.3
3A.3	20	6	3.6
3B	633	108	5.8
4A	553	77	7.2
4B	337	65	5.2
5A	157	93	1.7
5B	496	42	11.8
6A	411	94	4.4
6B	489	82	6.0
7A	221	67	3.3
7B	453	118	3.8
Total	6630	1247	5.4

When generating linkage groups for population Rusty/PI520392, 4,488 SNP markers were used, and 14 linkage groups were identified (Table 4.4). The total distance of all linkage groups spanned 1,742 cM and had an average marker density of 2.9 markers/cM. Each linkage group corresponded to a chromosome.

Table 4.4. Summary of linkage groups for Rusty/PI520392 population.

Chromosome	Number of Markers	Genetic Distance (cM)	Marker Density
1A	331	141	2.3
1B	580	81	7.2
2A	285	130	2.2
2B	347	181	1.9
3A	215	96	2.2
3B	503	78	6.5
4A	330	184	1.8
4B	110	92	1.2
5A	218	144	1.5
5B	309	99	3.1
6A	348	136	2.6
6B	107	38	2.8
7A	433	208	2.1
7B	372	133	2.8
Total	4488	1742	2.9

A total of 3,692 SNP markers were used to develop 14 linkage groups for population Rusty/PI636501 (Table 4.5). The total distance of the linkage groups was 2,037 cM with an average marker density of 1.3 markers/cM. When using the regression mapping algorithm, JoinMap was not able to find sufficient linkage for groups 2A, 3A, and 7A, therefore data in Table 4.5 is from the maximum likelihood mapping algorithm.

Table 4.5. Summary of linkage groups for Rusty/PI636501 population.

Chromosome	Number of Markers	Genetic Distance (cM)	Marker Density
1A	127	42	3.0
1B	512	73	7.0
2A*	203	284	0.7
2B	342	158	2.2
3A*	87	161	0.5
3B	322	134	2.0
4A	341	134	2.7
4B	207	90	2.3
5A	174	153	1.1
5B	248	61	4.1
6A	311	149	2.1
6B	419	147	2.9
7A*	181	264	0.7
7B	218	166	1.3
Total	3692	2037	2.3

Note: * represents data from maximum likelihood mapping algorithm.

QTL Identification in Rusty/PI192711 Population

Three QTLs were identified from the Rusty/PI192711 population, two with association to MCCF and TTKSK stem rust and the third associated with TRTTF stem rust. They were identified on chromosomes 3B, 6B, and 7A. QTL region *QSr.fgl-3B* located on chromosome 3B was associated with MCCF and TTKSK (Figure 4.1). PI192711 contributed the resistance of this QTL designated as *QSr.fgl-3B. QSr.fgl-3B* had an LOD of 6.5 when associated with MCCF and 6.7 when associated with TTKSK. This QTL is flanked by SNP markers *IWB70232* and *IWB72547*. This QTL explained 14.7% of the phenotypic variation of resistance to stem rust MCCF and 22.4% of the phenotypic variation of resistance to stem rust TTKSK (Table 4.6). The peak LOD marker of *QSr.fgl-3B* was SNP marker *IWB7940*. Rouse et al. (2014a) identified stem rust resistance gene *Sr12* between DArT markers *wPt6047* (3B:478559759) and *wPt0544* (3B:528464509). Based on the position of *IWB8629* (3B:478554776) and *IWB10086*

(3B:527678644), which are present within the *QSr.fgl-3B* region at 73 cM and 76 cM,

respectively, the gene underlying this QTL is possibly *Sr12* (Rouse et al. 2014a). There were no

significant markers identified from within the QTL region from GWAS analysis (-$\log_{10}$(FDR) >

1.0) (Figure 4.1).

Table 4.6. QTLs from Rusty/PI192711 population associated with stem rust resistance to races
MCCF, TTKSK, and TRTTF determined by composite interval mapping.

QTL	Chr	Marker Interval	Likely Sr gene	Interval Size (cM)	Interval Size (Mbp)	MCCF			TTKSK			TRTTF		
						LOD	R^2	AE	LOD	R^2	AE	LOD	R^2	AE
QSr.fgl-3B	3B	*IWB70232-IWB72547*	*Sr12*	25.12 cM	154.5 Mbp	6.472	0.147	-0.767	6.718	0.224	-1.277	-	-	-
QSr.fgl-6B	6B	*IWB33706-IWB46879*	Novel	48.30 cM	14.1 Mbp	-	-	-	-	-	-	13.81	0.406	-1.097
QSr.fgl-7A	7A	*IWB2539-IWA737*	*Sr22*	17.71 cM	13.6 Mbp	5.789	0.132	-0.725	4.748	0.164	-1.088	-	-	-

Note: No significant association with resistance found with RKQQ, JRCQC, and TTRTF. AE =
Additive effect, positive number shows resistance derived from Rusty and a negative number
shows resistance derived from PI192711. "-" are representative of no significant association with
resistance uncovered.
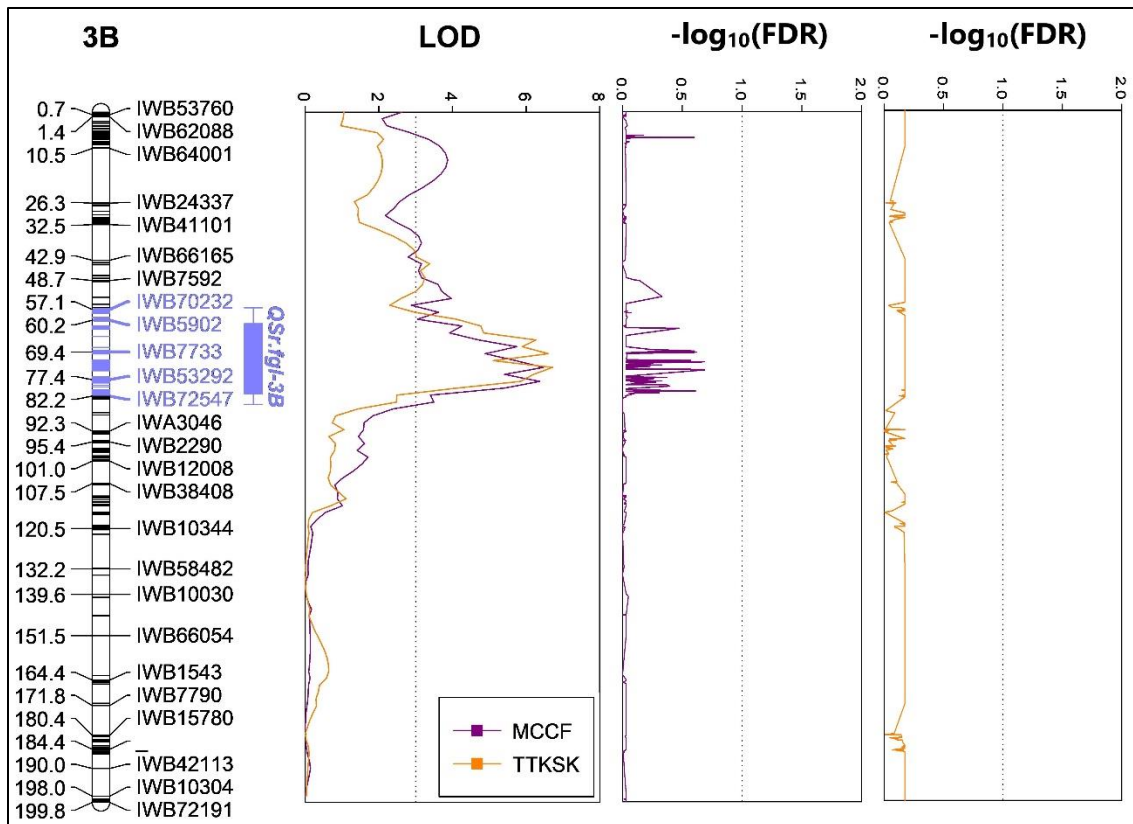
Figure 4.1. Linkage group on chromosome 3B from Rusty/PI192711 population with QTL region *QSr.fgl-3B* highlighted in blue. LOD scores as well as -log$_{10}$(FDR) values displayed for associated local stem rust race MCCF and foreign stem rust race TTKSK.

An additional QTL was identified with association to TRTTF resistance within the Rusty/PI192711 population located on 6B: *QSr.fgl-6B* (Figure 4.2). *QSr.fgl-6B* had an LOD score of 13.8 and accounted for 40.6% of phenotypic variation of resistance to TRTTF (Table 4.6). Zero significant markers were identified within this region from GWAS results. This region was flanked by SNP markers *IWB33706* and *IWB46879*. Based on previous literature, *Sr11* is located on chromosome 6B, however, not in the same region as *QSr.fgl-6B*.
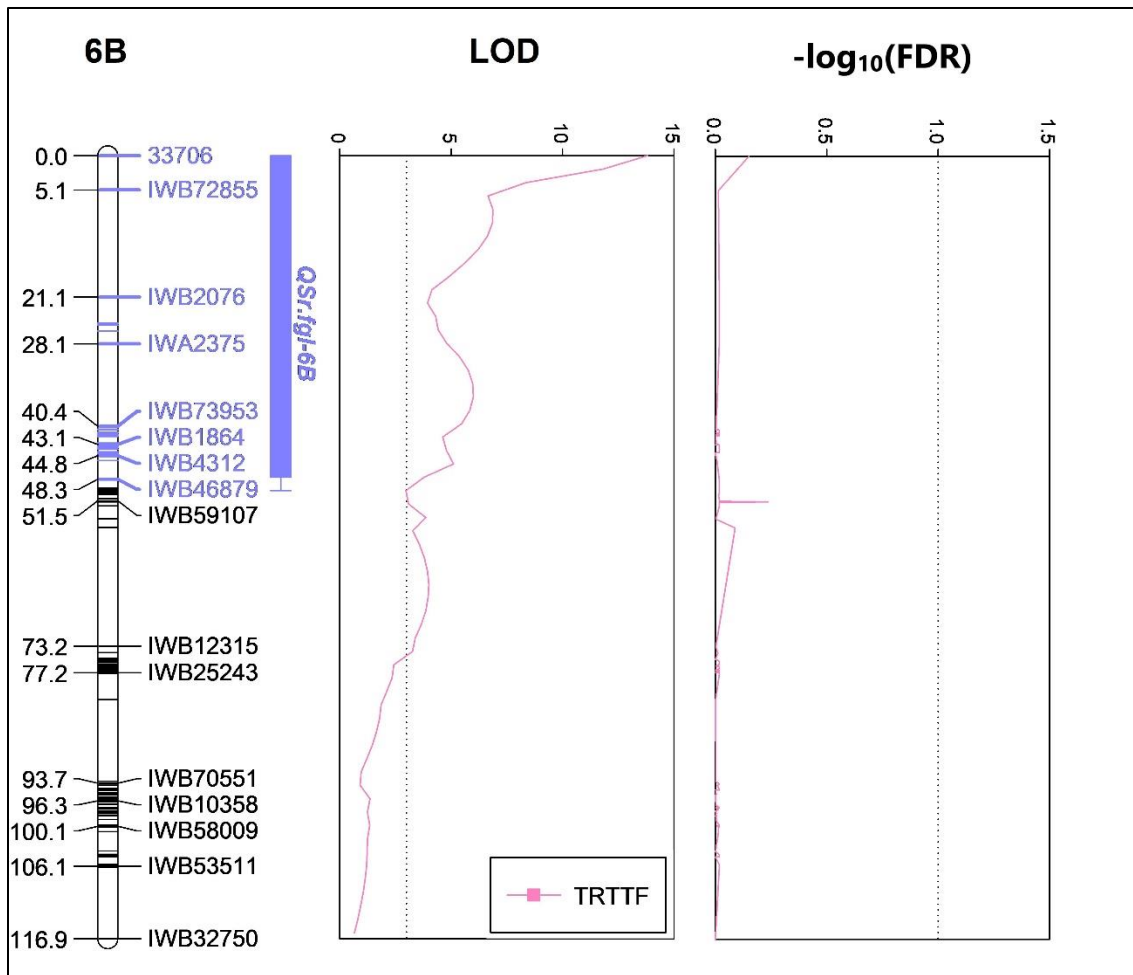
Figure 4.2. Linkage group on chromosome 6B from Rusty/PI192711 population with QTL region *QSr.fgl-6B* highlighted in blue. LOD scores as well as -log₁₀(FDR) values displayed for associated foreign stem rust race TTRTF.

The other QTL identified from Rusty/PI192711 population with association to MCCF and TTKSK stem rusts was located on chromosome 7A, designated as *QSr.fgl-7A* (Figure 4.3). *QSr.fgl-7A* had an LOD of 5.8 when associated with MCCF and 4.7 when associated with TTKSK. *QSr.fgl-7A* explained 13.2% (MCCF) and 16.4% (TTKSK) phenotypic variation of resistance (Table 4.6). Like *QSr.fgl-3B*, no significant markers were identified with association to MCCF or TTKSK from the *QSr.fgl-7A* region. This QTL was flanked by SNP markers *IWB2539* and *IWA737*. This region is known to be associated with *Sr22* and marker *cfa2040* (7A:717078361) was reported to be located 1.2 cM distal to *Sr22* (Yu et al., 2014). Based on the

positioning of SNP marker *IWB38711* that is present within the QTL region (7A:712058659 and

211 cM within QTL), *QSr.fgl-7A* was determined to correspond to *Sr22* (Yu et al. 2014).
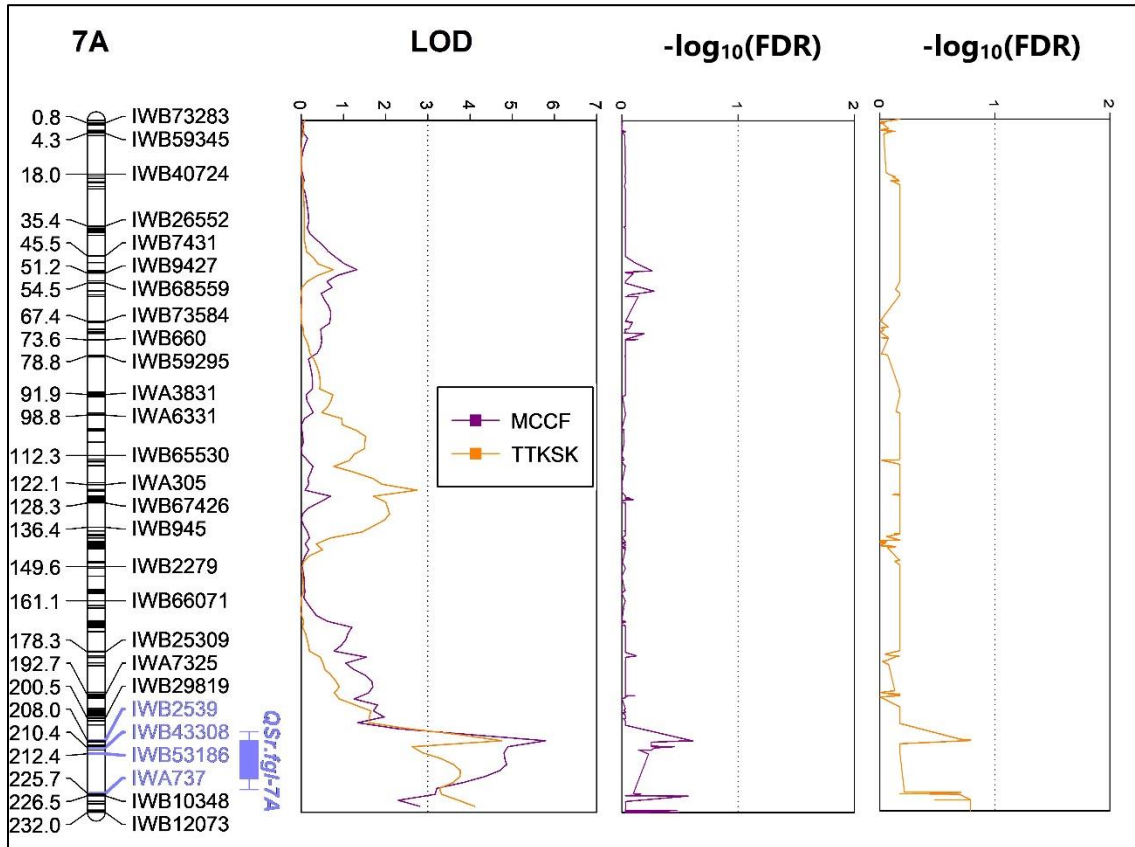
Figure 4.3. Linkage group on chromosome 7A from Rusty/PI192711 population with QTL region *QSr.fgl-7A* highlighted in blue. LOD scores as well as -log$_{10}$(FDR) values displayed for associated local stem rust race MCCF and foreign stem rust race TTKSK.

QTL Identification in Rusty/PI383416 Population

Four QTLs were identified within the Rusty/PI383416 population. *QSr.fgl-2B.1* was

associated with TRTTF resistance and had an LOD score of 26.5 and corresponded to 63.3% of

the phenotypic variation of resistance (Table 4.7). *QSr.fgl-2B.1* was also associated with RKQQ

with an LOD score of 5.9 and explained 15.2% of the phenotypic variation of resistance (Table

4.7). Of the 315 markers within the region, 84 were determined to be significant with RKQQ

stem rust and 179 were determined to be significant with TRTTF stem rust from GWAS

analysis. *QSr.fgl-2B.1* was flanked by SNP markers *IWB71922* and *IWA6122* and spanned an area of 17 cM (Figure 4.4). SNP marker *IWB26349* was positioned at 693740791 and at 51 cM on Figure 4.4, the region where *Sr9* is known to be associated (marker *wmc175* linked to *Sr9a* at 2B:67059647 (Tsilo et al. 2007)).

Table 4.7. QTLs from Rusty/PI383416 population associated with stem rust resistance to races MCCF, RKQQ, TRTTF, and JRCQC determined by composite interval mapping.

QTL	Chr	Marker Interval	Likely Sr Gene	Interval Size (cM)	Interval Size (Mbp)	MCCF			RKQQ			TRTTF			JRCQC		
						LOD	R^2	AE	LOD	R^2	AE	LOD	R^2	AE	LOD	R^2	AE
QSr.fgl-2B.1	2B	*IWB71922-IWA6122*	*Sr9**	20.4 cM	11.7 Mbp	-	-	-	5.938	0.152	-0.592	26.524	0.633	-1.763	-	-	-
QSr.fgl-2B.2	2B	*IWB55102-IWB7605*	*Sr28*	38.1 cM	18.7 Mbp	5.194	0.133	-0.544	-	-	-	-	-	-	-	-	-
QSr.fgl-4A.1	4A	*IWB68289-IWB33628*	*Sr7/SrND643*	40.6 cM	64.9 Mbp	3.675	0.096	-0.46	-	-	-	-	-	-	28.11	0.654	-1.896
QSr.fgl-5B.1	5B	*IWB45090-IWB26458*	Novel	7.1 cM	19.8 Mbp	4.235	0.11	-0.505	-	-	-	-	-	-	-	-	-

Note: No significant association with resistance found with TTRTF. AE = Additive effect, positive number shows resistance derived from Rusty and a negative number shows resistance derived from PI383416. "-" are representative of no significant association with resistance uncovered.

A second QTL region was observed on chromosome 2B with association to MCCF stem rust. *QSr.fgl-2B.2* was flanked by SNP markers *IWB55102* and *IWB7605* and had an LOD score of 5.2 (Table 4.7; Figure 4.4). Zero markers from GWAS analysis were deemed significant from withing this region. *QSr.fgl-2B.2* also contributed 13.3% of phenotypic variation of resistance with MCCF stem rust. *Sr28* has been determined to be positioned between SSR marker *wmc332* and DArT marker *wPt-7161*, *IWB55102* is located between the two. Based on the position of *IWB55102*, *QSr.fgl-2B.2* most likely corresponds to *Sr28* (Rouse et al. 2012).
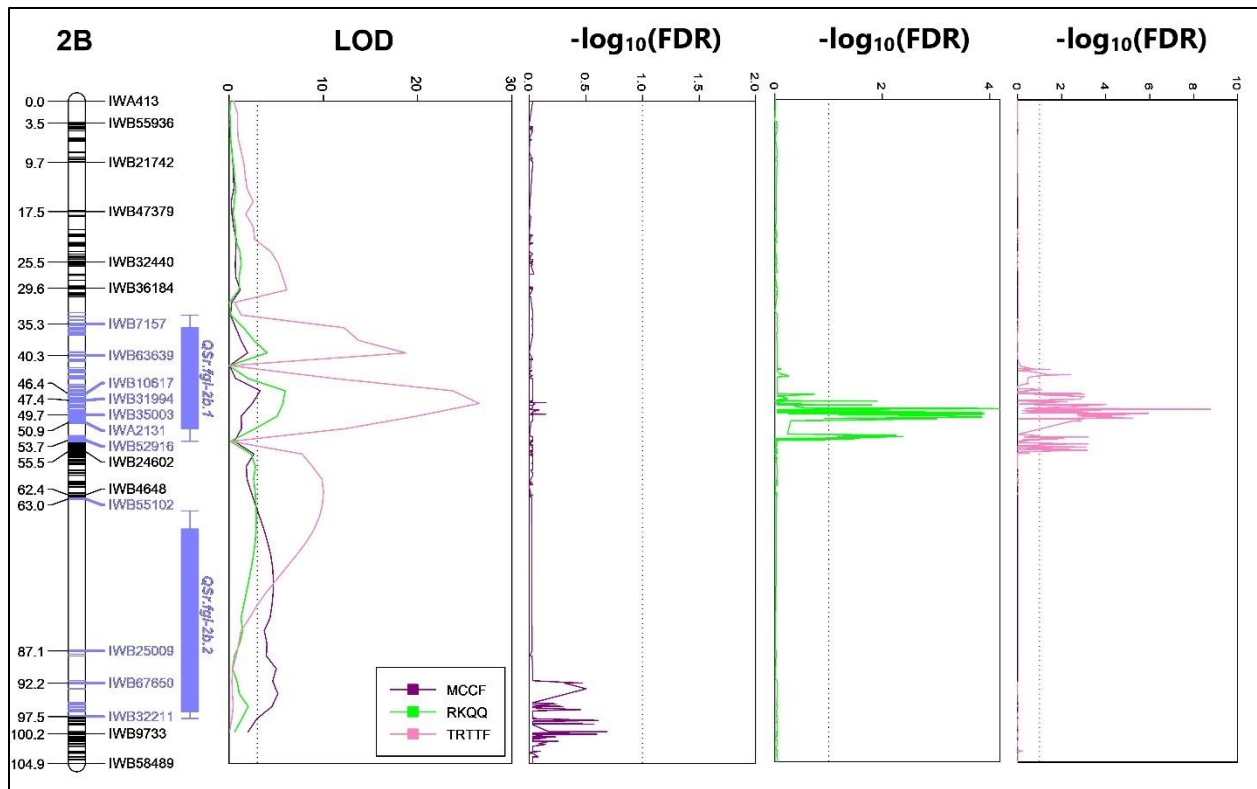
Figure 4.4. Linkage groups on chromosome 2B from Rusty/PI383416 population with QTL regions *QSr.fgl-2B.1* and *QSr.fgl-2B.2* highlighted in blue. LOD scores as well as -log₁₀(FDR) values displayed for associated local stem rust races MCCF and RKQQ and foreign stem rust race TRTTF.

A QTL region was identified on chromosome 4A with association to MCCF and JRCQC stem rust, designated as *QSr.fgl-4A.1* (Figure 4.5). LOD scores of 3.7 and 28.1 were observed fore MCCF and JRCQC (Table 4.7). *QSr.fgl-4A.1* contributed 9.6% phenotypic variation of resistance to MCCF and 65.4% resistance to JRCQC. Zero significant markers were identified with association to MCCF stem rust from within the region and 96 of the 388 SNP markers from within the QTL region were considered significant with association to JRCQC stem rust from GWAS. The region was flanked by SNP markers *IWB68289* and *IWB33628*. *QSr.fgl-4A.1* included marker *IWA4083,* which is associated with stem rust gene *Sr7* (Chao et al. 2017). *QSr.fgl-4A.1* also contained marker *IWB21159*, which is located relatively close by to SSR marker *wmc497* that corresponds to *SrND643* (Basnet et al. 2015). Based on the overall size of

62

QSr.fgl-4A.1 (40.6 cM) and the relative positions of markers *IWA4083* and *IWB21159*, *QSr.fgl-4A.1* likely correlates to *Sr7* and *SrND643*.
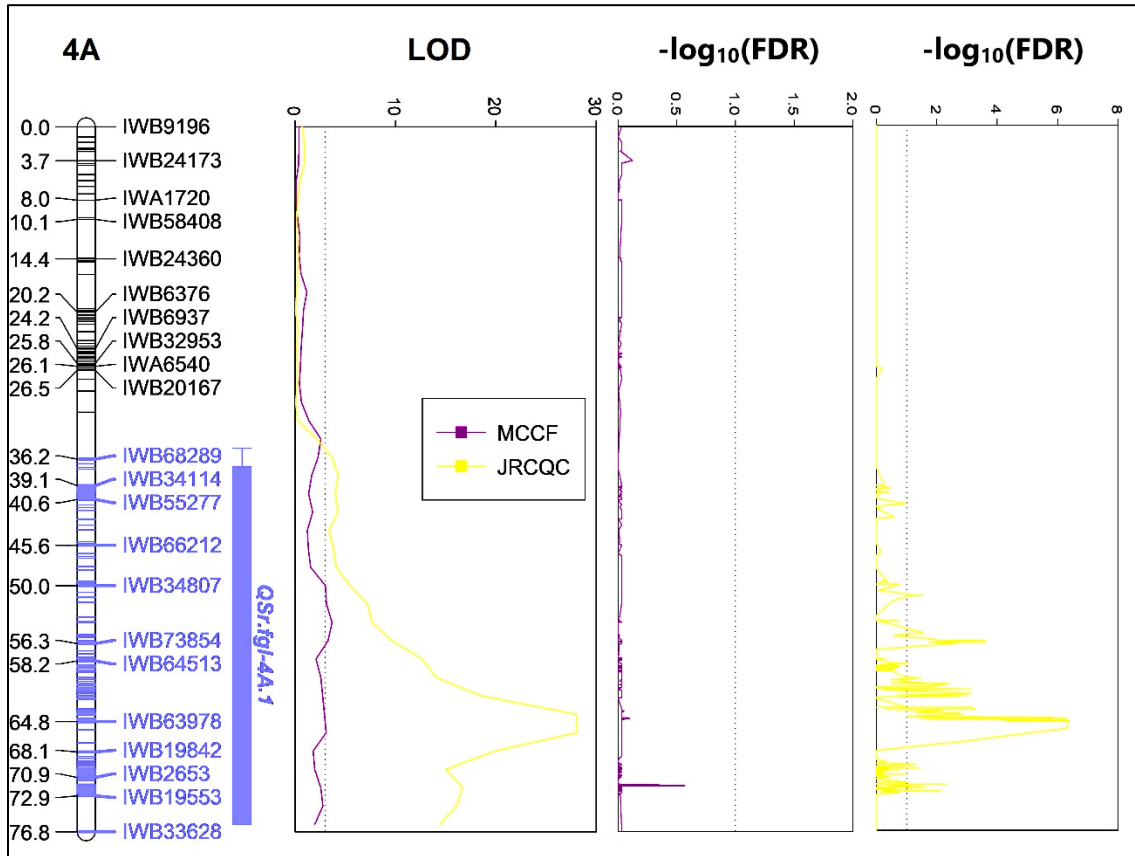
Figure 4.5. Linkage group on chromosome 4A from Rusty/PI383416 population with QTL region *QSr.fgl-4A.1* highlighted in blue. LOD scores as well as -log₁₀(FDR) values displayed for associated local stem rust races MCCF and foreign stem rust race JRCQC.

The final QTL region identified from the Rusty/PI383416 population was *QSr.fgl-5B.1* with association to MCCF stem rust and had an LOD score of 4.2 (Table 4.7). *QSr.fgl-5B.1* was flanked by SNP markers *IWB45090* and *IWB26458* and was responsible for 11% of phenotypic variation of resistance to MCCF (Figure 4.6). Zero significant SNP markers were identified within this region from GWAS. Very few stem rust resistance genes have been identified on chromosome 5B. One that has been identified is *Sr56* (Bansal et al. 2015). Of the significantly associated SNPs identified to be linked to *Sr56* by Bansal et al. (2015), none of the SNP markers

were present within the *QSr.fgl-5B.1* region, likely concluding that *QSr.fgl-5B.1* is not *Sr56*, but

rather a novel gene.
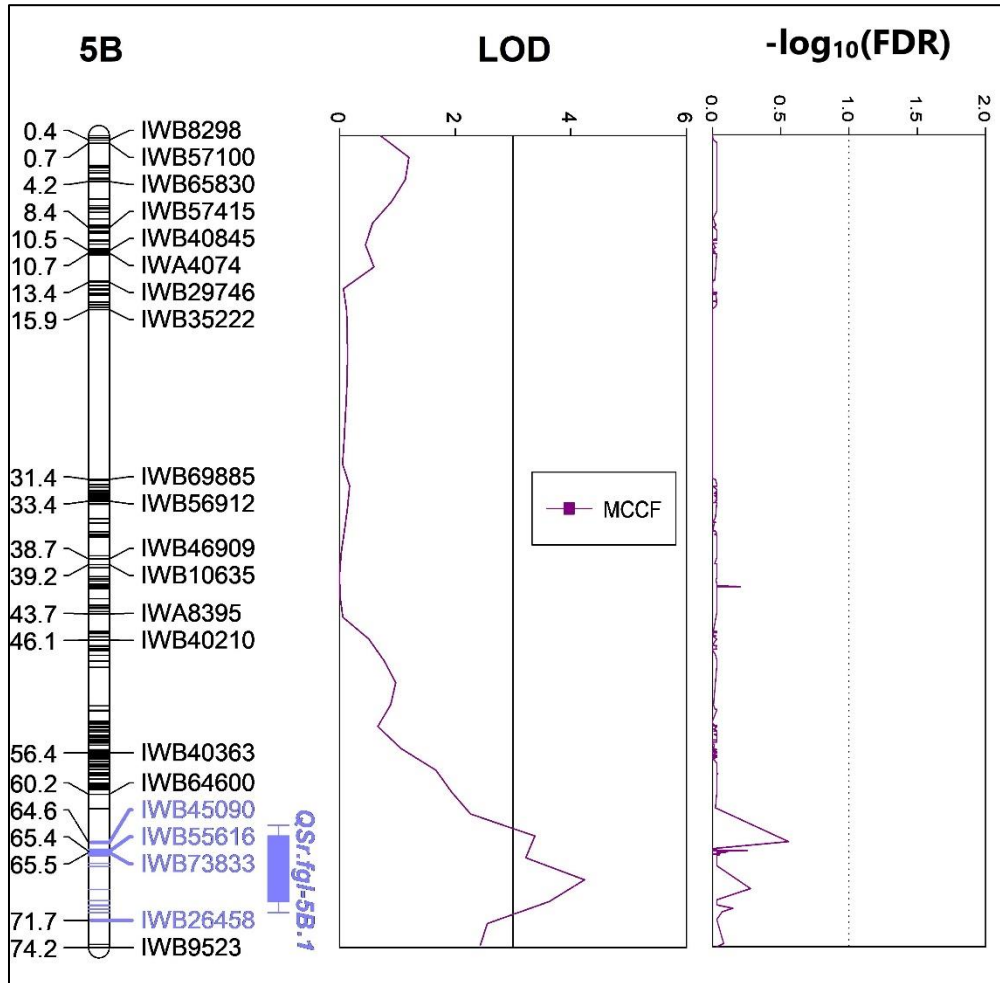
Figure 4.6. Linkage group on chromosome 5B from Rusty/PI383416 population with QTL region *QSr.fgl-5B* highlighted in blue. LOD scores as well as -log₁₀(FDR) values displayed for associated local stem rust races MCCF.

QTL Identification in Rusty/PI520392 Population

Four QTL regions were identified from the Rusty/PI520392 population. The QTLs

identified were located on chromosomes 2B, 4A, 5B, and 6A. The QTL region identified on

chromosome 2B was associated with the same races of rust as *QSr.fgl-2B.1* identified in the

Rusty/PI383416 population, local race RKQQ and foreign race TRTTF, and shared 55 markers

in common (Figure 4.7). This region had LOD scores of 5.2 for RKQQ stem rust and 4.2 for

TRTTF stem rust (Table 4.8). *QSr.fgl-2B.1* was flanked by SNP markers *IWB67251* and

IWA5461 and contributed 15.2% phenotypic variation of resistance to RKQQ and 15.6% of

phenotypic variation to TRTTF (Figure 4.7). Of the 91 SNP markers that make up *QSr.fgl-2B.1*

region, 54 were considered significant with RKQQ stem rust and 51 were considered significant

with TRTTF stem rust from GWAS. *QSr.fgl-2B.1* had SNP marker *IWB60406* (2B:693315227)

to place this QTL in the region of *Sr9*. Therefore, the gene underlying this QTL is also likely to

be *Sr9**.

Table 4.8. QTLs from Rusty/PI520392 population associated with stem rust resistance to races
RKQQ, TRTTF, JRCQC, and TTRTF determined by composite interval mapping.

QTL	Chr	Marker Interval	Likely Sr Gene	Interval Size (cM)	Interval Size (Mbp)	RKQQ			TRTTF			JRCQC			TTRTF		
						LOD	R^2	AE	LOD	R^2	AE	LOD	R^2	AE	LOD	R^2	AE
QSr.fgl-2B.1	2B	*IWB67251-IWA5461*	*Sr9**	18.7 cM	10.3 Mbp	5.208	0.152	-0.826	4.199	0.156	-0.647	-	-	-	-	-	-
QSr.fgl-4A.2	4A	*IWB41141-IWB44449*	Novel	21.6 cM	44.7 Mbp	-	-	-	-	-	-	8.844	0.30	-1.047	-	-	-
QSr.fgl-5B.2	5B	*IWB32873-IWB73873*	Novel	32.3 cM	39.9 Mbp	-	-	-	3.832	0.143	-0.577	8.596	0.293	-1.039	16.819	0.493	-1.018
QSr.fgl-6A	6A	*IWB69135-IWB73609*	Novel	8.3 cM	3.2 Mbp	-	-	-	4.625	0.17	-0.633	4.04	0.151	-0.735	3.758	0.141	-0.548

Note: No significant association with resistance found with MCCF. AE = Additive effect,
positive number shows resistance derived from Rusty and a negative number shows resistance
derived from PI520392. "-" are representative of no significant association with resistance
uncovered.

Figure 4.7. Linkage group on chromosome 2B from Rusty/PI520392 population with QTL region *QSr.fgl-2B.1* highlighted in blue. LOD scores as well as -log₁₀(FDR) values displayed for associated local stem rust races RKQQ and foreign stem rust race TRTTF.

A second QTL was identified on chromosome 4A from this study. *QSr.fgl-4A.2* was

found to only be associated with JRCQC having an LOD score of 8.8 and explained 30% of the

phenotypic variation of resistance to JRCQC (Figure 4.8). One hundred and eighty-six markers

were located within this QTL region with 54 being identified as significant from GWAS. This

region was flanked by *IWB41141* and *IWB44449* SNP markers (Figure 4.8). The previously

reported QTL located on chromosome 4A was representative of stem rust genes *Sr7* and

66

SrND643. This QTL does not coincide with either of those genes and there are no other known

QTLs in this region that have been discovered.

Figure 4.8. Linkage group on chromosome 4A from Rusty/PI520392 population with QTL region *QSr.fgl-4A.2* highlighted in blue. LOD scores as well as -log₁₀(FDR) values displayed for associated foreign stem rust race JRCQC.

The final two QTL regions identified from population Rusty/PI520392 were located on different chromosomes but associated with the same races of stem rust. QTLs *QSr.fgl-5B.2* and *QSr.fgl-6A* were both found to be associated with foreign stem rust races TRTTF, JRCQC, and TTRTF. *QSr.fgl-5B.2* region was flanked by *IWB32873* and *IWB73873* SNP markers and had LOD scores of 3.8 (TRTTF), 8.6 (JRCQC), and 16.8 (TTRTF) (Table 4.8; Figure 4.9). *QSr.fgl-5B.2* contributed 14.3% phenotypic variation of resistance to TRTTF, 29.3% to JRCQC, and 49.3% to TTRTF. Zero significant markers were identified within the region to race MCCF, however 64 and 56 of the total 128 SNP markers from within the QTL region with association to TTRTF and JRCQC were identified as significant markers by GWAS. This region was thought to be the same QTL region identified in population Rusty/PI383416; however, they are not associated with the same races of stem rust. Like previously mentioned, few stem rust resistance genes have been discovered on chromosome 5B and this QTL region likely corresponds to a novel stem rust resistance gene that provides resistance to TRTTF, TTRTF, and JRCQC.
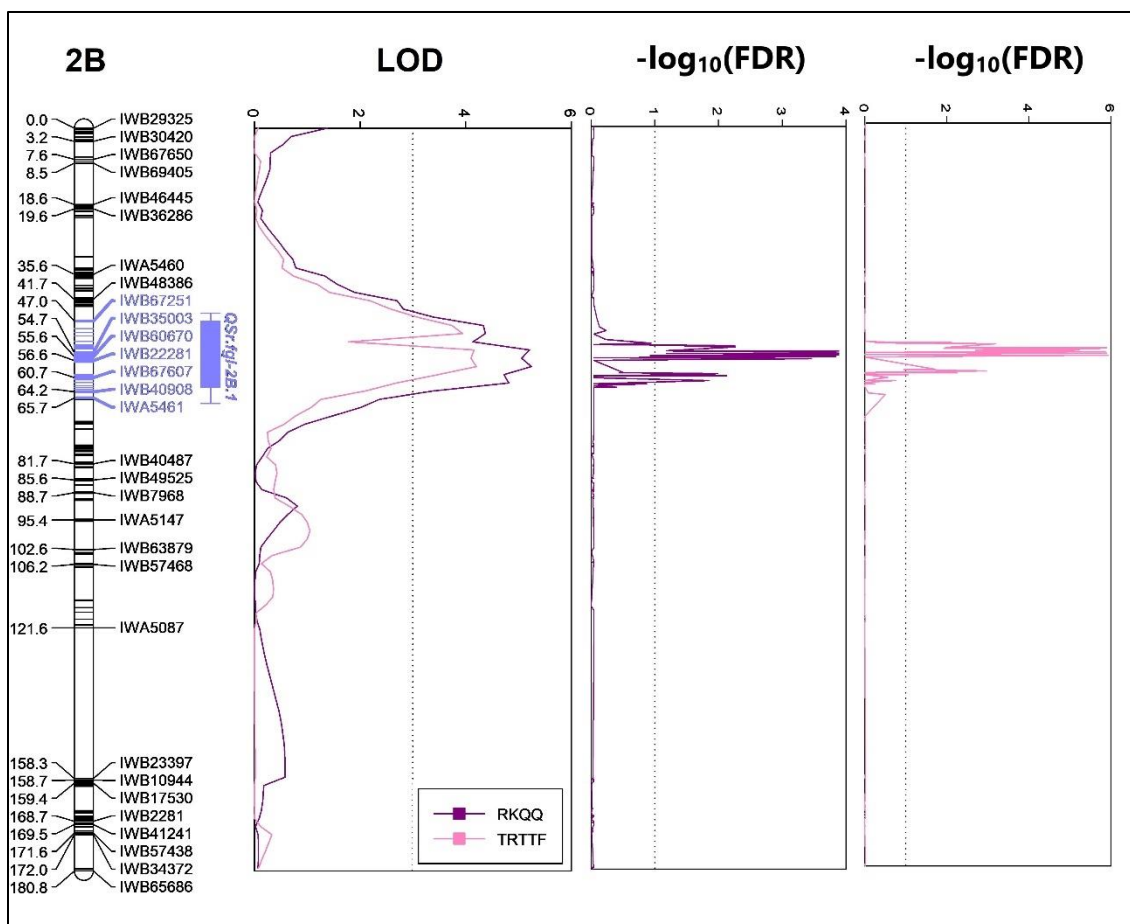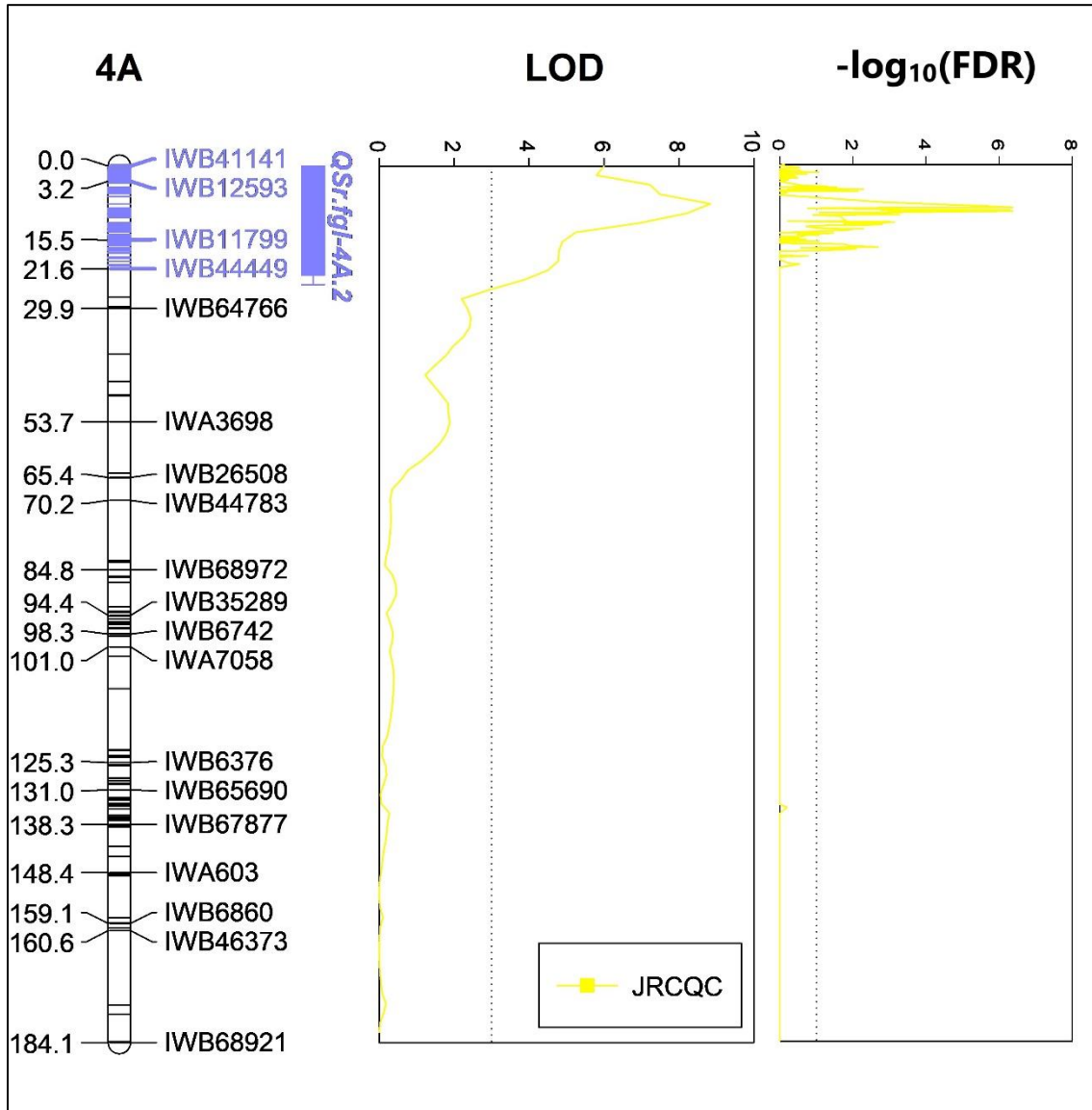
Figure 4.9. Linkage group on chromosome 5B from Rusty/PI520392 population with QTL region *QSr.fgl-5B.2* highlighted in blue. LOD scores as well as -log₁₀(FDR) values displayed for associated foreign stem rust races TRTTF, TTRTF, and JRCQC.

The other QTL region with association to TRTTF, TTRTF, and JRCQC foreign races of stem rust was located on chromosome 6A, *QSr.fgl-6A* (Figure 4.10). *QSr.fgl-6A* region was flanked by SNP markers *IWB69135* and *IWB73609* with LOD scores of 4.6 (TRTTF), 4.0 (JRCQC), and 3.8 (TTRTF) (Table 4.8). 17% of the phenotypic variation of resistance to TRTTF, 15.1% to JRCQC, and 14.1% to TTRTF can be explained by *QSr.fgl-6A*. Of the 102 SNP markers located within this QTL region, only 12 were considered significant from GWAS with association to TRTTF. No significant markers were identified with association to TTRTF and JRCQC. *Sr13* was located on chromosome 6A between EST markers *BE471213* and *CD926040* (Simons et al. 2010). SNP markers *IWB60699* (6A:472721858), *IWB31372*

69

(6A:472801768), and *IWB17069* (6A:473056392) were all present within the QTL region and located near EST marker *BE471213* (6A:472890810) that flanks *Sr13*.
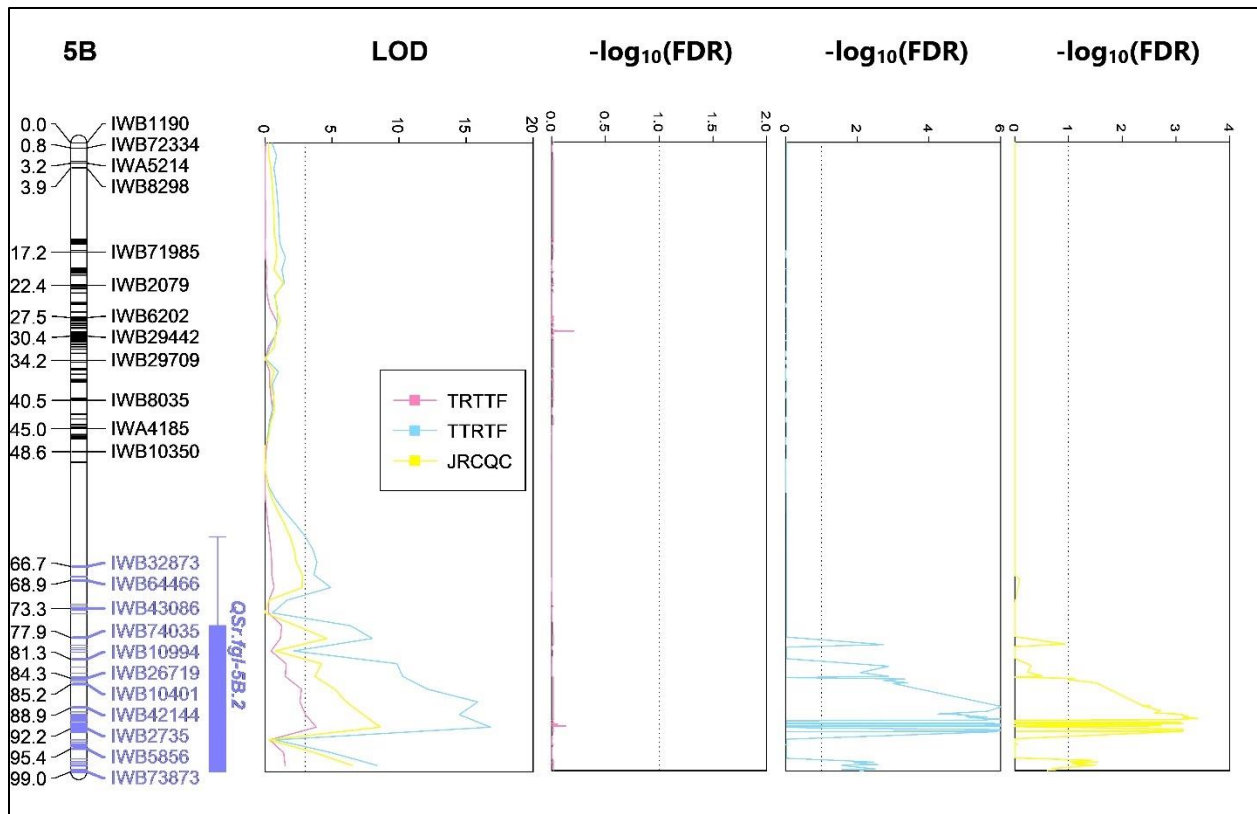

Figure 4.10. Linkage group on chromosome 6A from Rusty/PI520392 population with QTL region *QSr.fgl-6A* highlighted in blue. LOD scores as well as -log₁₀(FDR) values displayed for associated foreign stem rust races TRTTF, TTRTF, and JRCQC.

QTL Identification in Rusty/PI636501 Population

Only one QTL was identified in the Rusty/PI636501 population, and it was located on chromosome 2B (Figure 4.11). *QSr.fgl-2B.1* was associated with RKQQ stem rust and had an LOD score of 6.7 (Table 4.9). The region was flanked by SNP markers *IWA1305* and *IWB67760*. *QSr.fgl-2B.1* contributed 20.2% phenotypic variation of resistance to RKQQ and has 23 of the 69 SNP markers deemed significant from GWAS from within the region. *QSr.fgl-2B.1* is not in the same position as previously identified *QSr.fgl-2B.2* harboring resistant gene *Sr28,* however is the same as *QSr.fgl-2B.1* identified in the Rusty/PI383416 and Rusty/PI520392 populations. The

peak SNP marker *IWA3988* (2B:683043641) of this QTL was determined to be in the vicinity of

Sr9*.

Table 4.9. QTL from Rusty/PI636501 population associated with stem rust resistance to race RKQQ determined by composite interval mapping.

QTL	Chr	Marker Interval	Likely Sr Gene	Interval Size (cM)	Interval Size (Mbp)	RKQQ		
						LOD	R^2	AE
QSr.fgl-2B.1	2B	*IWA1305-IWB67760*	*Sr9**	10.3 cM	24.1 Mbp	6.7	0.202	-1.027

Note: No significant association with resistance found with MCCF. AE = Additive effect, positive number shows resistance derived from Rusty and a negative number shows resistance derived from PI636501.
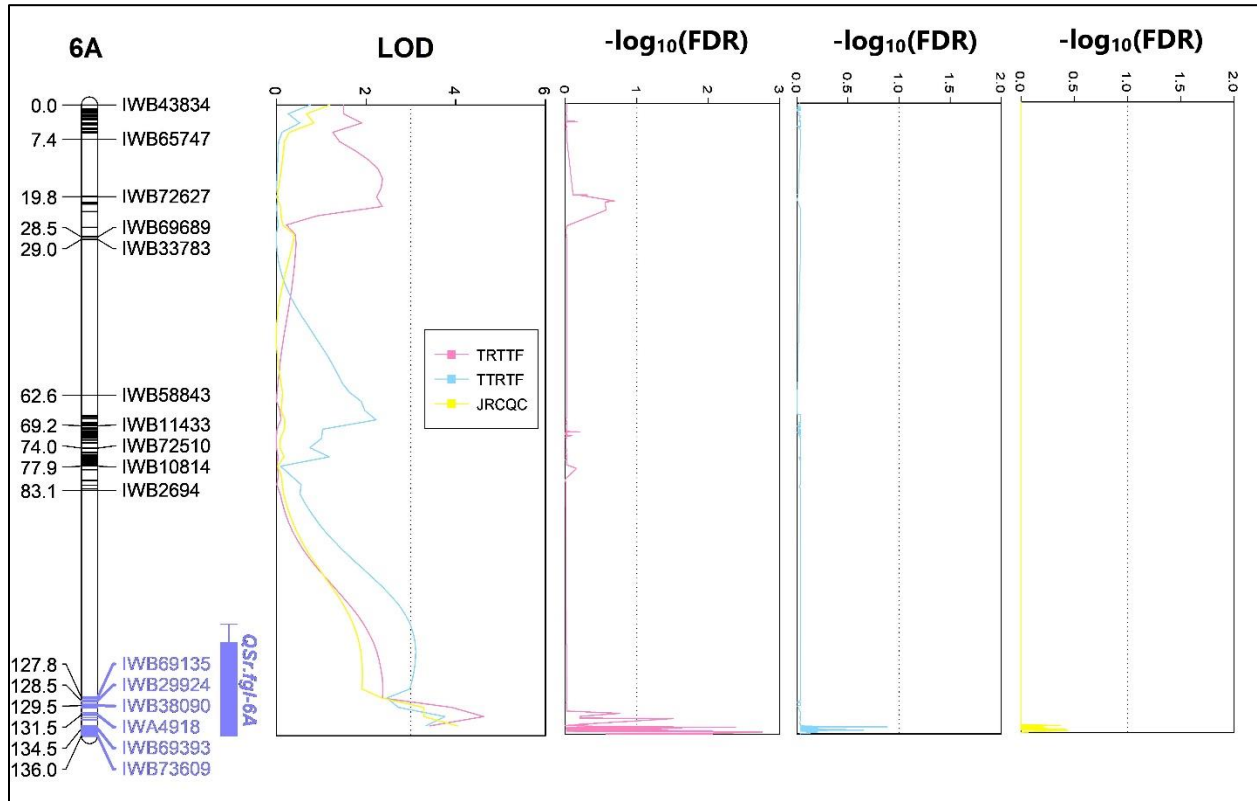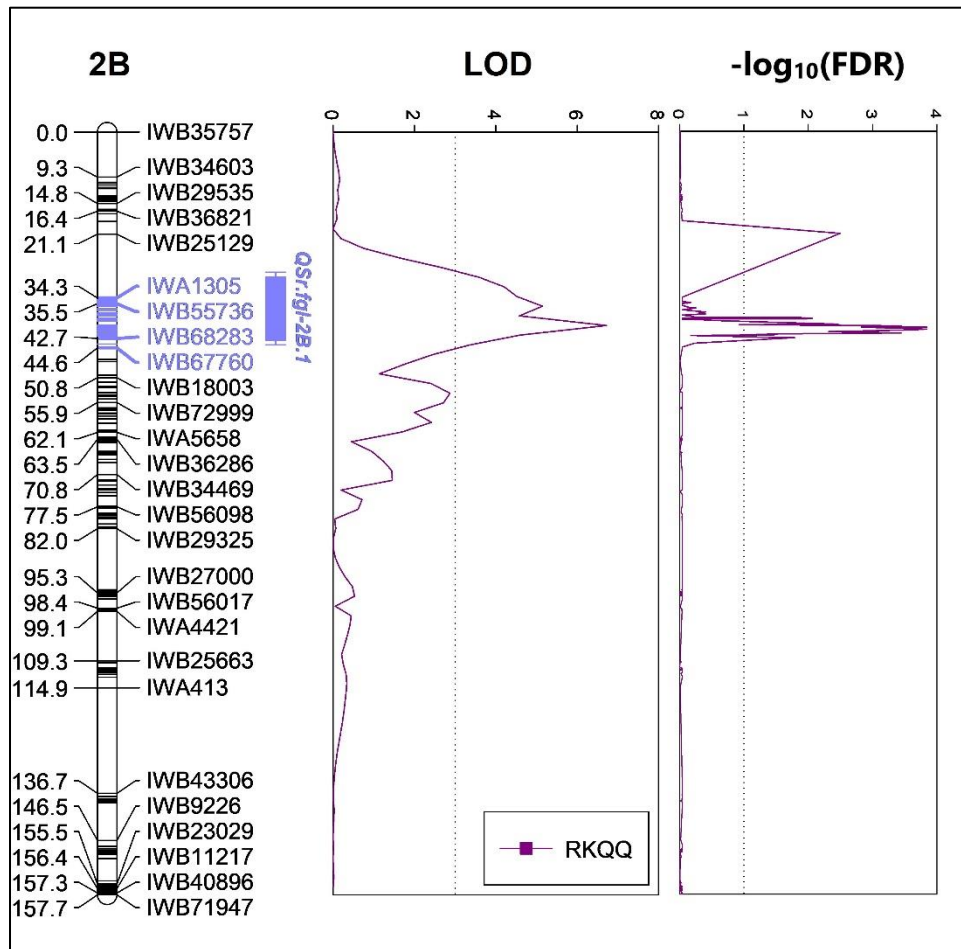

Figure 4.11. Linkage group on chromosome 2B from Rusty/PI636501 population with QTL region *QSr.fgl-2B.1* highlighted in blue. LOD scores as well as -log$_{10}$(FDR) values displayed for associated local stem rust race RKQQ.

GWAS Analysis

As a method to validate the QTL regions identified using QGene, a GWAS was performed with the same data. This method doesn't consider the *de-facto* parentage or the lines or the linkage map positions of the SNPs, but it can verify that the individual markers within an interval are associated with stem rust reaction scores. GWAS results from MCCF stem rust resulted in three significant markers being identified, none within identified QTL regions. TTKSK had zero significant markers identified. For QTL region *QSr.fgl-2B.1* (which was identified in three populations: Rusty/PI383416, Rusty/PI520392, and Rusty/PI636501), 85, 54, and 23 markers were found to be significant from within the region for local stem rust race RKQQ (Table 4.10). *QSr.fgl-2B.1* was also found to be associated with foreign stem rust race TRTTF in two populations (Rusty/PI383416 and Rusty/PI520392) and 179 and 51 markers were deemed significant by GWAS. Significant markers were identified with foreign stem rust race JRCQC with 96, 54, and 56 significant markers identified in QTL regions *QSr.fgl-4A.1* (*Sr7/SrND643*), *QSr.fgl-4A.2,* and *QSr.fgl-5B.2*. *QSr.fgl-5B.2* also contained 64 significant markers with TTRTF stem rust. Lastly, *QSr.fgl-6A* contained 12 significant SNP markers for foreign stem rust race JRCQC.

Table 4.10. The number of significantly associated and total SNP markers for identified QTL regions based on GWAS.

QTL Region	MCCF	RKQQ	JRCQC	TRTTF	TTRTF	TTKSK	Total
QSr.fgl-2B.1 (Sr9)* (Rusty/PI383416)	-	85	-	179	-	-	325
QSr.fgl-2B.1 (Sr9)* (Rusty/PI520392)	-	54	-	51	-	-	91
QSr.fgl-2B.1 (Sr9)* (Rusty/PI636501)	-	23	-	-	-	-	69
QSr.fgl-2B.2 (Sr28)	0	-	-	-	-	-	38
QSr.fgl-3B (Sr12)	0	-	-	-	-	0	197
QSr.fgl-4A.1 (Sr7/SrND643)	0	-	96	-	-	-	388
QSr.fgl-4A.2	-	-	54	-	-	-	186
QSr.fgl-5B.1	0	-	-	-	-	-	76
QSr.fgl-5B.2	-	-	56	-	64	-	128
QSr.fgl-6A	-	-	0	12	0	-	102
QSr.fgl-6B	-	-	-	0	-	-	47
QSr.fgl-7A (Sr22)	0	-	-	-	-	0	19

Note: "-" QTL region was not associated with race of stem rust.

Figure 4.12 shows the significance of SNPs based on stem rust race (local race RKQQ and foreign races TRTTF, TTRTF, and JRCQC) and chromosome location, with a -$\log_{10}$(FDR) > 1 being considered significant. Of the total 12 QTL regions identified using QGene, seven were validated from the GWAS results. The regions validated from GWAS are indicating by arrows in Figure 4.12.
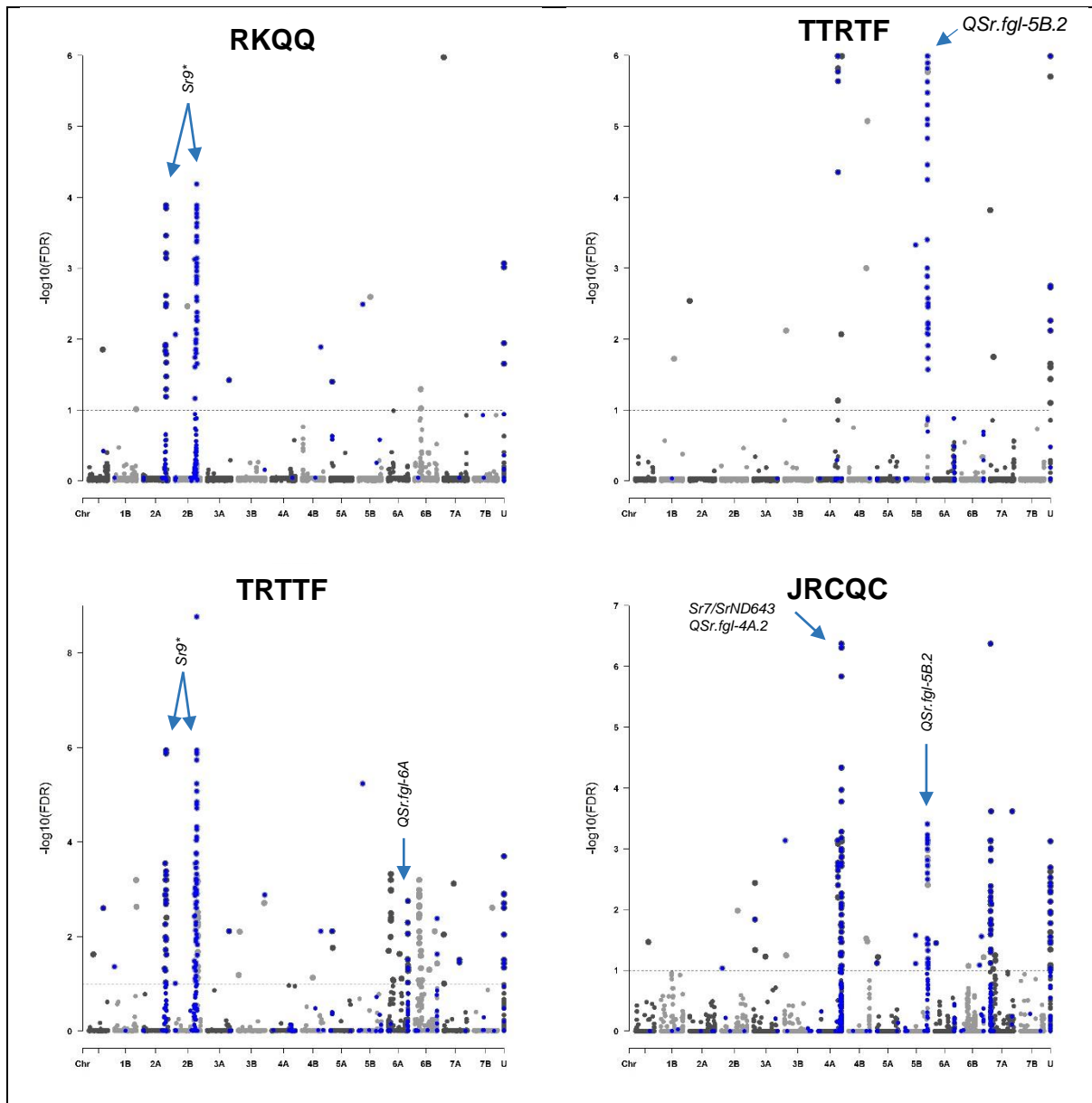
Figure 4.12. Manhattan plots of GWAS results for local race RKQQ and foreign races TRTTF, TTRTF, and JRCQC with SNP markers from QTL regions colored blue and validated QTL regions labelled and indicated with arrow.

Discussion

Twelve total QTL regions were identified after evaluation and analysis of the Rusty/PI192711, Rusty/PI383416, Rusty/PI520392, and Rusty/PI636501 populations. Pairing the number of QTL regions identified with rust association to the phenotype segregation ratios, the regions providing resistance to local stem rust races and foreign stem rust races can be identified.

74

Table 4.11 shows the phenotype segregation resistant to susceptible ratio as well an indication if any QTL regions that were identified to be associated to that race of rust.

Table 4.11. Observed resistant to susceptible ratio for each race of stem rust tested with all four populations.

Population	MCCF	RKQQ	JRCQC	TRTTF	TTRTF	TTKSK
Rusty/PI192711	40%:60%	1:7	1%:99%	1:3	1:3	1:3
Rusty/PI383416	95%:5%	3:1	1:1	1:1	3%:97%	-
Rusty/PI520392	97%:3%	3:1	1:1	7:1	1:1	-
Rusty/PI636501	94%:6%	1:1	-	-	-	-

Note: Shaded cells represent QTL identified from population with association to stem rust race. "-" Population not tested with race of stem rust.

Three QTL were identified after evaluation and analysis of the Rusty/PI192711 population. Even though two QTL regions were identified with association to MCCF, when Rusty/PI192711 was tested with MCCF a 40% resistant to 60% susceptible ratio was observed, not correlating to a single gene or two genes providing resistance. A 1:3 resistant to susceptible ratio was observed with races TRTTF, TTRTF, and TTKSK, with two QTLs being identified with association to TTKSK, one being associated with TRTTF, and no regions identified with association to TTRTF. A 1:7 resistant to susceptible ratio, indicating three genes required for resistance, was observed with local race RKQQ. A very distorted segregation ratio was observed when population Rusty/PI192711 was tested with foreign race JRCQC, and no QTL regions were identified that provide resistance.

The QTL identified in chromosome 3B, *QSr.fgl-3B*, is associated with MCCF and TTKSK stem rust and is predicted to be *Sr12* (Zurn, et al. 2018). The population Rusty/PI192711 that this QTL region was identified in had a distorted resistance to susceptibility ratio to MCCF stem rust and 1:3 ratio to TTKSK stem rust. The 190 markers within this QTL were not significantly associated with the TTKSK or MCCF races. This may be due to an undersized population for GWAS analysis with TTKSK or some segregation of different haplotypes in the

other populations within this region. The segregation ratio of this population with the stem rust races suggests more than a single stem rust resistance gene is providing resistance. TTKSK virulence and avirulence to *Sr12* is not clear, however, Rouse et al. (2014) shows that *Sr12* has weak effectiveness to TTKSK when alone (Zurn et al. 2018). The effectiveness of *Sr12* to TTKSK was increased when paired with *Lr34* or *Sr57* (Rouse et al. 2014; Zurn et al. 2018; Hiebert et al. 2016). No QTL was observed to signify *Sr57* within the population. Both races MCCF and TTKSK are avirulent to resistance gene *Sr22* located on chromosome 7A. Hatta et al. (2020) reported that *Sr22* is able to provide resistant to TTKSK. The QTL region identified on chromosome 7A from this study likely includes *Sr22*. When only the Rusty alleles are present at both the *Sr12* and *Sr22* loci, the lines are susceptible to TTKSK. When only the *Sr12* allele or *Sr22* allele is present, the lines remain susceptible to foreign race TTKSK. Moderate resistance is observed to TTKSK when both the *Sr12* and *Sr22* alleles are present, however, complete resistance is not achieved (Figure 4.13). An additional unknown genetic interaction must be taking place somewhere within the population that hinders resistance even when both resistance alleles are present. When associated with MCCF stem rust, there was a weak correlation between when both *Sr12* and *Sr22* resistance alleles are present and the line being resistant to MCCF. Just like with TTKSK stem rust, another unknown interaction must be taking place that provides resistance to MCCF in addition to *Sr12* and *Sr22*.
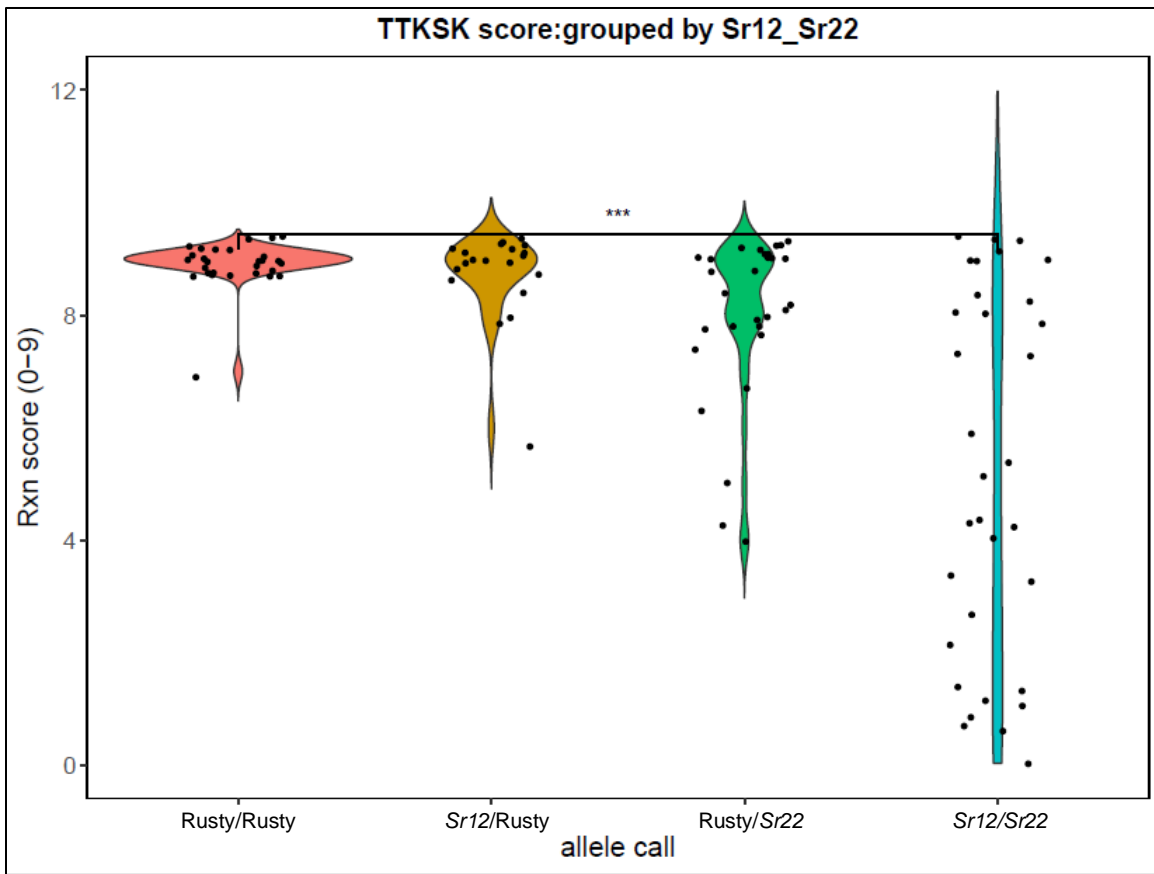
Figure 4.13. Violin plot of Rusty, *Sr12,* and *Sr22* alleles corresponding to infection severity to foreign stem rust race TTKSK.

The third QTL region identified from population Rusty/PI192711 was located on chromosome 6B with association to TRTTF stem rust. *Sr8a, Sr22, Sr24, Sr31,* and *Sr35* stem rust genes have been shown to be effective against this race, but these are not located on chromosome 6B. *Sr11* is located on chromosome 6B but not in the same region as *QSr.fgl-6B.* No previously published resistance gene resided in the area that *QSr.fgl-6B* was located in and was determined to be a novel stem rust resistance gene. Rusty/PI192711 had a 1:3 resistant to susceptible ratio observed when tested with race TRTTF, indicating two genes are required to provide resistance. When the Rusty allele was present at the locus, the line was susceptible. When the *QSr.fgl-6B* allele is present resistant lines are observed, as well as susceptible lines. A second QTL was not discovered within the population with association to TRTTF stem rust.

Identifying the second gene that is providing resistance to TRTTF and working with the *QSr.fgl-6B* would provide a clearer picture of how the alleles work together to provide resistance to TRTTF. Expected ratios were observed from Rusty/PI12711 with local race RKQQ (1:7) and foreign race TTRTF (1:3), however, no QTL regions were identified with association to either of those races of rust.

Four QTL were identified after evaluation and analysis of the Rusty/PI383416 population. Two QTLs were identified on chromosome 2B, *QSr.fgl-2B.1* and *QSr.fgl-2B.2*, one on chromosome 4A, *QSr.fgl-4A.1*, and one on chromosome 5B, *QSr.fgl-5B.1*. Distorted segregation ratios were observed for local stem rust race MCCF (95% resistant and 5% susceptible) and foreign stem rust race TTRTF (3% resistant and 97% susceptible). Expected 1:1 segregation ratio was observed with foreign races JRCQC and TRTTF indicating a single gene provides resistance and an additional 3:1 expected segregation ratio was observed with RKQQ indicating two genes provide resistance. QTLs were identified with association to all races tested with Rusty/PI383416 population except for TTRTF.

The first QTL region identified on chromosome 2B, *QSr.fgl-2B.1*, was discovered with RKQQ and TRTTF stem rust races and the second QTL region, *QSr.fgl-2B.2*, was discovered with MCCF stem rust. *QSr.fgl-2B.1* is in the region of *Sr9* which includes *Sr9a, Sr9b, Sr9d, Sr9e, Sr9g* and *Sr9h* (*SrWeb*), however TRTTF is virulent to *Sr9a*, *Sr9b, Sr9d, Sr9e,* and *Sr9g* and RKQQ is virulent to *Sr9b, Sr9d* and *Sr9g* and avirulent to *Sr9e*. Saini et al. (2018) reported that *Sr9e* has a minor effect against TRTTF stem rust. *QSr.fgl-2B.1* region had a very significant LOD score of 26.52 when associated with TRTTF indication a major resistance gene is present to provide resistance to TRTTF, so it is not *Sr9e* providing resistance or any other known stem rust resistance allele from *Sr9*, but rather a potentially novel allele of *Sr9*. *QSr.fgl-2B.2* was

discovered being associated with rust MCCF. MCCF is avirulent to resistance gene *Sr6, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr11, Sr21, Sr22, Sr24, Sr28, Sr30, Sr31, Sr36, and Sr38,* of which only *Sr9a, Sr9b, Sr9d, Sr9e, Sr28* and *Sr36* are located on chromosome 2B. This QTL is not in the region of *Sr9* or *QSr.fgl-2B.1* but rather located closer to the end of the chromosome. *QSr.fgl-2B.2* is located distal to *Sr9* or *QSr.fgl-2B.1* on 2B. *Sr36* is located proximal to *QSr.fgl-2B.1* which leaves *Sr28* within the region of this QTL. The markers flanking *Sr28* are SSR marker *wmc332* (2B:739396089) and DArT marker *wPt-7161* (2B:756189699). The SNP marker flanking this QTL, *IWB55102* (2B:743728622), is located between the flanking markers for *Sr28*, therefore *QSr.fgl-2B.2* likely is *Sr28*.

The third QTL identified with association to MCCF and JRCQC from Rusty/PI383416 population was region *QSr.fgl-4A.1*. Taking into consideration the total size of *QSr.fgl-4A.1*, 40.6 cM, there may be two genes within this region. *Sr7* and *SrND643* are located on chromosome 4A. SNP marker *IWA4083* (4A:742566219) is associated with stem rust gene *Sr7* (Chao et al. 2017) and is present within the QTL region and JRCQC is avirulent to *Sr7*. *SrND643* is flanked by SSR marker *wmc497* (4A:737292387) (Basnet et al. 2015) which is close to SNP marker *IWB21159* (4A:737296518) that is present within the *Qr.fgl-4A.1* region, therefore the genes underlying this QTL region are likely *Sr7* and *SrND643*. The final QTL identified in the Rusty/PI383416 population was *QSr.fgl-5B.1* associated with MCCF. Very few stem rust resistance genes have been mapped to chromosome 5B. *Sr56* is located on chromosome 5B and was identified by Bansal et al. (2014). When comparing the significant SNPs associated with *Sr56* with the *QSr.fgl-5B.1* region there was no overlap of SNP markers. *QSr.fgl-5B.1* likely contains a novel resistance gene that provides resistance to MCCF stem rust.

When observing a 1:1 resistant to susceptible ratio for Rusty/PI383416 population to foreign stem rust race TRTTF, the conclusion that a single gene is providing resistance can be drawn. TRTTF was only associated with *QSr.fgl-2B.1*, deemed a novel allele of stem rust resistance gene *Sr9* (*Sr9**). When only the Rusty allele was present at the locus the lines were susceptible, which the exception of six escape lines that received resistant scores despite the resistance allele being absent (Figure 4.14). When the *Sr9** allele was present at the locus the lines were resistant to TRTTF. Based on these findings, it is determined that *Sr9** provides resistance to TRTTF. Additionally, another 1:1 resistant to susceptible ratio was observed with race JRCQC. Again, only one QTL identified region was associated with JRCQC stem rust, *QSr.fgl-4A.1*, deemed *Sr7/SrND643*. When only the Rusty allele was present, the lines were susceptible to JRCQC. When the *Sr7/SrND643* allele was present, the lines were resistant to JRCQC. We can conclude that *Sr7/SrND643* provides resistance to foreign stem rust race JRCQC.
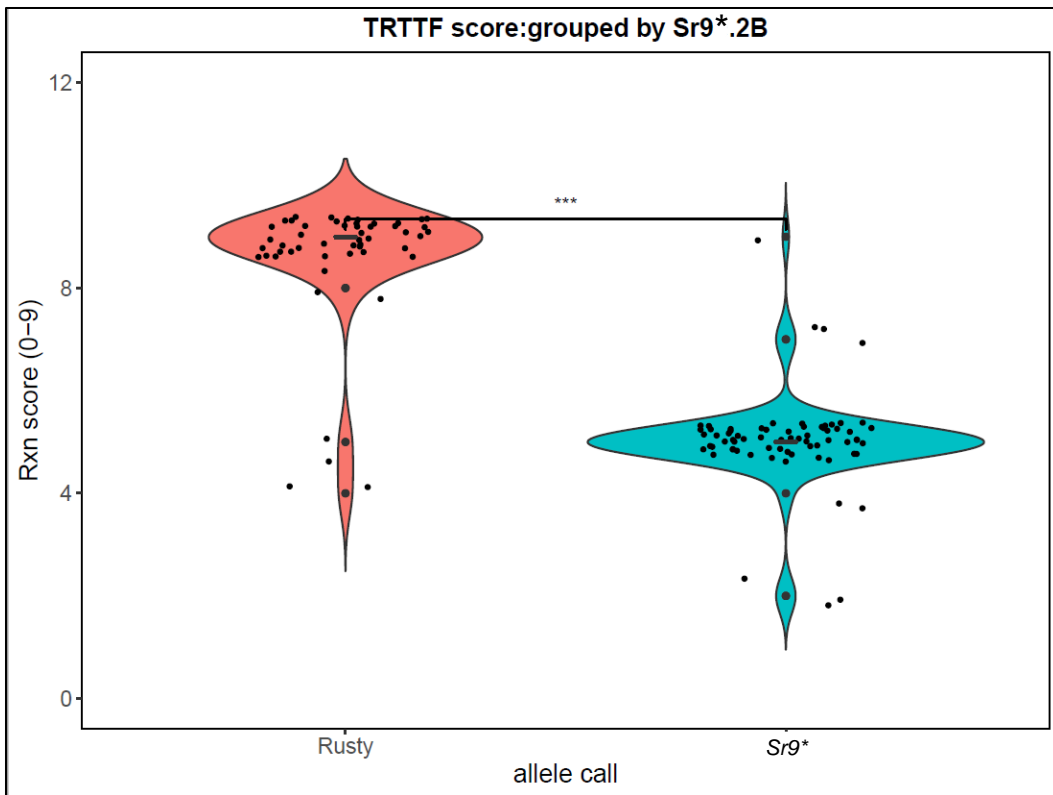
Figure 4.14. Violin plot of Rusty and *Sr9** alleles corresponding to infection severity to foreign stem rust race TRTTF.

A 3:1 resistant to susceptible ratio was observed with stem rust RKQQ, indicating two genes present that can independently provide resistance. Only one QTL region was identified with association to RKQQ, *QSr.fgl-2B.1* (*Sr9**). When comparing the infection severity scores from when the Rusty allele was present to when the *Sr9** allele was present, there is no indication that *Sr9** provides high levels of resistance to RKQQ but rather *Sr9** may be providing minor resistance. With neither the Rusty nor *Sr9** allele severely impacting infection severity, there must be another unknown genetic interaction taking place that provides a higher level of resistance to RKQQ. Three QTL regions were identified with association to MCCF (*Sr28, Sr7/SrND643,* and *QSr.fgl-5B.1*), but a distorted segregation ratio of 95% resistant and 5% susceptible does not fit the expected 7:1 ratio that there are three genes providing resistance to

MCCF. There must be an additional region providing resistance in addition to the three identified here.

Four of the five races of stem rust that Rusty/PI520392 was tested with gave expected segregation ratios. MCCF was the only population to give a distorted segregation ratio of 97% resistant and 3% susceptible. Foreign stem rust races JRCQC and TTRTF resulted in expected 1:1 ratios, indicating a single gene is providing resistance. Local stem rust race RKQQ resulted in a 3:1 ratio indicating two genes are providing resistance and lastly foreign stem rust race TRTTF resulted in a 7:1 resistant to susceptible ratio indicating three genes are providing resistance. No QTL region was identified with association to MCCF but at least one QTL region was identified for the other four races.

Four QTLs were identified in the Rusty/PI520392 population on chromosomes 2B, 4A, 5B, and 6A. *QSr.fgl-2B.1* was identified with rusts RKQQ and TRTTF and is the same QTL region identified in Rusty/PI383416 population, deemed a novel allele of *Sr9* (*Sr9**). The next QTL identified was *QSr.fgl-4A.2* associated with foreign stem rust race JRCQC. This QTL was not found to be associated with MCCF, like the previously identified QTL region on chromosome 4A, and therefore is a different gene that is providing resistance to JRCQC. There are not any known stem rust resistance genes within the region of *QSr.fgl-4A.2* and the gene underlying this QTL region is likely a novel gene.

The final two QTL regions identified after evaluation and analysis of the Rusty/PI520392 population were both associated with foreign stem rust races TRTTF, TTRTF, and JRCQC. The first QTL region identified on chromosome 5B, designated *QSr.fgl-5B.2*, was not the same as previously identified region *QSr.fgl-5B.1* from the Rusty/PI383416 population. As there are very few stem rust resistance genes mapped to chromosome 5B and no known resistance genes in the

region of *QSr.fgl-5B.2*, the gene providing resistance within this QTL region is likely a novel resistance gene. The other QTL region identified with association to TRTTF, TTRTF, and JRCQC was *QSr.fgl-6A*. There is one known stem rust resistance gene located on chromosome 6A that is also effective towards foreign stem rust races TRTTF, TTRTF, and JRCQC, which is *Sr8*. However, *Sr8* is not located within this QTL region. An additional stem rust resistance gene, *Sr13*, is located on chromosome 6A. The flanking marker of *Sr13* is located within the QTL region identified on 6A, however, JRCQC, TRTTF, and TTRTF are all virulent to *Sr13*. Even though this QTL cannot confirm to be *Sr13*, it can be confirmed that this QTL is located very close to *Sr13* and that *QSr.fgl-6A* provides moderate resistance to stem rust races TRTTF, JRCQC, and TTRTF and may potentially be a novel gene.

With 1:1 ratios being observed for JRCQC and TTRTF stem rust races, a single gene should be providing resistance to these races. Two QTL regions were identified with association to TTRTF stem rust race, *QSr.fgl-5B.2* and *QSr.fgl-6A*. When the Rusty allele was present at both loci the lines were susceptible (Figure 4.15). When only the *QSr.fgl-6A* allele was present the lines were moderately resistant with some susceptible lines as well. When only the *QSr.fgl-5B.2* allele was present the lines were resistant to TTRTF. When both the *QSr.fgl-6A* and *QSr.fgl-5B.2* alleles were present the lines exhibited the same amount of resistant as when only the *QSr.fgl-5B.2* allele was present. With *QSr.fgl-5B.2* providing the same level of resistance as when both alleles are present, we are able to determine that *QSr.fgl-5B.2* is providing the resistance observed to foreign race TTRTF.
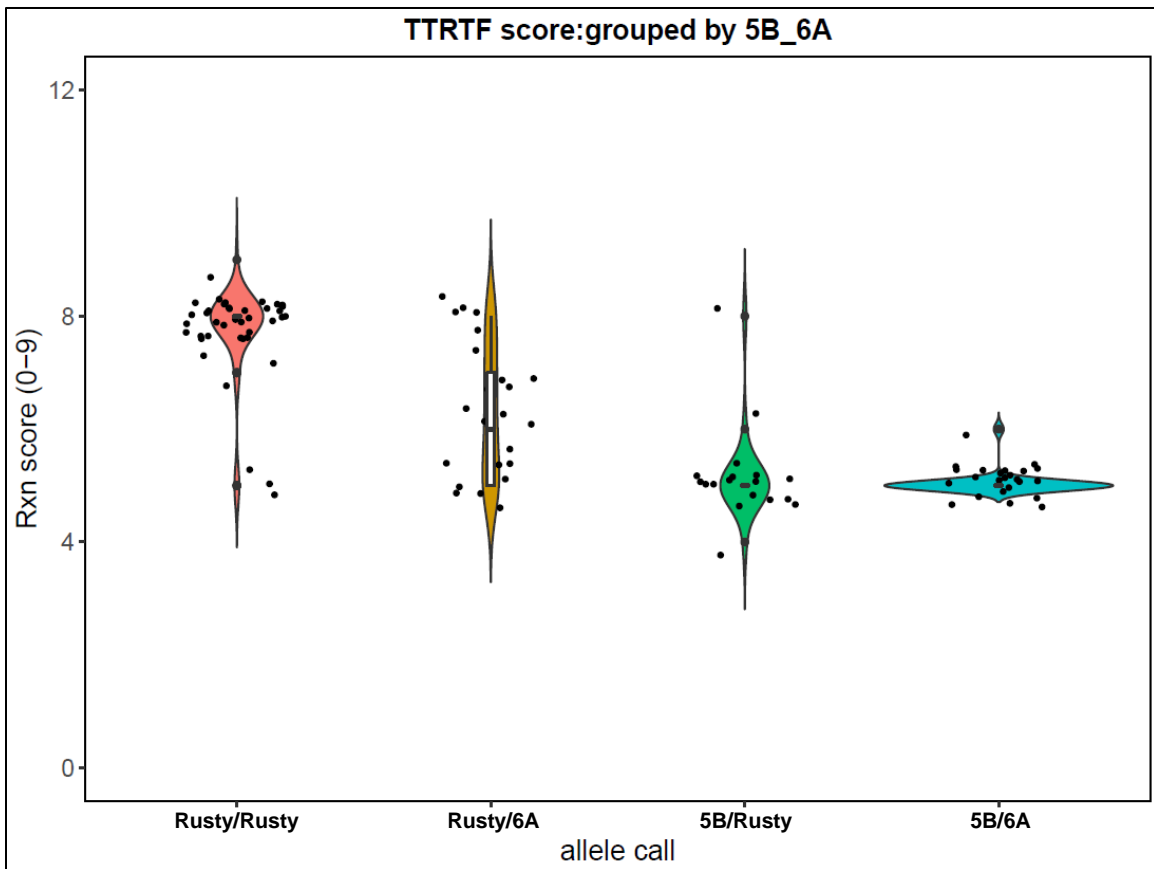
Figure 4.15. Violin plot of Rusty, *QSr.fgl-5B.2*, and *QSr.fgl-6A* alleles corresponding to infection severity to foreign stem rust race TRTTF.

The other 1:1 resistant to susceptible ratio observed was with race JRCQC, indicating a single gene is providing resistance. Three QTL regions were identified with association to JRCQC (*QSr.fgl-4A.2, QSr.fgl-5B.2,* and *QSr.fgl-6A*), however. When examining the resistance or susceptibility of lines based on the alleles present, there is an observed pseudo-additive effect which can be viewed in Figure 4.16. When only the Rusty allele is present at all three loci the line is susceptible. When only the *QSr.fgl-6A, QSr.fgl-5B.2,* or *QSr.fgl-4A.2* alleles are present the line is slightly less susceptible than when only Rusty was present. When a combination of alleles is present such as *QSr.fgl-6A* and *QSr.fgl-5B.2, QSr.fgl-4A.2* and *QSr.fgl-6A,* or *QSr.fgl-4A.2* and *QSr.fgl-5B.2,* the lines are more resistant than when only one of those alleles are present. When all three resistance alleles are combined, all the lines are resistant. Despite having

84

a resistant to susceptible ratio indicating a single gene is providing resistance, a combination of these three genes provides high levels of resistance to foreign stem rust race JRCQC.
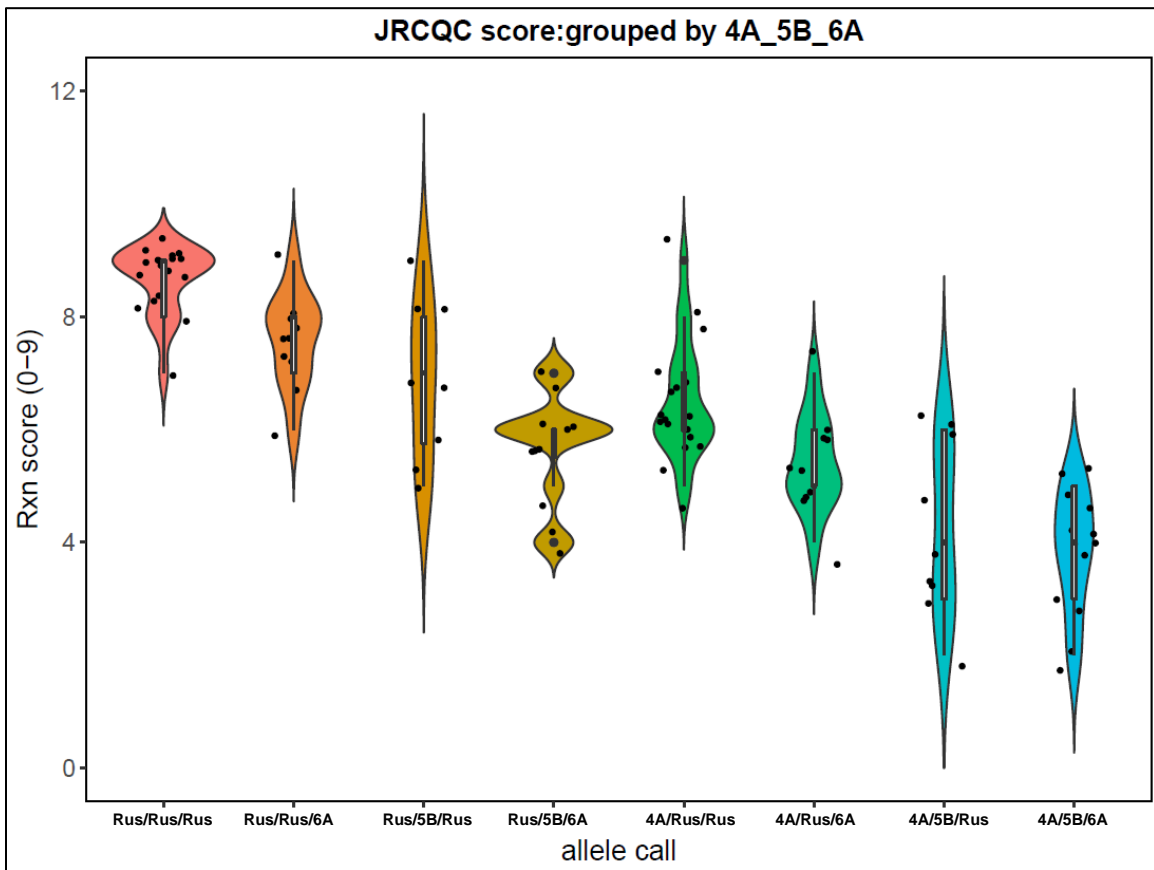

Figure 4.16. Violin plot of Rusty, *QSr.fgl-4A.2, QSr.fgl-5B.2,* and *QSr.fgl-6A* alleles corresponding to infection severity to foreign stem rust race JRCQC.

A segregation ratio of 7:1 was also observed in Rusty/PI520392 population with TRTTF stem rust. Correspondingly, three QTL regions were identified representing the three genes that are providing resistance to TRTTF. *Sr9*, QSr.fgl-5B.2,* and *QSr.fgl-6A* were all identified with associated with stem rust TRTTF. When only the Rusty allele was present at all three loci the lines were susceptible (Figure 4.17). When a single *Sr9*, QSr.fgl-5B.2,* or *QSr.fgl-6A* allele was present the line was resistant. Any combination of resistance alleles such as *Sr9** and *QSr.fgl-5B.2, Sr9** and *QSr.fgl-6A, QSr.fgl-5B.2* and *QSr.fgl-6A,* or *Sr9*, QSr.fgl-5B.2* and *QSr.fgl-6A* all resulted in lines having the same level of resistance as when a single resistance allele was

present. With no higher levels of resistance being observed when all three resistance alleles were present, this shows that there are three independent resistance genes providing resistance to TRTTF.
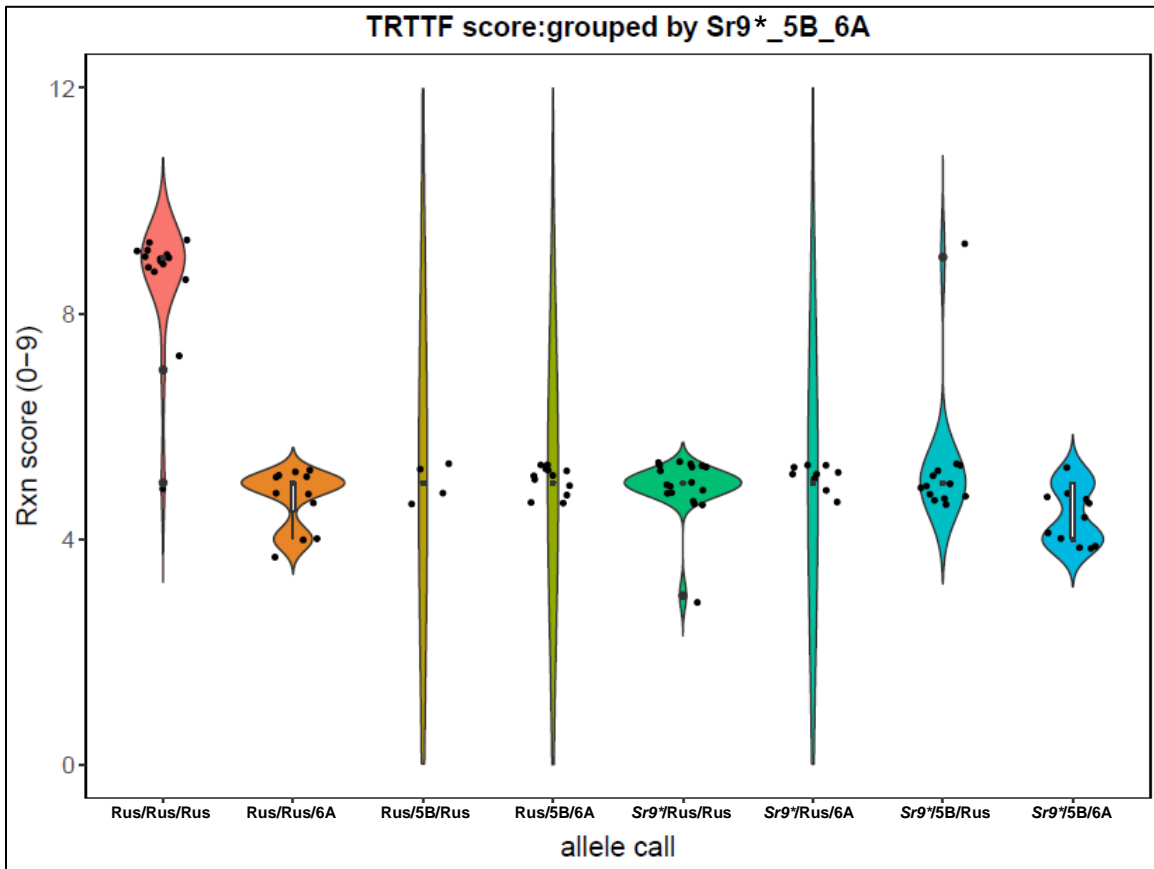

Figure 4.17. Violin plot of Rusty, *QSr.fgl-5B.2, QSr.fgl-6A*, and *Sr9** alleles corresponding to infection severity to foreign stem rust race TRTTF.

A final expected segregation ratio was observed with local stem rust race RKQQ from population Rusty/PI520392. With a 3:1 resistant to susceptible ratio, two genes are expected to provide resistance to RKQQ. Only one QTL region was identified with association to RKQQ, *QSr.fgl-2B.1* (*Sr9**). Similar to the observation made from *QSr.fgl-2B.1* providing resistance to RKQQ in the Rusty/PI383416 population, there was a very weak correlation between the *Sr9** allele being present with line resistance. Regardless of if the Rusty allele was present or the *Sr9** allele was present at the locus, there were resistance and susceptible lines observed. Being *Sr9**

does not provide high levels of resistance, it likely has a minor effect on RKQQ resistance, and an additional resistance gene is present within the population that was not uncovered here.

The final population, Rusty/PI363501 was only tested with local stem rust races MCCF and RKQQ. A distorted segregation ratio was observed with MCCF stem rust with 94% resistance and 6% susceptibility being observed. A 1:1 ratio was observed for local stem rust race RKQQ. A single QTL was identified with association to RKQQ stem rust, *QSr.fgl-2B.1*. This QTL region was the same as the QTL regions identified on chromosome 2B in Rusty/PI383416 and Rusty/PI520392 populations with association to RKQQ that was deemed a novel allele of *Sr9* (*Sr9**). When the Rusty allele is present at the loci there are resistant and susceptible lines observed and same goes for when the *QSr.fgl-2B.1* allele is present (Figure 4.18). Again, we find that *QSr.fgl-2B.1* is not a strong source of resistance for local stem rust race RKQQ and another undiscovered region that also provides resistance must be present within the population.
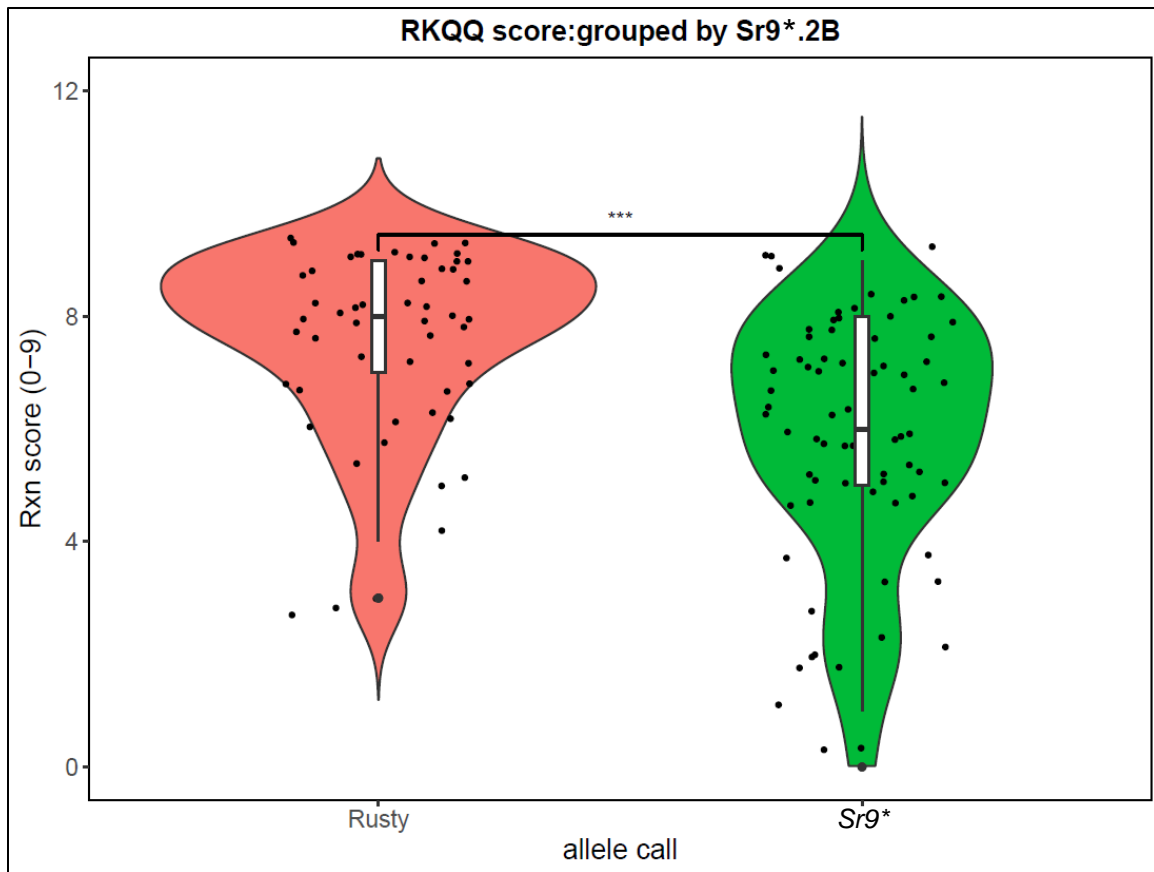
Figure 4.18. Violin plot of Rusty and *Sr9** alleles corresponding to infection severity to foreign stem rust race RKQQ.

Conclusion

From four populations twelve QTL regions were identified, seven of which corresponded to regions of already identified stem rust resistant genes. The other five QTL regions were deemed to possess novel genes. Seven of the QTL regions identified were validated with GWAS. A putative novel allele of stem rust resistance gene *Sr9* (*Sr9**) was identified in three of the four populations on chromosome 2B within QTL region *QSr.fgl-2B.1*. *Sr28* was identified in one population on chromosome 2B in QTL region *QSr.fgl-2B.2*. Stem rust resistance gene *Sr12* was identified on chromosome 3B in one population within the *QSr.fgl-3B* region. A combination of stem rust resistance gene *Sr7* and *SrND643* was identified on chromosome 4A in one population in region *QSr.fgl-4A.1*. An additional QTL region was identified on chromosome 4A in a

88

different population that did not coincide with *Sr7* or *SrND643* within region *QSr.fgl-4A.2* and is

potentially a novel resistance gene. Two QTLs were identified on chromosome 5B in two

different populations. Neither region correlated to any known stem rust resistance genes or one

another and therefore there are likely novel resistance gene in the regions of *QSr.fgl-5B.1* and

QSr.fgl-5B.2. A potential novel stem rust resistance gene was identified in one population on

chromosome 6A in *QSr.fgl-6A* that was near *Sr13* but was determined to not be *Sr13*, perhaps a

novel allele of *Sr13*. Another potential stem rust resistance gene was located on chromosome 6B

in one population, *QSr.fgl-6B*. Lastly, *Sr22* was identified on chromosome 7A in the *QSr.fgl-7A*

region of one population.

Table 4.12. Sources of resistance for populations with given races of stem rust identified from
phenotype data and identified QTL regions.

Population	MCCF	RKQQ	JRCQC	TRTTF	TTRTF	TTKSK
Rusty/PI192711	*Sr12 + Sr22 + Unknown*	-	-	*QSr.fgl-6B + Unknown*	-	*Sr12 + Sr22 + Unknown*
Rusty/PI383416	*Sr28 + Sr7/SrND643 + QSr.fgl-5B.1 + Unknown*	*Sr9* + Unknown*	*Sr7/SrND643*	*Sr9**	-	/
Rusty/PI520392	-	*Sr9* + Unknown*	*QSr.fgl-4A.2 + QSr.fgl-5B.2 + QSr.fgl-6A*	*Sr9* or QSr.fgl-5B.2 or QSr.fgl-6A*	*QSr.fgl-5B.2*	/
Rusty/PI636501	-	*Sr9* + Unknown*	/	/	/	/

Note: "-" No QTL identified with association to race of rust; "/" Population not tested with
stem rust race.

A combination of *Sr12, Sr22,* and an unknown genetic interaction provided resistance to

MCCF and TTKSK for Rusty/PI192711 population (Table 4.12). A combination of *Sr28*,

Sr7/SrND643, a novel gene in *QSr.fgl-5B.1,* and an unknown resistance gene provide resistance

to race MCCF in Rusty/PI383416 population (Table 4.12). *Sr7/SrND643* and *Sr9** can provide

resistance themselves to foreign stem rust races JRCQC and TRTTF (Table 4.12). When all three resistance alleles are present, *QSr.fgl-4A.2, QSr.fgl-5B.2,* and *QSr.fgl-6A* are able to provide the most consistent resistance to JRCQC (Table 4.12). The presence of a single resistance allele from *Sr9*, QSr.fgl-5B.2,* or *QSr.fgl-6A* are able to provide resistance to TRTTF, while a single *QSr.fgl-5B.2* allele is able to provide resistance to TTRTF (Table 4.12). *Sr9** was also found to be associated with local stem rust race RKQQ in the Rusty/PI383416, Rusty/PI520392, and Rusty/PI636501 populations and provided minor resistance. An additional source of resistance to RKQQ must be present in the populations but was not uncovered.

From the Rusty/PI192711 population two published stem rust resistance genes were identified and one potentially novel resistance gene on chromosome 6B (Figure 4.19). From four QTL regions, two stem rust resistance genes were identified with two potentially novel stem rust resistance genes located on chromosomes 2B and 5B from analysis of the Rusty/PI383416 population (Figure 4.20). A reoccurrence of the novel allele located on chromosome 2B, in addition to three novel genes, was identified in the Rusty/PI520392 population (Figure 4.21). A third occurrence of the novel allele located on chromosome 2B was discovered in the Rusty/PI636501 population (Figure 4.22).

A few published stem rust resistance genes were identified, and several novel stem rust resistance genes identified within the four populations tested in this study. Conclusions were able to be drawn about the gene(s) conferring resistance in some scenarios while other remains very unclean at this point. Additional crossing of resistant lines is necessary to separate the resistance genes from one another and will assist in decreasing the size of the identified QTL for introgression into adapted germplasm and map-based cloning of these genes. With simpler

phenotypic segregation ratios, it will be more clear of the source of resistance and susceptibility
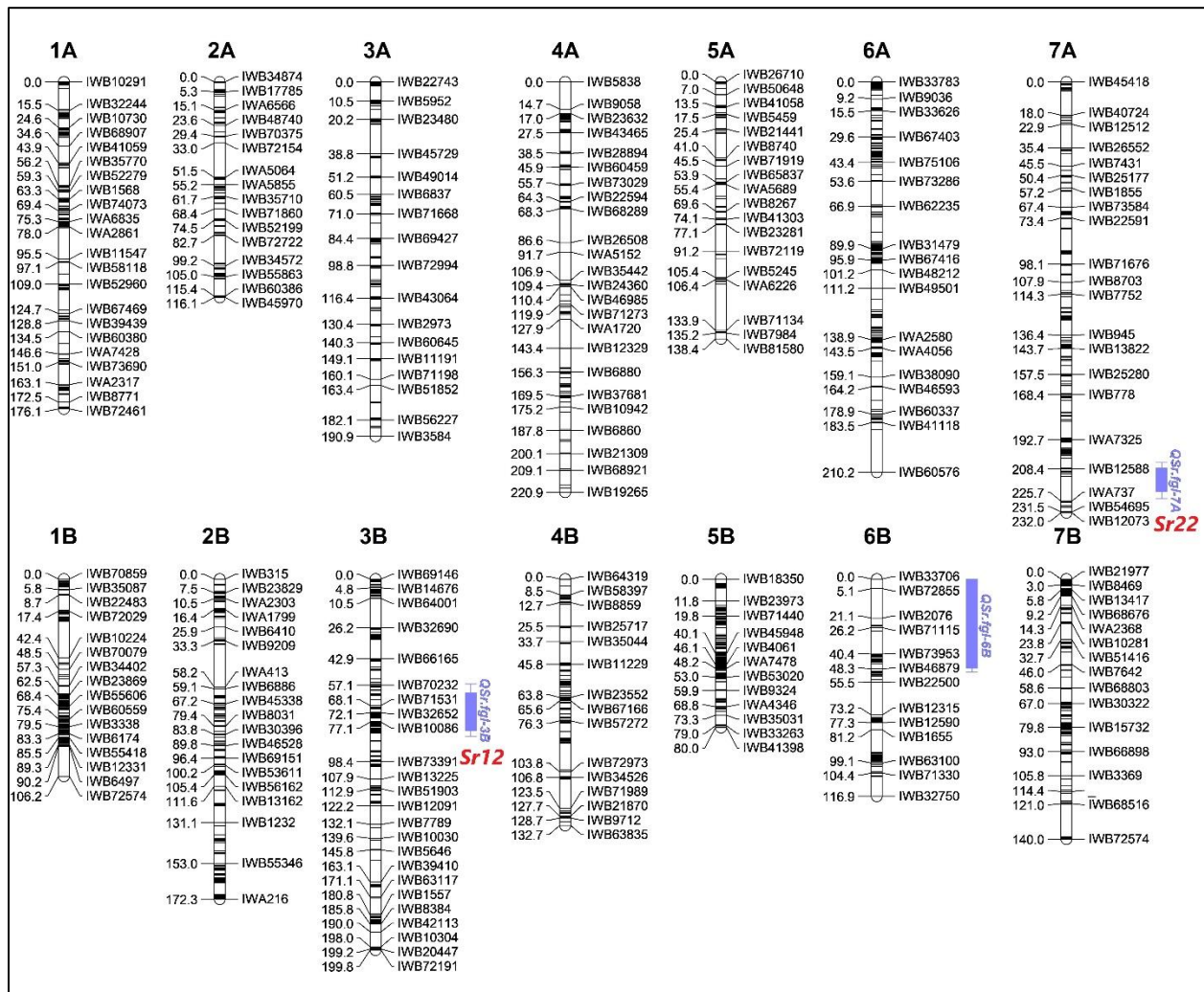
within the population to stem rust.

Figure 4.19. All 14 linkage groups identified within the Rusty/PI192711 population with QTL regions and predicted genes highlighted.
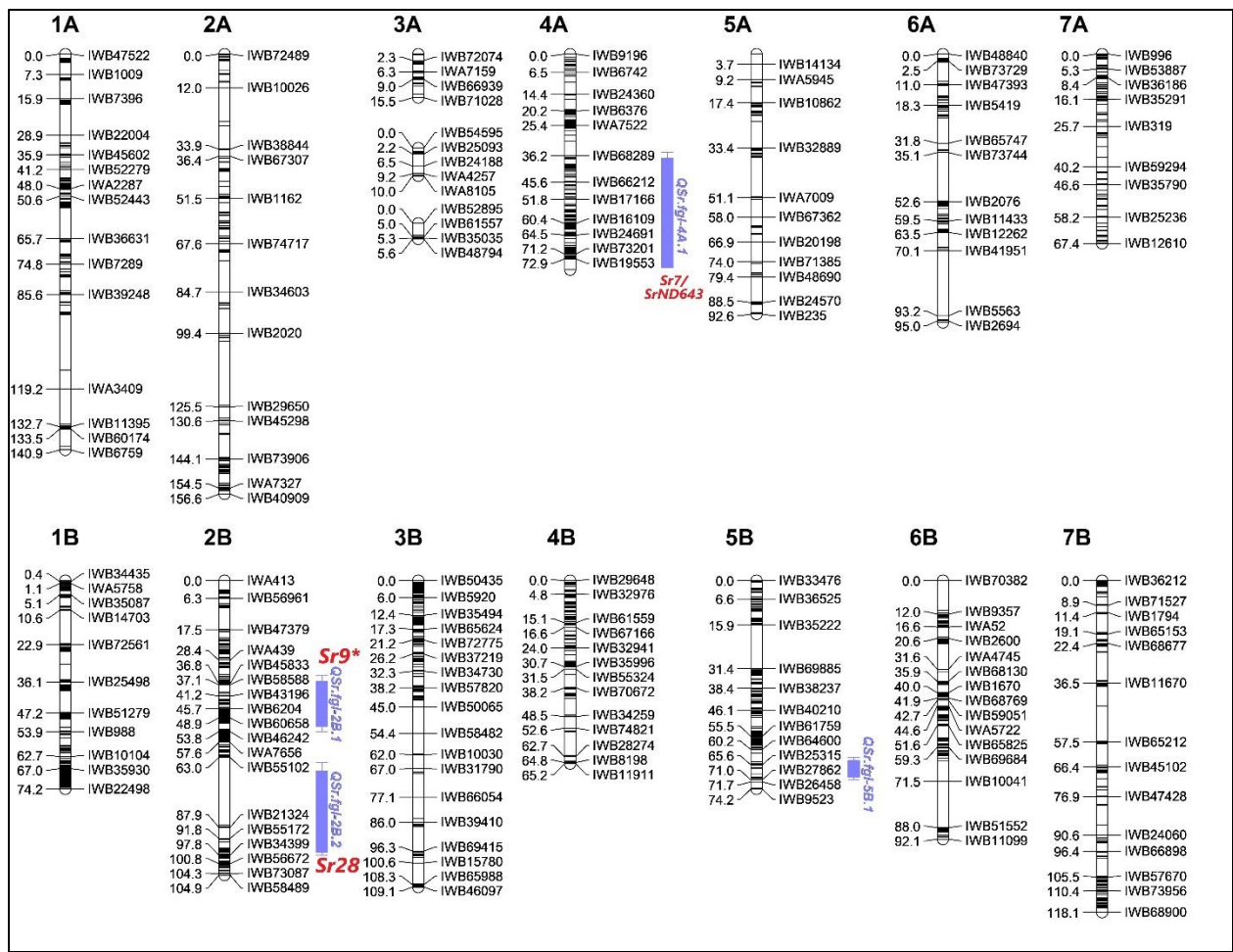
Figure 4.20. All 16 linkage groups identified within the Rusty/PI383416 population with QTL regions and predicted genes highlighted.
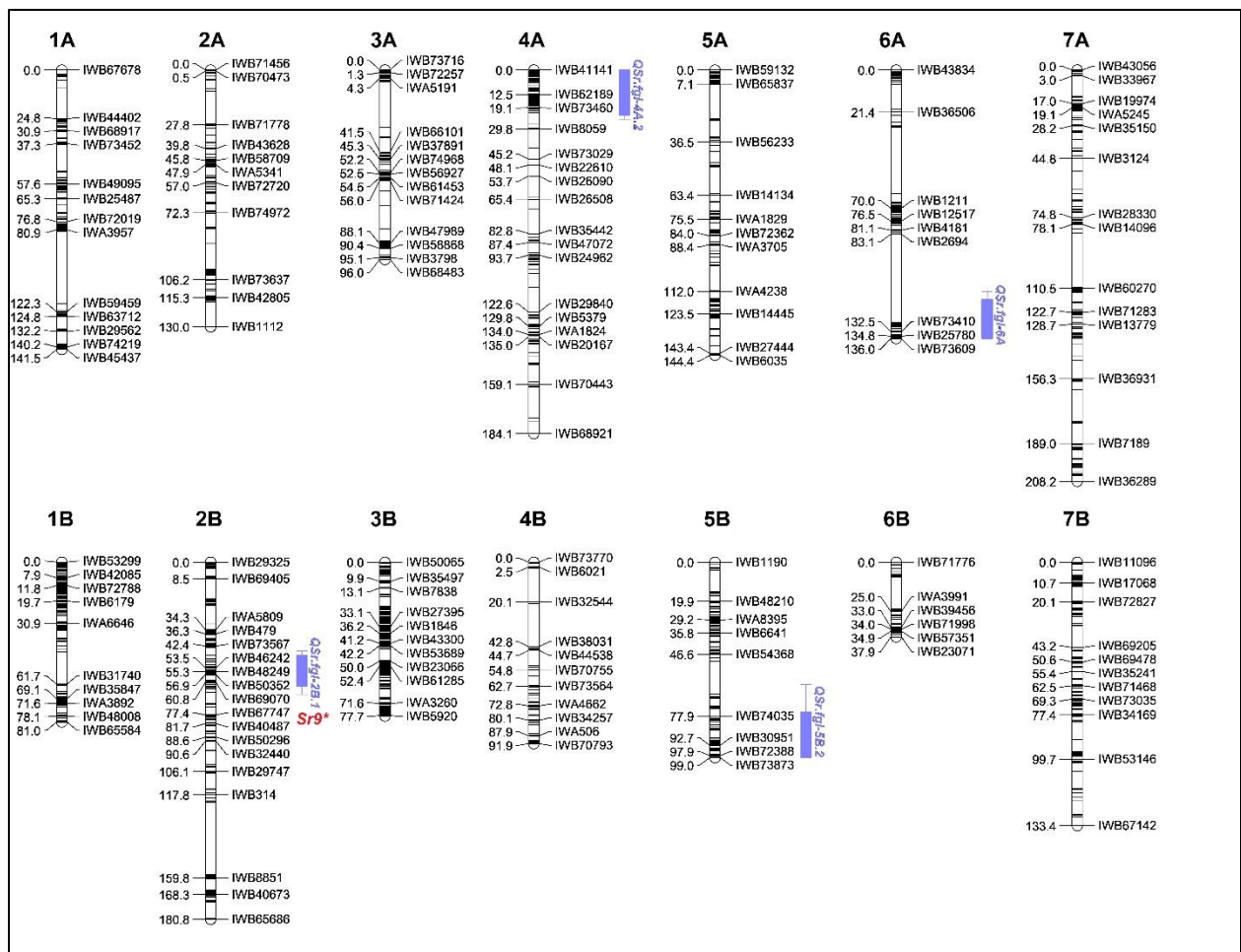
Figure 4.21. All 14 linkage groups identified within the Rusty/PI520392 population with QTL regions and predicted genes highlighted.
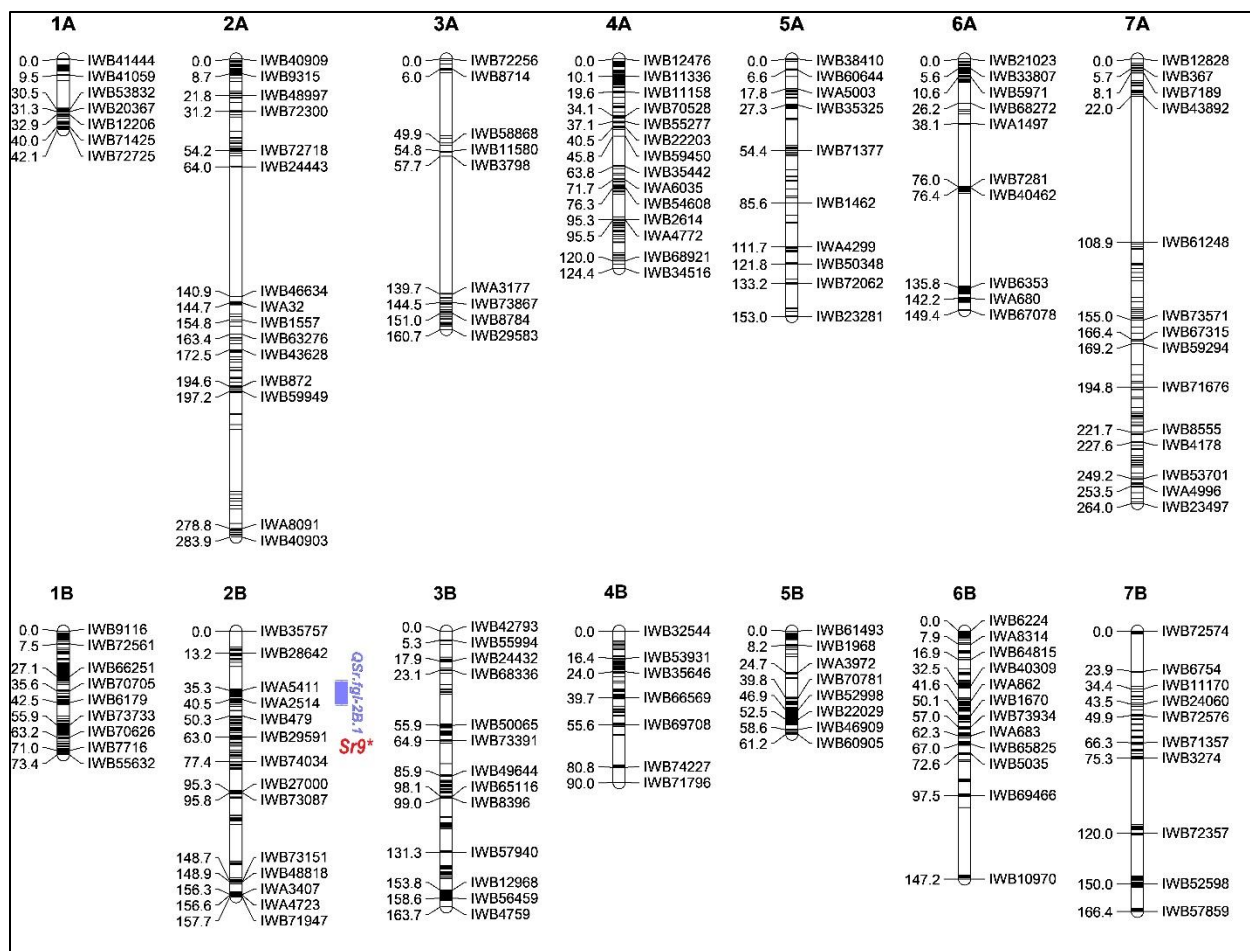
Figure 4.22. All 14 linkage groups identified within the Rusty/PI636501 population with QTL regions and predicted genes highlighted.

References

Bansal, U., Bariana, H., Wong, D., Randhawa, M., Wicker, T., Hayden, M., & Keller, B. (2014). Molecular mapping of an adult plant stem rust resistance gene *Sr56* in winter wheat cultivar Arina. *Theoretical and Applied Genetics*, *127*(6), 1441–1448. https://doi.org/10.1007/s00122-014-2311-1

Basnet, B. R., Singh, S., Lopez-Vera, E. E., Huerta-Espino, J., Bhavani, S., Jin, Y., Rouse, M. N., & Singh, R. P. (2015). Molecular mapping and validation of *SrND643*: A new wheat gene for resistance to the stem rust pathogen Ug99 race group. *Phytopathology*, *105*(4), 470–476. https://doi.org/10.1094/phyto-01-14-0016-r

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S.

    (2007). Tassel: Software for association mapping of complex traits in diverse samples.

    Bioinformatics, *23*(19), 2633–2635. https://doi.org/10.1093/bioinformatics/btm308

Chao, S., Rouse, M. N., Acevedo, M., Szabo-Hever, A., Bockelman, H., Bonman, J. M., Elias,

    E., Klindworth, D., & Xu, S. (2017). Evaluation of genetic diversity and host resistance

    to stem rust in USDA NSGC durum wheat accessions. *The Plant Genome*, *10*(2).

    https://doi.org/10.3835/plantgenome2016.07.0071

Hatta, M. M. A., Ghosh, S., Athiyannan, N., Richardson, T., Steuernagel, B., Yu, G., Rouse, M.

    N., Ayliffe, M., Lagudah, E. S., Radhakrishnan, G. V., Periyannan, S. K., & Wulff, B. B.

    (2020). Extensive genetic variation at the *Sr22* wheat stem rust resistance gene locus in

    the grasses revealed through evolutionary genomics and functional analyses. *Molecular*

    Plant-Microbe Interactions, *33*(11), 1286–1298. https://doi.org/10.1094/mpmi-01-20-

    0018-r

Hiebert, C. W., Kolmer, J. A., McCartney, C. A., Briggs, J., Fetch, T., Bariana, H., Choulet, F.,

    Rouse, M. N., & Spielmeyer, W. (2016). Major gene for field stem rust resistance co-

    locates with resistance gene *Sr12* in 'thatcher' wheat. *PLOS ONE*, *11*(6).

    https://doi.org/10.1371/journal.pone.0157029

Joehanes, R., & Nelson, J. C. (2008). QGene 4.0, an Extensible Java QTL-Analysis Platform.

    Bioinformatics, *24*(23), 2788–2789. https://doi.org/10.1093/bioinformatics/btn523

Kosgey, Z. C., Edae, E. A., Dill-Macky, R., Jin, Y., Bulbula, W. D., Gemechu, A., Macharia, G.,

    Bhavani, S., Randhawa, M. S., & Rouse, M. N. (2021). Mapping and validation of stem

    rust resistance loci in spring wheat line CI 14275. *Frontiers in Plant Science*, *11*.

    https://doi.org/10.3389/fpls.2020.609659

Rouse, M. N., Nava, I. C., Chao, S., Anderson, J. A., & Jin, Y. (2012). Identification of markers

    linked to the race Ug99 effective stem rust resistance gene *Sr28* in wheat (*Triticum*

    aestivum L.). *Theoretical and Applied Genetics*, *125*(5), 877–885.

    https://doi.org/10.1007/s00122-012-1879-6

Rouse, M. N., Nirmala, J., Jin, Y., Chao, S., Fetch, T. G., Pretorius, Z. A., & Hiebert, C. W.

    (2014b). Characterization of *Sr9h*, a wheat stem rust resistance allele effective to Ug99.

    Theoretical and Applied Genetics, *127*(8), 1681–1688. https://doi.org/10.1007/s00122-

    014-2330-y

Rouse, M. N., Talbert, L. E., Singh, D., & Sherman, J. D. (2014a). Complementary epistasis

    involving *Sr12* explains adult plant resistance to stem rust in Thatcher Wheat (*Triticum*

    aestivum L.). *Theoretical and Applied Genetics*, *127*(7), 1549–1559.

    https://doi.org/10.1007/s00122-014-2319-6

Saini, J., Faris, J. D., Zhang, Q., Rouse, M. N., Jin, Y., Long, Y., Klindworth, D. L., Elias, E. M.,

    McClean, P. E., Edwards, M. C., & Xu, S. S. (2018). Identification, mapping, and marker

    development of stem rust resistance genes in durum wheat 'Lebsock.' *Molecular*

    Breeding, *38*(6). https://doi.org/10.1007/s11032-018-0833-y

Simons, K., Abate, Z., Chao, S., Zhang, W., Rouse, M., Jin, Y., Elias, E., & Dubcovsky, J.

    (2010). Genetic mapping of stem rust resistance gene *Sr13* in tetraploid wheat (*Triticum*

    turgidum ssp. *durum* L.). *Theoretical and Applied Genetics*, *122*(3), 649–658.

    https://doi.org/10.1007/s00122-010-1444-0

Stakman, E. C., Stewart, D. M., & Loegering, W. G. (1962). *Identification of physiologic races*

    of Puccinia graminis var. tritici. U.S. Dept. of Agriculture Revised.

Stam, P. (1993). Construction of integrated genetic linkage maps by means of a new computer
package: Join Map. *The Plant Journal*, *3*(5), 739–744. https://doi.org/10.1111/j.1365-
313x.1993.00739.x

Tsilo, T. J., Jin, Y., & Anderson, J. A. (2007). Microsatellite markers linked to stem rust
resistance allele *Sr9a* in wheat. Crop Science, 47(5), 2013–2020.
https://doi.org/10.2135/cropsci2007.02.0087

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S.,
Milner, S. G., Cattivelli, L., Mastrangelo, A. M., Whan, A., Stephen, S., Barker, G.,
Wieseke, R., Plieske, J., Lillemo, M., Mather, D., Appels, R., … Akhunov, E. (2014).
Characterization of polyploid wheat genomic diversity using a high-density 90,000 single
nucleotide polymorphism array. *Plant Biotechnology Journal*, *12*(6), 787–796.
https://doi.org/10.1111/pbi.12183

Yu, L.-X., Barbier, H., Rouse, M. N., Singh, S., Singh, R. P., Bhavani, S., Huerta-Espino, J., &
Sorrells, M. E. (2014). A consensus map for Ug99 stem rust resistance loci in wheat.
Theoretical and Applied Genetics, *127*(7), 1561–1581. https://doi.org/10.1007/s00122-
014-2326-7

Zhu, T., Wang, L., Rimbert, H., Rodriguez, J. C., Deal, K. R., De Oliveira, R., Choulet, F.,
Keeble-Gagnère, G., Tibbits, J., Rogers, J., Eversole, K., Appels, R., Gu, Y. Q., Mascher,
M., Dvorak, J., & Luo, M. C. (2021). Optical maps refine the bread wheat *Triticum
aestivum* cv. Chinese Spring Genome Assembly. *The Plant Journal*, *107*(1), 303–314.
https://doi.org/10.1111/tpj.15289

Zurn, J. D., Rouse, M. N., Chao, S., Aoun, M., Macharia, G., Hiebert, C. W., Pretorius, Z. A., Bonman, J. M., & Acevedo, M. (2018). Dissection of the multigenic wheat stem rust resistance present in the Montenegrin Spring Wheat Accession PI 362698. *BMC Genomics*, *19*(1). https://doi.org/10.1186/s12864-018-4438-y