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Title: Identifying Quantitative Trait Loci for Resistance Against Stripe Rust and Septoria Tritici Blotch in Soft White Winter Wheat

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Stripe rust (*Puccinia striiformis* f. sp. *tritici*) and Septoria tritici blotch (*Zymoseptoria tritici*) are a constant and significant threat to wheat production, significantly reducing wheat quality and yield. Wheat is responsible for 20% of the world's human calorie intake, and wheat production must increase to supply the demand of the world's growing population. Both stripe rust and Septoria tritici blotch (STB) are critical foliar diseases of wheat in the Pacific Northwest (PNW). Increasing stripe rust and STB resistance through plant breeding is the most cost-effective, sustainable, and environmentally friendly approach to manage these diseases. A recombinant inbred line population was developed from a cross between 'Madsen' and 'Foote' soft white winter wheat cultivars to study stripe rust and STB resistance. Foote (PI 599663) was initially resistant to stripe rust but is now considered susceptible to new, virulent strains of the pathogen. However, Foote has maintained moderate resistance to STB in the PNW. Madsen (PI 511673) has provided effective resistance to stripe rust, but it is considered moderately susceptible to STB. The recombinant inbred line (RIL) population, consisting of 217 lines, was phenotyped across multiple environments for stripe rust and STB response and genotyped using Illumina HiSeq 3000 Sequencing. The 217 lines were also phenotyped for seedling resistance for stripe rust in growth chambers against a bulk population of spores collected from the field in 2012 and a single isolate of race Pstv-37. Pstv-37 has been the most abundant race in the PNW in the last six years. The STACKS and Bfctools programs were used for calling genotype variation. The best linear unbiased prediction (BLUP) was calculated across environments and used to detect QTL resistance. Results of quantitative trait locus (QTL) analysis indicated minor alleles for adult plant resistance to STB in wheat chromosomes 4B, 5A, 6B, 6D and 7DS. Stacking these

genes is the best strategy to develop durable resistance to STB. For wheat stripe rust in the field, major alleles for resistance were identified in wheat chromosome 2AS, which is likely the known stripe rust resistance gene Yr17, and in 1AS. Two minor QTL were found in 2AS/2DS and 4DL. For the growth chamber study, four QTL were found in 1B, 2B, 6B, and 7B, with the identified QTL dependent on the stripe rust race used for screening. Combining Yr17, 1AS, and the other QTLs will lead to developing durable resistance to individual cultivars. The QTL identified in this thesis could help to develop breeder-friendly molecular markers for use in genotypic selection for improved STB and stripe rust resistance in wheat.

Identifying Quantitative Trait Loci for Resistance Against Stripe Rust and Septoria Tritici Blotch in Soft White Winter Wheat

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

David Cobertera, Author

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Chapter 1 : General Introduction

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1.1 Introduction

Wheat (*Triticum* spp.) is a cereal with a high nutritive value[1] and supplies the human population with around 20% of its total caloric intake[2,3]. The grain of wheat is composed of 7% to 28% protein and 60% to 80% starch. The percentage of wheat protein determines the final product. The most notable wheat products are bread, noodles, and pasta. Additionally, wheat is also used for alcohol distillation, animal feed, and as raw material for biofuels[4].

Wheat has played an essential role in human civilization since its domestication ~ 10,000-12,000 years ago in the Near Eastern Fertile Crescent[5]. It was the main crop in the transition from hunting and gathering to settled agriculture[6]. The earliest wheat cultivated were einkorn, diploid wheat (genome AA), and emmer, tetraploid wheat (genome AABB)[7]. Plant domestication is an evolutionary process of speciation, natural selection, and adaptation of the plants guided by our ancestors. This process was the beginning of plant breeding. Plant breeding has been around since as early as the Neolith age, with humans changing and manipulating plant characteristics, such as structure, traits, and composition, making them more useful to humans[8]. Plant breeding became more sophisticated after Mendel proposed the two laws of heredity, establishing that plant traits are controlled by heritable factors (genes)[9]. The most crucial domestication traits in wheat were non-brittle rachis and free-threshing[10]. The brittle rachis results in seed loss due to the shattering of the spike at maturity. This trait is determined by the mutation of two major genes, brittle rachis 2 (*Br-A2*) in the short arm of chromosome 3A and brittle rachis 3 (*Br-A3*) in the short arm of chromosome 3B[11]. Free- threshing is the loss of glumes that enclose seeds, resulting in 'naked' seed. This trait is principally controlled by two homologous gene, *Tg* located in the short arm of chromosome 2B and the gene *Q* located in the large arm of chromosome 5A[12]. Other domestication traits were plant architecture, kernel size, and seed dormancy[13]. This process was the beginning of plant breeding.

Wheat has been distributed to all parts of the world except Antarctica, making it the commercial crop with the most land area globally with around 214 million hectares and an annual yield of 734 million tonnes[14]. Currently, most cultivated wheat are hexaploid bread wheat and tetraploid durum wheat, with 95% and 5% of the wheat grown worldwide respectively[14,15]. The biggest wheat producers are the European Union, Australia, Canada, India, the Russian Federation, Ukraine, and the United States[16]. Even with this level of wheat being grown in the world, wheat production must continue to increase to supply food for the expanding global population, which is expected to reach 9 billion by 2050 and about 11 billion by 2100[17]. At the same time, several factors, including climate change, lack of freshwater, decreasing availability of

suitable farmland, and unpredictable abiotic and biotic stresses, can compromise the ability to increase the yield of wheat enough to feed the planet. Therefore, wheat breeders must use new knowledge, techniques, and technology to counteract these factors to meet the demand.

Fungal diseases pose a severe and persistent challenge to wheat throughout the world. Pathogenic fungi represent a significant limitation for wheat production, significantly reducing wheat quality and yield, by up to 15-20%[18]. There are two types of plant pathogenic fungi, biotrophic fungi-obligate parasites which require living cells of the plants as a source of nutrients, such as stripe rust (caused by *Puccinia striiformis f. sp. Tritici*), and necrotrophic fungi - facultative parasites that feed on the host's dead cells and do not necessarily need living plants, such as Septoria tritici blotch (caused by *Zymoseptoria tritici*[19]). The fungal diseases that result in the most prominent and devastating loss of yield in wheat are rusts, blotches, and head blight/scab[18]. Of these, stripe rust is considered the most significant rust disease affecting winter cereal production across the world, causing yield losses of 5 to 50% depending on the year, representing an annual loss of 5.47 million tonnes worth $979 million[20]. Septoria tritici blotch (STB) is wheat's primary leaf blotch disease, decreasing crop yield by 10 to 50%, especially in humid and temperate areas. In recent years, STB prevalence has increased, having a significant economic impact due to loss of yield and quality and excessive use of fungicides, especially in Europe with losses of $1.2 billion[21].

Pesticides were one of the keys in the green revolution, allowing farmers to intensify production systems without incurring further losses leading to pesticides becoming the primary method of pest control[22]. Pesticides are usually an effective method, but they involve a high economic, social, and environmental cost. A viable and effective alternative is to use disease resistance genes to develop resistant cultivars[23]. In all breeding programs, one of the main objectives is breeding against disease. Ninety-eight percent of elite wheat cultivars in the U.S. have at least one resistance gene[8]. Fortunately, wheat has an efficient immune system, regulated by resistance genes. Wheat is the plant species with the largest number of resistance genes reported[24]. Resistance genes can be defined as a source of either full or partially heritable resistance. These genes can be used for breeding fungi-resistant wheat. Resistance genes can be classified by their resistance type: qualitative resistance - characterized by vertical resistance which generally depends on major genes with higher effects; and quantitative resistance characterized by unspecific horizontal resistance which generally depends on several-to-many genes with moderate-to-small effects. These two types of resistance can regulate a plant’s immune response either independent of the growth stage or dependent on the growth stage. Resistance genes can also be classified by the stage of growth in which the immune response appears. Seedling resistance is resistance effective at all plant growth stages, which is generally specific race-specific resistance gene and adult plant resistant, effective resistance only

at the adult stage. These genes usually provide resistance to more than one isolate or distinct pathogen species.

Plant breeding for resistance involves the knowing and manipulating two organisms, plants (host) and fungi (parasite). Resistance breeding is a perpetual challenge for breeders because plant pathogens (such as fungi) can rapidly overcome previously resistant crop cultivars due to the dynamic and elusive nature of the gene-for-gene interaction between the host plant and pathogenic system. Resistance breeding requires a constant supply of resistance genes that can be incorporated into new cultivars. Molecular markers are essential in both classical and modern plant breeding approaches to accelerate genetic gain[25]. Marker-assisted selection (MAS) and its variants are the most common method in resistance breeding. These methods allow the selection of resistance genes at the genotypic level in the absence of the pathogen, reducing time, cost and resulting in a higher gain per year compared to phenotypic selection[26]. MAS requires that identified molecular markers are linked to a resistance allele/gene, allowing breeders to deploy new genes for resistance faster and more effectively when disease undergoes race changes[27]. The common method to identify molecular markers linked to a resistance allele/gene is called quantitative trait locus detection (QTL analysis). This method detects the relationship between genotypes and phenotypes in order to explain the genetic variation[28]. QTL requires a variation in the genetic population in the trait of interest; quantifying, characterization, and determination of resistance reaction of crops against fungal pathogen; and high-resolution/fine genetic mapping. The common populations used are F_2 populations, backcrosses (BCs), near-isogenic lines (NILs), and recombinant inbred lines (RILs). The RIL population must consist of 100-250 individuals with genetic variation in the trait of interest to enable high resolution and fine mapping of the trait of interest[29,30]. Genotyping a population has historically been slow and difficult, especially in species with a large, complex genome with a high level of repetitive DNA.

In the last 10 years, new genome sequencing technologies have become faster and more economical, providing greater depth and increased sensitivity. These techniques allow identifying inter-individual variation at a genome-scale. There are two sequencing approaches: whole-genome sequencing, used when the species possess small genomes, such as fungi; and genome reduction and sequencing techniques such as genotyping-by-sequencing, transcriptome sequencing, and exome capture, which are used when the species possess large genomes, such as wheat. These techniques are valuable for the discovery, validation, and assessment of genetic DNA markers in populations[31]. Genetic DNA markers are genes or DNA sequences linked to a particular trait of interest with a known chromosomal position[28]. A high-quality wheat reference genome has been publicly available since 2018, providing an ordered and annotated wheat genomes sequence[32,33]. The publication of the wheat genome had been highly anticipated for wheat

breeders due to wheat having a large, polyploid, complex genome (haploid genome, 1C = 16 GB), which includes more than 85% repetitive DNA[32].

Phenotyping is essential in detecting resistance genes/alleles. Each plant can show symptoms and different resistance reactions during pathogenesis, such as hypersensitive response (HR) or systematic acquired resistance (SAR)[34]. There are different methods to quantify the individual variation. The general method is quantified visually by estimating the percentage symptoms (level of susceptibility) such as percentage of severity of the disease (% severity of disease), or percentage of leaf surface covered by lesions (PLACL), or the resistance reaction (such as hyperspectral reflectance). All of these require an infection with the pathogen of interest. The last step of quantifying variation is using statistical programs to identify genotypic-phenotypic interaction to explain the genetic variation. The most common method is a quantitative trait locus (QTL) analysis. This analysis is generally evaluated by analytical statistical software such as QGENE[35], MapChart[36], and R/qtl[37] to analyze the different variations of QTL analysis, such as single-markers analysis (SMA); interval mapping (IM), and composite interval mapping (CIM)[38].

The Pacific Northwest of the United States, commonly abbreviated as PNW, consists of three states: Idaho, Oregon, and Washington. It is one of the principal wheat producing regions in the United States, producing about 7.66 million metric tons each year, corresponding to $1,700 million. The primary market class of wheat grown in the PNW is soft white wheat, used for noodle products, crackers, cereals, and white crusted bread[39]. Wheat production in the PNW is concentrated in the Columbia and Snake River drainage systems and its production is primarily aimed at the Asian market[40]. The weather conditions such as humid climate with moderate temperature characterizes the Pacific Northwest. These conditions provide a favorable environment for fungal pathogens, especially stripe rust[41] and Septoria[19].

Wheat breeding is a vital aspect of world wheat production. Breeders' objectives are increasing yield and counteracting adverse factors setbacks to productivity. Successful modern wheat breeding uses new knowledge, techniques, and technology to achieve the goal. Therefore, this research aims to address counteracting fungal diseases, especially stripe rust and Septoria, which are the most important wheat leaf diseases worldwide and in the Pacific Northwest.

The objectives of this dissertation are:

1) Identify molecular markers for stripe rust resistance alleles/genes in field conditions in order to identify valuable markers and quantitative trait loci for future gene characterization and breeding of resistant cultivars against stripe rust.

2) Identify molecular markers for Septoria tritici blotch resistance alleles/genes in the field in order to identify valuable markers and quantitative trait loci for future gene characterization and breeding of resistant cultivars against Septoria tritici blotch.

3) Identify molecular markers of stripe rust resistance alleles/genes in the growth chamber in order to identify valuable markers and quantitative trait loci for seedling resistance for future gene characterization and breeding of resistant cultivars against stripe rust.

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Chapter 2 : Literature review

Chapter 2: Literature review

2.1 History of wheat

Domestication

Wheat (*Triticum* spp.) has been one of the principal food crops for human civilization since its domestication in the Fertile Crescent ~ 10,000-12,000 years ago[1]. Domestication of plants radically changed human societies, molding itself from a nomadic, gatherer, and hunter society to a sedentary, agricultural community[2]. The earliest cultivated wheat forms were einkorn (*Triticum monococcum L.*) and emmer (*T. turgidum ssp. dicoccum L*). Einkorn (diploid wheat) was domesticated from its wild form (*Triticum monococcum L. subsp. boeoticum*) in southeastern Turkey, between the Kartal–Karadağ and Karacadağ regions[3]. The progenitor of emmer (tetraploid wheat) is *Triticum turgidum L. ssp dicoccoides*[4]. Emmer and hexaploid wheat (*Triticum aestivum L.*) underwent a natural hybridization process. In the case of emmer hybridization occurred between *Triticum urartu* (2n =2x=14; AA)[5], which provides the A genome, and *Aegilops speltoides* (2n =14; SS)[6], which granted the B genome[3]. Instead, hexaploid wheat hybridization occurred in the agronomic field between two cultivated forms, emmer and *Ae. tauschii* (DD genome), 10,000 years ago[4].

Domestication traits

The early forms of cultivated wheat developed through a plant domestication process, such as hybridization, adaptation, and natural selection[7]. Spike morphology was the most significant domestication event, which involved two independent traits, non-brittle rachis and free-threshing[8,9]. The non-brittle rachis is the loss of function that promotes seed dispersal, favoring the collection of seeds at maturity[10]. Two genes control this trait, *Br-A2* (brittle rachis) located in the short arm of chromosome 3A and *Br-A3* (brittle rachis) located in the short arm of chromosome 3B[11]. Free-threshing is the loss of the enclosure of the seed by the lemma and palea, reducing the level of self-protection of the seed[10]. Two genes control this trait, *Tg* and *Q*, located in the short arm of chromosome 2B and the large arm of chromosome 5A, respectively[12]. These evolutionary traits with other traits such as kernel size, seed dormancy, and plant structure[9] are considered the beginning of plant breeding.

Wheat genetics

Bread wheat is an allohexaploid species consisting of three individual but related sub-genomes, A, *B and D* from three progenitor species, *Triticum urartu* ($2n = 2x = 14$; AA), *Aegilops speltoides* ($2n = 14$; SS)[6], and *Aegilops tauschii* ($2n = 2x = 14$; DD)[13–15]. Wheat has approximately 15.4 to 15.8 bp, with 107,891 gene loci across the 21 chromosomes[16]. It is estimated that the wheat genome has around 94,000-96,000 genes, and around 85% of the genome is repeated sequences[17], where genome A is

4.93pg DNA[18], genome B is 5.15 pg DNA[19], and genome D is 5.10 pg DNA[18]. A high-quality reference genome of hexaploid wheat has been publicly available since 2018[16]. In addition, there is a high-quality reference genome of the D genome[20], the AB tetraploid wild emmer (*Triticum diccocoides*) genome[21], and wild emmer's domesticated wheat relative (*Triticum durum)*[22]. Knowing the reference genome of wheat and its relatives facilitates the study of wheat's genetics, function, and evolution.

2.2 Cultivated wheat.

Wheat production

Wheat is one of the principal sources of the human diet due to its relatively long-term storage capacity, wide adaptability, and high nutritional value[23,24]. Wheat plays a vital role in world food security, being the most widely planted crop globally at about 214 million hectares per year with an annual yield of 734 million tons[25]. It contributes approximately 20% of total caloric intake[23,26]. The wheat grain comprises 7%-22% of protein and 60%-80% of starch, making it one of the most nutritive cereal crop[27]. The grain protein content and kernel hardness of wheat determine its grain market class, milling process, and final products[28]. Wheat is the only grain whose dough contains viscoelastic properties essential to producing leavened food such as bread[24]. Wheat is also used for other food products such as noodles, cakes, and pasta. Besides food products, wheat is also used for animal feed, alcohol distillation, and as a raw material for biofuel[29].

Wheat is produced on all continents, except Antarctica. The areas with the highest production are the European Union, India, Australia, Canada India, the Russian Federation, Ukraine, and the United States[30]. The flowering patterns of wheat genotypes are vastly different, allowing wheat to be grown in subtropical to temperate areas at different altitudes[31]. Wheat flowering is a complex trait regulated by photoperiod (*Ppd*), vernalization (*Vrn*), and earliness genes[32]. These genes are regulated by environmental stimuli such as daylength (*Ppd*), or temperature (*Vrn*), initiating or retarding flowering. This environmental response protects the wheat from flowering during either low or high-temperature extemes[33]. The photoperiod trait is regulated by three orthologous pseudo response regulator (PRR) genes located on the homoeologous chromosome 2 group (A, B, and D genomes) (*Ppd-A1*, *Ppd-B1*, and *Ppd-D1*)[34]. *Ppd-A1* is the most photoperiod sensitive locus followed by *Ppd-B1* and *Ppd-D1*[35]. Other genes with a photoperiod response such as *Ppd-B2* mapped on chromosome 7BS and the three recently discovered homeolog genes called *TaFT3*, located on the long arm on the chromosome 1 of each wheat sub-genome[36]. The photoperiod and vernalization genes differentiate wheat into winter and spring types. Winter wheat is planted in the fall and requires a chilling treatment for at least six to eight weeks with a temperature below 8°C (vernalization) to shift from vegetative to reproductive growth. These genes protects the wheat from flowering during the winter[37]. Spring wheat requires no vernalization so can be

planted in the spring[38]. Vernalization is controlled by genes at the *Vrn-1*, *Vrn-2*, *Vrn-3*, and *Vrn-4* loci. The *Vrn-1* loci involves three orthologous genes (*Vrn-A1*, *Vrn-B1*, and *Vrn-D1*), located on chromosome 5A, 5B, and 5D, respectively[39]. *Vrn-2* loci involves three genes across the wheat genome that have been mapped on chromosomes 5A, 4B and 4D[40]. *Vrn-3* loci involves the homologous chromosomes, 7B and 7D[40]. V*rn-D1* is found at a single locus on chromosome 5D[41]. *Vrn1* is the gene with a higher impact in the transition between vegetative to adult plant. *Vrn-A1* recessive is associated with winter wheat, while *Vrn-B1* and *VRN-D1* dominant are associated with spring wheat[42].

Cultivated wheat species include einkorn, spelt, hexaploid bread wheat, and tetraploid durum wheat. Hexaploid bread wheat is the most cultivated form of wheat, accounting for approximately 95% of the world's cultivated total wheat[25,43], followed by durum wheat representing around 5% of the total wheat grown worldwide[25,43]. Spelt and einkorn spelled represent a minuscule percentage of the total wheat worldwide, growing in only some areas of Spain, Turkey, the Balkans, and India[44].

Wheat in the Pacific Northwest (PNW) of the U.S.

The United States is eighth in the world for wheat production, producing around 63 million tons of wheat every year[45]. Wheat is the third-largest crop in the U.S, behind corn (*Zea mays*) and soybeans (*Glycine max*). Wheat has a wide production zone with every state involved in the nation's wheat production[46]. The Pacific Northwest (PNW), which consists of three states, Oregon, Idaho, and Washington, grows around 1.83 million hectares every year[45]. Wheat production in the PNW is concentrated in the Columbia and Snake Rivers, and their production is mainly for the Asian market. Soft white wheat is the principal wheat from the PNW; it's primarily used to produce noodles and crackers[47]. Wheat yield differs in the PNW due to agronomic practices such as irrigation/no irrigation or tillage/no-tillage. The farmers of the PNW produce 755 million metric tons of grain annually, rendering approximately 1.7 billion USD[45].

Future challenges in wheat

Food producers are having increasing competition for water, land, and energy from the expanding population predicted to be 9 billion by 2050 and about 11 billion by 2100[48], and from climate change. In addition, unpredictable abiotic and biotic stress can negatively affect production and compromise the ability to increase yield[48]. Thus, it is not a surprise the importance that wheat breeding has in meeting global production demands. Modern plant breeding techniques such as molecular assisted markers (MAS) and genomic selection are being applied to improve breeding efficiency and speed up cultivar development[49]. In all breeding programs, one of the main objectives is breeding for disease resistance. Ninety-eight percent of elite wheat cultivars carry at least one known resistance gene against one crucial disease[50].

The challenge for wheat producers and wheat breeders in the PNW is that producing wheat in an ideal climate for wheat production, with moderate to high humidity, mild winters, and moderate summer temperatures, is also ideal for fungal diseases. Fungal disease control is essential for wheat production in both the PNW and worldwide. The most critical diseases in the PNW are fungal diseases such as stripe rust caused by *Puccinia striiformis f. sp. tritici*), Septoria tritici blotch caused by *Zymoseptoria tritici*, diseases caused by fungal soilborne pathogens such as eyespot caused by *Tapesia yallundae*[51], and *Rhizoctonia cerealis*[52].

2.3 Fungal diseases

Plant diseases are the primary threat for global agriculture, reducing yields by over 10%[53,54] annually, with fungal diseases making up over 70% of plant diseases. Each year fungal diseases cause significant yield loss in crops such as wheat, rice (*Oryza sativa*), and barley (*Hordeum vulgare*), representing annual losses of more than 235 billion dollars[55]. Furthermore, In the United States alone, over 600 million dollars is spent annually on fungicides to control plant diseases[56]. Fungal diseases are present in all life cycles of the crop from germination through post-harvest. In addition, 15,000 fungi species cause disease in plants, where most of them are either Ascomycetes or Basidiomycetes[57].

The fungal infection cycle consists of establishing infection, colonization, growth, reproduction, dissemination of the pathogen, and pathogen survival in the host's absence[58]. However, each pathogen has a distinctive manner to accomplish each phase. The fungus produces survival structures from the end of the season to over-season and serves as the primary inoculum for the next disease cycle. Fungal plant pathogens can be divided into two groups, biotrophic and necrotrophic, based on how they utilize invaded plant tissues for nutrients[59]. Biotrophic fungi such as rust fungi infect the plant through effectors that modulate the plant defense system without being detected. It uses living host cells as a source of nutrients. Necrotrophic fungi such as blotch fungi use effectors and enzymes to kill the host cell or cell wall-degrading (CWDEs) to feed off the dead cells[60].

The success or failure of the pathogen depends on the interaction of three elements, pathogen, host, and environment. The interaction between host and pathogen depends on mutual recognition, the host's virulence effectors, and the plant's defense level. Two different theories explain this interaction, the gene-for-gene interaction theory, and the matching-allele theory. The gene-for-gene theory[61] is based on the concept of each resistance gene in the host having a corresponding gene in the pathogen with which it interacts. Therefore, the host’s resistance depends on both the gene's presence for resistance in the host and the corresponding genes for the pathogen's virulence. This theory has been demonstrated by genetic data and by host-pathogen isolation[62]. The matching-allele or inverse gene-for-gene theory differs in that the pathogen uses the plant's defense mechanisms to cause the disease[63]. This theory has been supported

by the necrotrophic fungal pathogen of plants such as tan spot caused by *Pyrenophora tritici-repentis* in wild emmer (*Triticum turgidum ssp. dicoccoides*)[64].

Pathogenic fungi pose a constant and significant threat to wheat production[54]. Fungal diseases cause substantial economic damage and are the major limitation for wheat production, reducing wheat quality and yield by 15-20%[53,54]. The most devasting fungal diseases in wheat are the rusts, blotch, and head blight/scab[54].

Stripe rust

Economic impact and control

Rust diseases of wheat are one of the oldest plant diseases known to humans. There are three different rust diseases in wheat, leaf rust (*Puccinia triticina*), stem rust (*Puccinia graminis f. sp. tritici*), and stripe rust (*Puccinia striiformis* f. sp. *tritici*). In the Pacific Northwest, the primary disease that attacks wheat is stripe rust[58]. An alternative name for stripe rust is yellow rust based on the orange to yellow color of the spores that appear in 'stripes' on infected plants. Favorable environmental conditions for stripe rust include moderate winter temperatures to allow over-wintering and cool, moist weather conditions in the spring and early summer. As a result, Stripe rust is the primary rust disease affecting winter cereal production across the world[57]. Currently, 88% of wheat elite cultivars are susceptible to stripe rust, resulting in a loss of about 5.5 million tons of wheat yearly, representing $979 million[65]. The continuing evolution of the pathogen in response to resistant wheat cultivars poses a persistent challenge to pathologists, breeders, and producers.

Currently, there are two strategies to control stripe rust, chemical control and genetic resistance. Chemical control requires dynamic monitoring to predict the infection to have an accurate chemical intervention and reduce environmental impact[66]. The fungicides most used to control stripe rust are demethylation inhibitors (DMI) such as propiconazole and fluquinconazole; quinone outside Inhibitor (QoI) such as azoxystrobin and pyraclostrobin; and succinate dehydrogenases (SDHIs) such as benzovindiflupy and bixafen[67]. The use of genetic resistance to control stripe rust is not based on monitoring of the pathogen but instead requires understanding pathogen distribution and resistance gene distributions in elite cultivars[68,69].

Cycle, host, and symptoms

In wheat, the stripe rust pathogen is caused by *P. striiformis*. f. sp.*tritici*[70]. *Puccinia striiformis* is a macrocyclic, heteroecious fungus with two hosts, wheat, and alternate host *Berberis or Mahonia spp*. This cycle comprises five stages that produce a different spore at each stage: basidiospores, pycniospores, aeciospores, urediniospores, and teliospores[71]. The urediniospores are essential spores infecting wheat each year. Teliospores and basidiospores cannot infect cereals and grasses but can infect *Berberis and Mahonia spp.* and produce pycniospores and aeciospores[71] (

Figure 2.1). The uredinial/telial stages infect bread wheat, emmer wheat and wild emmer, durum wheat and triticale (*Triticosecale*). Pycniospores and aeciospores infect the alternative hosts Barberry (*Berberischinensis, B. holstii, B. koreana B. shensiana, B. potaninii, B. vulgaris, B. dolichobotrys, B. shensiana, B. heteropoda*) and Oregon grape (*Mahonia aquifolium*)[72].

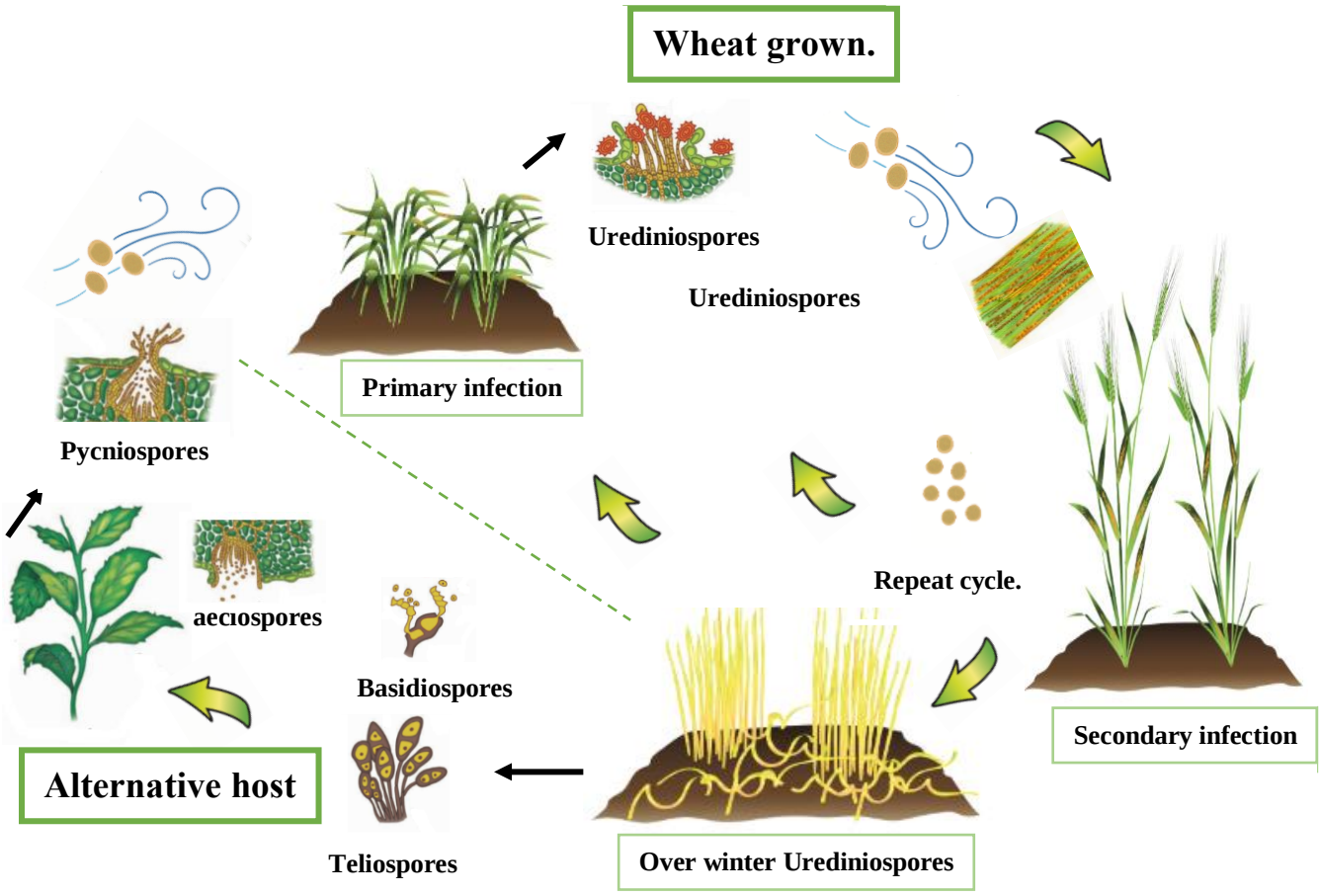

Figure 2.1. Stripe rust life cycle in wheat and its alternative hosts.

Stripe rust infection in wheat starts when urediniospores germinate and germ tubes grow into host tissues via stomates. Under favorable environmental conditions, the first symptoms (chlorotic patches on leaves) appear 6-7 days after inoculation, while the first uredinia appear 11-14 days after inoculation. In adult plants of susceptible wheat cultivars, the infection of stripe rust spreads longitudinally, forming stripes delimited by leaf veins. In seedlings, the pathogen spreads equidistantly within leaves and is not delimited by leaf veins. Pustules within uredinia are elongated, orange, and can produce thousands of urediniospores for many days until the infected leaf area senesces (Figure 2.2). Urediniospores predominantly infect leaves (usually between veins), leaf sheaths, glumes, and awns[73]. Resistant cultivars have various responses to infection, from the absence of visual symptoms to small hypersensitive spots to

Figure 2.2. Susceptible leaves symptoms

uredinia surrounded by a chlorotic or necrotic zone, all of which eliminate or limit the production of urediniospores[73].

Diversity and distribution

The stripe rust pathogen has a sizeable genetic variability. Researchers have detected DNA polymorphism both among races and between single-spores isolates within a race[74,75]. In the US, there have been over 126 different races of *P. striiformis* f. sp. *tritici* detected[76–78]. Genetic variability in stripe rust is due to mutation, somatic recombination, and probably sexual recombination[79]. Mutation is the primary theory to explain the formation of new races through stepwise evolution[65,80]. A mutation is a random event that occurs in any locus that can increase the number of virulences[81], or diminish virulences[82]. Mutations most likely to remain in the population are those that increase the amount of virulence[65,80]. Frequency of stripe rust race mutation is estimated to be from 1.4×10^{-6} to 4.1×10^{-6} per locus per generation in individual clonal lineages with an assumption of an average 15 generations per year[82]. Somatic recombination is an effective mechanism of stripe rust variation[83] and involves recombinant isolates by nuclear exchange, cell fusion, and/or chromosomal reassortment[84]. Sexual recombination occurs predominantly in the Himalayan region[85], when stripe rust infects the alternative host, and natural and human factors cause spore dispersal worldwide. Its rapid local adaptation via stepwise evolution contributes to the active resistance defeat in new varietal releases[79].

Resistance genes

The significant types of resistance to stripe rust in wheat are seedling (or all-stage) resistance (R) and adult plant resistance (APR) or high-temperature adult-plant resistance (HTAP)[86]. Wheat genes of both R and APR resistance to stripe rust use the designated nomenclature Yr (for yellow rust)[66]. The R genes refer to major gene resistance, seedling resistance, race-specific resistance, and gene-for-gene resistance[87]. Most wheat rust R genes encode proteins (NBS-LRR) that recognize a fungal protein and trigger a defense response that halts or suppresses pathogen reproduction[66,88]. An example of this is *Yr10,* the only cloned Yr seedling resistance gene that encodes a highly evolutionary-conserved and unique CC-NBS-LRR sequence[89]. R genes have less chance of being durable than APR genes due to the specificity of resistance. Adult plant resistance has been durable and therefore should be used in combination with race-specific resistance to prolong their life span[66]. Examples of these genes for stripe rust described in the literature are *Yr18* in chromosome 7D[90], *Yr46* in chromosome 4DL, and *Yr36* in chromosome 6B[91].

To date there are more than 78 permanently named, 67 proposed and/or designated Yr genes, and 327 quantitative trait loci (QTL)[92] for resistance to stripe rust identified in wheat, and some have been widely used in different areas of the world. Among a total of 78 Yr genes, 54 confer seedling resistance and 24 adult plant resistance[93,94].

Stripe rust in the PNW

The PNW has experienced different epidemics of stripe rust. The first epidemic occurred between 1959 to 1961. The losses of yield were estimated to be between 20% to 55%. The most severe epidemics occurred in 1960 in Washington, and 1961 in Oregon and Washington. The second period was from 1974 to 1978. In 1976, yield losses were 17% in Washington, 13% in Oregon, and 11% in Idaho. In 1980 and 1981, stripe rust epidemics were widespread, and yield losses in Washington were estimated to be 13% and 11%, and 5% to 9% in Oregon and Idaho[75]. After these periods, the yield losses caused by stripe rust were reduced due to widely grown resistant cultivars and the use of fungicides[75,95]. During the period 2002 to 2013, disease resistance was lost in many cultivars, with highly severe epidemics. The loss of resistance increased production costs. During this period, the disease became more of a problem in other parts of the United States, including the southeastern region and Midwest, where it had previously been infrequent or absent[96,97]. The year 2010 was the most extreme registered for the USA, with an estimated yield loss of over 10%[98]. Stripe rust disease continued to be severe in the PNW, equaling 1981 losses during 2011; in contrast, it was not extreme in the eastern Rocky Mountains region due to drought conditions[99].

In the last 15 years, new races of stripe rust appeared in the USA due to the accelerated evolution of the pathogen[99]. Simultaneously, the stripe rust population has increased in aggressiveness and adaptation to both high and low temperatures, reducing the effectiveness of disease resistance[100,101]. In

2010, races of stripe rust drastically changed virulence, having new virulence on Yr genes such as 27, 43, 44, 7, Exp2, Sp, Tr1, and Tye, reducing virulence on Yr gene such as 11, 16, 18, 19, 20, 3, and maintaining virulence on Yr genes such as 1, 10, 17, 8, 9 (Figure 2.4). These changes resulted in new races, such as PSTv-11, PSTv-14, PSTv-41, and PSTv-37[102] (Figure 2.3). The most dominant race since 2016 has been PSTv-37 with PSTv-39 starting to increase in frequency (Figure 2.3). In all these years, the winter wheat cultivar Madsen has remained highly resistant to stripe rust, as well as cultivars derived from Madsen, such as 'Cara' (PI 643435)[103] and 'ARS-Selbu' (PI 667744)[104]. Other cultivars have lost their resistance during this time, such as 'Eltan' (PI 536994)[105] and 'Xerpha' (PI 645605)[106] due to the appearance of new races in the PNW[107,108].

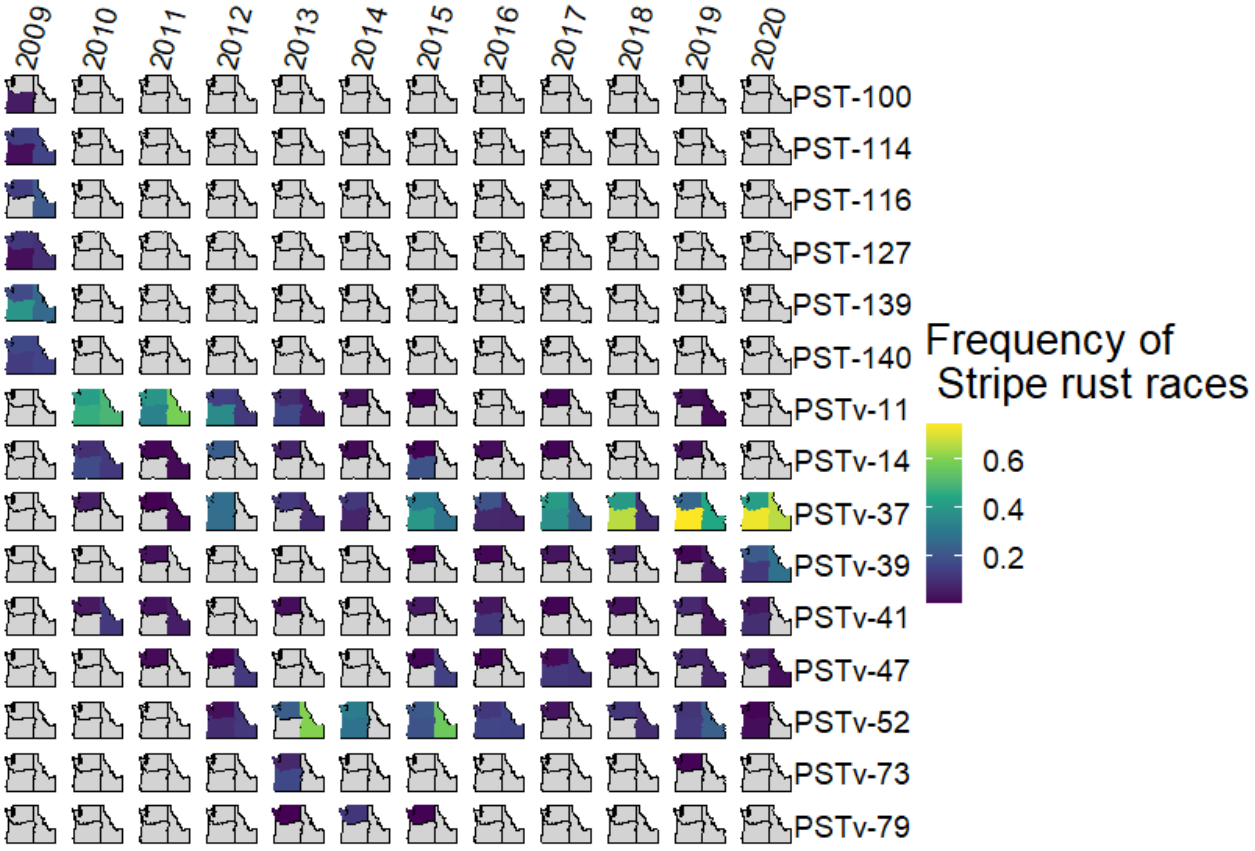

Figure 2.3. The most frequent races of stripe rust distributed in the PNW between 2009 to 2020, axis-x is the years, and axis-y is the races name, source: https://striperust.wsu.edu/races/data/.

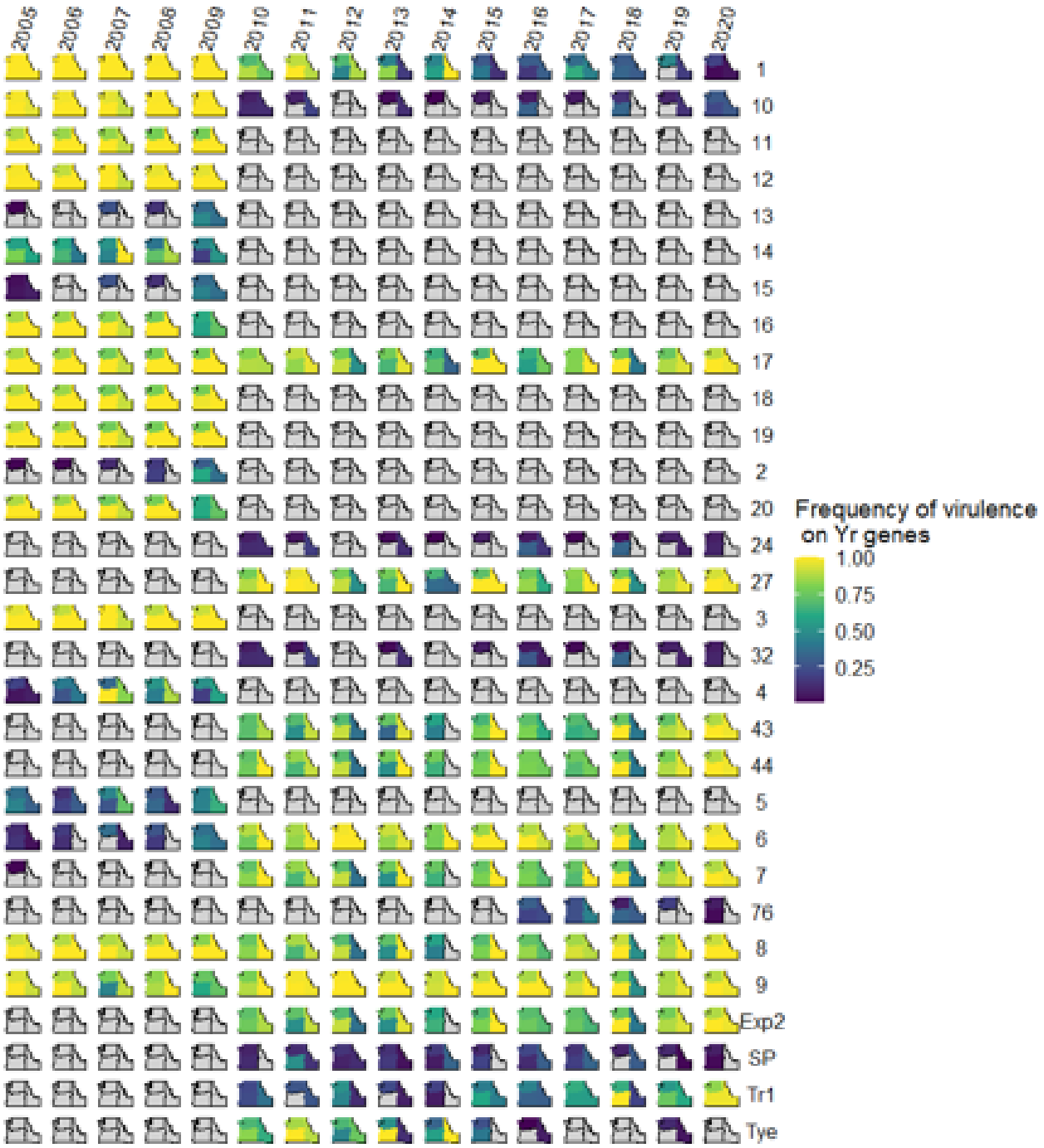

Figure 2.4. Frequency of virulence on Yr genes in PNW between 2005 to 2020, in the x axis is the years, and y axis are the different virulence on Yr genes. source: https://striperust.wsu.edu/races/data/

Septoria diseases

Economic impact

Septoria tritici blotch (STB) (*Zymoseptoria tritici*) can have a significant economic impact by decreasing yield by 10 to 50% (depending on the region and yearly disease pressure) and by reducing grain quality [109]. Agronomic practices to control STB, such as late seeding, growing resistant cultivars,[110] and biocontrol with bacteria such as *Bacillius megaterium* are not as prevalent as frequent fungicide application[111]. *Z. tritici* populations have evolved high levels of resistance to frequently used fungicides, such as demethylation inhibitor (DMI) and quinone outside inhibitor (QoI) fungicides[112]. Breeding for resistance is the most cost-effective and environmentally friendly approach to control STB[113,114].

Cycle, host, and symptoms

Septoria tritici blotch (STB) is caused by the ascomycete fungus *Zymoseptoria tritici* (formerly *Mycosphaerella graminicola*), is a foliar blotch disease and is considered one of the most devastating blotch diseases of wheat in the world[115]. *Z. tritici* has a heterothallic bipolar mating system with alleles *mat1-1* and *mat1-2*[116]. The sexual fruiting bodies (pseudothecia) contribute to the primary inoculum via air-borne ascospores. The asexual pycnidia release pycnidiospores that infect the wheat during the growing season are splash-dispersed. *Z. tritici* has two distinct phases of infection: the initial biotrophic phase, which occurs on the leaf surface where spores germinate under low temperature, humid conditions, and the final phase, which occurs between 7 to 14 days after infection. During the biotrophic phase, the ascospores and conidia produce hyphae that penetrate the leaf through stomata to access the apoplast[117,118], without damage, defense symptoms, or with feeble defense response[119–121]. The genetic factors that trigger the switch from a biotrophic state to a necrotrophic state are still unidentified[122]. The necrotrophic phase characterizes the final infection phase 7-14 days after infection. *Z. tritici* probably uses several mechanisms to induce necrosis, including the production of protein effectors[120,121,123–125]. During this phase, the host shows the first symptoms, chlorotic lesions, that eventually coalesce into larger necrotic blotches (Figure 2.6). The pathogen feeds on nutrients from the dying host tissue and produces asexual and sexual fruiting bodies[117,126,127]. At the end of the season, the pathogen produces pseudothecia to survive during the summer and fall until a new crop is planted and rains induce ascospore release (Figure 2.6).

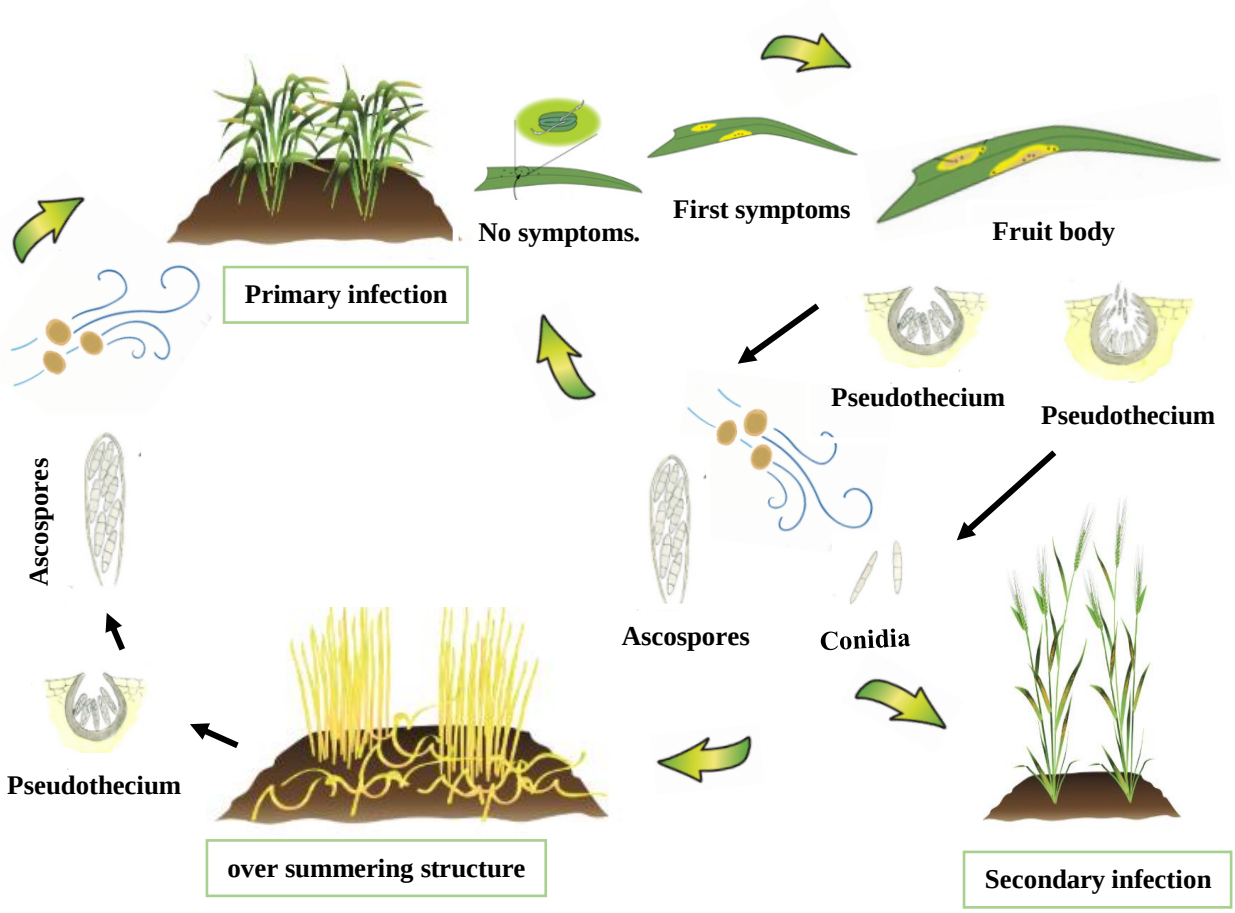

Figure 2.5 Septoria tritici blotch cycle

Figure 2.6 Septoria tritici blotch symptoms

Diversity and distribution

The *Z. tritici* population has high genotypic diversity and low clonality[122,128,129] with alleles in gametic equilibrium[130] and mating types at equal frequency[131]. A typical infection of 1 cm can contain two to six strains of pathogens, competing strongly for resources[128,132]. Asexual clones can increase the

virulence alleles and then be dispersed over long distances via airborne ascospores due to a strong selection either by the variability of the host or environment[133,134]. Virulence genes can move between 200 to 300 km every three growing seasons base on the stepping stone model of gene flow[135].

Resistance genes for STB

Twenty-six resistance genes to STB have been identified and genetically mapped as well as several quantitative trait loci (QTL) [129,136–151], including additive and epistatic effect genes[136,144]. Some studies confirm the specific interaction between bread wheat cultivars and STB isolates, such as: IN95-Lafayette-1196-WW-1-4 avirulent for Stb1[150] and Stv4[149], IPO323 avirulent Stb6[138], Paskeville avirulent for Stb2 and Stb3[152], IPO94269 avirulent Stb10[148], IPO89011 avirulent Stb9[147], MG2 avirulent Stb7[153] Stb13 and Stb14[143]. The first and the only gene cloned for STB resistance is *Stb6,* which encodes a wall-associated receptor kinase (WAK)-like protein[154]. Known individual Stb genes are not currently effective against *Z. tritici*. The efficacy of R genes is limited because of the versatility of STB, its active sexual cycle, and the strong selection favoring genes that can overcome adverse environmental (fungicides resistance) or biological (host resistance) factors during the asexual cycle.

2.4 Immune response for diseases.

The plant immune system consists of multiple layers of surveillance systems capable of recognizing pathogen effectors and has a rapid response to avoid causing severe damage[155]. The first defensive line is basal resistance, also called innate immunity or pathogen-associated molecular patterns (PAMP)-triggered immunity (PTI)[155]. This system consists of plant cells that identify microbe-associated molecular patterns (MAMPs) or pathogen-associated molecular patterns (PAMPs) by using toll-like receptors (TLRs), and pattern recognition receptors (PRRs) present on the surface of the plant cell. The plant's PTI response generates reactive oxygen species (ROS), for example, superoxide anion (O_2^-) or hydrogen peroxide (H_2O_2), after the plant detects MAMPs. Pathogens secrete proteins called effectors as countermeasures to suppress basal resistance. Pathogen effectors can suppress PAMPs, triggering susceptibility in the host; this process is called effector-activated susceptibility (ETS)[156]. However, when the plant identifies these effectors, either directly or indirectly, the effector-activated immunity (ETI) or the second level of defense, is triggered. The second line of defense is activated only after earlier localized exposure to a pathogen. There are two types of this response: hypersensitive response (HR) and systematic acquired resistance (SAR)[157]. Resistance genes limit the pathogen's access to water and nutrients by sacrificing plant cells by HR at the infection site to save the rest of the plant. SAR is an enhanced resistance that helps to initiate a quick and effective response involving the whole plant[157].

Resistance genes regulate the plant immune response. Resistance genes are a source of full or partially heritable resistance. There are two types of resistance genes: qualitative resistance genes (R

genes), generally major genes of large effect, and quantitative resistance genes (APR), which generally depend on several-to-many genes with moderate-to-small effects[158]. R genes are detected in seedling tests and remain effective throughout all growth stages. This kind of major gene resistance is generally not durable due to mutations in the pathogen, so durable resistance depends on the deployment of multiple resistance gene combinations, either inhomogeneous pure line cultivars[75,95], multi-line cultivars[159], or cultivar blends[160]. APR can express resistance in all stages of the plant or can express susceptible infection types in the seedling stage then develops varying levels of resistance in post-seedling stages in the field. Thus, they provide a non-complete level of resistance/immunity expressed only in the adult plant and in all stage, (except under particular conditions). It is characterized by a delay in infection and spore production. APR resistance has been durable and, therefore should be used in combination with race-specific resistance to prolong the effectiveness of R genes[66,75]. These genes are involved in general plant physiology and encode resistance allele-specific protein variants [66,161] that can recognize specific pathogen proteins[85].

R genes recognize pathogen effectors or damage, directly or indirectly, on the cell surface or inside the cell and activate the plant's 'immune system', playing an essential role in the gene-for-gene (GFG) interaction between pathogen and host. R genes are classified based on recognizing the effectors or damage, i.e., on the cell surface or inside the cell[162].

R genes on the cell surfaces are typically patterned recognition receptors (PRRs): receptor-like kinases (RLK) or receptor-like proteins (RLPs)[163]. RLK represents an extracellular domain, a transmembrane domain, and an intracellular kinase domain. The receptor-like proteins (RLPs) lack intracellular signaling domains and are made of ecto-ligand binding and transmembrane domains[164–166]. Both recognize PAMPs and host-derived DAMPs (Damage-Associated Molecular Patterns) during the infection directly (recognition of non-self) or indirectly (triggered Immunity or PTI). The wall-associated receptor kinase (WAK)-like protein is one type of RLKs that detect DAMPs that result from cellular damage during the initial infection. Five types of PRRs have been identified in wheat. They are: (1) the S-domain-type RK TaLRK10 that provides resistance to *P. triticina*[167], (2) TaLRRK-6D which provides resistance to *F. graminearum*[168], (3) LRR-RLP TaRLP1.1 and cysteine-rich domain-type RKs TaRLK-R1–3 that provide resistance to (*P. striiformis f. sp. tritici*[169], (4) the TaWAKL4 that provides resistance to STB (*Z. tritici*)[170], and (5) the NbBAK1 and NbSOBIR1 induced cell death in *Z. tritici* effector recognition[171].

The intracellular R genes typically encode proteins with a nucleotide-binding domain (NB) and leucine-rich repeats (LRR)[172]. The intracellular R genes (NLR) are the most common resistance genes and belong to the most extensive and most widespread group of resistance genes These genes can be subdivided based on the presence of the Toll-like/interleukin 1 domain (TIR-type-NLR) and coiled-coil

(CC-NB-LRR)[172] at the N-terminus. Many NLR genes are in the extra-pericentromeric regions[173] of the chromosome, which has high chromosomal duplications, recombination, and deletion rates. However, the wheat NLRs are found in the distal regions of the chromosomes, near the telomeres, and are close to each other[174,175]. The NLR function recognizes the effector and activates resistance responses such as hypersensitive response (HR), generating reactive oxygen species (ROS), or systematic acquired resistance (SAR). The NLRs are activated by the presence of a pathogen either binding to pathogen effectors[176], by sensing effector-induced modification of other host proteins (modified guardee)[177,178], by modification of target-mimicking decoy protein (modified decoy)[179], by modifying the NLR (integrated decoy)[180–182], or by the WRKY proteins that are a type of integrated decoy[183,184]. Over 2000 NB-LRR encoding genes have been reported in wheat, which is the largest number reported thus far in any plant speicies[185]. However, only 19 R genes (NLR) have been cloned for rust and powdery mildew resistance[88,186–196]. Wheat and its relatives have a rare, unique domain, ANK-NLR-WRKY, that also provides R genes[197].

2.5 Wheat Breeding

History of breeding

Breeding has been practiced since the domestication of wheat[198]. In the early centuries, farmers selected different traits of interest controlled by genes without knowledge of dominance, segregation, and heritability. Since Mendel's laws, plant breeding has become more sophisticated, utilizing the selection of pollen donors and artificial pollination [199]. These techniques are still being using for most cultivated crops. Artificial pollination creates diversity to improve traits such as disease yield, disease resistance, quality, morphology, and architecture.

The second significant advance in plant breeding was the discovery of rapid DNA sequencing invented by Frederik Sanger in 1977[200,201]. This allowed breeders to employ molecular markers to improve cultivars more rapidly[202,203]. Marker-assisted selection (MAS) is the strategy that involves the identification of the presence of gene or genes associated with a trait of interest through using molecular markers,[204] which are a sequence of DNA associated (linked) with this particular trait[205]. This technique can enhance the efficiency of breeding programs by identifying and quantifying genetic variation. It is considered very useful for low heritability traits, genes that are expressed in adult plants, and traits that are expensive to measure. In addition, molecular markers can also be used for plant variety protection, such as uniformity, distinctness, and stability testing processes[205].

Breeding for resistance

Wheat breeding programs aim to improve yield and disease resistance while maintaining quality for a specific end-use. Breeding for disease resistance in wheat requires a resistance source to start the

breeding program. Resistance genes can be provided by elite cultivars, traditional cultivars, or wheat relatives, either wild or crop forms. Recently, new technologies such as gene editing using CRISPR-Cas9 may also be used to induce disease resistance[205].

Developing elite resistant cultivars can be accomplished by classical breeding, molecular plant breeding, or a combination of both. The common methods in classical resistance breeding are backcross breeding, recurrent selection, and multi-stage selection. The goal of backcrossing (BC) is to transfer an R gene from distantly related sources, such as wheat relatives or landraces, to an elite cultivar through crosses between the elite cultivar and the resistance donor, followed by a series of crosses back to the elite cultivar as the recurrent parent. Selection of the resistant phenotype is required in each backcross generation. If the resistance is recessive, a selfing generation would be needed to select for resistance. On the other hand, if a molecular marker exists for the resistance gene, genotypic selection can be done and the selfing generation would not be required. The number of backcross generations needed depends on the genetic difference between the donor and the recurrent parent. Recurrent selection (RS) consists of developing various repeated cycles of recombination and selection without losing genetic diversity. In wheat, this method requires crosses and additional selfing steps to increase the additive effect. The main benefits are being able to test in multiple locations and improve multiple traits at once. However, this method is not efficient when the agronomic traits negatively impact quantitative resistance, such as the *Rht-D1* dwarfing locus having a negative effect on transfer of resistance to Fusarium head blight[205]. The multi-stage selection consists of a continuous selection process with multiple successive resistances in a single generation. The selection in each step depends on how the resistance is inherited. Using doubled-haploids (DH) allows starting the selection in the F_1 generation. The main advantages of the multi-stage selection approach are higher selection intensity, accuracy, and saving time. However, this method has high costs and only has one round of recombination[206].

Molecular plant breeding is the application of molecular biology or biotechnology to improve or develop new cultivars[207]. There are two major strategies, marker-assisted selection and genomic selection. Marker-assisted selection is the selection of genotypes based on a marker linked to a trait of interest. Marker-assisted selection can be applied in all the classical breeding methods increasing efficiency. Genomic selection (GS) uses all the genome markers available to create breeding values to improve selection efficiency. This method uses a training population with phenotypic and genotypic data to develop a model to predict performance based on marker data without phenotyping[208]. Both ways require new sequence technology such as genotyping-by-sequencing (GBS).

Molecular plant breeding technology

Over the past 20 years, new genome sequencing technologies have become more economical and progressively more efficient, increasing precision and sequencing speed[209]. This has allowed for a more

robust analysis of numerous plant and pathogen genomes. There are two sequencing approaches to identify inter-individual variation at a genome-scale. When the species possess small genomes, like fungi, whole-genome sequencing can be used. However, it is too expensive to generate whole-genome sequence data for species with large genomes, such as wheat. An alternative to whole-genome sequencing is to reduce genomic complexity by only sequencing a portion of the genome; this approach includes transcriptome sequencing, exome capture, genotyping-by-sequencing[209].

The transcriptome sequence or RNA sequence identifies and quantifies different expression levels of genes in particular conditions, allowing associated the genes with the specific trait[210]. The exome capture technique consists of the sequence of the exotic region of the genome. This technique consists of two phases, the first phase identifies the DNA encodes protein by probe hybridization, and the second is a high throughput DNA sequencing[211]. Genotyping-by-sequencing (GBS) is a technique that consists of construction of GBS libraries using restriction enzymes to reduce the genome complexity, and sequencing with the Illumina next-generation sequencing (NGS) platform[212]. GBS is characterized by low cost, efficient barcoding, and ease for calling up for PCR and purification steps[213]. In addition, Genotyping-by-sequencing generates multiple single nucleotide polymorphism markers (SNPs)[214] allowing efficient studies such as genome-wide association studies (GWAS) and/or quantitative trait loci analysis (QTL)[215].

Quantitative trait loci (QTL) analysis is a method to identify molecular markers associated with resistance genes. Quantitative trait loci (QTL) are a genome region associated with a specific trait linked to a particular polymorphic marker like SNPs identified by GBS. QTL analyses require a segregating population such as $F_{2,}$ backcross populations (BC), doubled haploids (DH), or recombinant inbred lines (RIL) and their and their phenotypic and genotypic data. Statistical methods include single marker analysis (SMA), interval mapping (IM), composite interval mapping (CIM), and multiple interval mapping (MIM) to identify QTL[216].

Single marker analysis (SMA) is the simplest method for QTL mapping. It analyzes individual markers and splits the population into groups according to the individuals' genotypes. Interval mapping (IM) uses intervals between each pair of markers, exploring for QTL between them[217]. Composite interval mapping (CIM) is a method that attempts to separate and isolate individual QTL effects by combining interval mapping with multiple regression analysis. The CIM aims to reduce residual variation and enhance QTL detection by considering markers near to putative QTL as covariates. This method identifies or discards putative QTL using forward or backward stepwise regression in combination with single interval mapping[218]. Multiple interval mapping (MIM) extends the methods for single interval mapping to develop a search for pairs of QTLs at a time[218].

Phenotyping disease

Phenotyping is essential to identify molecular markers associated with any trait in a crop and disease resistance is no exception. Each plant can show a range of symptoms or different resistance reactions during pathogenesis, such as systematic acquired resistance (SAR) or hypersensitive response (HR)[157]. Currently, the most used method to quantify the percent severity is based on human visual observations. Alternative visual methods include calculating the percentage of disease severity according to the modified Cobb scale[219], calculating the area under the disease progress curve (AUDPC)[220], or quantification using diagrammatic scales[220]. All these methods are labor-intensive, subjective, and have accuracy limitations. In recent years, researchers have studied different objective methods to quantify disease severity, by imaging technologies such as fluorescence imaging, hyperspectral imaging[221,222], multispectral imagery, and leaf color analysis through digital photography[223,224]. Fluorescence imaging, hyperspectral imaging[221,222], multispectral is based on the theory that healthy plants and infected plants differ in their ability to absorb, reflect, emit, transmit or fluorescent light. All use different visible spectrums to detect the severity of the pathogen. Fluorescence images use an overall average measurement of light quality in visible wavebands for individual pixels, multispectral images use between 3 and 10 different electromagnetic spectrum bands for each pixel, and hyperspectral image measures energy in narrower bands and uses 200 or more contiguous spectral bands. All three methods use the information of spectral bands to distinguish between diseases and healthy tissue[225]. An alternative approach is to analyze leaf color through digital photography, using the intensity of different colors to differentiate between disease area and leaf area.

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Chapter 3 Identifying Molecular Markers for Resistance to Septoria tritici Blotch

Chapter3: Identifying Molecular Markers for Resistance to Septoria tritici Blotch

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3.1 Abstract

Septoria tritici blotch (STB) is a foliar disease of wheat (*Triticum aestivum* L.) caused by the ascomycete fungus *Zymoseptoria tritici*. STB is a polycyclic disease and represents a significant threat to wheat production. High disease pressure can reduce the economic value of wheat by decreasing yield and grain quality. The control of STB relies primarily on the use of fungicides. Unfortunately, *Z. tritici* populations have evolved high resistance levels to some of these fungicides, causing them to lose their efficacy. Increasing STB resistance through plant breeding is the most cost-effective and environmentally friendly method for control. A recombinant inbred line (RIL) population was developed from a cross between 'Madsen' and 'Foote' soft white winter wheat cultivars to study STB resistance. Foote (PI 599663) has provided moderate resistance to STB in the Pacific Northwest (PNW), while Madsen (PI 511673) is moderately susceptible to STB. The RIL population, consisting of 217 lines, was phenotype across multiple environments for STB response and genotyped using Illumina HiSeq 3000 Sequencing. The STACKS program was used to select SNPs. Analysis of variance showed significant differences among phenotypes and genotypes in the RIL population ($p<0.01$). The best linear unbiased prediction (BLUP) value for each accession across different environments for STB severity was used for QTL mapping. Results of quantitative trait loci (QTL) analysis indicated minor genes in 4B, 5A, 6B, 6D, and 7DS. Stacking these QTL in one cultivar can reduce the severity by over 70% compared to not using any of these QTL. These QTL could develop breeder-friendly molecular markers for genotypic selection for improved STB resistance in wheat in the Pacific Northwest.

3.2 Introduction

Septoria tritici blotch (STB), caused by the ascomycete fungus *Zymoseptoria tritici*, is a devastating foliar disease of hexaploid wheat (*Triticum aestivum* L.), especially in humid and temperate areas. It has a significant economic impact by decreasing grain quality and crop yield by 10 to 50%, depending on the region and disease pressure[1]. The regions of the world where STB has become the most significant challenge in wheat production are the Pacific Northwest and the Northern Great Plains of the United States, Central, and Western Asia, and Europe[2]. *Zymoseptoria tritici* is a polycyclic pathogen, with

two types of spores, ascospores, and pycnidiospores. Ascospores are sexual spores driven by a heterothallic bipolar mating[3] system, and pycnidiospores are asexual spores. Both spores can contribute to the primary inoculum and the propagation of STB through the wheat growing season[4]. Infection is mainly initiated in the fall, after seedlings emerge, by air-borne ascospores from wheat debris the prior year, either from the same field or other fields within the region[5]. Septoria tritici blotch has two distinct phases, biotrophic and necrotrophic. The biotrophic phase starts when spores infect leaves through stomata and is characterized by an extended symptomless infection. The host shows the first symptoms during the necrotrophic phase, via small necrotic spots that expand into necrotic blotches on leaves[6]. The *Z. tritici* population in a wheat field is characterized by high genotypic diversity and low clonality[7–9]. A single wheat field contains about 92% of the global genetic STB diversity for neutral markers[7,10]. The variation in allelic frequency occurs by a strong selection of asexual clones[11,12] or by the gene flow from another field[13]. Management of STB relies primarily on fungicides, using between two to four applications per year. In Europe, the fungicide use for STB represents 70% of the annual use of fungicide in wheat and has a cost of approximately $1.2 bn/year[14]. In recent years, the global *Z. tritici* population has evolved high levels of resistance to some fungicides, such as quinone outside Inhibitors (Qols)[15]demethylation inhibitors (DMI)[16], and some resistance to the succinate dehydrogenase inhibitors (SDHIs) was recently reported in the UK [17]. One approach to reducing the use of fungicides to control STB is increasing host plant resistance to STB through plant breeding. The use of host plant resistance is not only an environmentally friendly approach to reduce the impact of STB on wheat production. It is also the most cost-effective way to control STB.

Plant resistance genes (PRGs) can be grouped into two types, qualitative and quantitative. Qualitative resistance genes are characterized by host genotype-pathogen genotype specificity and generally depend on a major gene with a large effect. Quantitative resistance genes are usually characterized by non-specific interactions, which generally depend on multiple genes with moderate-to-small effects. PRGs can regulate the resistance response either independent of the plant growth stage or ontogenetically. In wheat, 26 qualitative resistance genes against STB and several quantitative trait loci (QTL) have been identified and genetically mapped [9,18–33], showing additive and epistatic effects[18,26]. The first PRGs mapped against STB were *Stb1, Stb2,* and *Stb3*[34], mapped on chromosomes 5BL[32], 1BS[9], and 7AS[21], respectively, and were described as qualitative resistance genes. *Stb6* and *Stb15* are the most common STB genes in current European wheat germplasm[26]. A gene-for-gene relationship has only been demonstrated for *Stb6*[20]. *Stb6* encodes a wall-associated receptor kinase (WAK)-like protein and is the only PRG cloned for *Z. tritici*[35]. Each of these genes is useful against only one or a few isolates of STB but is not effective against current *Z. tritici* populations in the field. The limitation of these genes is due to the high genetic diversity of the pathogen, its active sexual cycle, large effective population size of *Z.*

tritici, and strong selection favoring genes that can overcome fungicides [36,37] or host resistance[38,39] during the asexual cycle. For example, the soft white winter wheat cultivar `Gene´, which has *Stb6* and *Stb10*[40], became highly susceptible to STB within 5 years of its release even when occupying only about 15% of the Willamette Valley, Oregon wheat-growing area[41]. It is not known which of the genes was overcome by the fungus, however. It is known that the soft white winter wheat cultivar, 'Foote', released in Oregon in 1998, remained moderately resistant to STB in Oregon[42] when it was abandoned due to susceptibility to stripe rust in 2004/05. In addition, there have been genes that have had lasting resistance, such as *Stb1,* which originated from the wheat cultivar Bulgari 88, but this cultivar has recently become ineffective against 10 different isolates[43]. *Stb4* was effective in California for more than 15 years until 2004[31], when resistance broke down. *Stb16* is considered the most promising durable resistance gene, providing resistance to more than 20 different *Z. tritici* isolates around the world[18], and maintains resistance against STB populations from Mexico and Uruguay[44]. Nonetheless, cultivar 'M3' carrying *Stb16* and *Stb17* was susceptible to four of 10 Iranian *Z. tritici* isolates[45].

Resistance to STB can also be quantitative. The chromosome regions with relevant quantitative trait loci for resistance against STB are 3BL, 6BS, and 7DL. At least one QTL or meta-QTL has been identified on all chromosomes, except 5D[46]. Additionally, wheat has PRGs with quantitative resistance to multiple diseases in addition to STB. Examples include *TaXA21* located in 5AL, which provides resistance to different stripe rust, powdery mildew, and Hessian fly biotypes BP[47], and *Tsn1* located in 5BL that provides susceptibility to necrotrophic pathogens such as *Stagonospora nodorum* and *Pyrenophora tritici-repentis*[48].

The STB population undergoes annual sexual reproduction and maintains a substantial effective population. Therefore, studying multiple environments can help us cover the evolution of the pathogen and increase the possibility of identifying durable resistance genes. Furthermore, identify the genes involved in resistance in the STS population. The Pacific Northwest is a perfect laboratory field to study STB due to its conditions like cool temperature, frequent rainfall, and high humidity. In addition, the STB is present yearly, with an effective substantial population in the PNW.

Resistance to STB can be confounded by factors such as plant height and flowering time. Plant height can influence the amount of inoculum splashed upwards as plants grow, increasing disease severity in shorter and earlier heading cultivars[49]. Heading dates are less relevant than plant height when cultivars were evaluated for STB at the same development stage under similar weather conditions[50]. Identified PRGs with non-specific resistance might improve the durability of resistance via marker-assisted selection (MAS).

The overall objective of this project is to identify QTL associated with STB resistance in a recombinant inbred line (RIL) population in the state of Oregon in the Pacific Northwest of the United States.

Specific objectives were:

1. Determine if plant height and flowering time are confounding factors influencing STB severity in a Foote by Madsen (FxM) RIL population.
2. Identify the QTL for adult plant resistance to STB in the Foote x Madsen RIL population-based for each five and a cross the environments.
3. Select a genomic model to predict STB severity.
4. Determine if markers for *Stb1* and *Stb6* contribute to resistance in the FxM population or are correlated with any QTL detected in the QTL analysis.

3.3 Materials and methods

Mapping population

The segregating population was a recombinant inbred line (RIL) F_7-F_9population, developed from the cross between the soft white winter wheat cultivars 'Foote' (PI 599663) and 'Madsen' (PI 511673)[51], followed by single seed descent. This population consisted of 216 RILs and was developed at Oregon State University (OSU). Foote was released by OSU in 2000 with the pedigree "Heima/Kalyansona/Bluebird/3/WWP7147, F1/4/Davis 6301/Heine VII/era/3/Buckbuck" and provided moderate resistance to STB in the Pacific Northwest (PNW) but was abandoned from production in the mid-2000s due to susceptibility to new stripe rust races. The wheat cultivar Madsen, with the pedigree "VPM1/Moisson 951//2*Hill 81", was developed by the USDA-ARS wheat genetics program in Pullman, WA. It is known to carry *Pch1* for *Pseudocercosporella herpotrichoides* foot rot resistance and *Yr17* for stripe rust (*Puccinia striiformis* f. sp. *Tritici*). Madsen was moderately resistant to STB when first released in 1988 but became moderately susceptible over a ten-year period[52].

Genotypic data

DNA extraction and sequencing

Parents and offspring were grown under greenhouse conditions, at 21 to 23 °C, a photoperiod of 16:8 hours (Light: Dark), avoiding pathogen or insect infection. Genomic DNA of parents and their RIL (F_6-F_7) progeny were extracted from seedlings at the three-leaf stage, with an automated extraction system (Thermo KingFisher Flex, Waltham, MA) at the Center of Genomic Research and Biotechnology (CGRB) at Oregon State University. Genotyping by sequencing (GBS) libraries were developed following a two-enzyme PstI (CTGCAG) and MspI (CCGG) restriction digestion GBS protocol described in Poland et

al.[53]. Barcode adapters were ligated to unique individual samples by (5 a 10 bp) based on oligonucleotides listed by Elshire et al.[54] and solely compatible with the two digested fragments (PstI and MspI). Four libraries were prepared and sequenced with an Illumina HiSeq 3000 (Illumina HiSeq 3000/HiSeq 4000 System, RRID:SCR_016386, Illumina, San Diego, CA, USA) at the Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR, USA.

SNPs data

The SNP calling was done using the STACKS v2.52 software packages.[55] All samples for each library for each enzyme were quality filtered, demultiplexed, and trimmed, using *process radtags*[55], with the rescue barcodes option (-r) and automatic clean data (-c), removing any reads with an uncalled base. The RIL population and both parents were identified from the raw data using the nucleotides barcodes. Both files from each enzyme were concatenated by sample. The raw reads for each library were aligned to the wheat indexed reference genome[56], using BWA[57] and sort and variants detection by SAMTOOLS[58]. The wheat reference genome (IWGSC RefSeq v1)[59] was previously indexed using BWA tools. SNPs for all populations were identified and genotyped at each locus and in each sample, gstacks STACKS v2.52 software packages[55]. The population program from gstacks was used to obtain the output files such as the locus consensus sequences in FASTA format, the SNPs, haplotypes in Variant Call Format (VCF), and the genotypes in PLINK format. The SNPs were filtered out by 20% of missing data, minor allele frequency MAS>5% missing rate per SNP (including SNPs with 80% genotype rate), using PLINK software[60].

Linkage map construction group

A linkage map was constructed using SNP data from GBS (GBS-SNP). Loci that were completely linked, i.e., displayed no segregation, were excluded from the dataset. Polymorphic markers were classified into two categories according to their segregation pattern. A test to detect markers with segregation distortion was performed. For each locus, the pattern of allelic segregation was tested using a χ^2 goodness-of-fit to fit test where expected frequencies responded to Mendelian segregation ratios.

Linkage group analysis was performed using Join Map v4.0[61] with the parameters set for a RIL population. Initial assignment to linkage groups was based on a logarithm of the odds (LOD) threshold of 6.0. We used a linkage with recombination rate (REC) <0.5 and a map LOD value of five for inclusion into the map and to calculate the linear order of the markers within a linkage group. Certain singletons were removed with the statistical method SMOOTH[62] and reorder the markers with the RECORD software[63].

Molecular markers

The RILs and their parents were genotyped using genetic markers associated with photoperiod(*Ppd-D1*)[64], vernalization https://maswheat.ucdavis.edu/protocols), height (*Rht-B* and *Rht-D1*)[65], and for two candidate resistance genes of Septoria tritici (*Stb1* and *Stb*6)[66,67].

Phenotypic data

The RIL population for the Foote × Madsen RIL population, along with its parents, was planted for evaluation of STB severity in five environments, which were combinations of three years (2016, 2018 and 2019) and two locations. Locations were the OSU Botany and Plant Pathology Field Laboratory (45.72 N, 122.54 W), Corvallis OR in 2016 and 2019, and the other three at the OSU Hyslop Crop Science Field Research Laboratory (44.63 N, 123.19 W), Corvallis OR in 2016, 2018, and 2019. Each trial is labeled by BF for trials located at the OSU Botany and Plant Pathology Field Laboratory or HF for trail located at the OSU Hyslop Crop Science Field Research Laboratory Farm, followed by the year. The experiments were in a randomized complete block design (RCBD) for each environment, with two replications. Each plot was 3 rows and 2m long. The parents were included twice in each replication.

Plots were treated with a strobilurin fungicide four times during late winter/spring to allow a reliable scoring of STB severity in the absence of stripe rust. The Willamette Valley population of *Z. tritici*. is now highly resistant to strobilurin fungicides[68], but the stripe rust population is still susceptible to strobilurin fungicides.

Ample natural STB infection occurred in all experiments. Two disease readings were taken in each field trial. The first reading was between stage 10.5 flowering wheat and stage 11 of the Feekes growth scale[69], and the second reading occurred two to three weeks later. STB severity was determined for each plot by estimating the percentage of the total leaf area that was covered by STB lesions, including all leaves on the plant.

Measurements for plant height and heading date were taken each year of the experiment. Plant height (ht) was measured for each plot as the distance from the ground to the spikes' top, excluding the awns. The heading date(hd) was scored for each plot using Julian days when approximately 50% of the spikes of each plot was emerged (above the collar of the flag leaf).

Statistical analysis

The Severity of STB.

All phenotypic data were analyzed using R software version 3.63[70]. Each combination between year and location was treated as an individual data set and analyzed separately using lme4 packages from R software version 3.63[70]. The model for each combination of year and location for STB was:

$$y_{ijk} = \mu + RIL_i + \text{Env}_j + e_{ijk}\ , \quad (1)$$

Where y_{ijk} was the severity response for STB for each RIL i and each environment (rep) j; mu (μ) is the population mean for the severity response (STB). RIL and Environments (rep) were treatments as random effects with zero mean and variance ($\sigma^2_{RIL}, \sigma^2_{env}$). Model errors were assumed to follow a normal distribution with zero mean and variance, i.e., $e_{ijk} \sim N(0, \sigma^2_{err})$. The likelihood ratio was calculated for each random effect, using the rand option in the lme4 packages from R package statistical[70]. This model was used to calculate heritability for each experiment, $h^2 = \sigma^2_g/\sigma^2_p = \sigma^2_g/(\sigma^2_g + \frac{\sigma^2 e}{r})$, where the RIL and rep were fitted as a random effect, using lme4 packages from R package statistical[70]. The variance components of the model are σ^2_g, genetic variance, and σ^2_e residual variance. From these two components, the phenotypic variance was calculated as $\sigma^2_p = \sigma^2_g + \frac{\sigma^2_e}{r}$ where r is the number of replications[71]. Phenotypic correlations were calculated to understand the concordance of STB scores measured at different time points and different years. Then, heading date and plant height were tested for the significant regression in the severity model.

General model

The general model for genotypes, environments, their interaction for STB severity was:

$$y_{ijk} = \mu + \text{u}_\text{i} + \text{u}_\text{j} + u_{ij} + e_{ijk}\ , \quad (2)$$

where y_{ijk} is the observed response for the k^{th} element of RIL i in environment (location x environment) j, mu is the population mean for the trait, and $\text{u}_\text{i} + \text{u}_\text{j}\ and\ u_{ij}$ are RIL, Environment, and RILxEnvironment random effects, and e_{ijk} is the unexplained model error. Model errors were assumed to follow a normal distribution with zero mean and variance, i.e., $e_{ijk} \sim N(0, \sigma^2_{err})$ and random effects were assumed to be independent of the model errors and normally distributed with zero means and variances σ^2_{Gen}, $\sigma^2_{Env}$and $\sigma^2_{Gen*Env}$. It was further assumed that $\text{u}_\text{i} + \text{u}_\text{j}\ and\ u_{ij}$ were mutually independent, i.e., $cov(u_i, u_j) = 0$. Variance components were determined and the significance of variance component

estimates were calculated by the restricted maximum likelihood, using lme4 packages from R package statistical[70]. Then, the plant heading date and plant height were added in the general model to test for significance of effects.

BLUPs

Best linear unbiased predictors (BLUPs) for STB for each RIL, Foote, and Madsen were determined across all the combinations of location/year using lme4 packages from R package statistical[70]. The model was:

$$y_{ij} = \mu + g_i + lg_j + e_{ij}\,, \tag{3}$$

where y_{ij} represents the estimated BLUPs of STB severity for each RIL line, μ is the overall mean, g_i and ly_j are the random effects of the i_{th}RIL and j_{th} location by year, respectively, and e_{ij} is the unexplained model error. It was assumed that g_i and lg_j were mutually independent, i.e., $cov(g_i, lg_j) = 0$, and were independent of the model errors and normally distributed with zero means and variance, i.e., $g_i \sim N(0, \sigma_g^2)$ and $lg_j \sim N(0, \sigma_{lg}^2)$, respectively. Model errors were assumed to follow a normal distribution with zero mean and variance, i.e., $e_{ijk} \sim N(0, \sigma_e^2)$. This model was fitted using lme4 packages from R package statistical[70]. The estimated BLUPs for each RIL were further used in QTL analysis to identify QTL resistance against Septoria tritici blotch.

QTL analysis

QTL analysis was performed for the combined five environments separately and the BLUPs estimated across environments, described before using R/QTL software[72]. In the first step, composite interval mapping (CIM) scans to identify putative QTL location along the genome were performed for each experiment and for the BLUPs obtained previously. The significance likelihood odd (LOD) threshold for CIM and MIM was obtained from a 1,000 permutation test[73]. Multiple interval mapping (MIM) was performed using forward selection and setting up the number of markers to find on the previously composite interval mapping (CIM) scan, using the Harley-Knott regression, method, and the windows for the CIM was set to 2 cM. Finally, each model was fitted, and each QTL were refined by using the function fitting and refine function on R/qtl[72].

The BLUPs estimated across each year by location, and their interaction were further analyzed. The addint function from R/QTL[72] calculated the epistasis interaction $QTL \cdot QTL$. The function addqtl[72] was used to scan for additional QTL to be added to the model. The additive or interaction effect for a second QTL for each chromosome was calculated by the addpair function in R/qtl[72]. The final QTL model was

fitted by stepwise function using an automated model search algorithm with a penalty calculated by using the scantwo functions in R/qtl[72] with 1,000 permutations test.

Genomic models

Three genomic prediction models were created using the R package lme. The models were compared using the maximum likelihood method. The accuracies of the models were evaluated by calculating the bias mean of residuals, and the precision was calculated by the root mean square error (RMSE). The general model (model 1) was:

$$\boldsymbol{Y} = \boldsymbol{X}\beta + \boldsymbol{Z}u + \epsilon \quad (4)$$

where $\boldsymbol{Y}$ is the vector of phenotypes, $\boldsymbol{\beta}$ is the vector of fixed markers effects selection in the QTL analysis and the Stb1 and Stb6 markers, u is the vector of random markers effects, $\boldsymbol{X}$ is the design matrices coded by (0,1) for QStb relating β to the observation in $\boldsymbol{Y}$, and $\boldsymbol{Z}$ is the design matrices for QStb, intercept, environment and interaction between QTL*environment, coded by (0,1) relating u to observation in Y. The $\boldsymbol{Z}$ matrix is considered a random effect, including intercept, for all QStb and location/year combinations with no variance and covariance restrictions.

Model 2 was:

$$\boldsymbol{Y} = \boldsymbol{X}\beta + \boldsymbol{Z}u + \epsilon \quad (5)$$

where $\boldsymbol{Y}$ is the vector of phenotypes, β is the vector of fixed marker effects selection in the QTL analysis, u is the vector of random markers effects, $\boldsymbol{X}$ is the design matrices coded by (0,1) for QStb relating β to the observation in Y, and $\boldsymbol{Z}$ are the design matrices for intercept and environment coded by (0,1) relating u to observation in Y. The $\boldsymbol{Z}$ matrix is considered a random effect with no restriction in variance and covariance.

Model 3 was:

$$\boldsymbol{Y} = \boldsymbol{X}\beta + \epsilon \quad (6)$$

where $\boldsymbol{Y}$ is the vector of phenotypes, β is the vector of fixed markers effects selection in the QTL analysis, u is the vector of random markers effects, $\boldsymbol{X}$ is the design matrices coded by (0,1) for QStb relating β to the observation in $\boldsymbol{Y}$.

The selection models were tested by environmental variables such as temperature, rainfall, relative humidity. The environmental variables were treated as fixed effects.

3.4 Results

Genotypic Data

A total of 11,587 SNPs were identified for the F×M population after filtering with PLINK software[60].. One line was removed after filtering due to a high proportion of missing SNP calls. The markers were further filtered based on Mendelian segregation ratios. A total of 8.658 SNPs were used to construct the linkage map, representing areas from all chromosomes of common wheat. Chromosome 2B was the chromosome with the highest number of markers (732), while chromosome 4D had the chromosome with lowest number of markers (88).

Phenotypic Analysis

The phenotypic analysis for each environment revealed a broad range of disease severity in the RIL population. The parents' reactions were consistent in rank, with Foote being moderately resistant and Madsen showing a moderately susceptible response to STB in all environments. The distribution of phenotypes was skewed toward lower disease severity in all environments. STB disease pressure varied across environments (Figure 3.1).

The correlation between both severities' reads in two different stages was significantly higher for all environments, ranking between 0.865 to 0.927. Thus, analyses were conducted using only the second reading for STB severity.

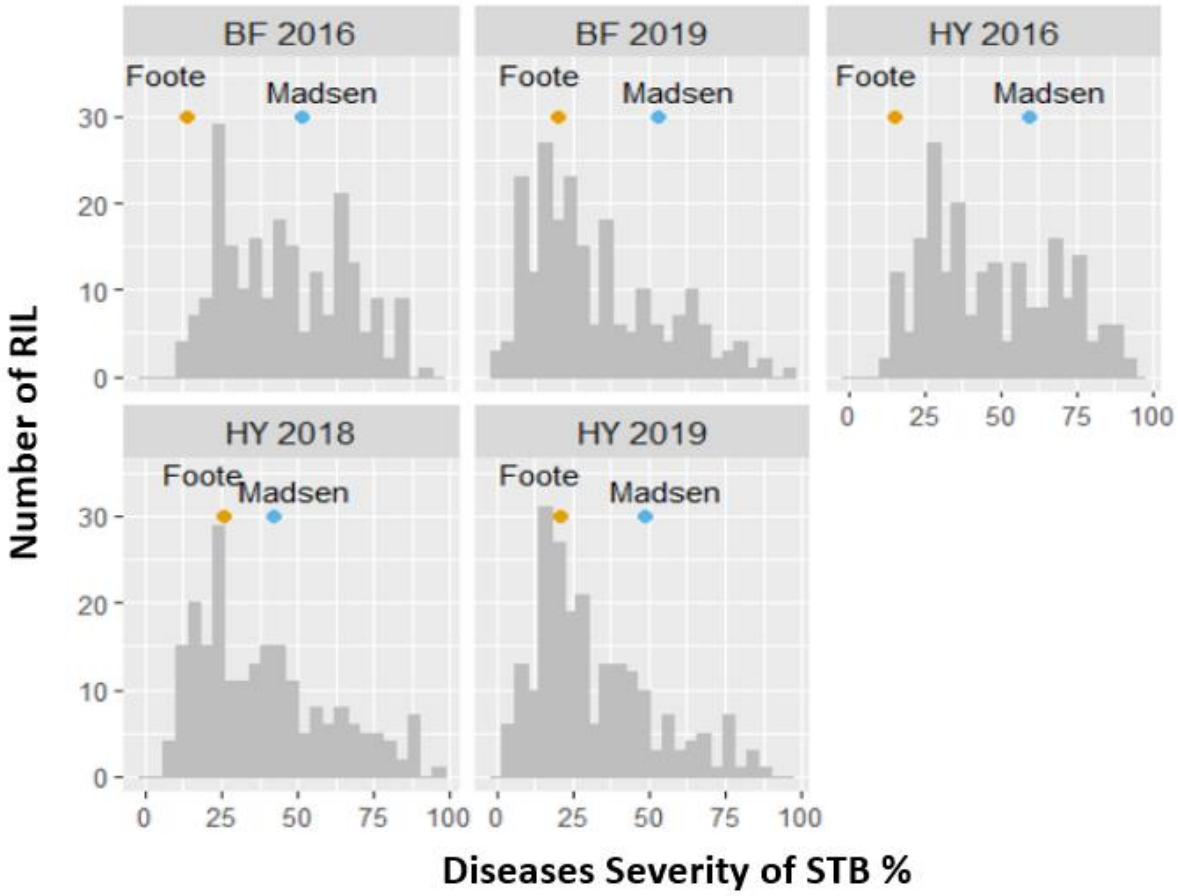

Figure 3.1 Recombinant inbred lines (RIL) histogram of the FxM RIL population with the colored points indicating the arithmetic mean of the percentage STB infection for the parents, orange for Foote, and the blue for Madsen. The y axis shows the number of RILs, and the y axis represents categories of percent severity of STB % for each environment: Botany Farm 2016 (BF 2016), Botany Farm (2019), Hyslop Farm (2016), Hyslop Farm (2018) and Hyslop Farm (2019).

STB was present in all experiments, with mean severity varying from 30.0% at the Hyslop Farm 2019 to 47.1% at the Hyslop Farm 2016 (Table 3.1). STB severity for the parent Foote ranged from 10.0% severity at Botany Farm 2015 to 29.4% at Hyslop Farm 2018 and disease severity of the parent Madsen ranged from 25.0% at Botany Farm 2015 to 66.3% at the Botany Farm 2016 (Table 3.1). Plots of the Foote parent were mistakenly planted to an old seed source in both 2016 trials and were replanted 2-3 weeks later with viable seed. This resulted in an underestimate of the susceptibility of Foote in these two trials.

STB severity for the RILs ranged from a low of 0-5%, depending on the experiment, and a high of 95-98% (Table 3.1). Heritability for each environment was high, ranging from 0.69 at the Hyslop Farm 2019 to 0.83 at the Botany Farm 2019 (Table 3.1).

Table 3.1. The severity of STB for parents and RIL population (mean, max and min, genetic variance σ_g^2, residual variance σ_e^2, number of replication r, and their heritability h^2 were calculated for each environment: Botany Farm 2016 (BF 2016), Botany Farm (2019), Hyslop Farm (2016), Hyslop Farm (2018) and Hyslop Farm (2019)

Loc/year	Foote	Madsen	RIL Population			$\sigma^2 g$	$\sigma^2 e$	r	h^2
			Mean	Max	Min				
HY 2016	12.9	59.4	47.13	98	5	377.9	213.4	2	0.78
BF 2016	14.4	66.3	44.77	95	2	300.6	276.5	2	0.69
HY 2018	29.4	38.1	38.29	98	2	346.3	282.5	2	0.79
BF 2019	27.5	47.8	31.16	98	0	414.4	167.1	2	0.83
HY 2019	25.7	45.4	30.04	95	0	293.1	264.1	2	0.70

The contribution of RIL and environment were further analyzed by the mixed model Eq (1). All genotypic (RIL) variances were significantly greater than 0 in each environment, and the variance ranged from 256.7 in Botany Farm 2016 to 368.0 in Hyslop Farm 2019. However, for variance between environments, only Hyslop Farm 2016 and Botany Farm 2019 were different than 0, where the environment variance was 10.95 for Hyslop Farm 2016, and 33.8 for Botany Farm 2019 (Table 3.2).

Table 3.2. ANOVA-like table, the variance of random effect for each environment, LRT (Likelihood Ratio Test), and the probability of variance test for each environment: Botany Farm 2016 (BF 2016), Botany Farm (2019), Hyslop Farm (2016), Hyslop Farm (2018) and Hyslop Farm (2019).

ANOVA-like table and variance of random effect						
Location	Factor	Variance	StdDev	LRT	Pr(>Chisq)	
BF 2016	Genotype (RIL)	256.7	16.0	59.29	1.36e-14	***
	Environment (rep)	0.0	0.0	0	1	
	Residuals	246.9	16.3			
HY 2016	Genotype (RIL)	352.9	18.8	109.08	2.2e-16	***
	Environment (rep)	11.0	3.3	8.64	0.003	**
	Residuals	206.5	14.4			
HY 2018	Genotype (RIL)	322.2	18.0	73.32	2e-16	***
	Environment (rep)	0.5	0.7	0.06	0.81	
	Residuals	277.5	16.7			
BF 2019	Genotype (RIL)	368.0	19.2	133.08	2.2e-16	***
	Environment (rep)	33.8	5.8	34.64	3.96e-09	***
	Residuals	173.7	13.2			
HY 2019	Genotype (RIL)	255.8	16.0	66.18	4.11e-16	***
	Environment (rep)	3.80	2.0	1.89	0.17	
	Residuals	241.2	15.5			

The Pearson correlations between severity of different environments revealed that the highest correlation was observed between Hyslop Farm 2019 and Botany Farm 2019 ($r = 0.68, p < 0.001$), and the lowest between Hyslop Farm 2019 and Botany Farm 2016 ($r = 0.34, p < 0.001$) (Table 3.3.)

Table 3.3. Pearson correlation coefficients for STB disease severity among environments (Botany Farm 2016, Botany Farm 2019, Hyslop Farm 2016, Hyslop Farm 201) and Hyslop Farm 2019), plant height (ht), and heading date (hd).

	Pearson correlation coefficients				
	BF 2016	BF 2019	HY 2016	HY 2018	HY 2019
BF 2016	1				
BF 2019	0.42	1			
HY 2016	0.64	0.49	1		
HY 2018	0.52	0.58	0.59	1	
HY 2019	0.34	0.68	0.50	0.59	1
ht	0.016	0.002	0.055	0.033	0.060
hd	0.061	0.051	0.058	0.060	0.057

*Note: all correlations were significant at P < 0.001 ***; Plant height (PH) was the distance from the ground to the spike's top for each plot.*

The mean plant height for each experiment was between 93.8 cm to 97.8 cm, with the range for RIL height being 75 to 115 cm. The mean heading date ranged between 131 to 134 Julian days. Heading date plant height, and their interaction showed no significant association with STB severity, p-value=0.65 and 0.64, respectively.

The analysis of the general model revealed that genotypic variance, environment variance, and genotype by environment interaction were highly significant with a Pr(>Chisq) of 2.2e-16, 2.2e-16, and 9.34e-14, respectively. Among the three sources of variation, the largest proportion was genotype, representing38% of variation in the model, followed by genotype by environment, which represented 13% of variation in the model. Environment accounted for 9% of the variation (Table 3.4). The ANOVA test based on the general model indicated that plant height and heading date were not significant with Pr(>Chisq)>0.37.

Table 3.4. Analysis-like table and variance of random effect for the general model, LRT (Likelihood Ratio Test).

Analysis-like ANOVA table, and variance of random effect					
Factor	Variance	% Var	LRT	Pr(>Chisq)	
Genotype (RIL)	231.50	0.38	442.41	2.2e-16	***
Environment	51.70	0.09	185.84	2.2e-16	***
Gen*Env	74.74	0.13	55.50	9.34e-14	***
Residuals	242.98	0.40			

Foote and Madsen had identical alleles for photoperiod sensitivity (*Ppd-A* (insensitive)and *Ppd-B* (wild type)) that influence heading date and for the dwarfing alleles *RHT-B* and *RHT-D* that directly affect plant height. The RIL population did differ for VrnB, with Foote donating the spring type allele and Madsen donating the winter type allele. However, in this study, the environment was the primary source of variation for heading date and plant height resulting in these two traits not affecting STB severity.

QTL analysis

Individual experiments

The QTL identified in each experiment (site/year) but at overlapping chromosomal positions were grouped and assigned the same name using the nomenclature QStb-followed by the chromosome location. Considering each year by location, seven different QTL were identified, contributing to STB resistance in the Foote × Madsen population (Table 7). The threshold of 1,000 permutations was 3.31 for composite interval mapping and 3.45 for multiple composite interval mapping using a significance level of 5%. All QTL had minor effects, accounting for 5.4% to 14.1% of the explained variance (Table 3.6). The QTL with the highest effect was QStb-7D, which was in the short arm of chromosome 7D (Table 3.6). QStb-7D was detected in all experiments (Table 8), except the Botany Farm 2016 with LOD scores for the QStb-7D ranging from 6.71 to 7.8. The phenotypic variance explained by QStb-7D ranged from 9.7% to 14.1%, with the additive effect ranging from -4.97 to -9.74 (Table 8). QStb-5A, QStb-6B, and QStb-6D were detected in three environments each (Table 3.6). QStb-2A, QStb-2B, and QStb-4B were each detected in only one environment each (Table 3.6). The resistance alleles for the QStb-2A, QStb-5A, QStb-6B and QStb-6D came from Madsen and QStb-2B, QStb-4B, QStb-7D came from Foote (Table 3.5).

Table 3.5. Summary of Septoria tritici blotch (STB) resistance QTL identified using the Harley-knot regression method in the Foote × Madsen population, under natural STB infection during 2016 to 2019.

QTL name	Chr	Physical map	Position (cM)	Donor
QStb-2A	2A	8735846-8735909	11.15	Madsen
QStb-2B	2B	87673492-87673429	150.44	Foote
QStb-4B	4B	597979606-597979669	161.02	Foote
QStb-5A	5A	509569535-509569472	137.24	Madsen
QStb-6B	6B	2609428-2609365	151.14	Madsen
QStb-6D	6D	61003540-61003598	102.38	Madsen
QStb-7D	7D	614103520-614103583	102.04	Foote

Table 3.6 Summary of the QTL detected using composite interval mapping (CIM) for the Foote x Madsen population response to Septoria tritici blotch under natural field infection, including likelihood odds (LOD) scores, explicative variance in the model (var_expli), and estimated additive effects (est_QTL), for all combinations of year by location.

Loc.Year	Chromosome	QStb -2A	QStb -2B	QStb -4B	QStb -5A	QStb -6B	QStb -6D	QStb -7D	QTL Model LOD^1	QTL Model Explained Variance (%)
BF 2016	LOD (CIM)			5.68	3.60				7.66	15.08
	LOD*			4.19	3.81					
	Explained variance			8.08	5.61					
	Additive effect			3.44	-3.36					
BF 2019	LOD (CIM)	4.79			3.66	4.26	4.26	4.10	21.41	36.65
	LOD*	4.59			3.84	5.05	4.16	7.71		
	Explained variance	9.29			5.41	7.21	5.88	9.74		
	Additive effect	-5.31			-4.03	-4.94	-4.25	7.44		
HY 2016	LOD (CIM)		4.25				4.30	9.46	14.39	26.42
	LOD*		4.02				3.89	7.69		
	Explained variance		6.58				6.37	13.1		
	Additive effect		4.22				-4.26	8.14		
HY 2018	LOD (CIM)				7.79	3.4	3.8	8.32	19.62	34.18
	LOD*				9.04	2.93	3.16	10.03		
	Explained variance				14.00	4.25	4.60	15.68		
	Additive effect				-5.69	-3.25	-3.44	8.06		
HY 2019	LOD (CIM)					4.79		8.01	11.94	22.46
	LOD*					4.18		7.83		
	Explained variance					7.23		14.1		
	Additive effect					-3.73		6.97		

LOD^1 is relative to the null model (with no QTL). LOD(CIM) for a single imputation for each marker, LOD is relative to the full model and the model with the term omitted. Explained variance is the estimated proportion of the phenotype variance explained by the term in the model. The estimates effects are derived by coding Foote at 0 and Madsen at 1.*

BLUPs models

The QTL identified in the BLUPs models overlapped in the same region of the chromosome as the QTL identified in the individual experiment analysis. A total of 5 QTL were identified by multiple interval mapping (MIM), QStb-4B, QStb-5A, QStb-6B, QStb 6D, and QStb-7D, using the BLUPs as phenotypic data. The QTL were located on chromosomes 4B, 5A, 6B, 6D, and 7D. The model explained 52.5% of the phenotypic variance (Table 3.7). A non- significant $QTL \cdot QTL$ interaction was found using the addint function in R/qtl. The highest LOD score was 1.10 from the interaction between QStb-6B and QStb-7D.

The other QTL interactions had LOD scores lower than 0.17. Two additional QTL were detected by addqtl function, but they were dropped during the stepwise QTL regression analysis.

Table 3.7. Summary of the QTL detected on by multiple interval mapping (MIM) in the Foote x Madsen population in response to Septoria tritici blotch under natural field infection, including likelihood odds (LOD) scores, explicative variance in the model (var_expli), and estimated additive effects (est_QTL), for all the estimated BLUPs.

							QTL Model	
	Chromosome	QStb **4B**	QStb **5A**	QStb **6B**	QStb **6D**	QStb **7D**	**LOD**	**Explained Variance (%)**
BLUPs RIL*Env	**LOD (MIM)**	4.17	4.27	5.23	5.11	10.97	27.02	52.5
	LOD*	4.67	6.26	5.91	6.40	15.33		
	Explained variance	6.01	8.19	7.71	8.40	22.19		
	Additive effect	3.45	-4.01	-4.08	-4.19	8.85		

LOD^1 is relative to the null model (with no QTL). LOD(CIM) for a single imputation for each marker, LOD is relative to the full model and the model with the term omitted. Explained variance is the estimated proportion of the phenotype variance explained by the term in the model. The estimated effects are derived by coding Foote at 0 and Madsen at 1.*

Genomic models

Model comparison and selection

The comparison between genomic models was based on the maximum likelihood ratio test. Stb1 and Stb6 markers do not reduce significantly against STB using these data, p-value= 0.98 and 0.96**,** respectively. The epistasis effect between QTL by environment was calculated by the maximum likelihood between model G and model 1. No epistasis effect was found in evaluating the genotype x environment interaction (p-value 0.519). The environment (site/year) affected STB severity (p-value 0.001) in the comparison between model 1 and model 2. Model 1 with the QTL and the environment effect was selected based on these results.

Results of the selected model.

The random effects for model 1 had a variance for the intercept of 41.1 and a residual of 43.1. All QStb were significant with a p-value <0.0001, and no correlation was found between QStb. The effect of each QStb was small, with QStb-7D having the highest effect at 7.60 followed by QStb-6D with -4.85, QStb-5A with -4.41, QStb-6B with -4.03, QStb-4B with 4.00, QStb7B 3.06, and QStb5B -2.6. The accuracy bias mean was -2.53e-13, and the precision was between 20.42 to 21.83 of the roots mean square error (RMSE) depending on the environment. All QTL had an additive effect. These additive effects differ among QTL and vary with the environment. The estimated disease severity for the parents was 38% for Foote and 52% for Madsen. The severity without QStb was estimated to be 66.2%. The lines carrying all

five significant QStb would have reduced disease severity to 16.7%, which was below the moderately resistant check cultivar Foote. The QTL with the highest estimated STB severity reduction is QStb7D,located on the 7D chromosome in a chromosomal region where four genes are considered resistance genes. Three of those are the leucine repeat domain superfamily, TraesCS7D02G502300, TraesCS7D02G505700, TraesCS7D02G509000, and one protein kinase-like domain superfamily, TraesCS7D02G503700. Both superfamily genes, protein kinase-like domain or leucine repeat domain superfamily, are considered resistance genes[74].

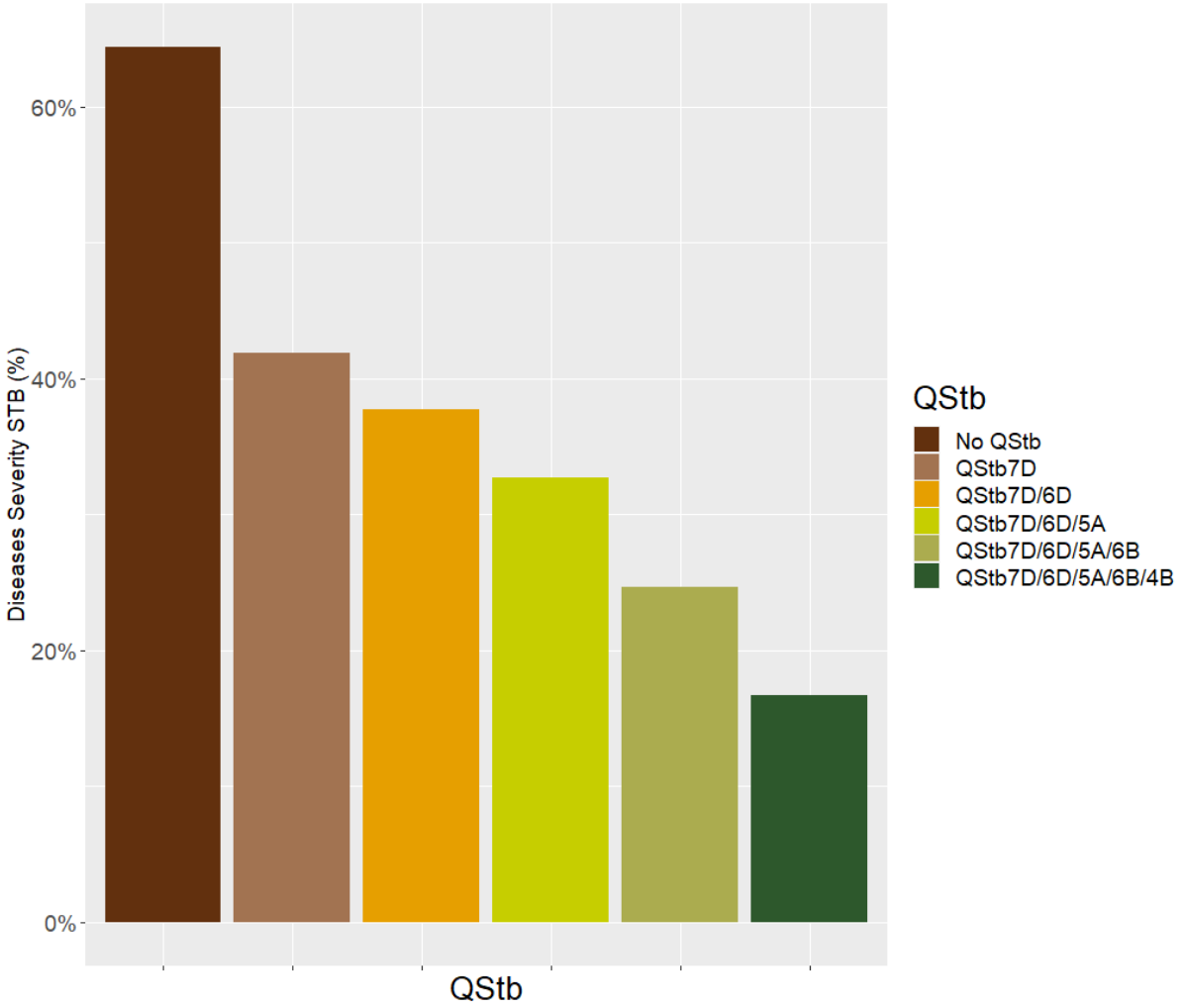

Figure.3.2 The STB severity prediction using the genomic model for individual lines where different QStb are present or absent.

The multiple environmental variables were tested using the maximum likelihood ratio statistical test separately with a range of p-values from 0.94 to 0.083.

3.5 Discussion

Septoria tritici blotch (STB) is a significant wheat disease worldwide, and wheat producers are currently reliant on multiple doses of fungicides to prevent yield losses and reduce of grain quality[75]. Chemical control makes the crop less sustainable and has led to STB populations being resistant to some fungicides. Traditional sanitation practices such as burning crop residue or crop rotation can reduce the level of primary inoculum, but ascospores from other fields can rapidly infect the previously sanitated[76,77]. An alternative is to develop cultivars with increased genetic resistance against STB, providing economic and environmentally sustainable management. The severity of STB depends on host resistance, the virulence and the aggressiveness of genetically diverse pathogen isolates, and the environment. STB resistance is a complex trait and usually depends on multiple genes with moderate-to-small effects[78]. Accumulation of independent quantitative resistance genes can reduce the severe infection of STB. In this study, a RIL population developed from a moderately susceptible parent and a moderately resistant parent was evaluated for STB severity in multiple environments under natural STB infection to identify QTL associated with resistance genes that reduce the STB severity. Infection by naturally occurring inoculum for each site/year led to a wide range of STB severity scores among the RILs, illustrating the wide range of resistance in the population. In this study, adult plant resistance was evaluated against multiple STB genotypes due to a natural STB population with a high genetic variation caused by the combination of large effective population size and yearly sexual recombination. Among these environments, seven QTL associated with STB resistance (QStb) with minor effects were identified. The QStb with the highest was QStb-7D, with an additive effect of 6.85.

The identification and utilization of molecular markers associated with STB resistance can facilitate the improvement of wheat resistance by marker-assisted selection[79]. The stepwise regression model identified five QTL, and the model explained 58.6% of the phenotypic variance. The QStb from chromosomes 4B, and 7D is derived from Foote, while the QStb is carried by chromosomes 5A, 6B, 6D are derived from Madsen. All these QTL may be associated with minor resistance and adult plant resistance.

Other traits, such as plant height and heading date, have sometimes been associated with STB severity[49]. The QTLs detected during the QTL analysis of STB resistance genes could be related to plant height or heading dates instead of resistance genes. Plant height can have an effect due to secondary cycles of infection of wheat by splash-dispersed pycnidiospores[80]. The pycnidiospores usually come from spores produced on lower leaves dispersed upwards through the canopy through rain splash. Levels of plant disease resistance often vary with plant developmental stage for a variety of reasons[81]. For this study, the variation in heading date and plant height did not affect disease severity. This is likely because Foote and Madsen do not substantially differ in plant height or heading date. For plant height, both parents carry the

same height genes (RHT-B and RHT-D). Heading date is a complex trait explained by three factors, photoperiod sensitivity, vernalization, and narrow-sense earliness[81]. Narrow-sense earliness was described by Lewis et al. (2008) as the factor which explained flowering time but is not the vernalization requirement or the photoperiod[78]. Although the RIL population segregated for the vernalization gene (VrnB), which classifies wheat as spring versus winter type, there was no significant difference for heading dates. This was partly due to the parents carrying the genes associated with photoperiod sensitivity that has a greater impact on heading date in places with moderate winters, such as western Oregon.

Best linear unbiased predictions (BLUPs) estimate the genotypic value of a trait, adjusting the empirical means toward the general means for each RIL and reducing the variability explained for the different environments (year/location). BLUPs analysis also reduces the bias of normality in the QTL model. Using the BLUPs in the QTL analysis increases the explained phenotypic variance compared to using the mean of each RIL. BLUPs analysis in this study created with or without the interaction (RIL* Env) effect detected five different QTL. The BLUPs analysis with the interaction term explained slightly more of the phenotypic variance. Only two of the seven QTL identified from individual experiments were not detected using BLUPs, i.e., QStb2A and QStb2B. These two QStb were each detected in only one location. No significant epistatic interactions or interactions among chromosomes were found. Only additive effects remained significant in the model after the stepwise analysis. The genomic model was used for testing QTL validation detected in the QTL analysis. The non-significant interaction between QTL confirmed what was observed in the QTL analysis. The genomic model showed that the QTLs identified in this study for STB resistance were stable across environments. The root means square error (RMSE) increased slightly if the random environment effects were not included. Although the environmental factor has a significant effect, it only increases the precision of the model slightly. The environment response is controlled by three factors: environmental variables, pathogen genotypes, and the interaction between pathogen genotypes and environmental variables. The model has an acceptable but considerable RMSE. These results agree with a similar prediction model for STB severity[82].

The QTL identified in our study have varying associations with those reported by others. Overall, 7DL, 3BL, and 6BS are the regions with the most QTL identified for STB resistance[85]. The markers from the candidate genes *Stb1* and *Stb6* were not associated with reduced STB severity in the FxM RIL population, even though the markers showed Mendelian segregation. QStb-7D had the most significant additive effect, and it is located on the long arm of chromosome 7DL. The QStb-7D comes from Foote and is the QTL with the highest phenotypic variance explained. The interval region on chromosome 7D where QStb-7D was detected is associated with four resistance genes from two resistance gene families. Three

genes are part of the leucine repeat domain superfamily, and one gene is from a protein kinase-like domain superfamily. One or more of these genes, or combinations among them, could reduce STB severity. Resistance genes Stb4[83] and Stb5[84] have been detected on the short arm of chromosome 7D[31], and several quantitative resistance genes have been identified on 7DL[85]. The region of chromosome 7D QTL that has been identified carrying STB resistance may also confer resistance against other necrotrophic pathogens such as Septoria nodorum blotch (*Phaeosphaeria nodorum*)[86] and Rhizoctonia root rot (*Rhizoctonia solani*)[87]. This opens the possibility that these four genes reduce STB severity and/or other necrotrophic pathogens.

Foote has another QTL on 4BL that slightly reduced STB severity. There is no known source of qualitative resistance for STB associated with the long arm of chromosome 4B (4BL), where QStb-4B is located. A QTL was detected on 4BL for STB resistance in 11 different environments in Europe[88]. Rht-b1, which is one of the dwarfing genes in wheat[89], is located on chromosome 4B. Plant height is considered one of the most confounding factors in determining resistance to STB[90]. The QTL detected in this study cannot be Rht-b1 because both parents carry the same dwarfing genes, and the Rht-b1 locus would not segregate in this population.

The other three QTL detected, Qstb-5A, Qstb-6B and Qstb-6D, had similar additive effects and came from Madsen. Qstb-5A was detected in chromosome 5AL. *Stb17* is associated with 5AL and provides resistance in only the adult plant stage, and it is considered a quantitative trait[18]. Consequently, Qstb-5A could be *Stb17* since it behaves as a quantitative adult plant resistance gene. QStb-6D is on the short arm of chromosome 6D. In the literature chromosome, 6DS is associated with *Stb18*, which confers genotype-specific resistance to multiple STB isolates (IPO323, IPO98022, IPO89011, and IPO098046)[91] for seedling resistance, but was inconsistently expressed at the adult plant stage[91]. Further study will be needed to determine if QStb-6D identified in this study is associated with *Stb18* or if QStb-5A is associated with Stb17. The chromosome 6BS is also one of the regions where QTL has been associated with STB resistance, so Qstb-6B could be a previously identified QTL or a novel QTL associated with STB resistance.

All QTL detected in this study are quantitative genetic loci that provide partial resistance to STB. This study confirms that breeding for Septoria tritici blotch resistance is challenging because it is a complex trait controlled by multiple quantitative genes with moderate-to-small effects. In addition, this study demonstrated that accumulating Qstb could reduce STB severity.

One of the most essential goals in plant breeding is to improve the durability of disease resistance. *Z. tritici* in the Willamette Valley is one of the most adaptable plant pathogens owing to a highly favorable

environment for infection, extremely large effective population size of the pathogen, and recurring sexual reproduction that recombines virulence effectors[13]. STB resistance in wheat cultivar 'Gene' conferred by *Stb6* and *Stb10*[40] was defeated five years after its release when it only occupied 15% of the wheat-growing area in the Willamette Valley[41]. Madsen lost much of its quantitative resistance against STB gradually over 10 year period during the 1990s[90]. Foote was one of the notable cultivars in the Willamette Valley in the early/mid-2000s[13] and showed resistance to STB when it was released, but rapidly declined in acreage due to becoming susceptible to stripe rust. Foote retained field resistance to STB[13] when it became stripe rust susceptible but was highly susceptible to some isolates of *Z. tritici* collected in 2004 and 2005[42], suggesting that it would have eventually become susceptible to STB. Other wheat cultivars such as 'Goetze' and 'Bobtail' with quantitative resistance have also shown evidence of erosion of their STB resistance several years post release[13]. Durability of STB resistance in the Willamette Valley, and perhaps elsewhere, will likely require multiple QTL as well as deployment of different QTL in time and space.

Resistance associated with QSt-7D was consistent over three years of field studies and provided a reduction of between 7% to 9% of STB severity, except at Botany Farm in 2016. This durability could be explained by the four associated resistance genes found on chromosome 7DL. Other independent studies of resistance of STB in the field detected different QTL with similar additive effects, around 10% or less[46]. The QSt-7D with the other QTL identified in this study can significantly reduce the severity of STB. Accumulating the most significant number of quantitative resistance genes increases the reduction of severity and may provide durable resistance. No major QTL effect was detected in this study, based on the concept of a major effect causing a reduction of severity of more than 30%[46]. This is because neither Foote nor Madsen carries a major resistance gene capable of defeating the high rates of infection found in the Willamette Valley. Madsen lost much of its quantitative resistance against STB during the early/mid 2000s[92]. Foote was one of the major cultivars in the Willamette Valley in the early/mid-2000s[13] and showed resistance to STB when released but lost acreage due to becoming susceptible to stripe rust. Though stripe rust susceptible, Foote still retained resistance to STB[13]. Foote did lose resistance against some isolates of STB that were collected during the 2004 and 2005[43] seasons but conserved some resistance against individual isolates collected from Madsen[43], and for the STB population, it was exposed to during this study. Our results suggest that QTL from Foote and Madsen can be combined to reduce STB severity and perhaps increase the durability of resistance.

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Chapter 4 : Identify Molecular Markers for Stripe Rust Resistance in Field Conditions

Chapter 4: Identify Molecular Markers for Stripe Rust Resistance under Field Conditions

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4.1 Abstract

Stripe rust, caused by *Puccinia striiformis f. sp. tritici*, is an important disease of wheat (*Triticum aestivum L.*) worldwide, and is the most important fungal disease of wheat in the United States Pacific Northwest (PNW). When environmental conditions are favorable for disease development, stripe rust can significantly reduce the economic value of wheat by decreasing both grain yield and quality. Foliar fungicides are adequate to control stripe rust, but chemical application adds substantial economic and environmental costs. The development of wheat cultivars with durable resistance to stripe rust is a priority due to the continuous evolution of new races of the pathogen. To study stripe rust resistance, a recombinant inbred line (RIL) population was developed from a cross between 'Madsen' and 'Foote' soft white winter wheat. Madsen (PI 511673) has provided effective resistance to stripe rust in the PNW, while Foote (PI 599663) was initially resistant to stripe rust but is now susceptible to the disease. This study aimed to identify molecular markers for stripe rust resistance in a 217 RIL population of Foote × Madsen. The population was evaluated across five environments, genotyped with GBS using STACKS, and compared with the reference genome of the International Wheat Genome Sequencing Consortium (IWGSC). Analysis of variance for phenotypic and genotypic data was significant for stripe rust severity among RILs ($P<0.01$). Results for quantitative trait loci (QTL) analysis indicated two major QTL, one in 2AS, which is likely associated with the known stripe rust resistance gene Yr17, and another in 1AS. Two additional minor QTL on 2AS/2DS and 4DL also were identified. The combination of the 1AS QTL and Yr17 reduced the stripe rust severity significantly. Adding the other two QTL detected in this study can reduce severity further and potentially contribute to resistance durability. The QTL identified in this study could help to develop breeder-friendly molecular markers for use in genotypic selection for improved stripe rust resistance in wheat.

4.2 Introduction

Stripe rust, caused by *Puccinia striiformis* f. sp. *Tritici*, is one of the most destructive diseases of wheat globally[1]. The Pacific Northwest (PNW) region of the U.S., composed of Oregon, Idaho, and Washington, has an ideal environment for stripe rust infection and development, with moderate to high

humidity, mild winters, and moderate summer temperatures. Since 1959, this region has experienced different levels of yield losses that have been characterized as low (<20%), moderate (20-40%), and severe (40-60%)[2,3]. The most severe epidemics occurred in the years 1960, 2005, 2007, 2010 and 2012[5]. The most recent epidemic was between 2010 and 2012, with the appearance of new stripe rust races such as PSTv-11, PSTv-14, PSTv-41, and PSTv-37[4]. The disease severity of stripe rust in this region depends on: temperatures in autumn (October-November); winter (December-January); and spring-summer (May-June); and precipitation during the spring (April)[5].

The stripe rust pathogen has sizeable genetic variability. In the U.S., PST races are identified annually, with 19 races being identified in 2020 (https://striperust.wsu.edu/races/data/). Genetic variability in stripe rust is due to mutation, somatic recombination, and possibly sexual recombination[6] if the alternative host (*Berberis or Mahonia spp)* is present [7]. Mutation is the primary mechanism to explain the formation of new races, which often occurs through stepwise evolution[8,9]. Frequency of stripe rust race mutation is estimated to be from 1.4×10^{-6} *to* 4.1×10^{-6} per locus per generation in individual clonal lineages[10]. Somatic recombination is the second most important mechanism to develop new races of stripe rust and involves recombination between genotypes by nuclear exchange, cell fusion, and chromosomal reassortment[11]. Sexual recombination is the rarest mechanism for creating variability. It occurs predominantly in the region near the Himalayas, where *Berberis* spp are ubiquitous, with spores being potentially dispersed worldwide by natural and human factors[12].

Stripe rust control is based primarily on fungicides and host plant resistance. In the PNW, fungicides have been widely used to control stripe rust, but add substantial cost to PNW farmers, who spend at least $10 million annually on fungicides[13]. Fungicides may also have negative environmental consequences. Thus, growing resistant cultivars is the most environmentally and economically sound method to control stripe rust[14]. Resistance to stripe rust has been a prime priority in wheat breeding programs in the PNW since the 1960s[14,15]. Wheat breeders have developed resistant cultivars with durable and adequate levels of resistance. Still, disease severity is not lowered sufficiently during heavy stripe rust years. Further, the underlying genes conferring resistance, and their durability, are not clearly characterized. Identifying QTL (quantitative trait loci) for resistance to stripe rust may help to increase the level of durable resistance in wheat.

Stripe rust resistance can be divided into two categories: qualitative and quantitative. Qualitative resistance is usually controlled by a single gene (major gene), often providing a high level of resistance, and tends to be specific to certain races of the pathogen (race-specific resistance)[16]. These genes are effective and can often fully protect the cultivar from rust damage even when the disease is very severe. However, these genes are not durable due to the development of new races in the pathogen population.

Most of these genes encode proteins (NBS-LRR), which identify fungal effectors and trigger a defense response that inhibits pathogen reproduction[17,18], such as Yr10, which is the only cloned gene for qualitative resistance[19]. Quantitative resistance is usually controlled by multiple genes with minor individual effects, does not usually provide complete resistance, and tends to be less race-specific than qualitative resistance. A particular category of quantitative resistance that has been highly effective for stripe rust control in the PNW is high-temperature adult plant resistance (HTAP)[15,20]. HTAP is quantitative resistance expressed only in the adult plant stage and only when a temperature threshold has been exceeded[15,20]. Currently, there are 67 qualitative genes and, 327 quantitative genes for resistance to stripe rust identified in wheat, and some have been used to provide resistance in commercial cultivars[21].

Traditionally, wheat breeders have used phenotypic selection to improve resistance against stripe rust. However, rust infection depends significantly on environmental effects such as temperature and humidity, making phenotypic selection difficult due to seasonal variation[22]. Marker-assisted selection (MAS) allows selection on the genotypes without stripe rust presence, making this technique potentially more effective[23,24]. The fastest and cheapest ways to identify single nucleotide polymorphisms (SNPs) associated with the trait of interest are through quantitative trait loci (QTL) analysis or a genome-wide association (GWAS) study[25]. These techniques evaluate the correlation between genotypic data and phenotypic data[26]. Genotype data are usually obtained through reduced genome sequencing technologies such as genotyping by sequencing (GBS)[27]. GBS is a technique that consists of construction of GBS libraries through restriction enzymes to reduce genome complexity, and allows sequencing with the Illumina next-generation sequencing (NGS) platform[28]. GBS is characterized by low cost, efficient barcoding, and ease of calling up SNPs[29].

New populations of stripe rust that have moved around the world in the last 15 years have increased aggressiveness and adaptation to both high and low temperatures, reducing the effectiveness of disease resistance[30,31]. During this time, the winter wheat cultivar ‘Madsen’ (PI 511673)[32] has remained highly resistant to stripe rust as well as cultivars derived from Madsen, such as 'Cara' (PI 643435)[34] and 'ARS-Selbu' (PI 667744)[35]. Though, other cultivars have lost their resistance during this time, such as 'Eltan' (PI 536994)[36], 'Xerpha' (PI 645605)[37] and ‘Foote’ (PI 599663), due to the appearance of new races of stripe rust in the PNW[38,39].

Madsen is a cultivar that was released in 1988 for production in the PNW[38]. Madsen carries effective resistance for fungal soilborne pathogens such as strawbreaker foot rot (caused by *Oculimacula yallundae Crous & W. Gams and O. acuformis Crous & W. Gams*), wheat blast (*Magnaporthe oryzae* Triticum pathotype MoT), root knot nematode (Rkn3) *(Meloidogyne spp*.), cereal cyst nematode (Cre5) (*Heterodera* spp.), and for fungal foliar pathogens such as stem rust (caused by *P. graminis subsp.*

graminis Pers.:Pers.), leaf rust (caused by *P. recondita Rob. Ex. Desm. f. sp. tritici*), and stripe rust (caused by *P. striiformis Westend. f. sp. tritici Erikss.*)[38]. Madsen has been widely used to develop new cultivars and for research. For example, a recombinant inbred line (RIL) population developed from a cross between Avocet S x Madsen has recently been used in a stripe rust study to identify seedling and adult plant resistance[33]. In this study, it was determined that Madsen carries *Yr17* plus five additional loci for stripe rust resistance[33].

The gene *Yr17* in wheat originated from the translocation 2NS/2AS from *Triticum ventricosum*[40]. The first wheat line with *Yr17* was VPM1, which was developed for strawbreaker foot rot resistance, and was widely used in countless European wheat breeding programs[41]. *Yr17* is closely linked with *Lr37* resistance from leaf rust and *Sr38* resistance from stem rust[42]. *Yr17* is a qualitative resistance gene used worldwide until virulence to *Yr17* was detected in the UK in 1994 and spread worldwide[1]. The researchers hypothesized that Madsen has maintained its resistance despite the presence of stripe rust races that are virulent to *Yr17* due to the presence of additional stripe rust resistance genes in combination with *Yr17* providing durable stripe rust resistance in Madsen in the PNW[16,33]. The objectives of this study were to 1) identify molecular markers for stripe rust resistance in a recombinant inbred line (RIL) population segregating for stripe rust susceptibility using genotyping by sequencing (GBS). 2) determine if the most effective QTL for resistance has changed over the six years.

4.3 Materials and Methods

Mapping population

The population for this study consisted of 217 F_{6-7} recombinant inbred lines (RILs) that were developed at Oregon State University (OSU). The population was developed between the soft white winter wheat cultivars 'Foote' (PI 599663)' and Madsen (PI 511673)[32], followed by single seed descent.

The wheat cultivar 'Foote' with the pedigree "Heima//Kalyansona/Bluebird/3/WWP7147,F1/4/Davis 6301/Heine VII/era/3/Buckbuck" is a soft white winter wheat developed by Oregon State University. Foote was initially resistant to stripe rust at the time of its release in 1998 but subsequently became susceptible. 'Madsen' with the pedigree "VPM1/Moisson 951//2*Hill 81", was developed by the USDA-ARS wheat genetics program in Pullman, WA, and carries *Yr17*. It also carries *Pch1* for strawbreaker foot rot resistance. Madsen is considered to have a moderate-to-high level of resistance to stripe rust. Since its release in 1988, Madsen has shown durable resistance to stripe rust in the PNW, indicating it carries stripe rust resistance genes in addition to *Yr*17 in its genome.

Genotypic data

DNA extraction and sequencing

Genomic DNA of the parents and their RIL (F_6-F_7) progeny were extracted from young leaf tissue, previously grown under greenhouse conditions to avoid pathogen or insect infestation. DNA extraction and quantification for GBS library construction were performed with an automated extraction system for acid isolation of DNA (ThermoKingFisher Flex, Waltham, MA), at the Center of Genomic Research and Biotechnology at Oregon State University (CGRB). The CGRB generated the GBS data following the protocol described in Elshire et.al[28]. The DNA was single digested by two restricted enzymes, PstI (CTGCAG) and MspI (CCGG). Barcodes adapters were unique for each sample (5 a 10 pb) based on oligonucleotides listed by Elshire et al.[28] The four libraries were prepared and sequenced with Illumina HiSeq 3000 at CGRB, Corvallis, OR, using 150 bp single-end high-output sequencing.

SNP callings

The raw data were quality filtered, demultiplexed, trimmed, and separated by sample based on their initial's barcodes, using the procces_radtags STACKS v2.52 software packages[43]. Only exact matches were considered. The parameters from procces_radtags was automatic clean data (-c), and rescue barcode options (-r), to remove any read with an uncalled base, and create an individual file for each RIL. The raw read for each RIL file was aligned to the wheat indexed reference genome[44], using the Burrows wheeler aligner (BWA)[45], previously indexed by using BWA tools[45]. The alignment output files were converted to binary version and sorted with view and sort algorithm, using the samtools v1.6[46]. The bcf files were generated with samtools mpileup and filtered out by quality score below 30, 25% missing data, minor allele frequency (MAF) between 30% to 70%, or more than 15% heterozygous calls. Only high-quality biallelic SNP for both parents and homozygous for opposite alleles were retained. The GBS-SNP markers were given the following format "TP23451231A". The first two letters correspond to a SNP (TP), or a SSR (SR). The following numbers correspond to the physical position on the RefSeq v1.0 assembly and the last two correspond where the marker is located.

Linkage map construction

Filtered GBS SNP (GBS-SNP) data were used to create a linkage map, and missing values were imputed using the ASMap packages from R/software[47]. The scaffolds pseudochromosome (ChrUn) were removed for QTL analysis. Initial group assignments were established using a p-value of 1E-10, and the maximum likelihood (ML) objective function. Recombination frequencies were calculated for each marker using the Kosambi function. During each interaction of linkage map construction, markers with excess of double recombination or markers not linked to any other marker were removed. For each locus, a χ^2 goodness-of-fit test was performed to detect distorted segregation markers, Bonferroni correction was used for

multiple testing. Markers that did not fit a Mendelian segregation proportion (χ^2 test p-value <0.01) were excluded from the analysis.

Molecular markers:

The RILs and their parents were genotyped by genetic markers associated with photoperiod and plant height. The photoperiod (*Ppd-A*, *Ppd-B*, and *Ppd-D1*) markers were genotyped by following the protocol of Yang et al.[48] and the plant height *Rht-B1* and *Rht-D1*) markers were genotyped following the protocol of Ellis et al[49].

The population was also genotyped for *Yr17* following the protocol of Cardozo et al. (not published), with the following primers: Ventriup (5'-AGG GGC TAC TGA CCA AGG CT- 3'), LN2 (5'-TGC AGC TAC AGC AGT ATG TAC ACA AAA-3'[40] used to detect the 2NS/2AS translocation and Yr17neg-F (5'-GATCCATGACGCGCATTTG-3') that indicates the absence of *Yr17* (Cardozo et al. Instituto Paraguayo de Tecnología Agraria (IPTA), not published).

Experimental fields

Field trial

The Foote and Madsen (FxM) RIL population with their parents was planted for evaluation of natural infection by stripe rust in five years (2014, 2015, 2017, 2018, 2020), at two different farms, the OSU Botany and Plant Pathology Field Laboratory (45.72 N, 122.54 W), Corvallis OR and the OSU Hyslop Crop Science Field Research Laboratory (44.63 N, 123.19 W), Corvallis OR. For each trial, the experiment was planted in a randomized complete block design (RCBD), with two replications. For the 2014 to 2020, each plot was 3 rows and 2 m long, and the parents were included twice in each replication. The exception was the experiment planted at the Hyslop farm in 2020 where, plots were 6 rows and 2 m long, and the parents were included four times in each replication.

Phenotypic data

Natural infection occurred for each experiment. The disease readings were taken at the flowering-milk stage (Zadoks 59-75). The percent rust severity for each plot was evaluated according to the modified Cobb scale[50]. Heading date was measured for each plot using the Julian calendar when around 50% of the spikes had emerged above the collar of the flag leaf. Plant height was measured at the ripening stage (Zadoks 92-98), for each plot as the distance between the ground to the spikes' top, excluding the awns.

Statistical analysis

Severity of rust for each combination of year and location.

All statistical analyses were conducted using R software version 3.63[51]. The model for each combination of year and location for stripe rust was treated as an individual data set and analyzed separately using lme4 packages from R packages statistical[51].

$$y_{ijk} = \mu + RIL_i + \text{Env}_j + e_{ijk}\,, \tag{7}$$

where y_{ijk} was the severity response for stripe rust (Yr) for each element of RIL i and each element of environment j, mu (μ) is the population mean for the severity response (Yr). RIL and Environments were treated as random effects with zero mean and variance ($\sigma^2_{RIL}, \sigma^2_{env}$), respectively. Model errors were assumed to follow a normal distribution with zero mean and variance, i.e., $e_{ijk} \sim N(0, \sigma^2_{err})$. All the variables were mutually independent, i.e., $cov(RIL_i, Env_j) = 0$. The random effects were calculated using the rand option in the lme4 packages from R package statistical[51]. Heritability was calculated for each combination between year and location, $h^2 = \sigma^2_g/\sigma^2_p = \sigma^2_g/(\sigma^2_g + \frac{\sigma^2 e}{r})$, where the RIL and rep were fitted as a random effect, using lme4 packages from R package statistical[51]. The phenotypic variance was calculated as $\sigma^2_p = \sigma^2_g + \frac{\sigma^2_e}{r}$ where r is the number of replications[52], and the genetic variance σ^2_g, and residual variance σ^2_e were the model's(7) variance components. Differences across environments were significant. The LSD test was performed to assess which environments were different from the others with a probability level P<0.05.

General model

The general model for genotypes, environments, and their interaction for stripe rust severity was:

$$y_{ijk} = \mu + \text{u}_\text{i} + \text{u}_\text{j} + u_{ij} + \beta_{\text{ph}}\text{PH}_{\text{ij}} + \beta_{\text{HD}}\text{HD}_{\text{ij}} + e_{ijk}\,, \tag{8}$$

where y_{ijk} is the observed response for the k^{th} element of RIL i in environment j, mu is the population mean for the trait, and $\text{u}_\text{i} + \text{u}_\text{j}\ and\ u_{ij}$ are RIL, Environment and RILxEnvironment random effects and e_{ijk} is the unexplained model error. β_{ph} is the effect of the plant height, β_{HD} is the effect of heading date. Both effects are considered fixed effects. Model errors were assumed to follow a normal distribution with zero mean and variance, i.e., $e_{ijk} \sim N(0, \sigma^2_{err})$ and random effects were assumed to be independent of the model errors and normally distributed with zero means and variances σ^2_{Gen}, σ^2_{Env} and $\sigma^2_{Gen*Env}$. It was further assumed that $\text{u}_\text{i} + \text{u}_\text{j}\ and\ u_{ij}$ were mutually independent, i.e., $cov(u_i, u_j) = 0$. Variance

components were determined and the significance of variance component estimates was calculated by the restricted maximum likelihood, using lme4 packages from R package statistical[51].

BLUPs

Best linear unbiased predictors (BLUPs) for stripe rust severity for each RIL, and for Foote and Madsen, were determined across all experiments, using lme4 packages from R package statistical[51].

The model was:

$$y_{ij} = \mu + g_{\mathrm{i}} + lg_{\mathrm{j}} + e_{ij}\,, \tag{9}$$

where y_{ij} represents the estimated BLUPs of stripe rust severity for each RIL line, μ is the overall mean, g_{i} and ly_{j} are the random effect of the $\mathrm{i_{th}}$RIL and $\mathrm{j_{th}}$ location by year, respectively, and e_{ij} is the unexplained model error. We assumed that g_{i} and lg_{j} were mutually independent, i.e., $cov(g_{\mathrm{i}}, lg_{\mathrm{j}}) = 0$, and to be independent of the model errors and normally distributed with zero means and variance, i.e., $g_{\mathrm{i}} \sim N(0, \sigma_g^2)$ and $lg_j \sim N(0, \sigma_{lg}^2)$, respectively. Model errors were assumed to follow a normal distribution with zero mean and variance, i.e., $e_{ijk} \sim N(0, \sigma_e^2)$. This model was fitted using lme4 packages from R package statistical[51]. The estimate BLUPs for each RIL were further used in genome-wide association mapping analysis (GWAS) to identify QTL resistance against stripe rust.

QTL analysis

QTL analysis was performed for each experiment using the BLUPs estimated across experiments, described above, using R/qtl package software[26]. In the first step, single-locus QTL were identified through composite interval mapping (CIM). The significance of QTLs was assessed using likelihood odds (LOD) thresholds obtained from a 1000 permutation test. The second step was multiple interval mapping (MIM). It was performed using forward stepwise regression, using an imputation method. The penalty was calculated using 1,000 permutation tests[53] with a significance level of 5%. Finally, the QTL was refined by the chromosome position using the refinqtl function in R/QTL software[26].

The BLUPs estimated across experiments were further analyzed. The function addint from R/QTL[26] was used to test the interaction between QTL, and the addqtl from R/QTL[26] was used to test additional QTL in the same chromosome. Finally, The QTL model was refined to a location by refinqtl function in R/QTL software[26].

Further analysis of *Yr*17

Genome-wide association mapping analysis (GWAS) was performed for the markers on chromosomes 2A, 2B, 2D and the BLUPs estimated across experiments, as described above. The GWAS model was performed based on the mixed model described in Yu et al.[54], using the rrBLUP package in the R software[55].

The model was:

$$y = X\beta + S\alpha + Z_u + e\ , \tag{10}$$

where y is a vector of phenotypic observation, where β is a vector of fixed effect from the principal component analysis, the variable u models the genetic background of each line as a random effect with $Var[g] = K\,\sigma^2$, and the variable α models the additive SNP effect as a fixed effect. The X, S, and Z are incidence matrices of 1s and 0s relating to y, β and u, respectively. The kinship matrix was the covariance between lines due to a polygenic effect. It was calculated with the A.mat function from the rrBLUP package in the R software[55].

A linkage map for the chromosome 2 was created by ASMap by bulking all the markers from the chromosome 2A, 2B and 2D and reconstructing the entire linkage map, using the Kosambi function.

4.4 Results

Genotypic Data

The FxM population lines were genotyped using genotyping-by-sequencing, the reads were aligned, and the variants from the genomes of the two parents were called and filtered for the whole population. The raw data had a GC content of 47.8%, with 156,043 SNPs. The first filtering data consist of 8,111 markers representing areas from all chromosomes of common wheat with a GC content of 49.8%. The chromosomes with the highest number of markers were chromosome 2B with 737 markers, and the chromosome with the fewest number of markers was chromosome 4D with 90 markers. A genetic linkage map was constructed using 6,110 markers, including 410 SSR and 5,700 SNPs, representing all chromosomes. The average marker distance in different linkage groups ranged from 0.3 cM to 9.8 cM. The B genome had the most markers with 2,382, followed by the A genome with 2,077, and the D genome was the smallest with 1,651. The map had reasonable contiguity, except for the chromosome 4D, having a large gap of 40 cM long.

Phenotypic Analysis

The RIL population and their parents were evaluated for stripe rust resistance. Foote and Madsen were consistent in disease severity, with Madsen being resistant and Foote susceptible. The distribution of phenotypes was skewed in all environments. Stripe rust disease pressure varied among environments (Figure 4.1).

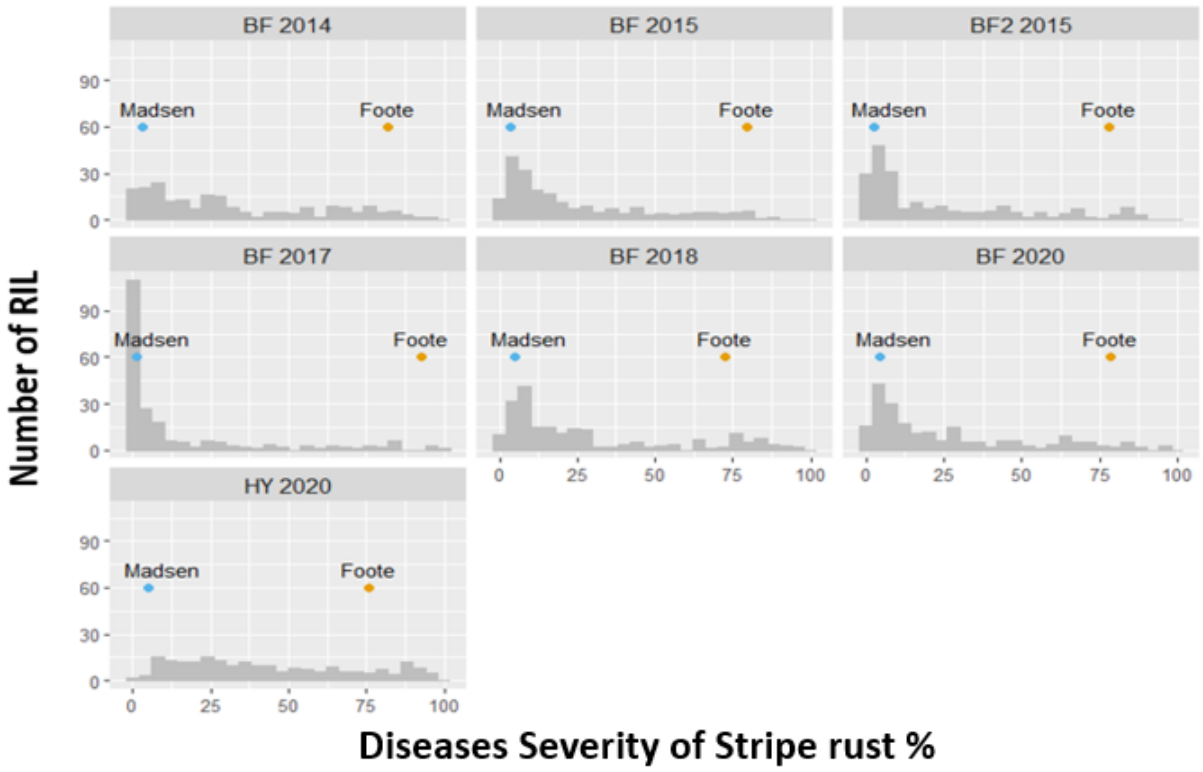

Figure 4.1 Recombinant inbred lines histogram showing distribution of stripe rust mean severity of the FxM population with Madsen and Foote.

Stripe rust was present at all site/year combinations and severity varied among experiments. The lowest pressure of stripe rust was the Botany Farm 2017, with an average of 14.6% of disease severity and lines ranging between 0 to 100%. The highest pressure of stripe rust was observed at the Hyslop Farm 2020 with an average of 46.0%, where RILs were between 0% to 99%. The parents were consistent in disease severity, with Foote ranging from 72.5% to 92.5% and Madsen ranging from 1.2% to 4.8% disease severity (Table 4.1). The range of the RIL population was consistent in all environments, between 0% to ~100%. Heritability for each environment was high, ranging from 0.95 at Botany Farm 2014 to 0.79 in Botany Farm 2020 (Table 4.1). The LSD test revealed no significant difference between the BF 2015 and BF2 2015 experiments. All environments were analyzed separately in the genomic association mapping,

except Botany Farm 2015 and Botany Farm2 2015 which were considered as a single dataset referred to as Botany Farm 2015 (BF2015).

Table 4.1 Severity of wheat stripe rust for parents and RIL population (mean, max, and min, genetic variance σ_g^2, residual variance σ_e^2, number of replication r, and their Heritability h^2.

Loc/year	Foote	Madsen	RIL Population			$\sigma^2 g$	$\sigma^2 e$	r	h^2
			Mean	Max	Min				
BF 2014	82.1	3.1	35.8 b	100	0	745.9	79.0	2	0.95
BF 2015	79.4	3.3	24.9 e	98	0	550.0	81.7	2	0.93
BF2 2015	77.9	2.6	26.5 e	95	0	616.6	80.8	2	0.94
BF 2017	92.5	1.2	14.6 f	100	0	576.0	126.5	2	0.90
BF 2018	72.5	4.8	33.9 c	98	0	746.9	97.4	2	0.94
BF 2020	75.6	4.8	30.2 d	98	0	527.6	287.3	2	0.79
HY 2020	78.7	4.2	46.0 a	99	0	643.1	200.0	2	0.87

Plant height (PH) and heading dates (HD) were not significantly different across environments (p-value = 0.54). They also were not significantly associated with stripe rust severity (p = 0.64 and 0.69 for PH and HD, respectively). The mean plant height among RILs varied between 92.4 cm to 98.2 cm. In all environments, RIL plant height ranged between 75 cm to 120 cm. The mean of heading date across environments ranged between 118 to 124 Julian calendar days. The variation for heading dates for RILs was approximately 15 to 20 days for each experiment. There was no segregation for the photoperiod markers (*Ppd-A*, *Ppd-B*, and *Ppd -D*) or height (*Rht-B1* and *Rht-D1*) markers.

The Analysis-like ANOVA table from the general model revealed that genotype variance was highly significant, as well as environment variance and genotype by environment with Pr(>Chisq) (2.2e-16) (Table 4.2). The proportion of variation was 61.0% for genotypes, 9.5% for environment, and 10.6% for the interaction genotype by environment (Table 4.2).

Table 4.2 ANOVA-like analysis table and variance of random effects for the general model, LRT (Likelihood Ratio Test)

Factor	Variance	% Var	LRT	Pr(>Chisq)	
Genotype (RIL)	525.09	61.0	1435.79	2.2e-16	***
Environment	81.41	9.5	473.45	2.2e-16	***
Gen*Env	91.07	10.6	195.74	2.2e-16	***
Residuals	162.67	18.9			

QTL analysis

For environment

The QTL identified for each environment were assigned names using the nomenclature Yr-following by the chromosome location. Multiple interval mapping (MIM) provided significant alternate QTL on 2AS and 2DS. The Pearson correlation between these two markers was 0.87, which could be linked to the same chromosome and named Yr2AS/2DS. These two markers were considered the same. Four different QTL were detected for stripe rust resistance in the FxM population, located on 1AS, 2DS, and 4DS with the composite interval mapping and 1AS, 2DS/2AS, and 2AL with multiple interval mapping. The threshold used was 4.29 for the additive and 5.02 for the interaction term, using 1,000 permutations. The 1AS and 2AS/2DS QTL contributed to stripe rust resistance in all environments and were present in CIM and MIM. The 1AS QTL was not detected in the Botany Farm 2014 data set. The phenotypic variation explained by 1AS increased between 2014 to 2020 from 12% to 24%. In contrast, the 2AS/2DS showed a reduction in the phenotypic variation explained from 48.5% to 15.08% (Table 4.4). The *Yr4D* was detected in three environments, Botany Farm 2017, Botany Farm 2018, and Botany Farm 2020 and only with the composite interval mapping (CIM). The phenotypic variation explained by Yr4D was 5.85% for BF 2018 and 6.05% for BF 2020 (Table 4.4).

Epistatic interaction was detected between two QTL, Yr1AS and Yr2AS/2DS, with a positive epistatic effect between QTL in chromosome 1AS and 2AS/2DS in four different environments (Table 4.6). The MIM had a higher explained variance than the CIM model in each environment except for Botany Farm 2015, which was 50.49 from CIM and 50.2 from MIM (Table 4.6). All the MIM models detected the combination of the two markers on 1AS and 2AS/2DS, except for the Botany Farm 2014 experiment. The QTL on 2AL was only identified in one location and only with multiple interval mapping, with a phenotypic variance response of 5.8% (Table 4.6). All the QTL were resistance alleles originating from the resistant parent Madsen, except for Yr4D which originated from the susceptible parent Foote (Table 4.3)

Table 4.3. Summary of stripe rust resistance QTL identified using the Harley-knot regression method in the Foote by Madsen population, under natural stripe rust infection during 2014 to 2020.

QTL name	Chr	Physical map	Position (cM)	Donor
Yr1AS	1AS	10130961….16855697	138	Madsen
Yr2AS	2AS	14417945….15899118	175	Madsen
Yr 4DS	4DS	1699017….1879144	41	Foote

Table 4.4. Summary of the CIM QTL model detected in FxM population associated with response to stripe rust under natural field infection, including likelihood odds (LOD) scores, explicative variance in the model (var_expli), and estimated additive effects (est_QTL), for all combination combinations of year by location.

Loc.Year	Chromosome	Yr1AS	Yr2AS	Yr4D	LOD^1	Model Explained Variance (%)
BF 2014	LOD*		31.15		31.15	48.53%
	Explained variance		48.53			
	Additive effect		-18.51			
BF 2015	LOD*	11.20	28.41		32.87	51.18%
	Explained variance	13.17	40.65			
	Additive effect	-8.71	-15.13			
BF 2017	LOD*	11.42	21.43	4.56	30.83	46.06%
	Explained variance	14.88	28.31	5.14		
	Additive effect	-8.65	-12.15	5.18		
BF 2018	LOD*	17.49	19.23	5.30	33.48	51.02%
	Explained variance	22.14	24.81	5.85		
	Additive effect	-12.57	-13.18	6.43		
BF 2020	LOD*	15.04	10.53	4.51	24.06	40.13%
	Explained variance	22.63	15.08	6.05		
	Additive effect	-12.24	-13.48	5.84		
HY 2020	LOD*	14.6	9.6		21.10	38.42%
	Explained variance	24.28	15.48			
	Additive effect	-11.94	-8.98			

LOD^1 is relative to the null model (with no QTL). LOD is relative to the full model and the model with the term omitted. Explained variance is the estimated proportion of the phenotype variance explained by the QTL in the model. The estimatq effects are derived by coding Foote at 0 and Madsen at 1.*

Table 4.5 Summary of the MIM QTL model under natural field infection for each environment, model of significant QTL in each chromosome, likelihood odds (LOD) scores, and the phenotype variance explained in the model.

Loc.Year	Model	LOD^1	Explained Variance (%)
BF 2014	$y = Yr_{2D}$	31.15	48.65%
BF 2015	$y = Yr_{1A} + Yr_{2A}$	32.63	50.24%
BF 2017	$y = Yr_{1A} + Yr_{2D} + Yr_{1A:2D}$	30.83	50.81%
BF 2018	$y = Yr_{1A} + Yr_{2D} + Yr_{1A:2D}$	33.48	53.19%
BF 2020	$y = Yr_{1A} + Yr_{2D} + Yr_{1A:2D}$	24.06	41.41%
HY 2020	$y = Yr_{1A} + Yr_{2A} + Yr_{2D}$	42.05	42.01%

LOD^1 is relative to the null model (with no QTL). Explained variance is the estimated proportion of the phenotype variance explained by the QTL in the model.

Table 4.6 Summary of the MIM model QTL detected in the FxM population associated with response to stripe rust under natural field infection, including likelihood odds (LOD) scores, explicative variance in the model (var_expli), and estimated additive effects (est_QTL), for all combinations of year by location.

Loc.Year	Chromosome	Yr1A	Yrchr2	Yr1A:2A	LOD^1	Model Explained Variance (%)
	LOD*		31.26			
BF 2014	**Explained variance**		48.65		31.15	48.5%
	Additive effect		-18.55			
	LOD*	9.94	25.57			
BF 2015	**Explained variance**	12.34	37.91		32.87	51.2%
	Additive effect	-8.31	-14.50			
	LOD*	18.86	28.88	10.78		
BF 2017	**Explained variance**	24.34	41.86	12.71	30.83	46.1%
	Additive effect	-8.89	-14.53	8.38		
	LOD*	22.41	26.40	10.87		
BF 2018	**Explained variance**	28.41	35.37	8.77	33.48	51.0%
	Additive effect	-12.51	-11.90	8.13		
	LOD*	18.19	14.62	9.45		
BF 2020	**Explained variance**	27.81	21.47	7.23	24.06	40.1%
	Additive effect	-9.41	-7.09	5.51		
	LOD*	23.26	23.26	8.78		
HY 2020	**Explained variance**	26.19	44.34	8.09	21.10	59.2%
	Additive effect	-7.33	-9.17	6.43		

LOD is relative to the full model and the model with the term omitted. Explained variance is the estimated proportion of the phenotype variance explained by the QTL in the model. The estimates effects are derived by coding Foote at 0 and Madsen at 1.*

BLUPs model

BLUPs were used with the phenotypic data to identify QTL in the CIM and MIM models. The QTL identified were overlapping in the same chromosomal position as the QTL detected for each individual environment. The CIM model shows three different significant QTL located on 1AS, 2AS/2DS and 4DL. The phenotypic variance explained by the model was 55.2%, and for each term was 19.4% for, Yr1AS, li 36.7% for Yr2AS/2DS, and 4.7% for Yr4DL (4.7%). The LOD scores between full model and individual QTL were 16.9, 28.1 and 4.4 for Yr1AS, Yr2AS/2DS and Yr4DL respectively (Table 4.7). The MIM model using BLUPs as the phenotypic data had the highest phenotypic explained variance (59.2%) and highest LOD score relative to the null model (42.0). This model has an intercept term, two additive QTL (Yr1AS and 2AS/2DS) and a positive epistatic interaction term between Yr1AS and 2AS/2DS. The additive effect was -9.98 for Yr1AS and -11.91 for Yr2AS/2DS, and the positive interaction term was

6.43. The phenotypic variance explained for each term of the model was 26.2% for Yr1A, 44.0% for Yr2AS/2DS and the interaction term explained 8.19% (Table 4.7).

Table 4.7. Summary of the QTL detected in the FxM population associated with response to stripe rust under natural field infection, including likelihood odds (LOD) scores, explicative variance in the model (var_expli), and estimated additive effects (est_QTL), for all the BLUPs estimated.

Location	Chromosome	Yr1A	Yr2A/2DS	Yr4DL	1AS:2AS	Model	
						LOD	Phenotypic variance (%)
BLUPs, CIM	LOD	16.91	28.08	4.43		37.65	55.19
	Explained variance	19.45	36.66	4.43			
	Additive effect	-10.16	-13.52	4.68			
BLUPs, MIM	LOD	23.26	34.34		8.48	42.05	59.20
	Explained variance	26.19	44.05		8.09		
	Additive effect	-9.98	-11.91		6.43		

LOD^1 is relative to the null model (with no QTL). LOD(CIM) for a single imputation for each marker, LOD is relative to the full model and the model with the term omitted. Explained variance is the estimated proportion of the phenotype variance explained by the term in the model. The estimates effects are derived by coding Foote at 0 and Madsen at 1.*

Further analysis of potential Yr17 markers

GWAS

GWAS was performed for all RILs using the BLUPs and the genotypic markers previously located on chromosomes 2A, 2B and 2D. The results for the GWAS analysis showed 12 significant QTL markers, eight referenced on 2A, two for 2B and two for 2D, associated with decreased stripe rust severity (Figure 4.2)

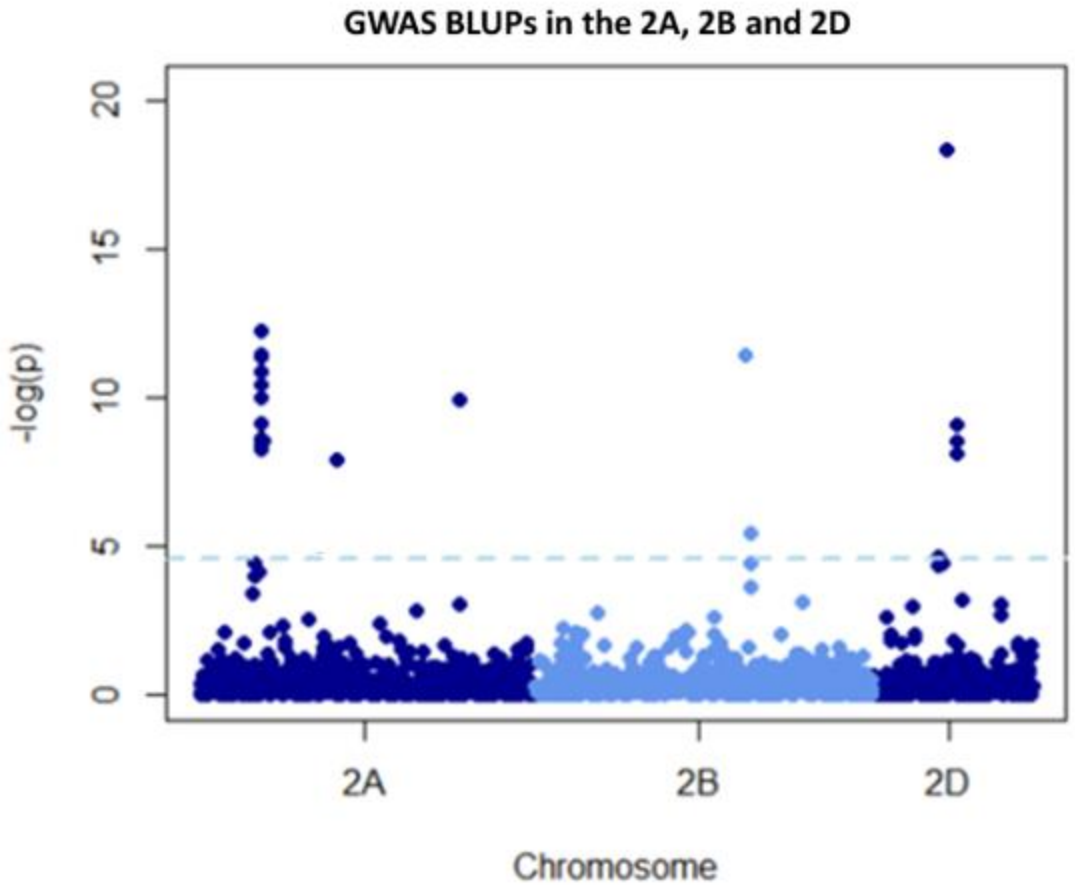

Figure 4.2 Manhattan plot for the GWAS analysis using the BLUPs as phenotypic data, and the two homologous chromosomes as the genotypic data. The horizontal axis is the chromosome 2A, 2B, and 2D. The vertical axis is the p-value for each single marker associated with the trait of interest. The dotted horizontal lines represent the Bonferroni correction $-log10(0.05/total(markers)$. *All colored dots are the markers predicted by rrBLUP package, and any dot above the dotted horizontal lines is a significant maker.*

Correlation markers

The pairwise recombination between the significant markers from GWAS and *Yr17* shows that 11 of the significant QTL identified on GWAS were significantly linked to *Yr17*,(χ^2> p. Value $0.0017, Bonferroni\ correction$). The rank distance between significant markers linked to *Yr17* was 1.89 to 40.09 cM (Table 4.8). The TP714206552A is more likely to be in 1AS because there is 19.40 cM distance between TP714206552A and the markers on 1AS. The markers TP129015422D, TP206187032D, TP158991182A suggest there are other QTL in the 2A chromosome.

Table 4.8 Markers significantly associated with stripe rust disease severity identified in the Manhattan plots. The Marker and Mark-short correspond to the Marker named. The LOD and the effect correspond on the GWAS model, where LOD is the p-value of the -log(p), and the effect is the effect of each individual

marker on stripe rust severity. The Pairwise analysis provides the R^2 (coefficient of correlation), cM distance in centimorgans, χ^2 corresponds to the p-value of testing significance of linkage disequilibrium, and Chr corresponds to the chromosome for each marker.

	GWAS model			Pairwise analysis			
SNP	Lod	Effect	Yr17	R^2	cM	χ^2	Chr
TPYr172A	0.055	-11.32	TPyr17	1.00	0.00	0	2A
TP319031972A	8.64	-11.34	TPyr17	0.93	1.89	0	2A
TP129020812D	18.06	-7.09	TPyr17	0.72	7.55	0	2A
TP212562682A	7.42	-5.83	TPyr17	0.70	8.49	0	2A
TP344404442B	11.4	-6.69	TPyr17	0.67	9.91	0	2A
TP41893962D	6.76	-4.66	TPyr17	0.66	10.38	0	2A
TP144187602A	10.85	-6.44	TPyr17	0.60	12.74	0	2A
TP91219272A	8.27	-3.51	TPyr17	0.53	13.68	0	2A
TP108471772A	11.38	-3.33	TPyr17	0.48	17.92	0	2A
TP218185162A	9.62	-5.22	TPyr17	0.48	17.92	0	2A
TP59721412B	5.72	-3.76	TPyr17	0.23	25.94	2.29E-12	2A
TP129015422D	9.09	-12.47	TPyr17	0.08	35.85	4.10E-05	2A
TP206187032D	9.22	-12.37	TPyr17	0.06	37.00	3.48E-04	2A
TP158991182A	8.08	-11.71	TPyr17	0.04	40.09	2.78E-03	2A
TP714206552A	9.15	-7.28	TPyr17	0.00	52.83	0.55	1A

4.5 Discussion

Madsen carries *Yr17* and other loci for stripe rust resistance[33] that have provided durable resistance against stripe rust since 1988[3,38] even though virulent races against *Yr17* are present in the region, such as Pstv-37 or Pstv-39[15]. The *Yr17* lines produced intermediate infection types (4-6) in the seedling stage, but can show drastically reduced severity depending on the temperature at the plant growth stage[56]. In this study, Madsen showed durable resistance to stripe rust ranging from 3.1 to 4.2% disease severity. The resistance from Madsen is mainly explained by two major QTL mapped on 1AS and 2AS. The resistance QTL on 2AS was present in all years. The 1AS QTL was not detected until 2015. The continuous evolution of stripe rust could explain these results. In 2015, the most prevalent race change was between Pstv-52 to Pstv-37. The difference between these two races is the virulence gene Tr1. The frequency of Tr1 among virulence genes has increased since its first report in 2010 (https://striperust.wsu.edu/races/data/). The wheat club cultivar Tres that has Madsen, as part of its pedigree, contains *YrTr1* and *YrTr2*[57]. Tres was used to develop the isogenic line NILYrTr1 to identify YrTr1 on the differential set for identifying races in the United Staes[58]. All evidence in this study indicates that the QTL detected in the 1AS could be associated with the virulence gene YrTr1[59]. However, YrTr1 was previously identified in chromosome 6D, but recent studies identified the YrTr1 on chromosome 1BS (Chen and associates, unpublished data). In the PNW, the resistance genes that have shown durable resistance have been identified with HTAP resistance genes[14]. Three QTL for adult-plant

resistance were reported in 1AS in three different cultivars, one Indian cultivar[60], and the other two cultivars from the PNW, an Idaho line IDO444[61], and Madsen[39].

In this study, disease severity differed among environments. These results can be explained by the evolution and change of stripe rust races, with a higher correlation between consecutive years and a lower correlation between longer periods of years. It also can be explained by the variability of environmental factors such as temperature, humidity relative, and pluviometry from year to year. In addition, other factors such as plant height (PH) and heading dates (HD) have been reported to have an effect on severity of stripe rust. However, no correlation was found between heading dates and plant height against diseases severity in this study.

The interaction between 1AS and 2AS had a positive epistatic effect. Yr1AS reduced disease severity by about 10%, and the Yr2AS reduced severity by about 12%, but their combination reduced the severity by only 15.5%. Other independent analyses for stripe rust had reported interactions between QTL, such as Vazquez et al. (2015) (2AS and 6DL)[16] and Zeng et al (2019) (2BL and 6BL)[62].

n additional QTL from Foote was detected on 4DL at the Botany Farm in the composite interval mapping. QYr4DL is a minor QTL present in three years 2017, 2018, and 2020, though its effect declined over the years. The QTL detected on 4DL could be *Yr46*, which confers adult plant resistance to stripe rust. Detection of this QTL only at the Botany Farm could be related to differences in stripe rust races. The dominant race of stripe rust for both sites was PSTv-37, which has also been the dominant race in the PNW since 2015 (https://striperust.wsu.edu/races/data/However, three additional races were detected at the Hyslop Farm: Pstv-39, Pstv-41, and Pstv-52. This difference could be due to the Hyslop site being planted to more wheat genotypes than at the Botany Farm.

The two major QTL in this analysis have been reported in other studies. Liu et al. (2018) used the Avocet S × Madsen RIL population to identify stripe rust resistance QTL. They found that Madsen carries *Yr17* and four additional QTL on 1AS, 1BS, 3BS, and 6BS, showing a high-level and durable resistance conferred by this combination of *Yr17* and multiple resistance QTL[33]. These results are supported by independent studies in different regions in the world in which *Yr17* combined with other QTL was associated with resistance durability[16,33,63,64]. In addition, *Yr17* may be linked to other resistance genes for high-temperature adult plant resistance[56]. Liu et al. (2018) detected more QTL than in this study, but most of the QTL not detected in our study had a relatively small effect in the work of Liu et al.[33], such as the QTL in 1BS and 3BS[33]. The 6BS QTL was not detected in our study despite the fact that the QTL on 6BS had an additive effect between -11.46 to -0.66 in the study of Liu et al.[33] It may be that Foote also carries

the 6BS QTL, which would result in a lack of polymorphism in the Foote x Madsen RIL population for this QTL.

Some markers on 2A, 2B, and 2D had a similar segregation pattern based on our linkage map. In addition, multiple interval mapping (MIM) provided alternative QTL locations on both 2AS and 2DS. GWAS provided an alternative analysis that does not require designation of linkage groups or parental data and is based only on pairwise linkage analysis to identify the correlations between markers. GWAS identified two groups in our study. The first group was markers linked to *Yr17*, and the second group was markers not linked to *Yr17*. Markers not linked to *Yr17* could be additional QTL on chromosome 2A or QTL on chromosome 2D. Additional QTL on 2A is supported by the more restricted construction linkage map. All those markers are segregated on the same linkage group, and by Liu et al[33], who suggested that *Yr17* is linked to another QTL for adult plant resistance. QTL on chromosome 2D is supported by the 2D marker close to the leucine-rich domain superfamily gene on that chromosome.

Durability of disease resistance is one of the most essential goals in plant breeding. All QTL detected in this study are from Madsen. Madsen was released in 1988[32] and has remained highly resistant to stripe rust[33]. The primary gene affecting wheat stripe rust severity in the Madsen x Foote population was *Yr17*. Virulence to *Yr17* in the U.S. has increased gradually from 27% in 2000 to 94% in 2007[65], and thus *Yr17* cannot alone account for the durability of stripe rust resistance in Madsen. As previously concluded in other studies of stripe rust resistance with *Yr17*[66–68], durability of resistance in Madsen likely resulted from the combination of *Yr17* and other QTL resulting in Madsen maintaining its resistance to stripe rust. The QTL detected on chromosomes 1AS and 2AS can be used as molecular markers to develop wheat cultivars with durable stripe rust resistance. A similar conclusion was suggested by Liu et al[33] based on their work using the Madsen x Avocet population. The results of their study combined with ours suggest that these two QTL were significant in populations with different genetic backgrounds. The Liu et al. study involved a spring (Avocet) parent, while the results presented in this study came from a population developed by a winter x winter cross. Thus, our research confirms the importance of the 1AS and 2AS QTL in a winter x winter cross.

4.6 References

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Chapter 5 : Identify Molecular Markers for Stripe Rust Resistance at the Seedling Stage in Wheat

Chapter 5: Identify Molecular Marker for Stripe Rust Resistance at the seedling stage in Wheat.

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5.1 Abstract

Stripe rust is a major disease of wheat (*Triticum aestivum L.*) worldwide and is caused by the fungus *Puccinia striiformis f. sp. tritici.* Genetic resistance to wheat stripe rust is often ephemeral owing to changes in the race composition of the pathogen. By contrast, the cultivar Madsen has maintained resistance to stripe rust in the Pacific Northwest (PNW) region of the U.S. since its release in 1988. The resistance gene *Yr17* interacts with other genes to provide resistance in the adult plant stage, even though it is no longer effective at the seedling stage. Accordingly, researchers have hypothesized that Madsen has other QTL that provides resistance in the seedling stage. The goal of this study was to identify stripe rust resistance quantitative trait loci (QTL) at the seedling stage in a recombinant inbred line (RIL) population between Madsen (PI 511673) and the susceptible cultivar 'Foote' (PI 599663). The 217 lines for the RIL population were evaluated in growth chambers against two types of stripe rust inoculum: a bulk population of spores collected from the field in 2012, and a single isolate of race PSTv-37, which has been the predominant stripe rust race recently in the PNW. The RILs were genotyped using Illumina HiSeq 3000 Sequencing and markers were called using the STACKS and Bfctools software. Stripe rust resistance QTL were found in chromosomes 1B and 2B using race PSTv-37 and in 6B and 7B when using the bulk population collected in 2012. These results suggest that durability of stripe rust resistance in cultivar Madsen could be associated with a suite of QTL that are expressed at different growth stages and in response to different pathogen races. The QTL identified in this study, could help develop breeder-friendly molecular markers, and be used in combination with the QTL detected at the adult plant stage to improve the durability of stripe rust resistance in wheat.

5.2 Introduction

Stripe rust, caused by *Puccinia striiformis* f. sp. tritici, is a globally devastating pathogen of wheat[1]. The Pacific Northwest (PNW) in the U.S. is one of the most at risk regions for stripe rust, with expected regional losses ranging from 5 to 25%, yearly[2] and observing new rust races annually[3]. In addition, the PNW is the region of the U.S. that has the highest diversity of stripe rust races, with frequent changes of predominant races[4]. Stripe rust races in U.S. are classified by the virulence and avirulence of isolates on a

set of 18 wheat genotypes with single, known resistance[5,6]. This differentiation of races is local because there has been no standardized and internationally accepted set of differential wheat cultivars to classify the stripe rust races[7]. New populations of stripe rust that have moved around the world in the last 15 years have increased aggressiveness and adaptation to both high and low temperatures, reducing the effectiveness of host plant resistance[3,8]. Currently, the most abundant stripe rust races in the PNW are PSTv-37 (50.7% in 2020) and PSTv-39 (21.1% in 2020) (https://striperust.wsu.edu/races/data/).

Stripe rust epidemics reduce the yield and quality of grain[9]. Having resistance genes that can protect the plant in the seedling stage significantly reduces disease severity. These genes are generally race-specific, but in the PNW are only effective for 3.5 years, on average, if used as single genes in cultivars[10] due to the frequent changes in the predominant stripe rust race. Historically, wheat breeders in the PNW have used a combination of qualitative and quantitative resistance genes[11]. Qualitative resistance tends to be specific to certain pathogen races (race-specific resistance), provides a high level of resistance, and often protects the plant during the whole growing season (all stage). Quantitative resistance tends to be less race-specific than qualitative resistance, is controlled by multiple genes with minor effects, and does not usually provide complete resistance. High-temperature adult plant resistance (HTAP) is a particular category of quantitative resistance [12,13] that is only expressed in the adult plant stage when a temperature threshold has been exceeded[12,13]. The combination of qualitative and quantitative resistance genes provides the most effective and durable resistance against stripe rust. Currently, there are 67 qualitative genes and 327 quantitative genes identified for resistance to stripe rust in wheat and many have been deployed in commercial cultivars[14].

The stripe rust resistance gene *Yr17* originated from a translocation between homologous chromosomes (2NS/2AS) with 2NS originating from *Triticum ventricosum*[15]. *Yr17* was widely used globally in cultivar development until a virulence race was detected in the UK in 1994 that overcame *Yr17* and was spread worldwide[2]. In the PNW, *Yr17* is present in multiple cultivars, such as 'Cara' (PI 643435)[16] , 'ARS-Selbu' (PI 667744)[17] and ‘Madsen’ (PI 511673)[18]. *Yr17* is considered a qualitative resistance gene, efficient, durable, non-race-specific in the PNW[19,20], but cultivars with *Yr17* have become more susceptible at low temperature and low light intensities in the seedling stage.

Most new races of stripe rust that have moved around the world in the last 15 years are virulent against *Yr17*[3,8]. However other cultivars with *Yr17*, such as Madsen maintained their resistance during this time. Accordingly, researchers hypothesized combining of other resistance genes with *Yr17* provides durable stripe rust resistance in the PNW[19,20]. Recent studies showed that Madsen was susceptible in the seedling stage to Pstv-4 and Pstv-14, (infection type 7-8) but resistant to PSTv-37 and PSTv-40, (infection type 2-3) at low-temperatures (4–20 °C)[19].

The objective of this study was to identify molecular markers for stripe rust seedling stage resistance in a recombinant inbred line (RIL) population segregating for stripe rust susceptibility using genotyping by sequencing (GBS).

5.3 Materials and Methods

Mapping population

The Recombinant Inbred Line (RIL) population used in this study was generated from the cross between the soft white winter wheat cultivars 'Foote' (PI 599663)' and Madsen (PI 511673)[18]. The population was developed at Oregon State University (OSU) by single seed descent for six generations.

The cultivar Foote was developed by Oregon State University with the pedigree "Heima//Kalyansona/Bluebird/3/WWP7147,F1/4/Davis 6301/Heine VII/era/3/Buckbuck". Foote is considered susceptible to stripe rust (ARS-GRIN, 2016), although it was resistant at the time of its release in 1998. Madsen was developed by the USDA-ARS wheat genetics program in Pullman, WA, with pedigree "VPM1/Moisson 951//2*Hill 81". Madsen has maintained a moderate-to-high level of resistance to stripe rust since its release in 1989.

Genotypic data

DNA extraction and sequencing

The parents and the RIL population were grown under greenhouse conditions at 21 to 23 °C with a photoperiod of 16:8 hours (Light: Dark). DNA extraction for each RIL and their parents was done at the three-leaf stage, with DNA being extracted from the leaf tissue with an automated extraction system for acid isolation of DNA (ThermoKingFisher Flex, Waltham, MA), at the Center of Genomic Research and Biocomputing (CGRB) at Oregon State University. The genotype by sequencing (GBS) libraries were developed following the protocol described in Poland et al.[21]. Unique individual-based barcodes adapters (5 a 10 bp) were ligated to individual samples[22]. The two restriction enzymes for the GBS were PstI (CTGCAG) and MspI (CCGG)[22]. Four libraries were prepared and sequenced with an Illumina HiSeq 3000 (Illumina HiSeq 3000/HiSeq 4000 System, RRID:SCR_016386, Illumina, San Diego, CA, USA) at the CGRB at Oregon State University, Corvallis, OR, USA

SNPs callings

The raw data were filtered, trimmed, and assigned to samples, using the unique individual barcodes adapters, by procces_radtags STACKS v2.52 software packages[23] following the parameters automatic clean data (-c) and rescue barcode options (-r). The wheat reference genome from IWGSC was indexed by the Burrows Wheeler aligner (BWA)[24]. Each sample was aligned to the wheat indexed reference gnome, using the Burrows Wheeler aligner (BWA)[24] and was sorted and aligned using SAMTOOLS v1.6

software, with the default parameters[25]. The bcf files for SNP calling were generated by bfctools mpileup and filtered by no more than 25% of missing data or more than 15% of heterozygous by markers or RILs, minor allele frequency 30 to 70% (MAF), and low-quality below 30%. The filter markers with biallelic position for both parents and homozygous for opposite alleles were retained. The markers obtained were named by two letters corresponding to a SNP (TP) or a SSR (SR), following with the physical position on the RefSeq v1.0 assembly, followed by ("_"), and the chromosome location. An example TP289123123_2A.

Linkage map construction

The linkage map was created by the ASMap packages from R/software[26], using the filtered data, except the pseudochromosome (ChrUn). The initials groups were established by maximum likelihood (ML) with a p-value of 1E-10. The Kosambi function was used by calculating the recombination frequency for each marker. Markers with an excess of double recombination (over 50%) and not linked to any other marker were removed during each interaction of linkage map construction. A χ^2 goodness-of-fit test and Bonferroni correction was performed for each maker. Markers not corresponding to the Mendelian segregation proportion (χ^2 test p-value <0.01) were excluded from the analysis.

Growth Chamber Screening

Growth chamber trial design and phenotypic data

The RIL population and their parents were evaluated with two different types of stripe rust inoculum; The first inoculum was a bulk population of stripe rust races collected from the cultivar 'Goetze' in 2012 in Corvallis, Oregon, and subsequently increased on plants of Goetze in growth chambers. Unfortunately, this population was lost in summer 2020 due to complications associated with a building renovation and the COVID-19 epidemic. Thus, the second set of experiments was conducted with a pure culture of race PSTv-37. This isolate was provided by Dr. Xianming Chen, USDA-ARS Pullman, Washington, and was originally collected in the Willamette Valley of Oregon in 2020. For this study, this race upon receipt was increased on plants of Foote wheat in growth chambers. Inoculum increases and the RIL evaluation were done in Percival model MB-60B growth chambers at 15°C within a photoperiod of 16:8 hours (Light: Dark). For each set, the experiment was arranged a randomized complete block design (RCBD) with two replications. A single seed of each RIL was planted in an individual cone and the parents were included 6 times. Three racks of 7 x 14 plots cones were used for each block. An extra ring of cones planted to the cultivar Madsen was placed as a border on the outside of each of the three racks. The second leaf of each RIL was inoculated with a mixture of urediniospores and talc by dusting the spores onto the second leave with a cosmetic brush when the plant was at the three-leaf stage. The inoculum concentration was a ratio of 1:5 spores: talc for the first inoculum and a ratio of 1:10 for the second inoculum. Immediately after

inoculation, inoculated plants were placed in a dew chamber at 13°C for 18 h without light and then moved to a growth chamber, with a constant temperature of 14-16 °C and with 16-h light /8-h darkness photoperiod. The infection type (IT) was recorded in the infection leave for each plant at three weeks after inoculation using a 0-9 scale[27].

Statistical analysis

Infection type of stripe rust for each set of races.

All statistical analyses were done using R software version 3.63[28]. The model for each set of races was treated as an individual data set and analyzed separately, using lme4 packages from R package[28].

$$y_{ijk} = \mu + RIL_i + \text{Rep}_j + e_{ijk}\,, \tag{11}$$

where y_{ijk} was the infection type response (IT) for each RIL i and each element of Rep j, mu (μ) is the population mean for the severity response (Yr). All variables were mutually independent, i.e., $cov(RIL_i, Env_j) = 0$. RIL, and Rep was treated as random effect with zero mean and variance ($\sigma^2_{RIL}, \sigma^2_{env}$). The e_{ijk} was the error term and was assumed to follow a normal distribution with zero mean and variance, i.e., $e_{ijk} \sim N(0, \sigma^2_{err})$. The genetic variance σ^2_g and residual variance σ^2_e were extracted for the model's (7) variance components. The phenotypic variance was calculated using the formula $\sigma^2_p = \sigma^2_g + \frac{\sigma^2_e}{r}$, where r was the number of replication (2), and σ^2_g and σ^2_e were the variances from the model. Heritability was calculated for each set of races as, $h^2 = \sigma^2_g/\sigma^2_p = \sigma^2_g/(\sigma^2_g + \frac{\sigma^2 e}{r})$, where the RIL and rep were fitted as a random effects, using lme4 packages from R package statistical[28].

QTL analysis

A composite interval mapping (CIM) analysis was performed independently based on the pathogen population in 2012 and the single race in 2020 using the R/qtl package software[29] to identify putative QTL locations along the genome. The significance of QTL was assessed using the likelihood odds (LOD) threshold of 5%, which was obtained by 1,000 permutation tests. The QTL detected on chromosome 2B was pairwise analyzed by each individual marker on chromosomes 2A and 2D due to some of the markers on those chromosomes having a similar segregation pattern based on our linkage map.

5.4 Results

Genotypic Data

A genetic linkage map was constructed using 6,110 markers, including 410 SSR and 5,700 SNPs, representing all chromosomes. Genome D had the lowest number of markers (1,651), and genome B had

the highest number of markers (2,382), followed by genome A (2,077). The map had reasonable contiguity, except for chromosome 4D, having a large gap of 40 cm long.

Phenotypic Analysis

Infection in both experiments was successful, having an infection type between 0 to 8.5 for both sets of stripe rust races. The infection type of parents was consistent in both experiments; Foote had an infection type between 4 to 6, and Madsen had an infection type between 2 to 4. The distribution of phenotypes was bimodal in both experiments, with transgressive segregation for both resistance and susceptibility (Heritability was 0.72 for the 2012 bulk inoculum and 0.74 for race PSTv-37 collected in 2020. ANOVA indicated no significant difference between experiments (P≥0.15) or between blocks with experiments (P≥0.54 for 2012 and 0.37 for 2020). Each inoculum type was evaluated separately due to the race(s) in 2012 being unknown (Figure 5.1).

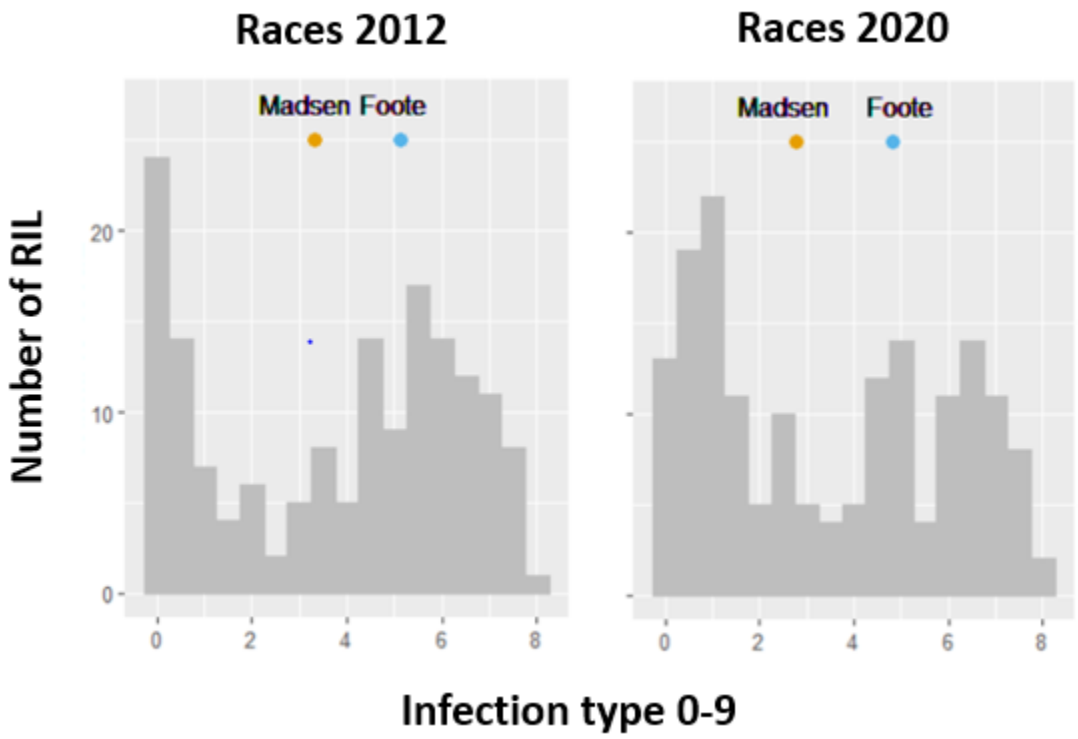

Figure 5.1. Recombinant inbred lines histogram showing the distribution of stripe rust mean severity of the two sets of stripe rust races in the FxM population with Madsen and Foote.

Table 5.1 Infection type for parents and RIL population (mean, max, and min, genetic variance σ_g^2, residual variance σ_e^2, number of replication r, and their Heritability h^2.

Inoculum	Foote	Madsen	RIL Population			$\sigma^2 g$	$\sigma^2 e$	r	h^2
			Mean	Max	Min				
Bulk 2012	5	3.5	4.5	8.5	0	6.78	5.08	2	0.72
PSTv-37 2020	5	3	4.0	8.5	0	7.78	5.01	2	0.74

QTL analysis

A name was assigned for each QTL identified in these two experiments using the nomenclature Yr- following by the chromosome location. Four QTL were detected in this study, Yr6B and 7B for the bulk inoculum collected in 2012, and Yr1B and Yr2B for the PSTv-37 race. The threshold used for additive effects was 4.2 for both inoculation experiments. All QTL detected had a minor effect, accounting for 5.1 to 8.6% of the variance. The QTL detected with inoculum from 2012 were Yr6B and Yr7B. Yr6B represents a resistance allele for Madsen and Yr7B was from Foote. The additive effect for those QTL were -1.0 and 0.9, respectively. The QTL detected with inoculum from 2020 were Yr1B and Yr2B, and both were from Madsen with additive effects of -1.2 for Yr1B and -0.6 for Yr2B. The QTL on 2B was not linked to any marker on chromosome 2A or 2D $\chi^2 > 0.55$(Table 5.2). The QTL detected on chromosomes 6 and 7 was not linked test because it did not identify any other significant marker in this population.

Table 5.2 Summary of the CIM model QTL detected in the FxM population-based on infection type response to stripe rust infected by bulk inoculum races collected in 2012, and race PSTv-37 collected in 2020, including likelihood odds (LOD) scores, explicative variance in the model (var_expli), and estimated additive effects (est_QTL), for all the combination year by the environment.

Inoculum	Chromosome	Yr1B	Yr2B	Yr6B	Yr7B	LOD^1	Model Explained Variance (%)
Bulk 2012	**LOD***			4.7	4.9		
	Explained variance			5.1	7.1	6.15	12.88%
	Additive effect			-1.0	0.9		
PSTv-37 2020	**LOD***	5.0	4.4				
	Explained variance	8.6	5.6			21.10	14.22%
	Additive effect	-1.2	-0.6				

LOD^1 is relative to the null model (with no QTL). LOD is relative to the full model and the model with the term omitted. Explained variance is the estimated proportion of the phenotype variance explained by the QTL in the model. The estimates effects are derived by coding Foote at 0 and Madsen at 1.*

5.5 Discussion

Madsen has provided durable resistance against stripe rust since 1988[30,31]. The resistance observed in Madsen is related to the presence of *Yr17* and additional QTL conferring resistance to stripe rust. The lines with *Yr17* produce an intermediate reaction of infection type (4-6) in the seedling stage but can show significantly reduced severity depending on the temperature at the adult stage[32]. The results from this study confirm that *Yr17* is only effective for adult plant resistance against most stripe rust races currently found in the PNW, as *Yr17* was not detected in the seedling assays. Two sets of inoculate were tested in this experiment. Race PSTv-37 has virulence against *Yr6, Yr7, Yr8, Yr9, Yr10, Yr17, Yr27, Yr43, Yr44, YrTr1, and YrExp2* (https://striperust.wsu.edu/races/data/). The bulk inoculum collected in 2012 most likely was composed of PSTv-11 and/or PSTv-14, which were the most predominant races in the PNW in 2012 (https://striperust.wsu.edu/races/data/). Reaction to PSTv-14 and PSTv-11 differ from Pstv-37 on *Yr1*, *Yr10*, Tye and reaction to PSTv-14 differs from that of PSTv-11 on Tr1 but not for PSTv-37. Therefore, itis most likely that all races tested in this experiment carry virulence to Yr17. The virulence has been present in around 95% of the races in the PNW since 2007. In this study, four different QTL were detected at the seedling stage, Yr1B, Yr2B, Yr6B, and Yr7B. Each QTL showed a minor effect. The minor effects of these QTL could be why they were not detected in the Foote x Madsen RIL population when evaluated for stripe rust in the field (Cobertera, 2021).

The most significant effect size QTL for resistance to the PSTv-37 race was the QTL detected on the short arm of chromosome 1B (1BS). The Yr genes mapped on 1BS are *Yr9* and *Yr10*. *Yr9* provides resistance to all three rust pathogens (stripe, leaf, and stem[34]), but does not provide resistance to PSTv-37[35]. Therefore, the QTL in this study detected on 1BS is not Yr*9*. On the other hand, *Yr10* is highly effective against PSTv-37[7]. *Yr10* is considered a global resistance gene[14]. In the PNW, *Yr10* was widely used in cultivar development since 1965. It was originally deployed in the club wheat cultivar Moro (PI 178383)[36], but it is currently only used with other genes in the resistance gene pyramids[14]. The QTL detected in this study is not likely to be the *Yr10* because 1) the pedigree of Madsen does not have a source for *Yr10*[19]; 2) *Yr10* is associated to the brown chaff color[37], and Madsen does not have this character and 3) *Yr10* has a significant effects on PSTv-37[7] and the QTL detected in this study had only minor effects. Ten QTL have been detected in chromosome 1BS associated with stripe rust resistance. Two of those QTL were detected in cultivars grown in the PNW, one from the cultivar Coda (PI 594372)[38] and the other from Madsen[19,39]. Both QTL had a minor effect against PStv-37[19,39]. Madsen is in the pedigree of the Coda[38]. Therefore, the QTL detected on 1BS in this study is likely to be detected in Madsen and Coda.

The other QTL detected using PSTv-37 race was on chromosome 2B. Chromosome 2B is a gene-rich region for stripe rust resistance. In the literature, 36 QTL and essential resistance genes such as *Yr5, Yr7, Yr27* have been reported on 2B. In the PNW, similar results have been found in several studies reporting resistance genes against stripe rust in chromosome 2B[20]. For example, Guo et al. 2008 identified two different loci for HTAP resistance on 2BS[40]. In addition, other cultivars such as 'IDO444'[41], 'Louise'[42], and 'Stephens'[43] have been reported to carry resistance genes on 2B. In two independent studies with PSTv-37, no QTL for resistance to this race were detected on 2BS. However, several studies have identified QTL on the chromosome 2BS in the seedling stage, using different races as inoculum, such as PSTv-40[12], PST-100[44], PSTv-11[44], and PSTv-51[44]. These results could be because not all Yr genes have been detected against stripe rust, or the population used in this study had no genomic variation in this area. Further analysis, with more replication, is required to verify the QTL detected in this study.

An additional two QTL were detected in the Foote by Madsen population using the bulk inoculum collected in 2012, one on chromosome 6BS, near the centromere, and the other in the long arm of chromosome 7. In the centromere region of chromosome 6B, six QTL have been mapped. Three QTL have been identified in the PNW in three different cultivars: 'Druchamp'[45], Stephens[17], and Madsen[19]. Druchamp is a French cultivar introduced to the United States in the late 1940s, and has been a source of stripe rust resistance in the PNW[45]. The QTL detected on Duchamp was detected in the seedling stage with three different races: PST-35, PST-100 and PST-114[45]. Stephens and Madsen are two varieties released in the PNW, in 1977 and 1988 respectively, Stephens is moderately susceptible, and Madsen is still resistant under field conditions[17,19]. The other two QTL have only been associated with HTAP resistance. Both studies suggest that the QTL detected on 6B is *Yr78*[17,19]. *Yr78* is known to confer partial resistance to PSTv-37 at the adult plant stage but provides no resistance at the seedling stage against PSTv-4, PSTv-14, PSTv-37, and PSTv-40[46]. Thus, this study does not contradict any results found in the literature, but further analysis is needed to determine if the QTL detected in this study is or is not *Yr78* due to the QTL having only a minor effect under growth chamber conditions at the seedling stage. In this chromosome region, there are also other Yr genes such as *Yr35* and *Yr36*. *Yr35* was transferred to bread wheat from *T. turgidum* ssp. dicoccoides[47] and shows resistance to PSTv-4, PSTv-14, PSTv-37, and PSTv-40[46]. Instead, by contrast *Yr36* confers only HTAP. This gene was previously cloned and encoded a kinase domain (WKS1)[48]. Although the QTL detected in this study is mostly likely to be *Yr35* or *Yr78*, further analysis is required to determine if the QTL identified in this study is *Yr35, Yr36,* or *Yr78.*

The other QTL identified using the 2012 inoculum was located on the long arm of chromosome 7B and was the only QTL provided by the cultivar Foote. In chromosome 7B, there are five major Yr genes *Yr52, Yr59, Yr67* (*YrC591*), *Yr39* and *YrZh84* and several QTL with low or moderate LOD and effect scores.

Weizhen Liu et al. identified a QTL on 7BL that provided resistance at the seedling stage to PSTv-51 but not in the adult stage[44]. The QTL detected in this study is close to this region and could be this QTL since it was only effective in the 2012 inoculum and not in the adult plant stage (Cobertera, 2021).

None of the QTL identified in this study provides sufficient resistance against the stripe rust races found in the PNW. However, all these QTL can be used in a resistance pyramid strategy against stripe rust with the combination of QTL detected in previous studies, such as the QTL on 1AS and *Yr17* (Cobertera, 2021). to provide durable resistance against stripe[19]. It is significant that different QTL were identified at the seedling stage when using different inoculum sources, and that a different set of QTLS were associated with adult plant resistance in the field (Cobertera, 2021). These results suggest that durability of stripe rust resistance in cultivar Madsen could be associated with a suite of QTL that has enabled it to respond to changes of the stripe rust pathogen over time.

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Chapter 6 : General Conclusions

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6.1 Conclusions

Durability of disease resistance is a fundamental goal of plant breeding. The most widely used strategy to develop durable resistance is by accumulating or pyramiding resistance genes in an individual cultivar[1]. The use of marker-assisted selection (MAS) can significantly reduce its time to develop new cultivars[2]. This technique requires knowing the chromosomal location of resistance markers associated with resistance genes and developing 'breeder friendly' molecular markers linked to these genes to ensure retention of the resistance genes during the development of a new cultivar[3,4]. This research aimed to identify molecular markers associated with resistance to Septoria tritici blotch (STB) and stripe rust of wheat. Attaining durable resistance also depends on the variability, adaptability, and virulence of the pathogen[1]. The two pathogens studied vary greatly in their life history and population structure, with the STB pathogen undergoing annual sexual reproduction and maintaining an immense effective population size. In contrast the stripe rust pathogen is clonal and undergoes severe, annual genetic bottlenecks.

This research utilized a molecular mapping population consisting of 217 recombinant inbred lines (RILs) derived from a cross between the wheat cultivars Foote and Madsen and was developed via single seed descent in the greenhouse. Madsen has maintained a high level of resistance to stripe rust since its release in 1988[5], though most of its initially moderate resistance to STB has eroded over time. Foote had moderate resistance to STB and a very high level of stripe rust resistance when released in 1998 but was highly susceptible to new stripe rust races that appeared in 2004-05. Foote was still moderately resistant to STB when it was abandoned owing to stripe rust severity, though pathogen isolates were identified with high virulence in Foote at that time.

The mapping population was grown in multiple environments. For both diseases, a randomized complete block design with two replications per environment was utilized, and infection was via naturally occurring inoculum. A specific fungicide was used to exclude stripe rust in the STB trials, while the rapid reproductive rate of stripe rust allowed it to exclude STB when that fungicide was not used substantially. Visual observations were used to estimate the severity of each disease. In addition to the field trials, the population was tested in growth chambers at the seedling stage using two types of stripe rust inoculum: a bulk population of spores collected from the field in 2012, and a single isolate of race PSTv-37, which had been the predominant race of stripe rust in the PNW in recent years. For all studies, the RILs were evaluated using genotyping-by-sequencing (GBS), and QTLs were identified using composite interval mapping (CIM) and multiple composite interval mapping. Best linear unbiased prediction (BLUPs) was used to analyze of the field trials results to reduce the variability explained for the different

environments. This technique estimates the genotypic value, adjusting the empirical means toward the general mean for each RIL. This technique has made it possible to analyze multiple locations/years in a single analysis, increasing the phenotypic value's precision, reducing environmental error, and reducing the normality bias in the QTL model.

Quantitative genes were detected for both STB and stripe rust. For STB resistance, five resistance QTL were identified after field testing in five environments. The Five QTL had small effect sizes ranging between 3.5 to 9.0, explaining 52.5% of the variance observed for STB resistance. For stripe rust resistance, four resistance QTL were identified after field testing in five environments. Two QTL (on chromosomes 1AS and 2AS (*Yr17)* with moderate effect sizes between 9.0 to 12.0 were identified, explaining 59.2% of the variance. Three of the four QTL (Yr1A, Yr2A/2D and Yr4D) for stripe rust resistance identified in the field trials are most likely high-temperature adult plant (HTAP) resistance genes. Four QTL were also identified in the growth chamber studies, two using the bulk inoculum and two different QTL using the single isolate inoculum. These pairs of QTLs provided a significant reduction of severity when found in the same plant. QTL detected in the field were not identified in the growth chamber or vice versa. This suggests that the QTL on chromosomes 1AS, 2AS/2DS and 4DL identified in the field trials may be high-temperature adult plant (HTAP) resistance genes and the four QTL that conferred stripe rust resistance on seedlings grown in the growth chamber may be all stage resistance genes. However, it is also possible that the seedling QTL with relatively small effects were masked by effects of the adult plant QTL in the field. Identifying different QTL using the bulk inoculum versus the single isolate in the growth chamber study suggests that these genes may be race-specific.

For both diseases, there was one QTL with a more significant effect than the others. The most effective QTL for STB was QStb-7D, which is probably one of the reasons why Foote is considered moderately resistant to STB. In the region of chromosome 7DL associated with QStb-7D there are four known resistance genes. These four genes could be functional against STB and provide durable resistance. In the literature, chromosome 7DL is one of the chromosomes with the most identified QTL associated with disease resistance in wheat. These QTL provide resistance to other diseases caused by other necrotrophic pathogens, such as Rhizoctonia root rot (*Rhizoctonia solani*)[6] and Septoria nodorum blotch (*Phaeosphaeria nodorum*)[7]. Further analysis is required to study the function of these four genes and their possible association with the QTL QStb-7D.

For stripe rust, the QTL with the largest effect was on 2A and could be associated with the resistance gene *Yr17,* most likely donated by Madsen. *Yr17* in wheat originated from the translocation 2NS/2AS

from *Triticum ventricosum*[8]. *Yr17* is one of the essential resistance genes in wheat worldwide. Therefore, *Yr17* has been widely used in wheat breeding programs[9], producing numerous cultivars with *Yr17* around the world. Cultivars with *Yr17* produce an intermediate infection type of 4-6, depending on the isolate inoculated and temperature[10]. Virulence to *Yr17* was the major cause of stripe rust epidemics in many countries in the last 20 years[10]. In the U.S., virulence to *Yr17* increased gradually from 27% to 94% between 2000 to 2007[11]. Consequently, *Yr17* cannot alone account for the durability of stripe rust resistance in cultivars such as Madsen. As previously concluded in other studies of stripe rust on wheat, the durability of stripe rust resistance for cultivars carrying *Yr17* is caused by interactions with other QTL.

During the development of the linkage map, it was observed that some markers on 2A, 2B, and 2D had similar segregation patterns for stripe rust resistance. For stripe rust, a more in-depth study was made of chromosomes 2A, 2B, and 2D using multiple interval mapping. This study identified alternative QTL locations on both 2AS and 2DS, with two groups being identified. The first group was markers linked to *Yr17* and the second group was markers not linked to *Yr17.* The markers in the second group (not linked to *Yr17*) could be on chromosome 2A or 2D. The suggestion that *Yr17* is linked to another QTL for adult plant resistance is supported by Liu et al.[5], and the additional QTL on 2D is supported because the 2D marker is close to the leucine-rich domain superfamily gene on that chromosome.

The highest level of reduction of disease severity for each of the two diseases occurred when all identified QTL were combined. This supports the concept of pyramiding resistance genes to maximize the level of disease resistance and increase the durability of resistance. A more significant number of QTL may be required for STB owing to their smaller individual effects and the high evolutionary potential of the causal pathogen to adapt to resistance. The regions on chromosomes 3BL, 6BS, and 7DL have the highest number of relevant QTL for STB resistance. Identifying the region on 7DL in Foote with multiple resistance genes may help develop new cultivars with durable resistance genes to STB. The combination of *Yr17 with* the QTL detected on 1AS, and other genes identified in this study should provide durable effective resistance to stripe rust. As durability of stripe rust resistance has often been associated with combinations of seedling and adult plant resistance genes, combining genes identified at different growth stages in this study may further contribute to durability of wheat stripe rust resistance.

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