AN ABSTRACT OF THE DISSERTATION OF

Maria Dolores Vazquez for the degree of Doctor of Philosophy in Crop Science presented on March 11, 2014

Title: Multi-location Analysis for the Identification of Quantitative Trait Loci Underlying Disease Resistance Against *Cephalosporium gramineum* and *Puccinia striiformis* f. sp. *tritici* by Linkage Mapping in Wheat (*Triticum aestivum* L.)

Abstract approved:

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Robert S. Zemetra Christopher C. Mundt

Wheat (*Triticum aestivum* L.) is one of the major crops produced in the U.S. Pacific Northwest, a region known for its wheat production for international export. Wheat production in the region is threatened by diseases such as Cephalosporium stripe and stripe rust. Cephalosporium stripe is a vascular wilt disease of wheat caused by the persistent soil-borne fungus and suspected necrotrophic pathogen *Cephalosporium gramineum* Nisikado & Ikata, and is a recurring disease in many localities when susceptible cultivars are grown. Stripe rust, a foliar disease of wheat caused by the air-borne biotrophic fungus *Puccinia striformis* f. sp. *tritici* is a disease present in every region around the world where commercial wheat is grown. Attaining durable resistance to stripe rust would greatly benefit wheat producers in the region. Combining stripe rust

resistance with resistance to other diseases, such as Cephalosporium stripe, is challenging. Wheat cultivars with high levels of resistance to several diseases are favorable candidates for genetic studies to determine the inheritance of resistance and facilitate the development of a method to genotypically select for disease resistance.

Two populations of recombinant inbred lines were developed from 'Tubbs'/'NSA-98-0995' (TxN) and 'Einstein'/'Tubbs' (ExT) with population sizes of 271 and 259 $F_{(5:6)}$, respectively. Tubbs is susceptible to stripe rust and Cephalosporium stripe while Einstein and NSA-98-0995 demonstrate moderate to high resistance to both diseases. Both populations were assessed across seven environments (combinations of locations and years) for stripe rust resistance under natural infection and four environments for Cephalosporium stripe resistance under artificial inoculation. The populations were mapped using diversity array technology (DArT) and simple sequence repeat (SSR) markers for quantitative trait loci (QTL) analysis. Results for Cephalosporium stripe resistance was quantitatively inherited with several QTL detected (>5), including some QTL in the same chromosome location in both populations. For stripe rust resistance, seven QTL were identified in the TxN population, suggesting quantitative resistance contributed by several minor genes. In the ExT population two QTL with major effects and with epistatic interactions between them were identified. One of them, a major QTL from Tubbs on chromosome 2AS that may be Yr17, was not expressed in the TxN population or in Tubbs, perhaps owing to suppressor(s). Expression of the 2AS QTL in the ExT population may be due to interaction with the QTL on chromosome 6AL from the resistant parent Einstein or to any other gene in the background of the population.

QTL on chromosomes 2AS, 5AL, and 6BS were associated with resistance to both Cephalosporium stripe and stripe rust.

These results highlight a complex set of interactions among major genes, minor genes, the presence of different stripe rust races, epistasis, genetic background, and possibly a suppressor of resistance. Results from this study are expected to assist in selecting molecular markers to genotypically select for resistance to these diseases, improving the chances of developing wheat cultivars with durable resistance to both diseases in the future.

# Multi-location Analysis for the Identification of Quantitative Trait Loci Underlying Disease Resistance Against *Cephalosporium gramineum* and *Puccinia striiformis* f. sp. *tritici* by Linkage Mapping in Wheat (*Triticum aestivum* L.)

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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.

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Maria Dolores Vazquez, Author

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## CONTRIBUTION OF AUTHORS

Dr. Robert Zemetra and Dr. Chris Mundt advised all aspects of the research conducted while providing valuable feedback throughout the research project and were actively involved in the preparation and improvement of the chapters. Dr. James C. Peterson and Dr. Jari von Zitzewitz designed and developed the populations for this study. Ms. Kathryn Sackett, Mr. Adam Heesacker, Dr. Jeff Leonard, and Dr. Xianming Chen (USDA-WSU) coordinated field-testing, DNA extraction, genotyping, and conducted disease evaluations for some locations. Dr. Alfonso Cuesta-Marcos provided valuable feedback for the QTL and association analysis.

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Dedication

To my father Sergio Arturo Vazquez Moguel,
porque yo soy lo que tu me enseñaste a ser. Gracias por estar orgulloso de mi.
Te Quiero Papa

and to

Elena Sanchez-Olguin
“Porque los amigos son la familia que uno escoge”

Que la luz los guie en ese camino que es parte de la vida y Dios nos permita volvernos a ver asi sea en sueños.

# CHAPTER 1

# GENERAL INTRODUCTION

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# GENERAL INTRODUCTION

## Wheat (*Triticum sp)* and wheat production

Cereals are the world's most important sources of food for direct human consumption and livestock feed. Wheat is one of the top three staple crops in the world. The global total wheat production in 2012 was 786,408,310 metric tonnes. In the United States of America (USA), wheat is a fundamental food crop for the security and economy of the country. Total wheat production in 2012 in the United States was 61,755,240 metric tonnes, around 10% of the world wheat production. The USA is one of the world's biggest exporter (FAOSTAT, 2013; USDA, ERS, 2013).

The wide genetic adaptability of wheat allows production in different regions around the world under different climatic conditions. Appropriate genetic adjustments allow wheat to avoid winter damage from cold temperatures, and escape drought and high temperatures in the warmer summer months. Timing of planting is environmentally determined but most of the growth components, which vary between different varieties, are under genetic control (Snape et al., 2001). A complex group of genes in any wheat genotype influences flowering and maturity. Those gene groups are vernalization (Vrn genes), the exposure to low, non-freezing temperatures; photoperiod response (Ppd genes), which is the plant response to daylight-length, and earliness per se (Eps genes) that regulates flowering time independent of environment (Braun and Sãulescu, 2002; Kamran et al., 2013; Streck et al., 2003 and Worland and Snape, 2000). Based on

vernalization response, wheat varieties can be broadly divided into spring, winter and an intermediate group known as facultative (Worland and Snape, 2000).

Wheat is a polyploid with two main species in commercial production, durum wheat (*Triticum turgidum* L. var *durum*) and common wheat (*Triticum aestivum* L). Common wheat is a hexaploid, with 42 chromosomes and genomes designated as ABD. The origin of common wheat is considered to be in northwest Iran or northeast Turkey as a result of a hybridization of tetraploid wheat *Triticum turgidum* (AB genome), and diploid *Aegilops tauschii*, the donor of the D genome (Feldman, 2000; Bernardo, 2002).

**Wheat (*Triticum sp*) and Cephalosporium stripe disease (*Cephalosporium gramineum*)**

Cephalosporium stripe is the name for a vascular wilt disease of wheat (*Triticum aestivum* L.) and other grasses. The pathogen responsible is *Cephalosporium gramineum* Nisikado & Ikata, a soil-borne fungus (Nisikado et al., 1934). It has been suggested by Baaj and Kondo (2011) that there are at least four evolving populations of *C. gramineum*, with no evidence of substantial pathogenicity variability (Cowger and Mundt, 1998). The primary source of inoculum for Cephalosporium stripe is infected crop debris that remains after harvest, although seed could be an important source of inoculum in some situations (Lai and Bruehl, 1967; Murray, 2006).

Cephalosporium stripe was first reported in Japan in 1931 (Nisikado et al., 1934) and has been reported in the UK, Canada, and other regions of Europe and East Asia (Richardson and Rennie, 1970) . In the USA, it was discovered during the mid-1950's (Bruehl, 1956) and, currently, it is known to be a recurring disease in the wheat-growing

regions of Kansas, Montana, Idaho, eastern Washington and Oregon (Bockus and Sim, 1982; Bockus et al., 1994; Morton and Mathre, 1980a; Quincke et al., 2012). Under conducive conditions, the disease can negatively impact yield, with important economic losses to growers, largely due to reduced seed weight and seed number per head (Johnston and Mathre, 1972; Richardson and Rennie, 1970). The method used for evaluating disease severity in Cephalosporium stripe is by visual symptoms at the late stage of the disease, although a polymerase chain reaction (PCR)-based assay for diagnosis of the presence of *C. gramineum* infection in wheat is under evaluation (Baaj and Kondo, 2011; Klos et al., 2012) .

The fungus over-winters in its sporodochium-producing saprophyte stage (*Hymenula cerealis*), colonizing dead substrate (Lai and Bruehl, 1967). The asexual fruiting bodies, sporodochia, release to the soil numerous conidia that under favorable weather conditions will infect the host cereal or grass (Howell and Burgess, 1969; Wiese and Ravenscroft, 1978). Infection of plants begins when inoculum is abundant in the fall and continues through winter. Conidia enter the xylem through damaged areas when they are washed down into the root zone, or by active penetration leading to colonization of the crown tissues and vascular bundles (Douhan and Murray, 2001; Mathre and Johnston, 1975). There is evidence suggesting that, under Pacific Northwest conditions, crown roots appear to be the most important infection sites. Cool weather and wet soil are favorable conditions for the pathogen to colonize the host. Low soil pH (4.5-5.5) and high moisture further exacerbate the symptoms of the disease (Anderegg and Murray, 1988; Blank, 1998; Bockus and Claassen, 1985; Douhan and Murray, 2001; Love and Bruehl, 1987; Stiles and Murray, 1996).

Diseased wheat plants present chlorotic leaf striping, although severe symptoms such as wilting, leaf necrosis, stunting, shorter culms, small heads and prematurely ripening heads (whiteheads) are seen in the spring and summer after abundant colonization by *C. gramineum* (Johnston and Mathre, 1972; Morton and Mathre, 1980a; Morton et al., 1980). *C. gramineum* produces a toxin (Graminin A) that under laboratory conditions induced chlorosis and browning of leaves and vascular tissues of wheat cuttings (Kobayashi and Ui, 1979; Rahman et al., 2001). In addition it is reported that the pathogen produces a glucopolysaccharide that has been hypothesized as a major contributor to vascular dysfunction (Pool and Sharp, 1969) although Vanwert and Fulbright (1986) stated that neither the toxin nor the polysaccharide are major contributors to the pathogenicity of *C. gramineum* in wheat.

Cultural controls for Cephalosporium stripe include delayed planting, burning of crop residue, deep plowing, crop rotation, and the addition to lime to the soil to increase soil pH. However these methods are not economically or environmentally feasible and no chemicals are registered for control of the disease (Bockus and Claassen, 1985; Martyniuk et al., 2006; Raymond and Bockus, 1983). There is no complete resistance in commercial wheat cultivars, although the use of moderately resistant cultivars reduces the amount of inoculum and hence the disease severity in the next planting season (Morton and Mathre, 1980b; Mundt, 2002; Shefelbine and Bockus, 1989). In addition it has been reported that progeny from the cross between the winter wheat relative *Thinopyrum ponticum (Agropyron elongatem)* and *Triticum aestivum* present moderate to high resistance to soil diseases, including Cephalosporium stripe (Cox et al., 2002; Mathre et al., 1985). Currently, the method available to identify resistance in breeding programs is

based on field screening and is time consuming and spacelimited given that field testing requires artificial inoculations at planting.

**Wheat (*Triticum sp*) and stripe rust disease (*Puccinia striiformis* f. sp. *tritici*)**

Rusts have been a problem for small grain cereals probably since domestication. According to the USDA Agricultural Research Service (ARS), wheat rusts are the most common diseases in the USA and worldwide, causing millions of dollars in losses annually in all wheat market classes (McIntosh, 2009; USDA, ARS, 2013). Rust pathogens adapt to many different types of environments, evolve rapidly, and the airborne spores spread quickly over long distances (Hovmøller, 2001). Rust fungi are known as specialized pathogens; each rust species is divided into specialized forms having a specific host genotype to attack under particular environmental conditions. Yield losses due to wheat rusts can be substantial depending on the crop development stage, the level of resistance, as well as the environmental conditions (McIntosh, 2009). The genus *Puccinia* includes three important species of rust fungi that attack wheat; *Puccinia striiformis* f. sp. *tritici* is one and causes stripe (yellow) rust that occurs mainly in high rainfall, cooler regions (McIntosh, 1998). The characteristic symptom is the development of yellow uredinia along upper leaf veins with the appearance of yellow stripes. These uredinia release wind-dispersed spores known as urediniospores that can be wind-blown over long distances. It is an obligate fungus, which means it is completely dependent on living tissue for reproduction (Brown and Hovmøller, 2002). The fungus can develop virulent pathotypes rapidly to infect wheat cultivars with new sources of resistance (Hovmøller, 2001). The mechanisms by which new genetic variants are created

in *P. striiformis* f. sp. *tritici* are not fully understood, but according to a recent discovery, sexual recombination and mutation from avirulence to virulence could occur (Zheng et al., 2013). New strains have recently have been reported proliferating in warmer and drier areas (Hovmøller et al., 2008; Jin, 2012; Milus et al., 2009).

In the United States, *P. striiformis* f. sp. *tritici* can overwinter and over-summer in the region of eastern Washington, northern Idaho, and northeastern Oregon. This region has its own local inoculum, but is also influenced by inoculum from outside of the region. The central and northern areas of the Great Plains (Kansas, Nebraska, South Dakota and North Dakota) usually receive inoculum of stripe rust from the southern Great Plains (Texas and Louisiana). The timing, type, and direction of winds determine the earliness, scale, and development rate of epidemics of stripe rust (Chen, 2005).

**Genetics of Pathogen-Host Resistance**

Two types of resistance have been recognized in plant-pathogen interactions, first by van der Plank (1968) and then by Parlevliet (2002). These are designated as vertical or race-specific resistance and horizontal or non-race specific resistance (Parlevliet, 2002). Vertical resistance corresponds to single genes with major effects that are simply inherited and follow the "gene-for-gene" hypothesis (Flor, 1971) in which an interaction between a dominant resistance allele (R gene) in the host plant and a dominant avirulance allele (Avr gene) of the pathogen induces a rapid activation of a defense mechanism often called the hypersensitive response (Parlevliet, 2002). Plants expressing horizontal resistance are believed to be under different mechanisms based on the additive effects of

some to several genes with small effects. This resistance may or may not be the same as those governing the hypersensitive reaction (Poland et al., 2008).

Cloning of major genes for resistance has revealed two distinct pathogen-sensing mechanisms to detect pathogens and to elicit resistance responses. In the first category, conserved microbial elicitors called pathogen-associated-molecular patterns (PAMPs) are recognized by receptor proteins called pattern recognition receptors (PRRs) located in the external face of the host cell. PAMPs are typically essential components of whole classes of pathogens, such as flagellin, chitin and lipopolysaccharides. Plants also respond to endogenous molecules released by pathogen invasion, such as cell wall or cuticular fragments called danger-associated molecular patterns (DAMPs). Stimulation of PRRs leads to PAMP-triggered immunity (PTI). PTI is generally effective against non-adapted pathogens in a phenomenon called non-host resistance. Extracellular recognition by PRRs fall into one of two receptor classes: transmembrane receptor kinases and transmembrane receptor-like proteins. Although the PAMP concept encompasses the idea that all PAMPs should be recognized by all species, this has been found to not always be the case (Dodds and Rathjen, 2010; Michelmore et al, 2013).

The second category involves recognition by intracellular receptors of pathogen virulence molecules called effectors; this recognition induces effector-triggered immunity (ETI). Recognition events are mostly mediated by a class of receptor proteins that contain nucleotide-binding (NB) and leucine-rich-repeat (LRRs) domains. Plant NB-LRR proteins confer resistance to diverse pathogens, including fungi, oomycetes, bacteria, viruses and insects. There is high diversification of ETI receptors and pathogen effectors within and between species, whereas some PRR functions are conserved widely across

families. ETI is active against adapted pathogens and is qualitatively stronger and faster and often involves a form of localized cell death called the hypersensitive response (HR). Generally, PTI and ETI give rise to similar responses but vary in magnitude; these include a rapid influx of calcium ions from external stores, a burst of active oxygen species, activation of mitogen-activated protein kinases (MAPKs), reprogramming of gene expression, deposition of callosic cell wall appositions at sites of attempted infection and, often, localized cell death (HR) (Dodds and Rathjen, 2010; Jones and Dangl 2006; Michelmore et al, 2013).

Some of the downstream responses to ETI and PTI are better understood than the signalling pathways. The salicylic acid (SA) and jasmonic acid (JA) – ethylene (ET) hormone pathways are important regulators of defense-gene expression. These two pathways act antagonistically to some extent but still with considerable overlapping between them. The SA pathway involved in resistance to biotrophic pathogens, while the JA–ET pathway is involved in responses to necrotrophic pathogens and chewing insects (Dodds and Rathjen, 2010; Métraux, 2001; Senthil-Kumar and Mysore, 2013; Oostendorp et al., 2001).

*Puccinina striiformis* f. sp. *tritici* is an obligate fungus (biotrophic), which means it is completely dependent on living tissue for reproduction, while *Cephalosporium gramineum* is a saprophyte and may be necrotrophic, which means it gets its nutrients from dead tissue. The genetic interaction of necrotrophic pathogens with a host major gene is different because, while effective R genes provide resistance to the biotrophic pathogen eliciting programmed host death cell, necrotrophic pathogens exploit this interaction by causing an overreaction, killing the plant to extract their nutrients. The

ability to induce necrosis is central to successful virulence of necrotrophs. Toxins from necrotrophs are comparable to effectors in their ability to suppress immune responses, to induce immune-like responses, to target host proteins, and to enhance disease through the manipulation of host physiology, as well as in their intraspecific variation (Guest and Brown, 1997; Mengiste, 2012).

**Breeding for resistance and quantitative trait mapping**

Breeding for disease resistance has been a main goal in crop improvement programs. Breeding and releasing cultivars with effective genetic resistance is a preferred means of disease control, along with applying proper cultural practices. Various aspects contribute to the development of new wheat cultivars with increased resistance to diseases, such as understanding pathogen biology, characterization of pathogen avirulence, identification of plant disease resistance genes as resistance sources, and obtaining information on wheat genetic diversity and relationships among elite experimental lines and cultivars (Kaur et al., 2008; Mahmood et al., 2004).

The use of molecular approaches, particularly molecular markers, has allowed better characterization of the genetic resistance diversity in wheat germplasm (Hulbert and Pumphrey, 2014). The availability of high-throughput molecular markers linked to resistance genes and their genetic location could make the selection process faster and more cost effective. Molecular markers are known to be useful in the process of identification of disease resistance genes. Those markers are based on differences in the DNA sequence of individuals and provide guide points that are useful to pinpointing the location of specific genes. Simple sequence repeat (SSR), or microsatellite markers, rely

on the use of PCR, a technique used to amplify a sequence piece of DNA, generating as a product millions of copies of that specific DNA piece. These types of markers work by identifying primers that flank a tandem repeat. A tandem repeat is a repetitive DNA sequence made up of very short motifs with a size of 1 to 6 base pairs (Weising et al., 2005). Tandem repeat sequences constitute 80% of the wheat genome, and are widely distributed across the genome, and therefore highly abundant. Diversity Array Technology (DArT) it is a high-throughput, robust system with minimal DNA sample requirements capable of providing comprehensive genome coverage without any DNA sequence information needed. DArT markers are based on a microarray hybridization technology that detects the presence versus absence of individual DNA fragments in a genomic representation of an organism or a population of an organism (Akbari et al., 2006; Jaccoud et al., 2001). A SNP is a DNA sequence variation occurring when a single nucleotide in the genome differs between members of a biological species or paired chromosomes in an individual (Gupta et al., 2001). It is believed to be the most abundant marker type, promising a nearly unlimited supply of markers (Rafalski, 2002). The genomic distribution of SNPs is not homogenous; SNPs usually occur in non-coding regions more frequently than in coding regions. Due to the increased amount of sequence information and the determination of gene function in cereals the use of these bi-allelic molecular markers is increasing (Trick et al., 2012).

The above-mentioned markers are commonly used for genetic mapping. Genetic mapping, also known as linkage mapping, is one of the various applications of molecular markers in any species. It refers to the determination of the relative position of markers and of the distance between them (Semagn, 2006). A genetic map indicates the position

and relative genetic distance between markers along a chromosome. Genetic maps are used to identify regions within genomes associated with a particular quantitative trait, known as quantitative trait loci (QTL). Important agronomic traits such as yield, quality and some forms of disease resistance are under the control of multiple genes and they are known as quantitative or polygenic traits. QTL mapping involves a systematic search for linkage disequilibrium between QTL and genetic markers. This association analysis therefore requires both marker information and phenotypic values measured for each individual in the mapping population (Silva et al., 2012; Xu, 2002).

Mapping of QTL is of increasing importance in research and breeding programs, since it can help to identify the respective roles of specific resistance loci versus partial resistance genes and the interactions between the genes and the environment. It also is expected to serve breeders as a tool for marker-assisted selection of complex disease resistance traits; although progress in characterizing genes underlying QTL has been slow (Kaur et al., 2008; Salvi and Tuberosa, 2005). It is common for QTL with partial effects to not be detected consistently across environments, and very little is known about additive and epistatic interactions among rust resistance QTL (Singh et al. 2013; Yu et al. 2011). Environmental conditions, pathogen population structure, timing of epidemic development, and genetic background all seem to significantly influence the expression of partial resistance phenotypes. None of the rust R genes identified to date have properties of the characterized pattern recognition receptors (PRR) that detect PAMPs. This is probably because these more conserved functions are not typically variable in cereal species. R genes that detect conserved effectors may provide more durable resistance (Hulbert and Pumphrey, 2014)

**Dissertation objectives**

The main objective of this research was to identify QTL linked to stripe rust and Cephalosporium stripe resistance in wheat, both important diseases of wheat in the USA Pacific Northwest. This objective was accomplished through detection of chromosomal regions for resistance to Cephalosporium stripe and stripe rust in two winter wheat populations and one winter wheat germplasm diverse set.Phenotypic data were collected from field trials over several environments (combinations of years and locations) with the aim to understand the genetic basis for resistance to Cephalosporium stripe and stripe rust Chromosomal locations of QTL were mapped and compared among populations and with previous QTL studies  to identify common regions of interest.

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# CHAPTER 2

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### Abstract

Cephalosporium stripe is a vascular wilt disease of winter wheat (*Triticum aestivum* L.) caused by the soil-borne fungus *Cephalosporium gramineum* Nisikado & Ikata. In the USA it is known to be a recurring disease when susceptible cultivars are grown in the wheat-growing region of Midwest and Pacific Northwest. There is no complete resistance in commercial wheat cultivars, although the use of moderately resistant cultivars reduces the disease severity and the amount of inoculum in subsequent seasons, making it important to breed for resistance to Cephalosporium stripe. The goal of this study was to detect and to validate chromosomal regions for resistance to Cephalosporium stripe in two winter wheat populations. Field inoculation was performed and Cephalosporium stripe severity was visually scored as percent of prematurely ripening heads (whiteheads) per plot. 'Tubbs'/'NSA-98-0995' and 'Einstein'/'Tubbs', each comprising a cross of a resistant and a susceptible cultivar, with population sizes of 271 and 259 F(5:6) recombinant inbred lines, respectively, were phenotyped across four environments and mapped with diversity array (DArT) technology and simple sequence repeat (SSR) markers, covering polymorphic regions of ≈1,480 and 1,117 centimorgans, respectively. Phenotypic data revealed significant ($P < 0.01$) genotypic differentiation for Cephalosporium stripe among the recombinant inbred lines and genotype-environment and QTL-environment interactions. Entry-mean heritabilities ($h^2$) for Cephalosporium

stripe were 0.80 for 'Tubbs'/'NSA-98-0995' and 0.30 for 'Einstein'/'Tubbs'. In the quantitative trait loci (QTL) analysis, six and nine QTL were found, explaining in total, around 30% and 50% of the phenotypic variation in 'Tubbs'/'NSA-98-0995' and 'Einstein'/'Tubbs', respectively. The largest effect QTL, from ‘NSA-98-0095’ and ‘Einstein’ on chromosome 5AL1 linked to marker gwm29, was detected consistently across environments in both populations, making it a good candidate for use in marker assisted selection. Several QTL with smaller effects were identified in both populations on chromosomes 5AL, 6BS, and 3BS, along with others QTL identified in just one population. These results indicate that resistance to Cephalosporium stripe in both mapping populations was of a quantitative nature. Epistatic interactions were detected in both populations among the identified QTL in 5AL1 and 6BS as well as 5AL1 and 4DS. In both cases the alleles come from the resistant parents.

**Introduction**

Cephalosporium stripe is a vascular wilt disease of winter wheat (*Triticum aestivum*) and other grasses that is caused by the soil-borne fungus *Cephalosporium gramineum* Nisikado & Ikata (Nisikado et al., 1934). Characteristic symptoms include leaves with one-to-three broad, yellow-to-brown stripes that extend to the leaf sheaths and stems. Severe symptoms such as wilting, leaf necrosis, stunting, shorter culms, small heads and prematurely ripening heads (whiteheads) are seen in the spring and summer after abundant colonization by *C. gramineum* (Johnston and Mathre, 1972; Morton and Mathre, 1980a; Morton et al., 1980). Under the appropriate environmental conditions, the disease can negatively impact yield with important economic losses to growers, largely due to reduced seed weight and seed number per head (Johnston and Mathre, 1972; Richardson and Rennie, 1970). *C. gramineum* exists in at least four evolving populations (Baaj and Kondo 2011), with no evidence of substantial pathogenic variability (Cowger and Mundt, 1998). The primary source of inoculum for Cephalosporium stripe is infected crop debris that remains after harvest, although seed may be an important source of inoculum in some situations (Lai and Bruehl, 1967; Murray, 2006). Cephalosporium stripe is favored by short crop rotations, early fall planting, presence of crop debris on the soil surface, cool and wet fall seasons, and root damage caused by soil freezing (Mundt, 2010).

Cephalosporium stripe was first reported in Japan in 1931 (Nisikado et al., 1934) and has been found in the UK, Scotland, Canada and other regions of Europe and East Asia (Richardson and Rennie, 1970). In the USA, it was discovered during the mid-1950's (Bruehl, 1956) and now it is known to be a recurring disease in the wheat-growing

region of Midwest and Pacific Northwest when susceptible cultivars are grown (Bockus and Sim, 1982; Bockus et al., 1994; Morton and Mathre, 1980a; Quincke et al., 2012). Cephalosporium stripe is an emerging problem in Scotland under conditions of short rotations, reduced tillage, and wet soils (Oxley, 2009).

Cultural controls for Cephalosporium stripe include delayed planting, burning of crop residue, deep plowing, crop rotation, and the addition of lime to increase the soil pH, but these practices all have significant economic and/or environmental impacts, and no chemicals are currently registered for control of the disease (Bockus and Claassen, 1985; Martyniuk et al., 2006; Raymond and Bockus, 1983). There is no complete resistance in commercial wheat cultivars, although the use of moderately resistant cultivars reduces disease severity in the current season and the amount of inoculum in subsequent seasons (Morton and Mathre, 1980b; Mundt, 2002; Shefelbine and Bockus, 1989). In addition, it has been reported that progeny from crosses between the winter wheat relative *Thinopyrum ponticum (Agropyron elongatem)* and *Triticum aestivum* provide moderate-to-high resistance to Cephalosporium stripe (Cox et al., 2002; Mathre et al., 1985).

Field methods currently used to identify resistance to Cephalosporium stripe in breeding programs are time-consuming and space-limited. The identification of molecular markers associated with Cephalosporium stripe resistance would allow for genotypic selection of resistant genotypes. In a previous study, Quincke et al., (2011) performed a QTL analysis on a recombinant inbred line (RIL) population with two commonly grown Pacific Northwest USA winter wheat cultivars that varied for resistance to Cephalosporium stripe. They identified seven QTL for resistance, indicating that molecular markers may be useful for the identification of lines resistant to *C.*

*gramineum*. The present study was undertaken to further explore the genetic resistance and identify QTL linked to Cephalosporium stripe resistance under artificially inoculated field conditions, using two mapping populations derived from crosses between European and Pacific Northwest USA winter wheat parents and see if chromosomal regions associated to resistance go across populations.

## Materials and Methods

### Mapping populations

Two populations of $F_5$-derived $F_6$ recombinant inbred lines (RILs) developed at Oregon State University were studied. The first population, consisting of 271 RILs, was derived from a cross between an awnless, hard red winter wheat experimental line 'NSA-980995' (Limagrain, UK), with a moderate-to-high level of resistance to Cephalosporium stripe, and the awned, soft white winter wheat cultivar 'Tubbs' (PI 651023), which is highly susceptible to Cephalosporium stripe. The second population, consisting of 259 RILs, was derived from a cross between the awnless, hard red winter wheat cultivar 'Einstein' (Limagrain, UK) with a high level of resistance to Cephalosporium stripe and the cultivar Tubbs. The initial crosses for both populations were done in 2003. The cultivar Einstein, bred by Nickerson Seeds and commercialized by Limagrain UK, is widely grown in Western Europe and has the pedigree (NHC 49/UK Yield Bulk) x (Haven/(Moulin/Galahad)) (Limagrain, 2013). Tubbs is a cultivar released in 2000 that was widely grown in the Pacific Northwest until it became susceptible to stripe rust (*Puccinia striiformis*), and has the pedigree Madsen/Malcom (USDA-AMS, 2009). NSA-

98-0995 is an experimental line developed by Limagrain, UK, with no publically available pedigree.

Plant DNA extraction and genotyping

For both populations, DNA of parental and $F_5$-derived progeny was extracted from young leaves of greenhouse-grown plants using the DNeasy 96 Plant Kit (QIAGEN Science, Maryland, USA) Group). DNA concentration was tested using NanoDrop ND-1000 UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc. Wisconsin, USA.). A final volume of 15 ng/ul was sent to Triticarte Pty. Ltd Canberrra, Australia to be genotyped with DArT (Diversity Array Technology) markers (Akbari et al., 2006). Additional simple sequence repeat (SSR) markers were screened for polymorphism in the Tubbs x NSA-98-0995 (TxN) and Einstein x Tubbs (ExT) populations in facilities at the USDA ARS Wheat Genetics, Quality, Physiology and Disease Resistance Unit at Pullman, WA, USA, using approximately 50 ng genomic DNA extracted from young leaves at Oregon State University.

Map construction and molecular analysis

For the populations used in this study, genotypic data were used to create the genetic linkage map with the software JoinMap v.4.0. (Van Ooijen, 2006). Genetic distances were calculated using the Haldane function (Haldane, 1919). For each linkage group, the best marker locus order was determined using the maximum likelihood in Join Map v.4.0. The TxN map was constructed with 229 markers, 13 SSR and 216 DArT comprising 49 linkage groups, representing areas from all chromosomes of common wheat except 4D. The total genome length covered was 1481 cM. The ExT map was constructed with 198 markers, 18 SSR and 180 DArT comprising 32 linkage groups,

representing areas from all chromosomes of common wheat but 6D and 7D. For both populations, final linkage groups were assigned to each chromosome with data provided by Triticarte wheat map alignment (Triticarte, 2013) and maps available on the database GrainGenes 2.0 (2013).

Field trials and phenotyping

The $F_6$-derived seed harvested from the greenhouse was used to establish plots in the field. For each population, the experimental design used was a randomized complete block with two replications. The parental cultivars, the RIL progeny, and two cultivar checks 'Stephens' (Kronstad et al., 1978) and 'Xerpha' (Jones et al., 2010) were included in the field trial. The cultivars Stephens and Xerpha were the high and low disease severity checks to Cephalosporium stripe, respectively. Experiments were conducted at the Columbia Basin Agricultural Research Center field station near Pendleton in 2010, 2011, and 2012 and in Moro, OR in 2010. Both locations are in semi-arid wheat producing areas of the Columbia Plateau with mean annual precipitation of 279 mm in Moro and 406 mm in Pendleton.

Plots consisted of two rows 2.5 m long that were later trimmed to 1.8 m long post-heading and prior to collecting phenotypic data. Fertilization and weed control were appropriate for commercial winter wheat production in eastern Oregon. Spring application of fungicide (Bumper® Makhteshim Agan Industries, Ltd. Israel) was applied to avoid eyespot and stripe rust. For all locations, Cephalosporium stripe was established by artificial inoculation to ensure disease uniformity and high disease pressure. Before planting, oat kernels infested with *C. gramineum* (Mathre and Johnston, 1975) were added to the seed envelopes in an amount equal to the volume of wheat seed. Planting

dates were in early September to increase chances of high disease incidence. One reading was taken for each location in each year. Cephalosporium disease incidence was recorded every year during the last week of June on a plot basis by visual estimation of the percentage of tillers that were ripening prematurely (whiteheads) (Mathre and Johnston, 1975; Quincke et al., 2012). The examination of lower stems and roots and observation of known check cultivars provided confidence that whiteheads were caused by *C. gramineum*. Tubbs and Stephens (the susceptible parent and susceptible check, respectively) showed above 40% whiteheads, usually two-three weeks after heading. Developmental state of the lines ranged from early milk to early dough (Zadoks 50-60). Presence of awns, heading date, and height were recorded for possible association with Cephalosporium stripe resistance. Presence of awns and height were recorded in Pendleton 2010.

QTL and statistical analyses

The square-root transformation of whitehead percentage on a plot basis was used to calculate analysis of variance and heritability, with the transformation being used to better satisfy the assumptions of analysis of variance. The PROC GLM procedure in SAS version 9.1.3 (SAS Institute Inc., 2000) was used to calculate least squares means and to determine effects for RILs, environment, and RILs x Environment. The PROC MIXED procedure was used to calculate family heritability ($h^2$) on a plot basis as $h^2 = \sigma^2_g / \sigma^2_p = \sigma^2_f/(\sigma^2_f + \sigma^2_e/r)$, where the variance components are $\sigma^2_g$, genetic variance; $\sigma^2_p$, phenotypic variance; $\sigma^2_f$, family variance; $\sigma^2_e$, error variance; and r, number of replications (Holland et al., 2010). For all tests, a probability level of $P<0.05$ was used.

The least squares means of the whitehead percentage on a plot basis as a

measurement for disease incidence was used to perform QTL analyses. QTL analysis was performed using composite interval mapping (CIM) in WinQTL Cartographer v.2.5 (Wang et al., 2007 ). For both populations, the QTL analyses were done individually per location and with the arithmetic mean across environments to deduce balanced values for each RIL. Likelihood-odds (LOD) thresholds for declaring statistical significance were calculated by 1000 permutations (Churchill and Doerge, 1994). Window size was set at 5 cM for each dataset section using forward and backward stepwise regression. The additive effects (a) and phenotypic variance coefficients of determination ($R^2$) for each QTL were estimated by CIM for each individual environment and for the arithmetic mean across environments. Epistatic interactions analyses were performed with multiple interval mapping (MIM) in WinQTL Cartographer v.2.5 using the option "Scan through QTL mapping results file" and later refined using the option "Testing for existing QTLs" under the AIC-based selection criteria (Silva et al, 2012, Wang et al., 2007).

## Results

Phenotypic values and statistical analysis

Significant disease pressure was obtained each year in each location for both populations. For the TxN population, the disease severity values for the susceptible parent Tubbs ranged from 31.2% whiteheads in Pendleton 2012 to 69.9% in Pendleton 2010. Disease severity for the resistant parent NSA-98-0995 ranged from 0.6% in Moro 2010 to 3.6% in Pendleton 2012. With the ExT population the resistant parent Einstein disease severity scores ranged from 2.1% in Pendleton 2012 to 24.7% in Pendleton 2010. The resistant and susceptible check cultivars Xerpha and Stephens, were present in both

populations and had disease severity scores range from 0.9 to 9.4% and 26.1 to 79.1% respectively (Table 2.1 and Table 2.2).

Disease severity values in each environment for RIL in both populations suggest that the response is that of a quantitative trait. When data are square root transformed, disease severity responses were normally distributed in all environments. All statistical analyses were performed using the transformation square root for whiteheads percentage values. *P*-values in ANOVA test for both populations suggest line by environment

Table 2.1 Mean disease severity values (% whiteheads on a plot basis) for the 272 recombinant inbred lines in the TxN population, the parental lines and two cultivar checks exposed to Cephalosporium stripe disease in four environments

| **TxN population** | **Varietal checks** | | **Parents** | | **RILs population** | |
|---|---|---|---|---|---|---|
| **Environment** | **Xerpha** | **Stephens** | **NSA 98-0995** | **Tubbs** | **Mean** | **Range** |
| **Pendleton 2010** | 5.8 | 76.7 | 2.2 | 64.8 | 25.9 | 0-90 |
| **Moro 2010** | 1.5 | 49.0 | 0.6 | 41.9 | 14.6 | 0-80 |
| **Pendleton 2011** | 6.0 | 41.3 | 1.8 | 39.0 | 12.8 | 0-75 |
| **Pendleton 2012** | 2.8 | 33.5 | 3.6 | 38.8 | 15.3 | 0-80 |

Table 2.2 Mean disease severity values (% whiteheads on a plot basis) for the 259 recombinant inbred lines in the ExT population, the parental lines and two cultivar checks exposed to Cephalosporium stripe disease in four environments

| **ExT population** | **Varietal checks** | | **Parents** | | **RILs population** | |
|---|---|---|---|---|---|---|
| **Environment** | **Xerpha** | **Stephens** | **Einstein** | **Tubbs** | **Mean** | **Range** |
| **Pendleton 2010** | 9.4 | 79.1 | 24.7 | 69.9 | 24.8 | 0-95 |
| **Moro 2010** | 0.9 | 76.7 | 2.8 | 44.1 | 10.0 | 0-80 |
| **Pendleton 2011** | 7.8 | 34.2 | 2.2 | 35.7 | 5.7 | 0-50 |
| **Pendleton 2012** | 1.1 | 26.1 | 2.1 | 31.3 | 7.0 | 0-70 |

interactions; significant differences among genotypes in each environment and that replication accounted for some of the variation (Table 3; Table 4). Heritabilities ($h^2$) were moderate-to-high depending on the environment with the exception of one location in the ExT population. For the TxN population, the values ranged from 0.5 to 0.6 per individual location and coefficients of variation (CVs) ranged from 29 to 45%. When TxN data for all locations were combined, the heritability was 0.8. In the case of the ExT population, heritabilities ranged from 0.3 to 0.7; coefficients of variation (CVs) ranged from 31 to 51% and when all data were combined, the heritability was 0.3. In the TxN population, there is little indication of transgressive segregation, as severity ratings of RILs generally fell within parental values, but this was not the case for the ExT population, where disease severity values for 25% of the population fell below the average value of the resistant parent Einstein, thus suggesting transgressive segregation (Fig 2.1 and Fig 2.2).

QTL analysis

*TxN population*

The six QTL contributing to disease resistance in the TxN population were identified in chromosomes 3BS, 5AL (two QTLs), C5BL, 6BS, and 7BS (Table 2.5). The QTL *QCsns.orz-6BS* and *QCsns.orz-5AL.1* were identified in every environment and in the arithmetic mean across environments (Table 2.5). The QTL in the short chromosome of 6B linked to marker wPt2726, showed the highest phenotypic variance response (5.5 to 21.7%). Also, QTL *QCsns.orz-5AL.1* linked to the marker gmw291, relative to the other QTL, explained a high percentage of the phenotypic variance (7.4 to 13.6). The

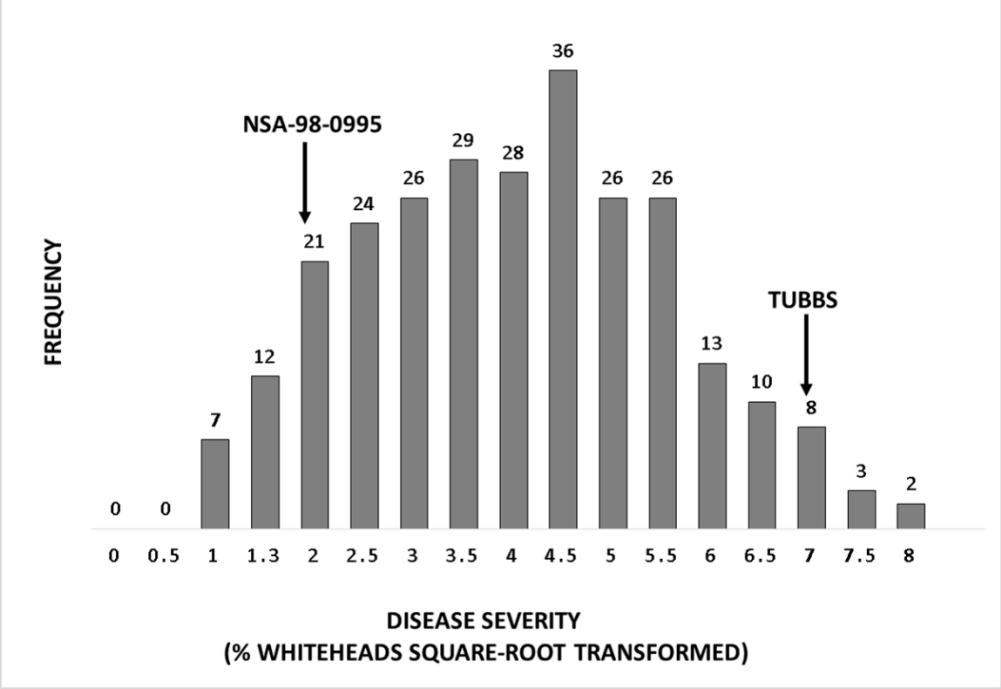


Fig 2.1 Recombinant inbred lines histogram of the TxN population with the arrows indicating the arithmetic mean of the percentage whiteheads for the parents. Numbers on top of the bars are frequency for each bin.

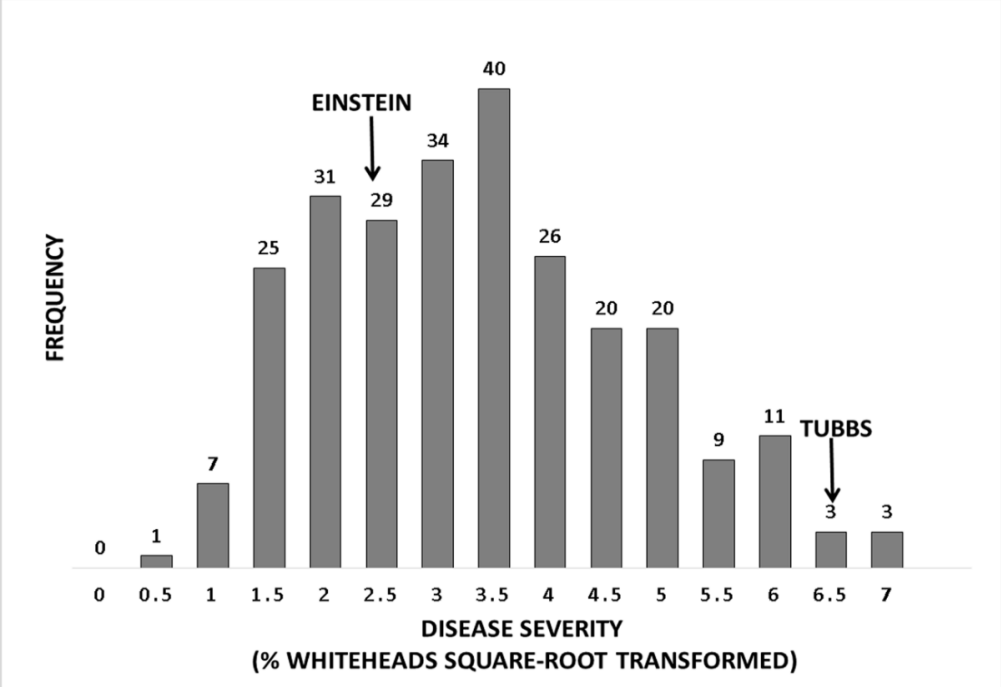


Fig 2.2 Recombinant inbred lines histogram of the ExT population with the arrows indicating the arithmetic mean of the percentage whiteheads for the parents. Numbers on top of the bars are frequency for each bin.

Table 2.3 Analyses of variance (Type III SS), coefficient of variation (CV), and heritability estimates ($h^2$) for disease severity in TxN population (272 recombinant inbred lines) exposed to Cephalosporium stripe disease in four and combined environments

| **TxN population** | **Source of variation** | |
|---|---|---|
| **Environment** | **DF** | **Mean square** |
| **Combined** | | |
| Rep (Env) | 3 | 115.3** |
| RIL | 271 | 18.4** |
| RIL x Env | 808 | 1.1** |
| Error | 1047 | 0.8 |
| CV (%) | 34 | |
| $h^2$ (SE) | 0.8 (±0.017) | |
| **Pendleton 2010** | | |
| Rep | 1 | 91.7** |
| RIL | 267 | 9.9** |
| Error | 263 | 1.6 |
| CV (%) | 29 | |
| $h^2$ (SE) | 0.6 (±0.037) | |
| **Moro 2010** | | |
| Rep | 1 | 13.8** |
| RIL | 270 | 6.5** |
| Error | 245 | 2.1 |
| CV (%) | 45 | |
| $h^2$ (SE) | 0.5 (±0.047) | |
| **Pendleton 2011** | | |
| Rep | 1 | 5.1** |
| RIL | 271 | 5.3** |
| Error | 270 | 1.2 |
| CV (%) | 40 | |
| $h^2$ (SE) | 0.6 (±0.037) | |
| **Pendleton 2012** | | |
| Rep | 1 | 4.1* |
| RIL | 271 | 4.2** |
| Error | 265 | 1.3 |
| CV (%) | 32 | |
| $h^2$ (SE) | 0.6 (±0.039) | |

* Significant at the 0.05 probability level
** Significant at the 0.01 probability level

Table 2.4 Analyses of variance (Type III SS), coefficient of variation (CV), and heritability estimates ($h^2$) in the ExT population (259 recombinant inbred lines) exposed to Cephalosporium stripe disease in four and combined environments.

| **ExT population** | **Source of variation** | |
|---|---|---|
| **Environment** | **DF** | **Mean square** |
| **Combined** | | |
| Rep (Env) | 3 | 93.2** |
| RIL | 258 | 8.6** |
| RIL x Env | 773 | 1.1** |
| Error | 1003 | 0.6 |
| CV (%) | 42 | |
| $h^2$ (SE) | 0.3 (±0.025) | |
| **Pendleton 2010** | | |
| Rep | 1 | 12.6** |
| RIL | 258 | 10.3** |
| Error | 256 | 1.7 |
| CV (%) | 31 | |
| $h^2$ (SE) | 0.7 (±0.027) | |
| **Moro 2010** | | |
| Rep | 1 | 23.7** |
| RIL | 258 | 5.6** |
| Error | 258 | 1.3 |
| CV (%) | 45 | |
| $h^2$ (SE) | 0.6 (±0.041) | |
| **Pendleton 2011** | | |
| Rep | 1 | 10.5** |
| RIL | 258 | 3.2** |
| Error | 253 | 0.8 |
| CV (%) | 48 | |
| $h^2$ (SE) | 0.5 (±0.044) | |
| **Pendleton 2012** | | |
| Rep | 1 | 24.7** |
| RIL | 257 | 3.1** |
| Error | 232 | 1.2 |
| CV (%) | 51 | |
| $h^2$ (SE) | 0.3 (±0.057) | |

* Significant at the 0.05 probability level
** Significant at the 0.01 probability level

QTL identified in the short arm of chromosome 3B, *QCstb.orz-3BS* linked to the marker wPt943, was identified in three locations and the arithmetic mean across environments. The QTL located in the 5B centromeric-long arm region, *QCsns.orz-C5BL,* was linked to marker barc04. It was identified in two environments and the combined mean analysis. The region where the C5BL QTL is located is known for the translocation 5BS:7BL present in several west European wheat cultivars (Badaeva, 2007). Two QTL were identified in just one environment, QTL in 5AL, *QCsns.orz-5AL.2,* linked to marker tPt3642 and in the short arm of 7B, *QCsns.orz-7BS,* linked to marker *wPt0963.* The resistant parent NSA-98-0995 was the allele donor for five of the six identified QTL, while the susceptible parent Tubbs was the resistance allele donor for the QTL on chromosome 3BS. The phenotypic variance explained by these six QTL varied from 28.4 to 54.6%, depending on the environment. Epistatic interaction was detected between the QTL on *QCsns.orz-5AL.1* and *QCsns.orz-6BS,* with an effect of 5.7% (Table 2.7)

*ExT population*

The low heritability in the ExT population (Table 4) is likely due to greater variation in disease severity among environments and missing values in the Pendleton 2012 experiment. Nine QTL contributing to disease resistance in the ExT population were identified in chromosomes 1B, 2AS, 2BL, 3BS, 4BS, 4DS, 5AL (two QTL) and 6BS (Table 6). The QTL identified in just one location are in chromosomes 1BS, 2AS, 3BS, with the susceptible parent Tubbs as the allele donor and each explaining a phenotypic variance around 5%. Also identified in one location are QTL in chromosomes 4BS and 6BS, with the parent allele donor Einstein and each explaining a phenotypic variance of 12.0 and 8.7%, respectively. The QTL in chromosome 5AL1, *QCsen.orz-*

*5AL.1* linked to marker gwm291, was identified in every environment and in the combined means analysis, explaining a phenotypic variance between 6.6 and 11.6%, depending on the environment. The QTL in chromosome 4DS, *QCsen.orz-4DS,* linked to marker wPt0472 and with the resistance allele donor from Einstein, was identified in two environments and in the combined means analysis, explaining a phenotypic variance between 7.5 and 17.0%, depending on the environment. The second QTL in 5AL, *QCsen.orz-5AL.2,* linked to marker wPt3563 and with the resistance allele derived from Einstein, was identified in one environment and in the combined means environment. For the QTL in chromosome long arm 2B, *QCstb.orz-2BL* linked to marker wPt9736, the allele donor was Tubbs and was identified in one environment and in the combined means analysis, explaining a phenotypic variance of around 5%. Total phenotypic variance explained by the identified QTL in the ExT population ranged between 16.2 to 48.9%, depending of the environment. Epistasis was detected between QTL *QCsen.orz-5AL.1* and *QCsen.orz-4DS,* with an effect of 5.3%; both alleles were from parent donor Einstein (Table 2.7).

**Discussion**

In the case of the TxN population, three main QTL (6BS, C5BL, 5AL1) accounted for around 30% of the total phenotypic response and were found to be present in lines where disease severity was below 15% whiteheads. The presence of these three QTL in combination reduced disease incidence substantially, but the presence of just one of these QTL showed little effectiveness in reducing disease (Fig 2.3 and Fig 2.4).

Table 2.5 Summary of the QTL detected in the TxN population associated with disease response to Cephalosporium stripe, including closest linked markers, likelihood odds (*LOD*) scores, phenotypic coefficients ($R^2$), and estimated additive effects (*a*).

| QTL | | QCsns.orz -5AL.1 | QCsns.orz -6BS | QCstb.orz -3BS | QCsns.orz -C5BL | QCsns.orz -5AL.2 | QCstb.orz -7BS |
|---|---|---|---|---|---|---|---|
| Closest marker | | gwm291 | wPt2726 | wPt9432 | barc4 | tPt3642 | wPt0963 |
| Moro 2010 | LOD | 11.9 | 4.0 | 2.8 | 3.0 | . | . |
| | $R^2$ | 18.0 | 5.5 | 3.6 | 4.0 | . | . |
| | a | 6.2 | 78.8 | 76.5 | 74.3 | . | . |
| Pendleton 2010 | LOD | 6.8 | 7.2 | . | . | 3.2 | . |
| | $R^2$ | 8.5 | 11.2 | . | . | 3.9 | . |
| | a | 6.0 | 6.8 | . | . | 4.1 | . |
| Pendleton 2011 | LOD | 7.4 | 13.0 | 3.5 | . | . | 3.3 |
| | $R^2$ | 9.7 | 19.6 | 4.1 | . | . | 4.2 |
| | a | 3.6 | 5.1 | -2.3 | . | . | 2.3 |
| Pendleton 2012 | LOD | 7.9 | 16.7 | 3.2 | 7.1 | . | . |
| | $R^2$ | 10.0 | 21.7 | 3.3 | 7.7 | . | . |
| | a | 3.9 | 5.8 | -2.3 | 3.5 | . | . |
| Combined environment | LOD | 13.6 | 13.1 | 4.7 | 5.3 | . | . |
| | $R^2$ | 16.2 | 17.3 | 4.9 | 5.7 | . | . |
| | a | 5.1 | 5.2 | -2.8 | 3.0 | . | . |

Negative additive effect values (a) indicate that the resistance allele is derived from parent 'Tubbs'
Positive additive effect values (a) indicate that the resistance allele is derived from parent 'NSA-98-0995'

Table 2.6 Summary of the QTL detected in the ExT population associated with disease response to Cephalosporium stripe, including closest linked markers, likelihood odds (*LOD*) scores, phenotypic coefficients ($R^2$), and estimated additive effects (*a*).

| | QTL | QCsen.orz-5AL1 | QCsen.orz-4DS | QCsen.orz-5AL2 | QCstb.orz-2BL | QCsen.orz-4BS | QCsen.orz-6BS | QCstb.orz-1BL | QCstb.orz-2AS | QCstb.orz-3BS |
|---|---|---|---|---|---|---|---|---|---|---|
| Environment | Closest marker | gwm291 | wPt0472 | wPt3563 | wPt9736 | tPt-0602 | cfd1 | wPt2315 | cfd36 | wPt9432 |
| Moro 2010 | LOD | 5.3 | . | . | 3 | . | . | . | . | . |
| | R2 | 8.8 | . | . | 5.9 | . | . | . | . | . |
| | a | 3.4 | . | . | -2.9 | . | . | . | . | . |
| Pendleton 2010 | LOD | 4.6 | . | 5.9 | . | 6.6 | . | 3.9 | . | . |
| | R2 | 7.3 | . | 9.3 | . | 12 | . | 5.7 | . | . |
| | a | 5.6 | . | 6.2 | . | 7.3 | . | -4.9 | . | . |
| Pendleton 2011 | LOD | 4.2 | 8.2 | . | . | . | 6.2 | . | . | 3.4 |
| | R2 | 6.6 | 17 | . | . | . | 8.7 | . | . | 4.5 |
| | a | 1.7 | 2.8 | . | . | . | 2.4 | . | . | -1.4 |
| Pendleton 2012 | LOD | 4 | 4.3 | . | . | . | . | . | 3.9 | . |
| | R2 | 6.2 | 7.6 | . | . | . | . | . | 5.8 | . |
| | a | 2 | 2.1 | . | . | . | . | . | -1.9 | . |
| Combined environment | LOD | 7.2 | 5 | 5.4 | 4.1 | . | . | . | . | . |
| | R2 | 11.6 | 7.9 | 8.6 | 6.2 | . | . | . | . | . |
| | a | 3.4 | 2.7 | 2.9 | -2.6 | . | . | . | . | . |

Negative additive effect values (a) indicate that the resistance allele is derived from parent ‘Tubbs’
Positive additive effect values (a) indicate that the resistance allele is derived from parent ‘Einstein’

Table 2.7 Summary of the epistatic interactions detected using multiple interval mapping (MIM) in the TxN and ExT populations among identified QTLs, phenotypic variance by locations, and arithmetic means cross locations.

| Population | Location | MIM Phenotypic variance (R%) | Epistatic interaction | Markers interacting | Epistatic effect (%) |
|---|---|---|---|---|---|
| TxN | Moro 2010 | 50.0 | 5AL1x6BS | gwm291*wP2726 | 4.6 |
| | Pendleton 2010 | 28.4 | . | . | . |
| | Pendleton 2011 | 54.6 | 5AL1x6BS | gwm291*wP2726 | 5.7 |
| | Pendleton 2012 | 43.0 | . | . | . |
| | Mean across locations | 50.0 | 5AL1x6BS | gwm291*wP2726 | 3.6 |
| ExT | Moro 2010 | 16.3 | . | . | . |
| | Pendleton 2010 | 31.9 | . | . | . |
| | Pendleton 2011 | 48.8 | 5AL1x4DS | gwm291*wPt0472 | 5.3 |
| | Pendleton 2012 | 29.8 | 5AL1x4DS | gwm291*wPt0472 | 4.3 |
| | Mean across locations | 30.7 | . | . | . |

A similar result was found for the ExT population, where a combination of the three main QTL (5AL1, 4DS and 5AL2) reduced disease severity substantially (Fig 2.5 and Fig 2.6). Approximately 25% of the ExT population showed transgressive segregation, which may be caused by recombination of additive alleles, epistatic effect between alleles or overdominance (Rieseberg et al., 1999).

In both populations, we detected epistatic interactions, with the interacting alleles derived from the resistant parents. One major QTL in chromosome 5AL linked to marker gwm291 was detected consistently across environments in both populations, making it a good candidate for use in marker-assisted selection. This region was identified previously (Quincke et al., 2011), located close and probably pseudo-linked to the B1 gene conditioning the awnless trait (Kato et al., 1998). In addition, several QTL for resistance to Cephalosporium stripe were found in common among the TxN population, ExT population, and the Coda x Brundage population reported previously by Quincke et al. (2011) (Table 2.8). The models used in this study only accounted for additive effects and interaction among detected QTL. It would also be expected that epistatic interactions between non-detected QTL and other loci would have a role in the levels of disease resistance observed in this study. In both populations of the current study, there were QTL detected from the susceptible parent Tubbs that contributed to resistance in the recombinant inbred line populations. Although in both populations the combinations of three QTL from the resistant parent reduced disease incidence of Cephalosporium stripe substantially, QTL from the susceptible parent could play a role in the genetic background of the RILs so as to provide disease resistance, even when such resistance is not functional in the susceptible parent Tubbs.

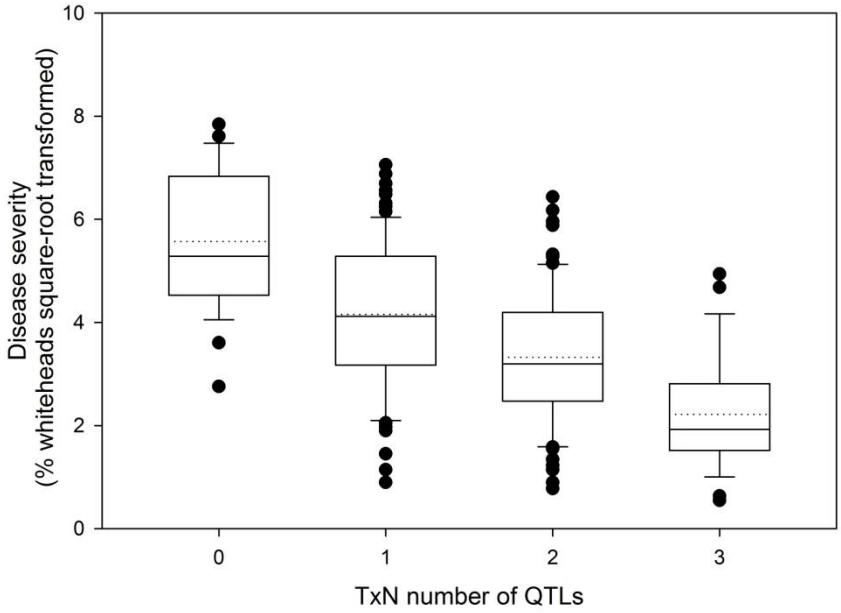


Fig 2.3 TxN population boxplots (medians are thick lines, means are dotted lines, quartiles are boxes, whiskers extend to the farthest points that are not outliers, and outliers are black bullets) for disease severity associated with the number of the three most frequently identified QTL.

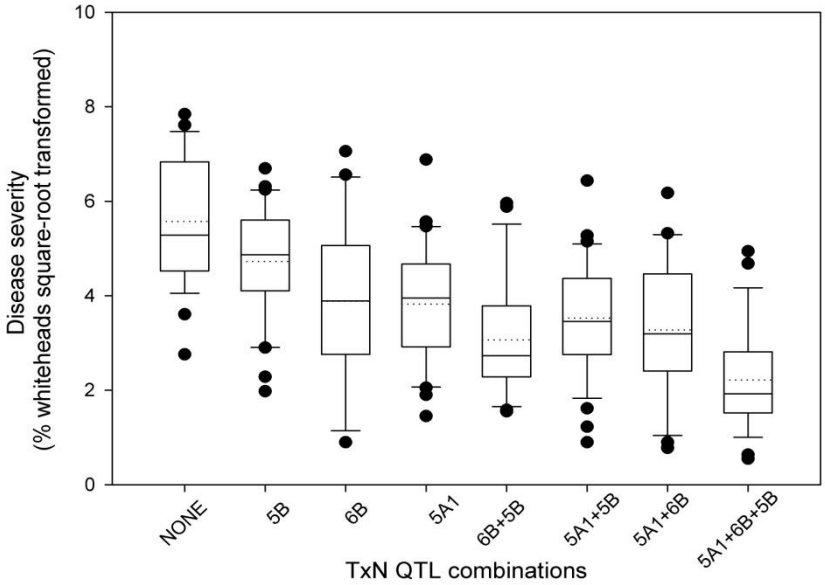


Fig 2.4 TxN population boxplots (quartiles are boxes, medians are continuous lines, means are dotted lines, whiskers extend to the farthest points that are not outliers, and outliers are black dots) of the three most frequently identified QTL in the (5BS, 6BS and 5AL1) and specific QTL combinations among them.

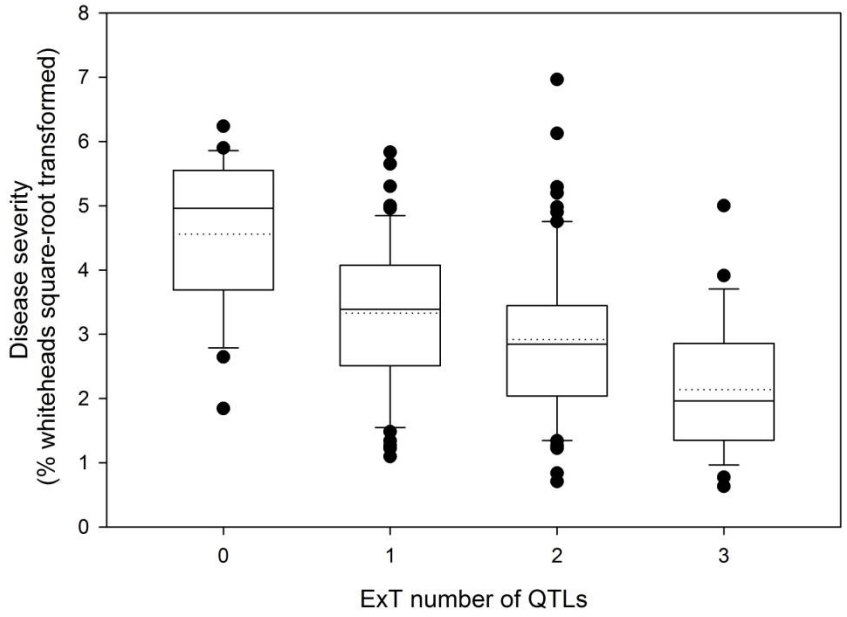


Fig 2.5 ExT population boxplots (quartiles are boxes, medians are continuous lines, means are dotted lines, whiskers extend to the farthest points that are not outliers, and outliers are black dots) for disease severity associated with number of the three most frequently identified QTL (5AL1, 4DS and 5AL2).

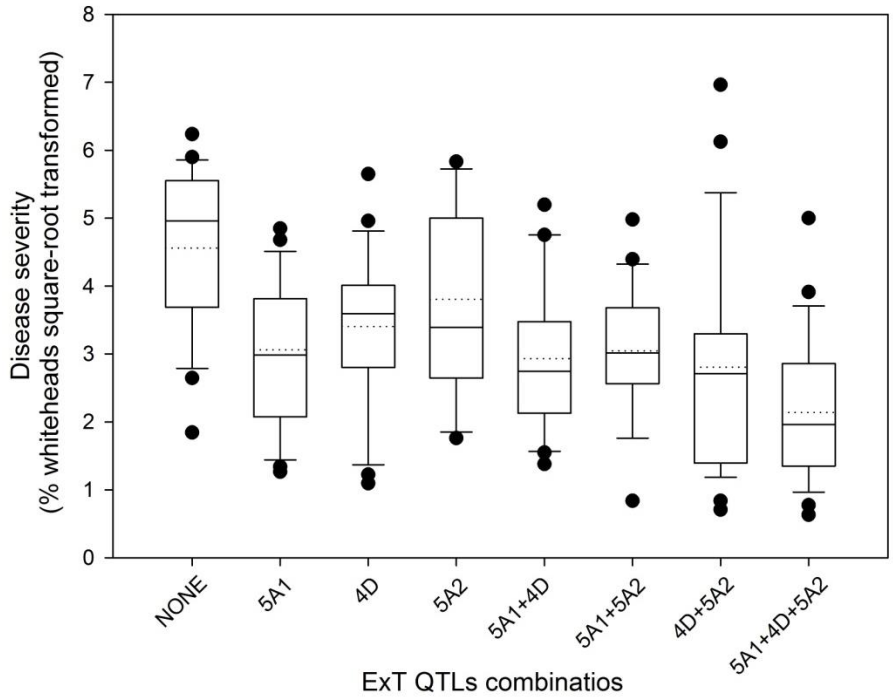


Fig 2.6 ExT population boxplots (quartiles are the boxes, medians are the continuous lines, means are dotted lines, whiskers extend to the farthest points that are not outliers, and outliers are black dots) for disease severity associated with the three most frequently identified QTLs (5AL1, 4DS and 5AL2), and combinations among them.

Table 2.8 Summary of QTL regions detected in this study and their similarities with the Coda x Brundage (CxB) population (Quincke et al, 2011).

| **Chromosome region** | **Bin#** | **Marker associated** | **Population** | **Parent donor** |
|---|---|---|---|---|
| 5AL1 | 5AL23-0.87-1.00 | gwm291 | TxN, ExT, CxB | NSA-98-0995, Einstein, Brundage |
| 5AL2 | 5AL12-0.35-0.57 | wPt-3563 tPt3642** | TxN**, ExT, CxB | NSA-98-0995, Einstein, Brundage |
| 6BS | sat 0.00-1.00 | wPt-2726 | TxN, ExT, CxB | NSA-98-0995, Einstein, Brundage |
| 3BS | 3BS1-0.33 | wPt-9432 | TxN, ExT | Tubbs |
| 4BS | 4BS4-0.37 | tPt-0602 | ExT, CxB | Einstein, Brundage |
| C-5BL | C-5BL6-0.29 | barc4 / gwm639 | TxN, CxB | NSA-98-0995, Coda |

** Regions in the chromosome identified by comparing position of nearby markers using other populations' maps (Graingenes 2.0 and cmap).
#Identification of bin using as reference Marone et al., 2012

One potential explanation for this phenomenon is the presence of suppressor genes. Knott (2000), in a study using isolates of stem rust (*Puccinia graminis* f. sp. *tritici* Eriks. & Henn.), suggested that the cultivar 'Medea' possessed suppressors for some of its genes for resistance and the suppressors were lost during the backcrossing to 'LMPG', a susceptible wheat line, allowing progeny of the cross to display resistance that was not detected in Medea. In addition, Helguera et al., (2003) found that *Lr37*, which confers

resistance to leaf rust (*Puccinia triticina* Eriks), was not functional in the cultivar 'Anza-Lr37', suggesting the presence of a suppressor factor.

Interactions among QTL and background effects lead to questions regarding how to successfully validate QTLs in several wheat backgrounds and environments and how to estimate the best QTL combinations for their use in marker-assisted selection when pyramiding genes for resistance. Miedaner et al., (2006) highlighted the unexpected outcomes that can arise when combining QTLs for resistance in a different genetic background than their original source with Fusarium head blight (FHB). They introgressed two donor-QTL located in chromosomes 3B and 5A from 'CM82036', a non-adapted line, and one donor-QTL in chromosome 3A from 'Frontana', a Brazilian cultivar, into elite European spring wheat. Individual and combined QTL effects were estimated for FHB disease severity and Fusarium exoantigen content (DON). All three individual donor-QTL alleles significantly reduced DON. However, the donor-QTL allele 3A had no significant effect in FHB severity, either individually or in combination with other QTL. The highest effect was from the stacked donor-QTL alleles 3B and 5A for both traits.

The mechanisms of action of the QTL identified in this study are unknown. However, it has been suggested that host-selective toxins may be a mechanism of action of *Cephalosporium gramineum* (Kobayashi and Ui, 1979; Rahman et al., 2001). There is evidence that necrotrophic pathogens produce effectors (host-selective toxins) that interact with defense-associated proteins eliciting a resistance-like response that confers susceptibility. Defense-associated proteins, to which the host selective toxins may interact, belong to the class of resistance proteins used in the resistance mechanism that

follow the gene-for-gene interaction system. Such resistance proteins consist of the N-terminal nucleotide-binding site (NBS) C-terminal leucine-rich repeat (LRR) proteins characterized as NB-LRR. Another class of defense-associated proteins is composed of serine/threonine protein kinase (S/TPK) domain (Faris et al., 2010; Lorang et al., 2007). Lorang et al., (2012) reported that necrotrophic pathogens that make use of host-selective toxins as determinants of pathogenicity may do so by interacting with the same genes that biotrophic pathogens use to induce resistance reactions (Dangl and Jones, 2001; Wolpert et al., 2002). Lillemo et al., (2013) reported a QTL for the necrotrophic pathogen spot blotch (*Bipolaris sorokiniana*, telemorph *Cochliobolus sativus*) that co-locates to the locus *Lr34,* which provides resistance to leaf rust *(Puccinia triticina* Eriks*).* Joshi et al., (2004) reported the phenotypic marker leaf tip necrosis linked to at least three different loci of biotrophic disease resistance *L34/Yr18/Pm38* is associated with moderate resistance to spot blotch. Adhikari et al., (2012) reported a QTL in 3BS linked to resistance to spot blotch in the same location where Poole et al., (2012) identified a QTL linked to resistance to Fusarium crown rot (*Fusarium pseudograminearum*) and where Chen et al., (2013) identified a QTL for resistance to sharp eyespot (*Rhizoctonia cerealis)*. This is the same region where the QTL in 3BS from ExT and TxN (from Tubbs) was detected in this study, but it was not a QTL with strong effect and was detected in only one location for both populations. In addition, Lowe et al., (2011) evaluated a cross between UC1110, an adapted California spring wheat, and PI610750, a synthetic derivative from CIMMYT's Wide Cross Program, for its response to current California races of stripe rust (*Puccinia striiformis* f. sp. *tritici*). They reported a QTL in chromosome 5AL that is located in the same region as 5AL1 for Cephalosporium stripe

found in ExT, TxN populations and in CxB (Quincke et al., 2011). In addition, there is one QTL identified in 5AL from Cappelle Desprez that confer resistance to eyespot-strawbreaker foot rot (*O. yallundae* and *O. acuformis*) that is in a similar region of 5AL2 that was identified in the ExT, TxN and CxB (Quincke et al. 2011) studies for Cephalosporium stripe. Some of these regions have also been related to disease resistance to other diseases, such as Fusarium head blight (*Fusarium gramineum*) and Septoria tritici blotch (*Zymospeptoria graminicola*), suggesting that either these regions are hot spots for multiple specific genes or for general genes that give resistance to multiple pathogens (Bovill et al., 2006; Buerstmayr and Anderson, 2009; Cuthbert et al., 2007; Liu et al., 2013; Miedaner et al., 2012; Muhovski et al., 2012 and Risser et al 2011). Results of this study are part of the first step to develop genotypic markers for their use in breeding for resistance to Cephalosporium stripe. The next step is to saturate those chromosomal regions of interest, which will allow for the identification of markers that are closely linked with the Cephalosporium stripe resistance QTL.

Results of this study have identified potential QTL for resistance to Cephalosporium stripe that have now been identified in several populations indicating they may be useful QTL for breeding for Cephalosporium stripe resistance across and array of breeding combinations. The discovery of epistatic interactions among the QTL for resistance provides an explanation on the variability in disease resistance response when combining QTL among different combinations. Further work is needed to improve the molecular markers identified in this study by saturating the chromosomes regions of interest to identify markers more closely linked with the Cephalosporium stripe resistance QTL.

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# CHAPTER 3

## A TALE OF TWO POPULATIONS: STRIPE RUST, MAJOR GENES, MINOR GENES, AND GENETIC BACKGROUND

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## A TALE OF TWO POPULATIONS: STRIPE RUST, MAJOR GENES, MINOR GENES, AND GENETIC BACKGROUND


### Abstract

Stripe rust is a foliar disease of wheat (*Triticum aestivum* L.) caused by the air-borne fungus *Puccinia striiformis* f.sp *tritici.* The disease is present in every region around the world where commercial wheat is grown. The U.S. Pacific Northwest is known for its production of wheat for international export, making it an important economic commodity for the region. For wheat breeding programs, breeding for durable resistance to stripe rust continues to be a priority and a challenge due to the complexity in the interaction between the different genes, types of genes involved in the resistance response and to the wide diversification and continuous evolution of stripe rust races. The goal of this study was to detect chromosomal regions for resistance to stripe rust in two winter wheat populations evaluated over several years and locations to aid in the understanding of the genetic basis for resistance to stripe rust. Two recombinant inbred line populations 'Tubbs'/'NSA-98-0995' (TxN) and 'Einstein'/'Tubbs' (ExT), each comprising of a cross of a resistant and a susceptible cultivar, with population sizes of 271 and 259 $F_{(5:6)}$ recombinant inbred lines, respectively, were phenotyped across seven locations/years and mapped with diversity array technology (DArT) and simple sequence repeat (SSR) markers, covering polymorphic regions of ≈1,480 and 1,117 centimorgans, respectively. The seven environments for the stripe rust study were under natural epidemics and stripe rust severity was visually scored as percent on a plot basis according

to the modified Cobb Scale. Results for phenotypic data analysis revealed significant ($P < 0.01$) genotypic differentiation for stripe rust among the recombinant inbred lines. Entry-mean heritabilities ($h^2$) for stripe rust were 0.9 for both 'Tubbs'/'NSA-98-0995' and 'Einstein'/'Tubbs' for data averaged over environments. In quantitative trait loci (QTL) analysis, two major QTL located in chromosomes 2AS and 6AL were detected in the ExT population, with epistatic interaction detected among them plus three other minor QTL identified. Eight QTL were identified in the TxN population, with two accounting for a larger percentage of the phenotype variance than the others, with some evidence for loss of effectiveness of these two major QTL during the course of this study. Epistatic interactions were detected in both populations between the alleles from the susceptible and the resistant parent in the ExT population and only between alleles from the resistant parent in the TxN population. Each population revealed a very different behavior in their response to stripe rust, providing useful information on the genetic basis for stripe rust resistance on wheat.

**Introduction**

Stripe (or yellow) rust caused by the biotrophic fungus *Puccinia striiformis Westend* f. sp. *tritici Erikss,* is an important disease that threatens worldwide production of wheat. Releasing cultivars with genetic resistance is an effective way to control the disease and is preferred over the application of fungicides. Planting resistant cultivars reduces the amount of inoculum produce thus minimizing the disease pressure that could lead to potential future epidemics (Guest and Brown, 1997). Two types of resistance have been recognized for rust pathogens, one designated as major gene resistance, vertical resistance or race-specific resistance and the other as minor gene resistance, horizontal resistance, partial resistance, adult plant resistance, high-temperature adult plant resistance or non-race specific resistance (Lin and Chen 2007; Parlevliet, 2002). The mechanism involving host response in race-specific resistance is eliciting a programmed cell death known as the hypersensitivity reaction. Resistance genes to stripe rust in this category are considered non-durable given the high selection pressure put on the pathogen and the ease of attaining mutations that result in lack of recognition of the pathogen effectors by the plant receptors. (Guest and Brown, 1997; Jones and Dangl, 2006). Minor resistance genes generally do not provide the immunity, or high level of resistance, that a single major gene often does. The mechanisms by which fungal disease is inhibited by minor resistance are unclear (Poland et al., 1988), but are manifested through an increase in the latency period, reduced uredinia size, reduced infection frequency and reduced spore production. (Caldwell 1968; Parlevliet 1979). These different components of resistance may be pleitropically controlled (Parlevliet, 1986; Richardson et al., 2006).

The stripe rust pathogen can survive cool summers in most regions of the Pacific Northwest over 40°N latitude (Chen et al., 2013). Moderate winters in these regions favor the survival of the pathogen, resulting in the Pacific Northwest (Idaho, Oregon and Washington) being a region where both over-summering and over-wintering can occur. Thus, epidemics can be frequent in the presence of susceptible cultivars and virulent races (Chen, 2005).

Over the last 20 years, more than 30 studies have been published involving quantitative trait loci (QTL) analysis for stripe rust in wheat (Roserwane et al., 2013; Chen, 2013). With the advance in high-throughput marker technologies such as diversity array technology (DArT) and single nucleotide polymorphism (SNP) the genetic location of these QTL is being better documented through many different mapping studies. The goals of this study are to map the chromosome locations of QTL in two recombinant inbred line populations, compare and contrast the genetics of resistance between these two populations, and to identify common regions of interest with previous QTL studies of wheat stripe rust.

## Materials and Methods

### Mapping populations

Two populations of $F_5$-derived $F_6$ recombinant inbred lines (RILs) developed at Oregon State University were studied. The first population, consisting of 271 RILs, was derived from a cross between an awnless, hard red winter wheat experimental line 'NSA-980995' (Limagrain, UK), with a moderate-to-high level of resistance to stripe rust, and the awned, soft white winter wheat cultivar 'Tubbs' (PI 651023), which is highly

susceptible to stripe rust. The second population, consisting of 259 RILs, was derived from a cross between the awnless, hard red winter wheat cultivar 'Einstein' (Limagrain, UK) with high level of resistance to stripe rust and the cultivar Tubbs. The initial crosses for both populations were done in 2003. The cultivar Einstein, bred by Nickerson Seeds and commercialized by Limagrain UK, is widely grown in Western Europe and has the pedigree (NHC 49/UK Yield Bulk) x (Haven/(Moulin/Galahad)) (Limagrain, 2013). Tubbs is a cultivar released in 2000 that was widely grown in the Pacific Northwest until it became susceptible to stripe rust and has the pedigree Madsen/Malcom (USDA-AMS, 2009). NSA-98-0995 is an experimental line developed by Limagrain, UK, with no publically available pedigree.

Plant DNA extraction and genotyping

For both populations, DNA of parental and $F_5$-derived progeny was extracted from young leaves of greenhouse-grown plants using the DNeasy 96 Plant Kit (QIAGEN Science, Maryland, USA). DNA concentration was tested using NanoDrop ND-1000 UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc. Wisconsin, USA.). A final volume of 15 ng/ul was sent to Triticarte Pty. Ltd Canberrra, Australia to be genotyped with DArT (Diversity Array Technology) markers (Akbari et al., 2006). Additional simple sequence repeat (SSR) markers were screened for polymorphism in the Tubbs x NSA-98-0995 (TxN) and Einstein x Tubbs (ExT) populations in facilities at the USDA ARS Wheat Genetics, Quality, Physiology and Disease Resistance Unit at Pullman, WA, USA, using approximately 50 ng genomic DNA extracted from young leaves at Oregon State University following the protocol described by Riera-Lizarazu et al. (2000).

Map construction and molecular analysis

For the populations used in this study, genotypic data were used to create the genetic linkage map with the software Join Map v.4.0. (Van Ooijen, 2006). Genetic distances were calculated using the Haldane function (Haldane, 1919). For each linkage group, the best marker locus order was determined using the maximum likelihood in Join Map v.4.0. The TxN map was constructed with 229 markers, 13 SSR and 216 DArT comprising 49 linkage groups, representing areas from all chromosomes of common wheat except 4D. The total genome length covered was 1481 cM. The ExT map was constructed with 198 markers, 18 SSR and 180 DArT comprising 32 linkage groups, representing areas from all chromosomes of common wheat except 6D and 7D. For both populations, final linkage groups were assigned to each chromosome with data provided by Triticarte wheat map alignment (Triticarte, 2013) and maps available on the database GrainGenes 2.0 (2013).

Field trials and phenotyping

The $F_6$-derived seed harvested from the greenhouse was used to establish plots in the field. For each population, the experimental design used was a randomized complete block with two replications. The parental cultivars, the RIL progeny, and two cultivar checks Madsen' (Allan et al., 1989), and 'Xerpha' (Jones et al., 2010) were included in the field trial. The cultivars Xerpha and Madsen were the high and low stripe rust disease severity checks respectively. From years 2009 to 2013, experiments were conducted in seven different environments. At the OSU Hyslop Crop Science Field Research Laboratory, Corvallis OR in 2010 and 2011; the OSU Botany and Plant Pathology Field Laboratory, Corvallis, OR in 2013; the USDA Experimental Station, Mt. Vernon WA in 2009; Pullman WA in 2010; and at a site near the Columbia Basin Agricultural Research

Center field, Pendleton, OR in 2010 and 2011. Natural infection established in all locations. In Mt. Vernon 2009, the natural population was a mixture of 14 races. In Pendleton 2010, the prevalent race was PsTv-8. In Pullman 2010 and in Corvallis 2010 and 2011 the races PsTv-11 and PsTv-14 were predominant. By 2012 the presence of races PsTv-48 and PsTv-53 were reported in the U.S. Pacific Northwest, with the prevalent races being PsTv-11 and PsTv-14 (Chen, 2013).

Plots consisted of two rows 1 m long in Mt. Vernon 2009, Pullman WA 2010 and Corvallis 2011 and 2013. Plots established in Hyslop Farm Corvallis 2010 and in Pendleton 2010 and 2011 were six rows 5 m long. Fertilization and weed control were appropriate for commercial winter wheat production in their respective location. The percent rust severity for each plot was evaluated according to the modified Cobb Scale (Roelfs et al. 1992). Depending on the timing of the epidemics in the different environments, disease readings were taken at early jointing stage (Zadoks 30-31), and/or flowering- milk (Zadoks 59-75) stages. Data used for statistical analysis and QTL analysis was the last note taken in every environment that was between flowering-milk (Zadoks 59-75) stages.

QTL and statistical analyses

The PROC GLM procedure in SAS version 9.1.3 (SAS Institute Inc., 2000) was used to calculate least squares means and to determine effects for RILs, environment, and RILs x environment. The PROC MIXED procedure was used to calculate family heritability ($h^2$) on a plot basis as $h^2 = \sigma^2_g / \sigma^2_p = \sigma^2_f/(\sigma^2_f + \sigma^2_e/r)$, where the variance components are $\sigma^2_g$, genetic variance; $\sigma^2_p$, phenotypic variance; $\sigma^2_f$, family variance; $\sigma^2_e$, error variance; and r, number of replications (Holland et al., 2010). For all tests, a

probability level of $P<0.05$ was used.

The least squares means of disease severity were used for QTL analysis, performed using composite interval mapping (CIM) in WinQTL Cartographer v.2.5 software (Wang et al., 2007 ). For both populations, the QTL analyses were done individually per location and with the arithmetic mean across environments to deduce balanced values for each RIL. Likelihood-odds (LOD) thresholds for declaring statistical significance were calculated by 1000 permutations (Churchill and Doerge, 1994). Window size was set at 5 cM for each dataset section using forward and backward stepwise regression. The additive effects (a) and phenotypic variance coefficients of determination ($R^2$) for each QTL were estimated by CIM for each individual environment and for the arithmetic mean across environments. Epistatic interactions analyses were performed with multiple interval mapping (MIM) in WinQTL Cartographer v.2.5 software using the option "Scan through QTL mapping results file" and later refined using the option "Testing for existing QTLs" under the AIC-based selection criteria (Silva et al, 2012, Wang et al., 2007).

## Results

Phenotypic values and statistical analysis

Significant disease pressure was obtained each year in each location for both populations. Epidemics in 2011 were particularly severe in the experiments and in commercial wheat production fields throughout the major wheat growing areas of the Pacific Northwest. For both populations, the disease severity values for the susceptible parent Tubbs ranged from 22.0% in Pullman 2010 to 98.3% in Pendleton 2011. Disease

severity for the resistant parent NSA-98-0995 ranged from 0.0% in all 2010 locations to 38.3% in Pendleton 2011. With the ExT population the resistant parent Einstein disease severity scores ranged from 0.0% in Pullman 2010 and Corvallis 2010, to 5.3% in Pendleton 2011. The resistant and susceptible check cultivars Madsen and Xerpha were present in both populations and had disease severity scores ranging from 0.0 to 35.6% and 20% to 100%, respectively (Table 3.1 and Table 3.2).

Table 3.1 Mean disease severity values (% on a plot basis) for the 271 recombinant inbred lines in the TxN population, the parental lines, and two cultivar checks exposed to natural inoculation in seven locations.

| **TxN population** | **Varietal checks** | | **Parents** | | **RILs population** | | |
|---|---|---|---|---|---|---|---|
| **Environment** | **Madsen** | **Xerpha** | **NSA-98-0995** | **Tubbs** | **Mean** | **Range** | **$h^2$ (SE)** |
| **Mt. Vernon 2009** | 35.6 | 45.0 | 9.8 | 53.8 | 40.0 | 1-95 | 0.6 (±0.04) |
| **Pullman 2010** | 0.0 | 31.2 | 0.0 | 25.8 | 9.0 | 0-75 | 0.8 (±0.02) |
| **Corvallis 2010** | 0.0 | 42.5 | 0.0 | 50.8 | 10.9 | 0-80 | 0.7 (±0.03) |
| **Corvallis 2011** | 5.6 | 68.8 | 6.6 | 74.2 | 46.6 | 0-90 | 0.8 (±0.02) |
| **Corvallis 2013** | 12.5 | 78.8 | 18.0 | 83.3 | 66.0 | 9-90 | 0.5 (±0.05) |
| **Pendleton 2010** | 0.6 | 37.5 | 0.3 | 70.8 | 16.2 | 0-100 | 0.8 (±0.02) |
| **Pendleton 2011** | 1.9 | 96.6 | 38.3 | 98.3 | 83.7 | 0-100 | 0.7 (±0.03) |
| **Environments mean** | 9.1 | 58.9 | 11.7 | 68.6 | 42.2 | 9-86 | 0.9 (±0.01) |

Heritabilities ($h^2$) were generally high, with Mt Vernon 2009 and Corvallis 2013 tending towards moderate values (Table 3.1 and Table 3.2), with the exception of Pullman 2010 location, where no heritability was calculated since disease data was taken from just one repetition.

Table 3.2 Mean disease severity values (% on a plot basis) for the 259 recombinant inbred lines in the ExT population, the parental lines, and two cultivar checks exposed to natural inoculation in seven location.

| **ExT population** | **Varietal checks** | | **Parents** | | **RILs population** | | |
|---|---|---|---|---|---|---|---|
| **Environment** | **Madsen** | **Xerpha** | **Einstein** | **Tubbs** | **Mean** | **Range** | **$h^2$ (SE)** |
| **Mt. Vernon 2009** | 28.5 | 47.0 | 1.5 | 47.0 | 7.2 | 0-80 | 0.6 (±0.037) |
| **Pullman 2010** | 0.0 | 19.0 | 0.0 | 22.0 | 6.0 | 0-70 | . |
| **Corvallis 2010** | 1.2 | 35.0 | 0.0 | 78.4 | 6.8 | 0-90 | 0.8 (±0.025) |
| **Corvallis 2011** | 10.0 | 96.6 | 5.2 | 93.3 | 28.2 | 0-100 | 0.9 (±0.013) |
| **Corvallis 2013** | 2.0 | 51.5 | 1.6 | 44.0 | 6.9 | 0-50 | 0.8 (±0.020) |
| **Pendleton 2010** | 1.0 | 66.0 | 2.0 | 85.0 | 12.3 | 0-100 | 0.8 (±0.027) |
| **Pendleton 2011** | 6.5 | 100.0 | 5.3 | 97.7 | 32.0 | 0-100 | 0.9 (±0.012) |
| **Environments mean** | 7.6 | 62.8 | 2.3 | 69.6 | 14.8 | 0-83 | 0.9 (±0.006) |

In the ExT population 93 RILs (35% of the population) fell in the same bin as the resistant parent Einstein (Fig 3.1 and Fig 3.2). ANOVA results for the combined analyses indicate significant effects of environment, RIL and line by environment interaction for both populations, though the mean squares were small relative to the main effect of RIL (Table 3.3; Table 3.4). Coefficients of variation (CVs) among environments ranged from 12 to 65% for the TxN population and from 32 to 96% for the ExT population. CVs for the arithmetic means across environments were 1% for TxN and 8% for ExT (Tables 3.3 and 3.4).

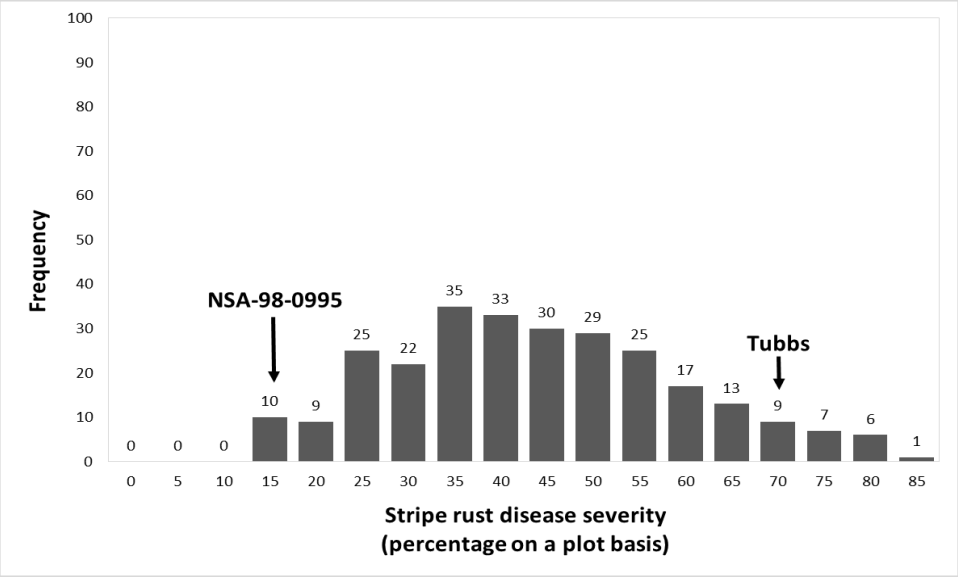


Fig 3.1 Recombinant inbred lines histogram of the TxN population with the arrows indicating the arithmetic mean of the percentage rust infection for the parents. Numbers on tops of the bars are frequency for each bin.

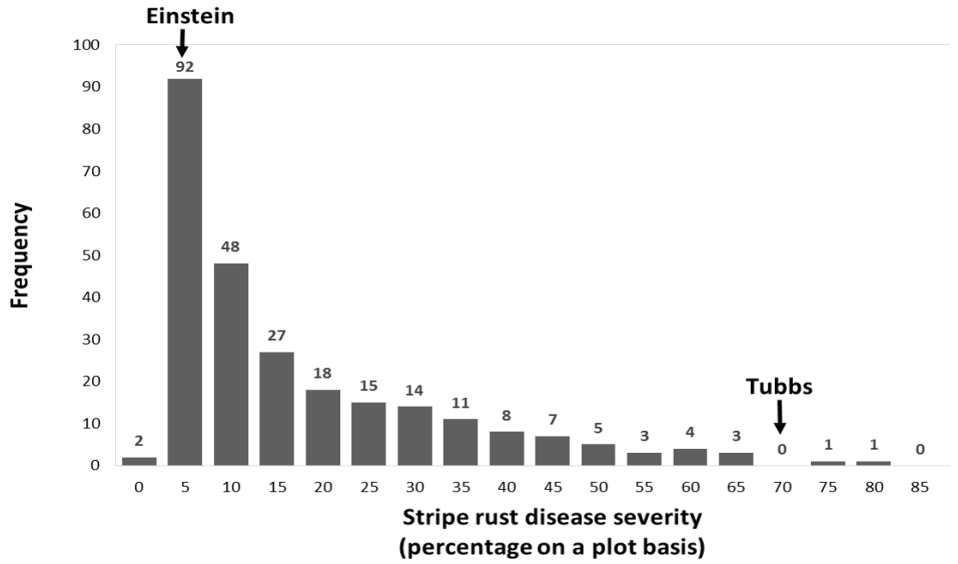


Fig 3.2 Recombinant inbred lines histogram of the ExT population with the arrows indicating the arithmetic mean of the percentage rust infection for the parents. Numbers on top of the bars are frequency for each bin.

Table 3.3 Analyses of variance (Type III SS), and coefficient of variation (CV), for stripe rust disease severity in TxN population (271 recombinant inbred lines) in six and across environments

| **TxN population** | **Source of variation** | | **CV (%)** |
|---|---|---|---|
| **Environment** | **DF** | **Mean square** | |
| **Across environments** | | | 1.1 |
| Environment | 6 | 335.5** | |
| RIL | 270 | 4.8* | |
| RIL x Environment | 1624 | 0.5** | |
| Error | 1088 | 0.2 | |
| **Mt. Vernon 2009** | | | 34.1 |
| Rep | 1 | 10151.7** | |
| RIL | | 748.2** | |
| Error | | 176.7** | |
| **Corvallis 2010** | | | 13.3 |
| Rep | 1 | 0.0* | |
| RIL | 270 | 1.0* | |
| Error | 270 | 0.2 | |
| **Corvallis 2011** | | | 25.7 |
| Rep | 1 | 3011.8 | |
| RIL | 270 | 1436.3** | |
| Error | 270 | 142.9 | |
| **Corvallis 2013** | | | 31.6 |
| Rep | 1 | 3.0* | |
| RIL | 270 | 168.6** | |
| Error | 270 | 56.8 | |
| **Pendleton 2010** | | | 65.0 |
| Rep | 1 | 208.8 | |
| RIL | 270 | 856.4** | |
| Error | 270 | 110.7 | |
| **Pendleton 2011** | | | 12.6 |
| Rep | 1 | 524.2 | |
| RIL | 270 | 725.4** | |
| Error | 270 | 111.1 | |

*Significant at the 0.05 probability level

** Significant at the 0.01 probability level

Table 3.4 Analyses of variance (Type IIISS), and coefficient of variation (CV), for stripe rust disease severity in ExN population (259 recombinant inbred lines) in six and across environments

| **ExT population** | **Source of variation** | | **CV (%)** |
|---|---|---|---|
| **Environment** | **DF** | **Mean square** | |
| **Across environments** | | | 8.7 |
| Environment | 6 | 398.3** | |
| RIL | 258 | 39.3** | |
| RIL x Environment | 1548 | 4.5** | |
| Error | 1552 | 1.4 | |
| **Mt. Vernon 2009** | | | 48.5 |
| Rep | 1 | 70.4** | |
| RIL | 259 | 6.3** | |
| Error | 258 | 3.0** | |
| **Corvallis 2010** | | | |
| Rep | 1 | 284.3* | 93.6 |
| RIL | 258 | 307.2** | |
| Error | 257 | 40.2 | |
| **Corvallis 2011** | | | 33.1 |
| Rep | 1 | 30.4 | |
| RIL | 258 | 1496.3** | |
| Error | 256 | 85.9 | |
| **Corvallis 2013** | | | 50.7 |
| Rep | 1 | 44.6 | |
| RIL | 258 | 126.4** | |
| Error | 258 | 12.2 | |
| **Pendleton 2010** | | | 88.3 |
| Rep | 1 | 3099.0** | |
| RIL | 258 | 854.7** | |
| Error | 258 | 118.8 | |
| **Pendleton 2011** | | | 32.0 |
| Rep | 1 | 200.2 | |
| RIL | 258 | 1975.1** | |
| Error | 258 | 104.9296 | |

*Significant at the 0.05 probability level

** Significant at the 0.01 probability level

QTL analysis

*TxN population*

From the eight QTL identified contributing to stripe resistance in the TxN population, seven were identified in the arithmetic means analysis, in chromosomes 2AL, 2BL, 5BL, 5AL, 3BL, 4AL and 6BS with all of the resistance QTL originating from the resistant parent NSA-98-0995 (Table 3.5). The two QTL identified in most (five of seven) locations were in chromosome 2AL and 2BL. The phenotypic variance explained by the QTL in chromosome 2AL ranged from 4% in Pendleton 2010 to 30% in Mt. Vernon. For the QTL in chromosome 2BL, the phenotypic variance ranged from 7% in Pullman 2010 to 30% in Corvallis 2011. The phenotypic variance for both QTL from the arithmetic mean analysis was 16 and 21% respectively, suggesting that both QTL contribute to the resistance with a major effect. The QTL in chromosome 5A was identified in four of seven locations with a phenotypic response variance of around 5% in each location. The QTL in chromosome 5BL, 3BL, and 4AL were identified in three of seven locations. The QTL in chromosome 5BL showed a phenotypic response of 10 to 24%, the QTL in chromosome 3BL and 4AL showed a phenotypic response of around 7 and 5%, respectively at every location where they were identified. The QTL in chromosome 6BS and 5DL were identified in just one location with a phenotypic variance response of 4%. The QTL in chromosome 6BS was also identified in the arithmetic mean analysis, while the QTL in chromosome in 5DL was not. Only the QTL in chromosome 5DL presented a resistance allele from the susceptible parent Tubbs. Epistatic interactions were detected between QTL in chromosome 2AL and 2BL with a negative effect and between QTL in chromosome 2BL and 5BL with a positive effect (Table 3.6).

Table 3.5 Summary of the QTL detected in the TxN population associated with disease response to stripe rust under natural field inoculations, including closest linked markers, likelihood odds (LOD) scores, phenotypic coefficients ($R^2$), and estimated additive effects (a).

| Environment | QTL | QYren.orz-2AL | QYren.orz-2BL | QYren.orz-5AL | QYren.orz-5BL | QYren.orz-3BL | QYren.orz-4AL | QYren.orz-6BS | QYrtb.orz-5D |
|---|---|---|---|---|---|---|---|---|---|
| | Closest marker | wPt7011 | wPt0950 | gwm291 | wPt8285 | wPt3107 | wPt6440 | wPt2726 | wPt580 |
| Mt. Vernon 2009 | LOD | 25.8 | 15.7 | 8.0 | . | . | 3.1 | . | . |
| | $R^2$ | 33.6 | 17.1 | 7.4 | . | . | 3.0 | . | . |
| | a | 11.3 | 8.2 | 5.3 | . | . | 3.4 | . | . |
| Pullman 2010 | LOD | 3.5 | 3.4 | . | 7.4 | . | 3.0 | . | . |
| | $R^2$ | 4.7 | 7.2 | . | 10.1 | . | 3.7 | . | . |
| | a | 3.0 | 3.8 | . | 4.8 | . | 2.7 | . | . |
| Corvallis 2010 | LOD | 6.4 | 4.4 | . | 10.2 | . | . | 2.9 | . |
| | $R^2$ | 7.5 | 9.5 | . | 12.0 | . | . | 3.9 | . |
| | a | 5.2 | 5.9 | . | 7.2 | . | . | 3.7 | . |
| Pendleton 2010 | LOD | 3.1 | 4.2 | . | 18.8 | . | . | . | . |
| | $R^2$ | 4.2 | 7.7 | . | 23.6 | . | . | . | . |
| | a | 4.2 | 5.8 | . | 10.3 | . | . | . | . |
| Corvallis 2011 | LOD | 15.4 | 20.1 | 3.2 | . | 3.9 | . | . | . |
| | $R^2$ | 18.4 | 30.0 | 4.0 | . | 5.1 | . | . | . |
| | a | 11.6 | 14.7 | 5.4 | . | 6.1 | . | . | . |
| Pendleton 2011 | LOD | . | . | 3.8 | . | 6.5 | 4.6 | . | . |
| | $R^2$ | . | . | 5.5 | . | 11.5 | 6.0 | . | . |
| | a | . | . | 4.5 | . | 6.5 | 4.7 | . | . |
| Corvallis 2013 | LOD | . | . | 4.2 | . | 4.6 | . | . | 3.2 |
| | $R^2$ | . | . | 5.9 | . | 9.2 | . | . | 4.5 |
| | a | . | . | 4.7 | . | 5.8 | . | . | -4.1 |
| Environments mean | LOD | 12.6 | 12.2 | 5.5 | 4.0 | 5.3 | 3.9 | 4.1 | . |
| | $R^2$ | 16.7 | 21.7 | 6.6 | 4.2 | 8.5 | 4.9 | 4.8 | . |
| | a | 6.4 | 7.3 | 4.0 | 3.2 | 4.6 | 3.4 | 3.4 | . |

Negative additive effect values (a) indicate that the resistance allele is derived from parent 'Tubbs'
Positive additive effect values (a) indicate that the resistance allele is derived from parent 'NSA-98-0995'

Table 3.6 Summary of the epistatic interactions detected using multiple interval mapping (MIM) in the TxN and ExT populations among identified QTLs, phenotypic variance by locations, and arithmetic means cross locations.

| Population | Location | Epistatic interaction | Markers interacting | Epistatic effect | Epistatic effect variance (%) | MIM Phenotypic variance (R%) |
|---|---|---|---|---|---|---|
| ExT | Mt. Vernon 2009 | . | . | . | . | . |
| | Pullman 2010 | 2ASx6AL | cfd36*wPt4229. | -3.3 | 8.0 | 0.3 |
| | Corvallis 2010 | 2ASx6AL | cfd36*wPt4229. | -3.9 | 11.9 | 0.4 |
| | Pendleton 2010 | 2ASx6AL | cfd36*wPt4229. | -5.1 | 9.4 | 0.5 |
| | | 2ASx6AL | wPt6105*wPt4229. | -3.9 | 7.1 | |
| | Corvallis 2011 | 2ASx6AL | cfd36*wPt4229. | -8.7 | 13.0 | 0.7 |
| | Pendleton 2011 | 2ASx6AL | cfd36*wPt4229. | -9.4 | 9.5 | 0.5 |
| | Corvallis 2013 | 2ASx6AL | cfd36*wPt4229. | -2.2 | 8.9 | 0.4 |
| | Across environment | 2ASx6AL | cfd36*wPt4229. | -5.7 | 13.6 | 0.7 |
| TxN | Mt. Vernon 2009 | 2ALx2B1 | wPt7011*wPt0950 | -4.4 | 5.5 | 0.5 |
| | Pullman 2010 | 2B1x5B1 | wPt0950*wPt8285 | 4.5 | 9.8 | 0.7 |
| | Corvallis 2010 | 2B1x5B1 | wPt0950*wPt8285 | 8.4 | 16.6 | 0.8 |
| | Pendleton 2010 | 2B1x5B1 | wPt0950*wPt8285 | 9.2 | 18.1 | 0.8 |
| | Corvallis 2011 | 2ALx2B1 | wPt7011*wPt0950 | -8.5 | 10.3 | 0.6 |
| | Pendleton 2011 | . | . | . | . | 0.2 |
| | Corvallis 2013 | . | . | . | . | 0.2 |
| | Across environment | 2B1x5B1 | wPt0950*wPt8285 | 2.3 | 3.1 | 0.6 |
| | | 2ALx2B1 | wPt7011*wPt0950 | -2.7 | 3.5 | |

Table 3.7 Summary of the QTL detected in the ExT population associated with disease response to stripe rust under natural field inoculations, including closest linked markers, likelihood odds (LOD) scores, phenotypic coefficients (R2), and estimated additive effects (a).

| | QTL | QYrtb.orz-2AS | QYren.orz-6AL | QYren.orz-7BL | QYren.orz-5BL | QYren.orz-4AL |
|---|---|---|---|---|---|---|
| Environment | Closest marker | cfd36 | wPt-4229 | wPt2356 | wPt6105 | wPt1007 |
| Mt. Vernon 2009 | LOD | 10.5 | 2.8 | 5.1 | . | 3.2 |
| | R2 | 14.5 | 3.6 | 6.7 | . | 4.3 |
| | a | -4.5 | 2.1 | 2.9 | . | 2.3 |
| Pullman 2010 | LOD | 3.7 | 7.9 | . | . | . |
| | R2 | 4.9 | 10.5 | . | . | . |
| | a | -3.2 | 4.2 | . | . | . |
| Corvallis 2010 | LOD | 4 | 13.7 | . | . | . |
| | R2 | 5 | 16.6 | . | . | . |
| | a | -3 | 9.1 | . | . | . |
| Pendleton 2010 | LOD | 5.7 | 14.6 | . | 3.2 | . |
| | R2 | 7.8 | 18.2 | . | 3.7 | . |
| | a | -6.3 | 8.9 | . | -4.6 | . |
| Corvallis 2011 | LOD | 24.9 | 33.7 | . | . | . |
| | R2 | 21.6 | 31.4 | . | . | . |
| | a | -13.4 | 15.5 | . | . | . |
| Pendleton 2011 | LOD | 27.5 | 24.8 | . | . | . |
| | R2 | 25.6 | 26.6 | . | . | . |
| | a | -17.1 | 16.4 | . | . | . |
| Corvallis 2013 | LOD | 12.8 | 13 | 2.6 | . | . |
| | R2 | 17.2 | 16.2 | 3.2 | . | . |
| | a | -3.4 | 3.2 | 1.4 | . | . |
| Environments mean | LOD | 17.9 | 25.9 | . | . | . |
| | R2 | 57.7 | 57.3 | . | . | . |
| | a | -7.7 | 8.6 | . | . | . |

Negative additive effect values (a) indicate that the resistance allele is derived from parent 'Tubbs' Positive additive effect values (a) indicate that the resistance allele is derived from parent 'Einstein'

*ExT population*

For the five QTL detected in the ExT population, two were identified in all seven locations, in chromosome 2AS and 6AL, showing high phenotypic responses (<20%) and a stable epistatic interaction between them in all locations except Mt. Vernon (Table 3.6,

Table 3.7). Minor QTL were identified in just one environment, in chromosome 5BL, 4AL, and 7BL. The resistance allele for QTL in chromosome 2AS and 5BL came from the susceptible parent, while QTL in chromosome 6AL and 4AL and 7BL came from the resistant parent.

**Discussion**

The data does not suggest transgressive segregation in either population, although in the ExT population 35% of the population fell in the same bin as the resistant parent Einstein. Histograms suggest quantitative inheritance of resistance for the TxN population (Fig. 3.1) and the effects of major genes for the ExT population (Fig. 3.2). Two QTL were identified in chromosome 2A. One in the long arm of chromosome 2A with the allele derived from the resistant parent NSA-98-0095 located close to marker *wPt-7011,* which is in the region of marker gwm382 that has been previously reported to be linked to stripe rust resistance in the French cultivar Camp Remy (Boukhatem et al., 2002; Mallard et al., 2005). The other QTL located in chromosome 2A is in the short arm close to marker cfd36 and the allele came from the susceptible parent Tubbs. Many studies have identified QTL in a similar region in the cultivars Camp Remy, Apache, Stephens, Cappelle-Desprez and Recital (Agenbag et al., 2012; Boukhatem et al., 2002; Paillard et al., 2012; Vazquez et al., 2012). The alien introgressed gene *Yr17* from *A. ventricosa* is located in this region and is most likely the resistance gene originated from Tubbs since is known that one of Tubbs parents, cv. Madsen, carries this gene from its parent VPM1 (Allan, 1989). Further evaluation would be needed to confirm it is Y17 because other studies have reported QTL with LOD scores consistent with major genes

that do not correspond to the *Yr17* gene and these QTLs are in a location homeologous to the introgression (Agenbag et al., 2012; Hao et al., 2011).

The cultivar 'Recital' is known to be susceptible to stripe rust, as is Tubbs. If the QTL identified in 2AS is the same for Tubbs and Recital, in neither case does it provide a high resistance. This QTL could be a minor gene, but LOD scores are consistent with major effects. When the effect of the 2AS QTL from Tubbs is removed in a subsequent analysis, a QTL was detected in the exact same region with a high LOD score (~ 12), but from the resistant parent Einstein (data not shown). It is thus possible that the combination of both QTL in chromosome 2AS, one from Tubbs and one from Einstein, provide the high resistance effect seen in the RIL from the ExT cross.

One parent of Tubbs, the cultivar Madsen, shows a high level of resistance to wheat stripe rust that has been stable since release of that cultivar in 1989 (Mundt, unpublished). That resistance was either not transferred to Tubbs or not expressed, as Tubbs was only marginally resistant against stripe rust races present at its release, and is highly susceptible to the newer, highly aggressive races (Hovmøller et al., 2008) of wheat stripe rust now prevalent in the Pacific Northwest. It also is of significance that the 2AS QTL of Tubbs was not found in the TxN population. Such genetic background effects are not rare. For example, backcrossing of two QTL for resistance to barley stripe rust into the susceptible winter feed barley cultivar Steptoe resulted in resistance, but backcrossing the same QTL into the highly susceptible, spring malting cultivar Colter did not (Hayes et al., 2006). Similarly, Ma et al. (1995) reported a high frequency of stripe rust resistance gene suppression in synthetic hexaploid wheats, suggesting the presence of suppressor genes.

In the case of the ExT population, where only two QTL with high phenotypic

variance responses were detected in six of seven locations, it appears that both QTL are major genes or at least provide high resistance effects. In addition, we identified an epistatic interaction between these two QTL, one in chromosome 2AS with the allele from the susceptible parent Tubbs and the other in chromosome 6AL with the allele from the resistant parent Einstein that is located in a similar location to a QTL for resistance to stripe rust in the cultivar 'Platte' (Vazquez et al., 2012). This epistatic interaction is regarded as the reason for the presence of many RIL in the ExT populations with disease severity values as low as those of the resistant parent Einstein (Figure 3.3). The durability of the resistance provided by the interaction between these two QTL is unknown but given it is an interaction it may be more difficult to the pathogen to overcome the resistance and by hence more durable that major genes acting alone. Three additional QTLs were identified in the ExT population. One of these QTL is in chromosomes 7BL and was identified in two of the seven environments. The other QTL were in chromosome in 4AL and 5BL with the later having the allele originating from the susceptible parent (Table 3.7).

The TxN population results suggest a more quantitative disease response, with several QTL identified with the resistance allele from the parent NSA-98-0995. For a RIL to achieve the level of resistance recorded from the resistant parent NSA-98-0995, seven QTL were required (Figure 3.4, Table 1). Results suggest that these QTL may be a combination of major and minor genes. Vazquez et al (2012) reported a similar result for the cultivar Stephens, with 13 QTL being identified, probably a combination of major and minor genes. In .the Vazquez et al (2012) study, Stephens showed a similar resistance level to the disease as the parent NSA-98-0095 (around 35%).

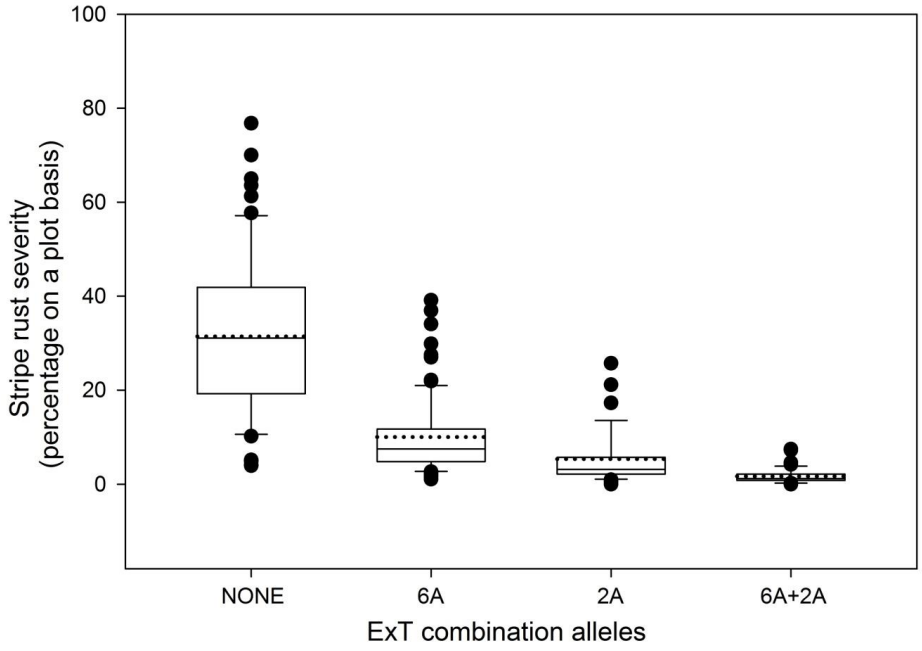


Fig 3.3 ExT population boxplots (quartiles are boxes, medians are continuous lines, means are dotted lines, whiskers extend to the farthest points that are not outliers, and outliers are black dots) for disease severity associated with number of the two identified QTL (2A and 6A) .

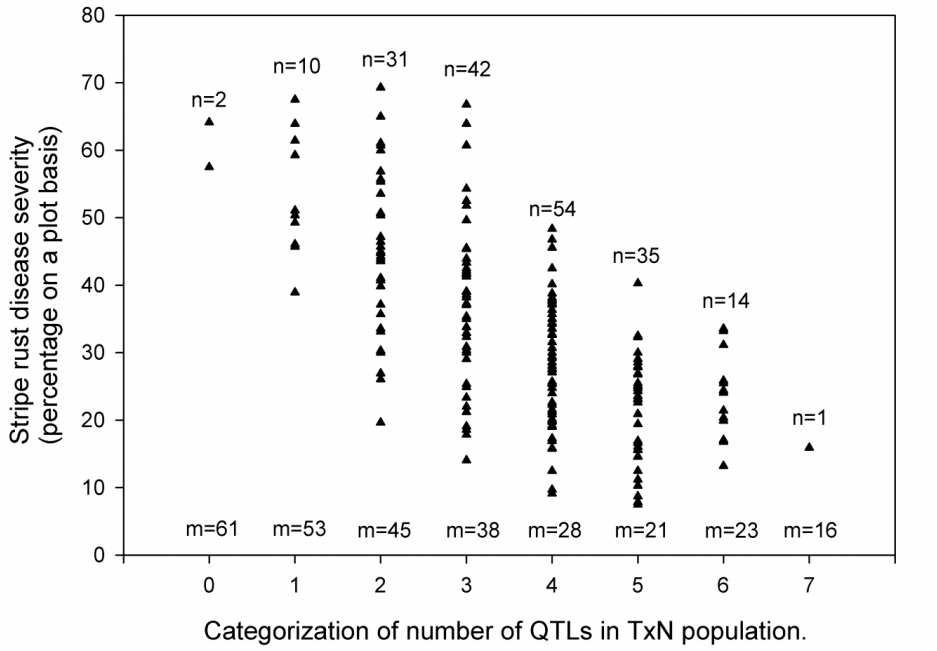


Fig 3.4 Number of QTL identified in the TxN population in categories and their corresponding array of disease severity response. Each triangle is the mean of a single progeny averaged over all locations. The letter indicates the median disease severity for a given number of QTL and n indicates the number of lines in each category.

.Stephens has been considered a cultivar with moderate but durable resistance to stripe rust, prompting the question of what could be the acceptable level of disease susceptibility in commercial cultivars to provide more durable resistance. The QTL in 2BL in the TxN population is positioned in the same region where Mallard et al. (2005) identified a QTL linked to marker barc101, suspected to be gene *Yr7.* In addition, Guo et al. (2008) identified a seedling QTL in the same region from the cultivar 'Aquileja'. In the TxN population was identified a QTL in 5BL close to marker wPt-8285, which has been linked to tan spot (*Cochliobolus sativus*) resistance in another population (Singh et al. 2010). Another QTL was identified in 5AL linked to marker gwm297 and which is linked to *Yr34* (Bariana et al., 2010). An additional QTL was identified in 3BL in the same position where Lin and Chen (2009) reported a QTL for HTAP resistance in the cultivar 'Express'. The QTL in chromosome 4AL has been reported before by Vazquez et al. (2011) and Ramburan et al. (2004). Although there is a QTL identified in chromosome 4AL in the ExT population with the resistance allele originating from Einstein, it does not seem to be located in a similar region of the long arm to the one observed in the TxN population based on the results of this study. The QTL identified in 6B in the TxN population is located in a similar location to one identified by Santra et al. (2008) in cultivar Stephens. The QTL in the TxN population in chromosomes 2AL, 2BL and 5BL with relative high phenotypic response (~12%) were not detected in Pendleton 2011 and Corvallis 2013 environments, raising the question of whether these QTL became ineffective due to the presence of new races of stripe rust. In the TxN population there was also detected two epistatic interactions between alleles from the resistant parent NSA-98-0095, but none of them were identified in Pendleton 2011 and Corvallis 2013,

possibly due to the QTL being involve in the epistatic interaction. The QTL in chromosome 5DL, although identified in one location, was linked to marker wPt-5870, which is known to be linked to the gene *SbmTmr1* which provide resistance to soil-borne wheat mosaic virus (McIntosh, 2010).

This study highlights the complexity of resistance to stripe rust in wheat and the roles specific combinations of genes, genetic background, and interactions among genes play in conferring stripe rust resistance. The results of this study reinforce the importance of combining minor and major genes to provide resistance that may be durable (Chen, 2013; Paillard et al.,2012; Rosewarne et al., 2013; Vazquez et al., 2012). The discovery of epistatic interactions among the QTL for resistance provides an explanation on the variability in disease resistance response seen in both populations. Further work is needed to improve the molecular markers identified in this study by saturating the chromosomes regions of interest to identify markers more closely linked with the stripe rust resistance QTL, although it is important to keep in mind that controlling epistastis is not possible at this point and this phenomenon could not result in level of resistance expected.

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# CHAPTER 4

## SUMMARY

# CHAPTER FOUR

## SUMMARY

Substantial increases in food demand are expected by the year 2050 (Alexandratos and Bruinsma, 2012; Tilman et al., 2011). Along with changes in climate and land use, outbreaks of disease are especially limiting to food production, as crop diseases can lead to significant losses in yield and quality (Anderson et al., 2004; Strange and Scott, 2005) and pathogens are constantly adapting to overcome resistance in new cultivars (McDonald and Linde, 2002). Thus, attaining durable disease resistance is a main goal of most plant breeding programs. Breeding for quantitative resistance, along with increasing genetic diversity in and out of the breeding programs's germplasm pool, are approaches that have long been proposed to slow the development of epidemics and reduce the disease severity in crops (Mundt, 2014).

Wheat, *Triticum aestivum* L., is one of the major agronomic crops produced in the world. The U.S. Pacific Northwest is known for its wheat production for international export and each year, depending on the environment, wheat production in the region is threatened by diseases such as Cephalosporium stripe and stripe rust. Environmental conditions, pathogen population structure, timing of epidemic development, and genetic background all seem to significantly influence the expression partial resistance phenotypes. No resistance gene has been identified for Cephalosporium stripe in wheat and none of the rust R genes identified to date have properties of the characterized pattern recognition receptors (PRR) that detect PAMPs. R genes that detect conserved effectors may provide

more durable resistance. Attaining durable resistance to stripe rust has been challenging and no complete resistance has been identified in to Cephalosporium stripe.

The primary goal of this study was to gain further insight into the genetic basis of resistance to these two diseases to facilitate the development of a method to genotypically select for resistance for Cephalosporium stripe and stripe rust. For that reason, two winter wheat biparental populations were developed that differed for level of resistance/susceptibility to these two diseases. To study the response of the germplasm to the diseases, field trials were conducted over several environments (combinations of years and locations) and the individuals of this study were genotyped with diversity array technology (DArT), simple sequence repeat (SSR) to perform mapping by linkage. The results from each population disease combination were compared and contrasted to identify common chromosome regions of interest with previous QTL studies.

For Cephalosporium stripe, the disease resistance seen in both biparental populations (ExT and TxN) was more of a quantitative nature. The lack of genetic variability of the pathogen *Cephalosporium gramineum* may play a role in the type of resistance observed. On the other hand, the disease response reported for stripe rust in one population (TxN) was the typical quantitative inheritance of resistance, result of several QTL that each explained a small phenotypic response variance. For the population (ExT) in the stripe rust study the phenotypic response seen was explained by mainly two QTL with epistatic interaction between them with a possible suppressor playing a role in the lack of resistance seen in one of the parents.

This study identified the effect of genetic background and possible suppressors of resistance as in the case of the QTL located in chromosome 2AS and derived from the

susceptible parent Tubbs. Further research it is needed to better understand the role epistasis plays in the expression of transgressive segregation seen in the ExT and TxN population and the high levels of resistance seen in the ExT population. Several regions were found in common between the two biparental populations and the two diseases in chromosomes 2AS, 5AL and 6BS.

Chromosomal regions similar to those identified in this study have been reported for Fusarium crown rot, Fusarium head blight, stripe rust and Septoria tritici (Adhikari et al., 2012; Bovill et al., 2006; Buerstmayr and Anderson, 2009; Cuthbert et al., 2007; Gervais, 2003; Kato et al., 1998; Liu et al., 2013; Miedaner et al., 2012; Muhovski et al., 2012). In some cases (Lorang et al., 2012; Wolpert et al., 2002), pathogens that make use of host-selective toxins as pathogenicity factors co-opt the same resistance mechanisms as biotrophic pathogens that follow the gene-for-gene interaction system (Dangl and Jones, 2006). Mengiste (2012) reported that plants respond to the attack of necrotrophic and biotrophic pathogens through both common and contrasting mechanisms.

In conclusion, this study demonstrated the importance of performing QTL analysis over several locations, years, and plant populations. Considering more than one disease helped to focus on common chromosomal regions relevant to the multiple traits that must be considered in a successful plant breeding program. This study highlights the importance of considering the pathogen biology, the action of minor and major genes, possible epistatic interactions and the individual genetic background in the disease resistance response seen for every specific population/disease.

The results of this research also leads to several avenues of research that should be explored to further explain disease resistance response observed in wheat. These future

research areas include; 1) determining if the resistance response mapped to common chromosome regions for both diseases is quantitative; 2) determining if specific combinations of resistance QTL tend to be more effective than others when combined in agronomically relevant genetic backgrounds and 3) determining the levels of quantitative resistance that will optimize reduced selection pressure on pathogen populations without compromising the economic return to the wheat producer.

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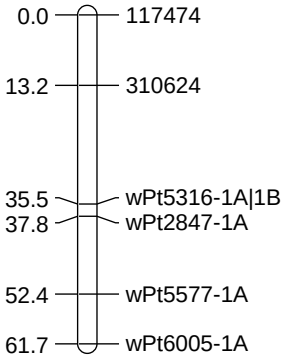
# APPENDIX 1
## Linkages maps used in this study for Tubbs x NSA-98-0995 population

**1A1**

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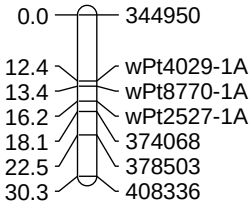


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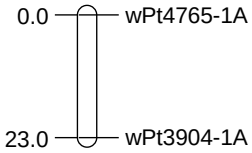


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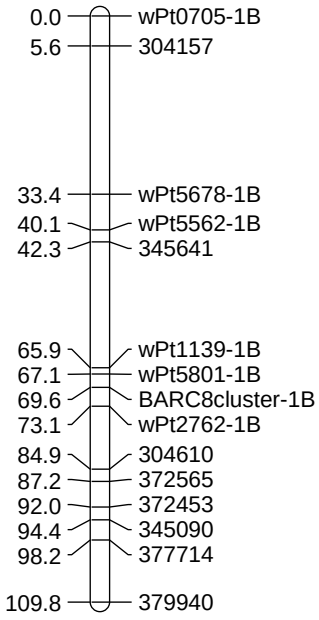


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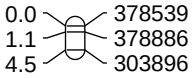


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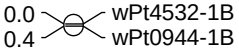

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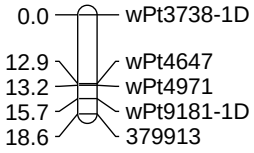


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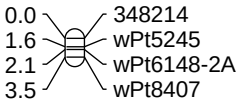


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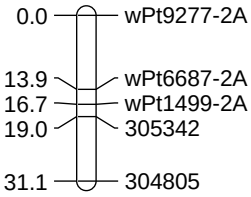


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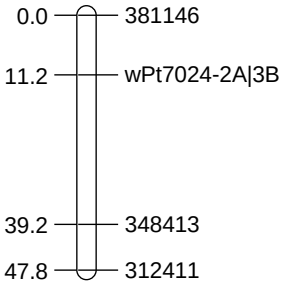

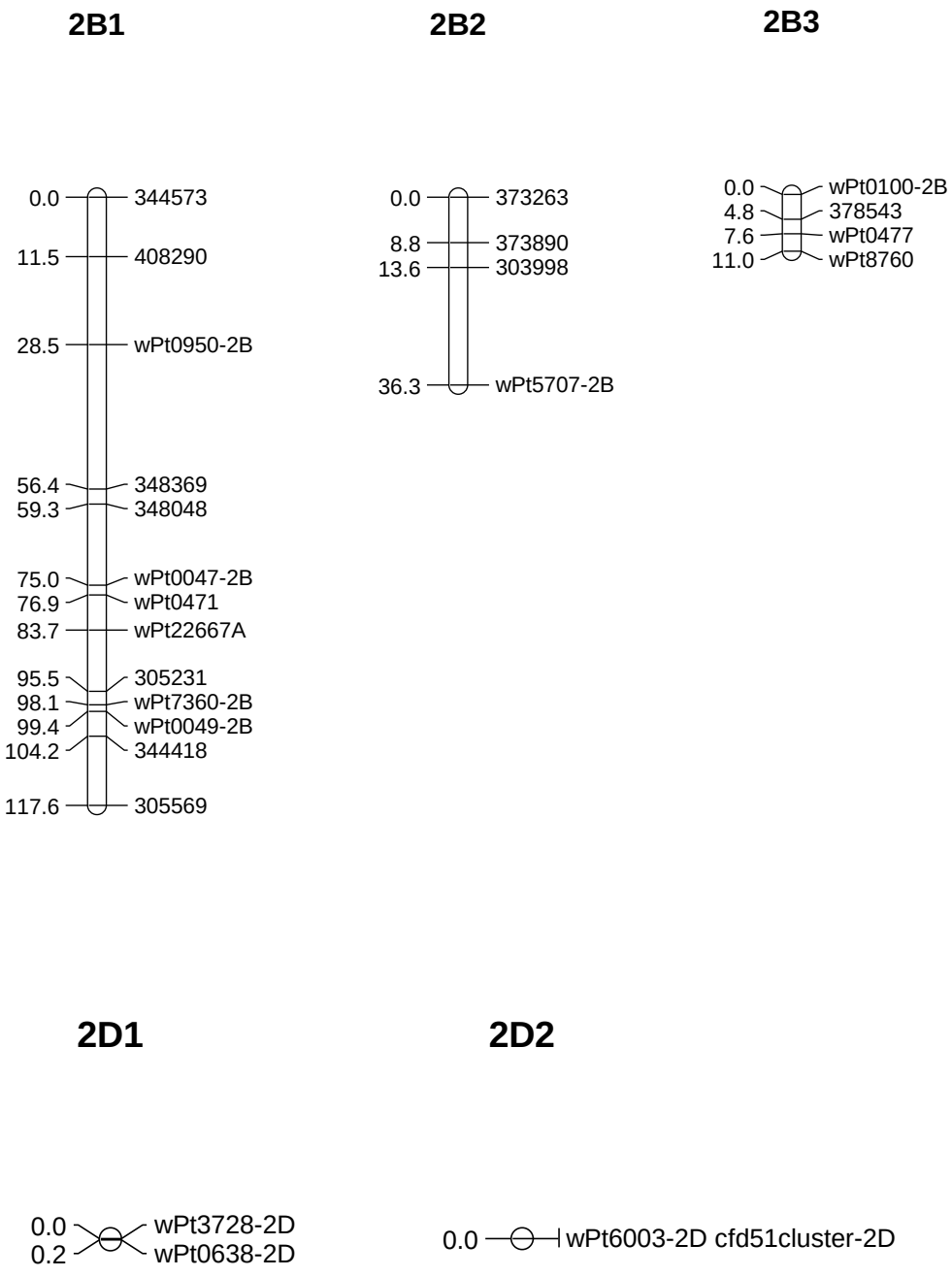
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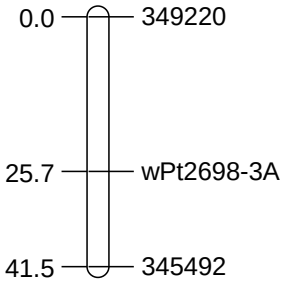


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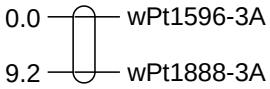


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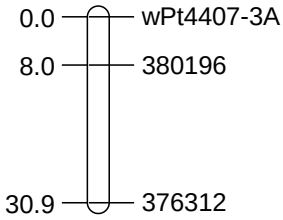


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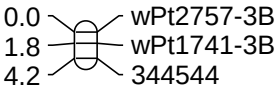


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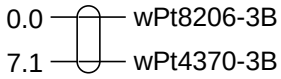


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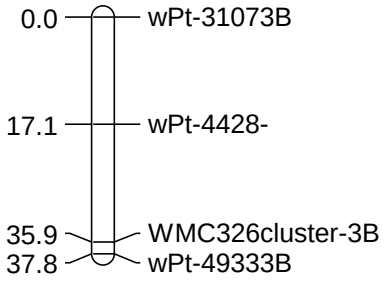


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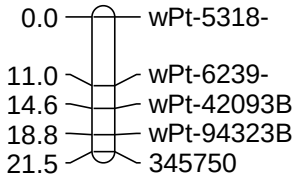


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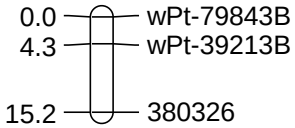

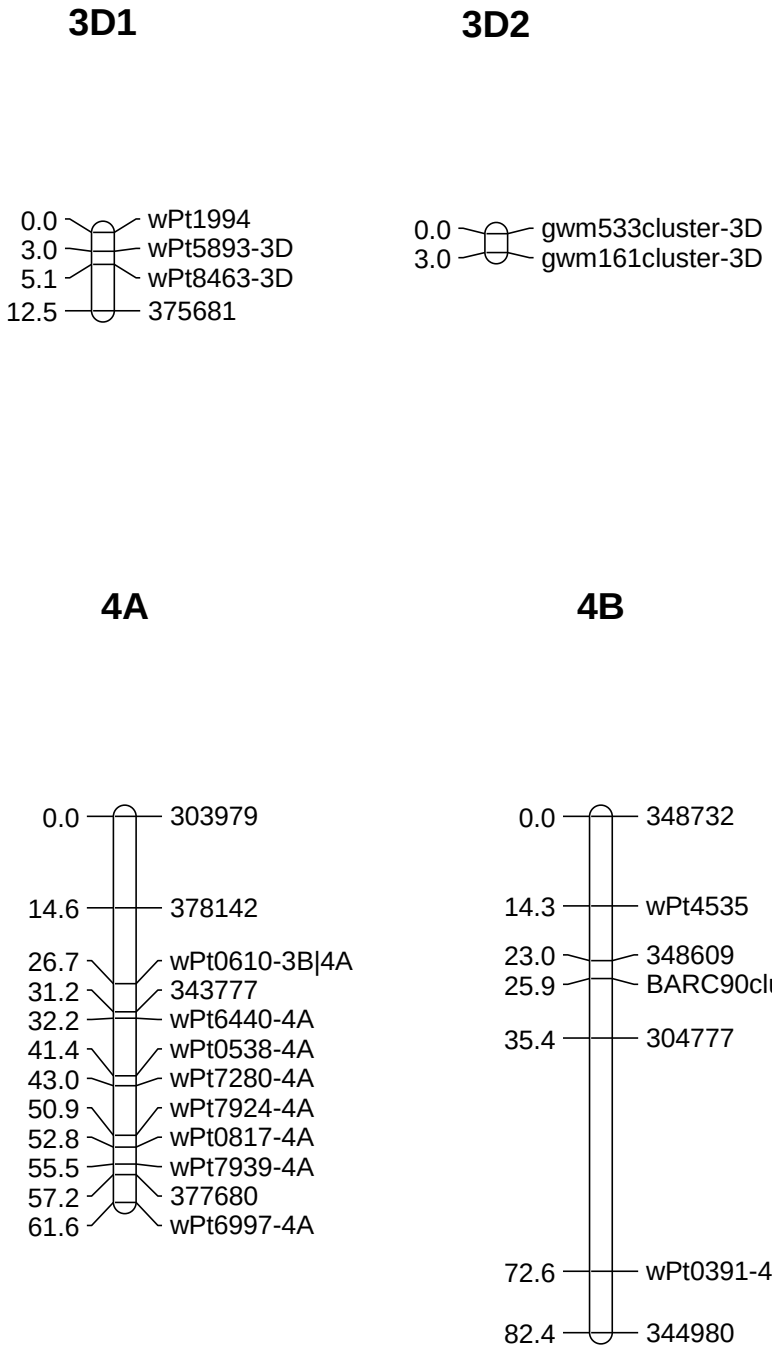
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3.0 gwm161cluster-3D
4A
0.0 303979
14.6 378142
26.7 wPt0610-3B|4A
31.2 343777
32.2 wPt6440-4A
41.4 wPt0538-4A
43.0 wPt7280-4A
50.9 wPt7924-4A
52.8 wPt0817-4A
55.5 wPt7939-4A
57.2 377680
61.6 wPt6997-4A
4B
0.0 348732
14.3 wPt4535
23.0 348609
25.9 BARC90cluster-4B
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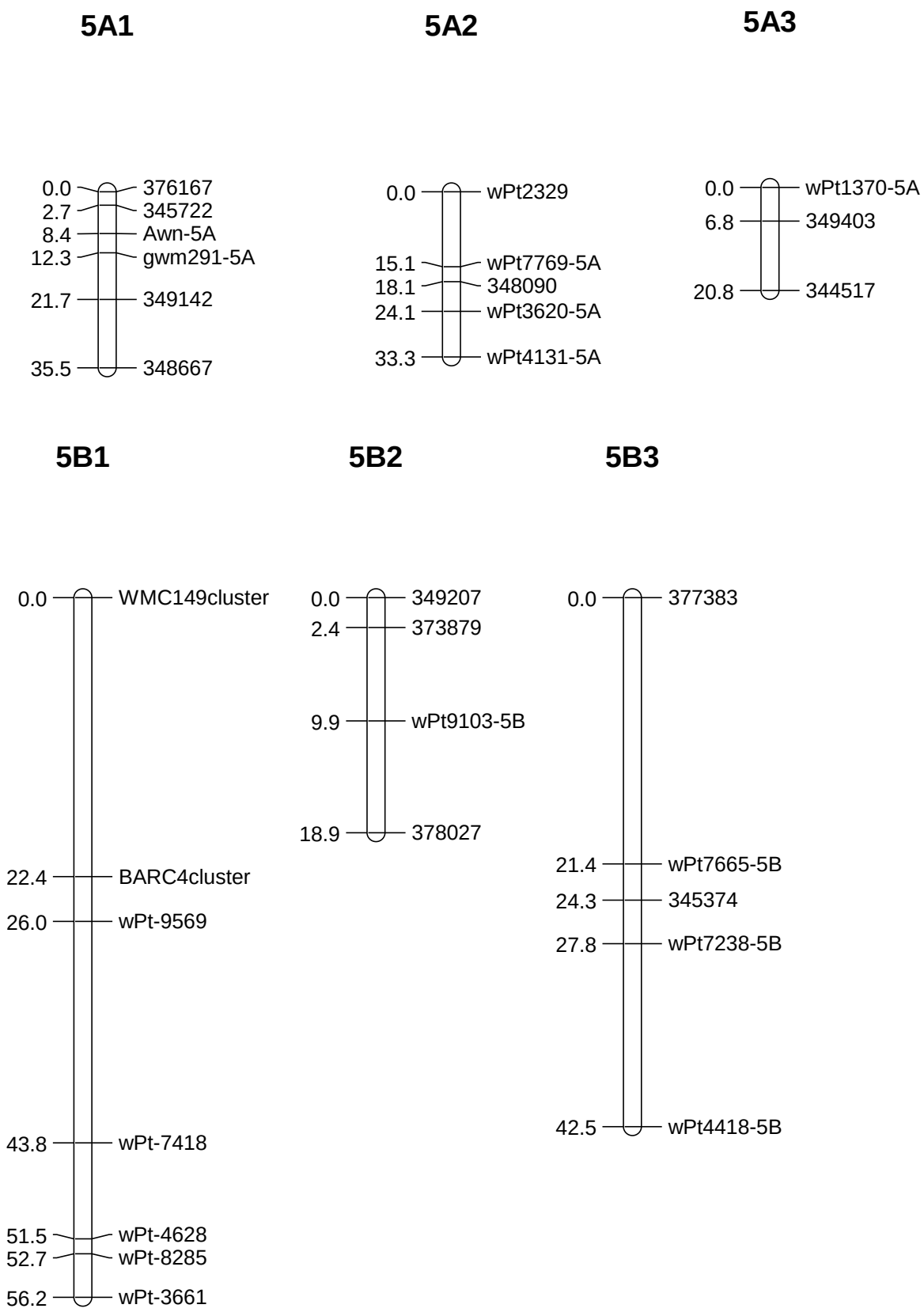
82.4 344980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0.0 376167
2.7 345722
8.4 Awn-5A
12.3 gwm291-5A
21.7 349142
35.5 348667
5A2
0.0 wPt2329
15.1 wPt7769-5A
18.1 348090
24.1 wPt3620-5A
33.3 wPt4131-5A
5A3
0.0 wPt1370-5A
6.8 349403
20.8 344517
5B1
0.0 WMC149cluster
22.4 BARC4cluster
26.0 wPt-9569
43.8 wPt-7418
51.5 wPt-4628
52.7 wPt-8285
56.2 wPt-3661
5B2
0.0 349207
2.4 373879
9.9 wPt9103-5B
18.9 378027
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27.8 wPt7238-5B
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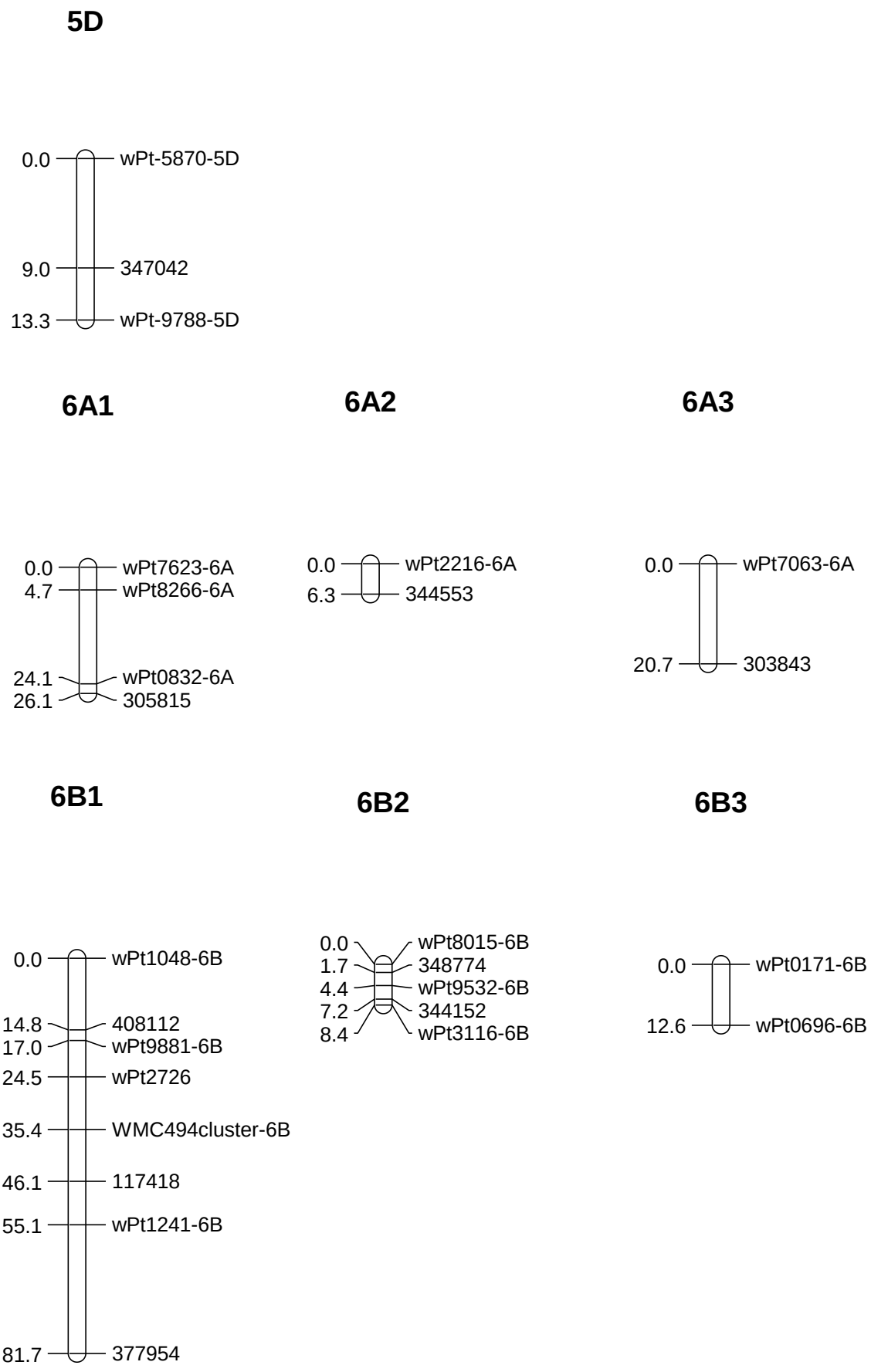
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347042
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wPt-9788-5D
6A1
6A2
6A3
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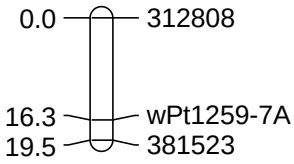
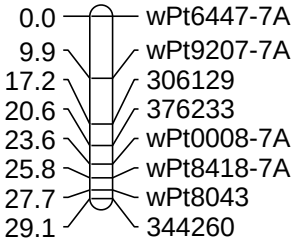
wPt7623-6A
4.7
wPt8266-6A
24.1
wPt0832-6A
26.1
305815
0.0
wPt2216-6A
6.3
344553
0.0
wPt7063-6A
20.7
303843
6B1
6B2
6B3
0.0
wPt1048-6B
14.8
408112
17.0
wPt9881-6B
24.5
wPt2726
35.4
WMC494cluster-6B
46.1
117418
55.1
wPt1241-6B
81.7
377954
0.0
wPt8015-6B
1.7
348774
4.4
wPt9532-6B
7.2
344152
8.4
wPt3116-6B
0.0
wPt0171-6B
12.6
wPt0696-6B

**7A1**




**7A2**

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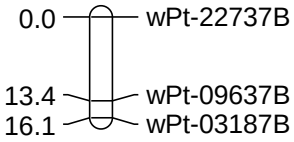
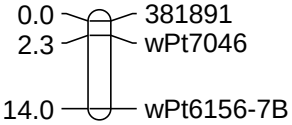
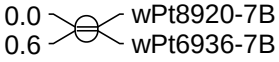
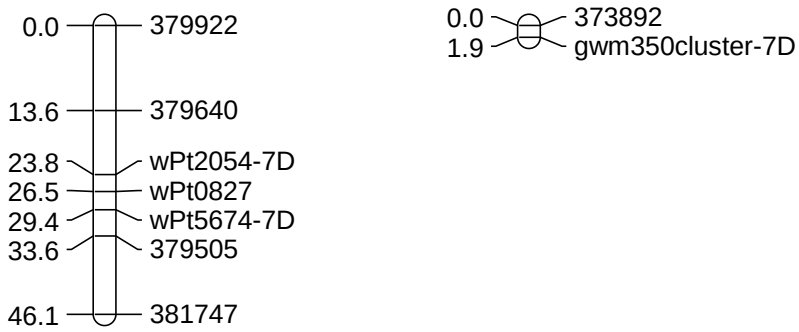



**7B2**




**7B3**




7D1
7D2
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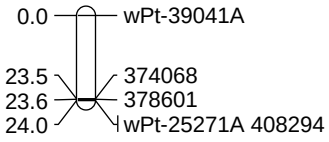
379922
13.6
379640
23.8
wPt2054-7D
26.5
wPt0827
29.4
wPt5674-7D
33.6
379505
46.1
381747
0.0
373892
1.9
gwm350cluster-7D

**APPENDIX 2**
**Linkages maps used in this study for Einstein x Tubbs population**

**1A1**

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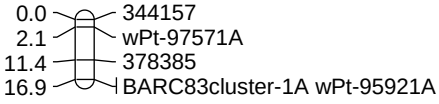
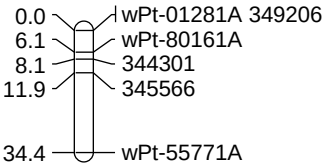
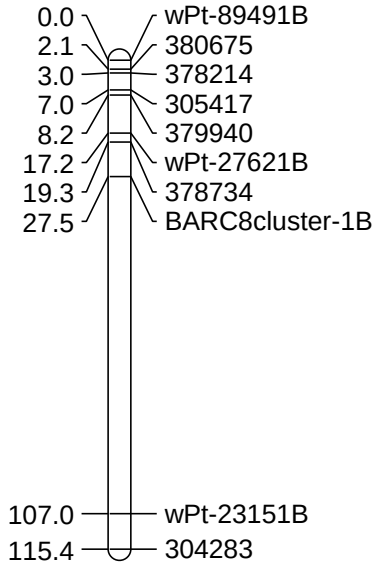


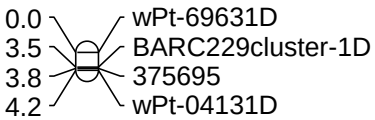

**1A3**




**1B**



## 1D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3.5
3.8
4.2
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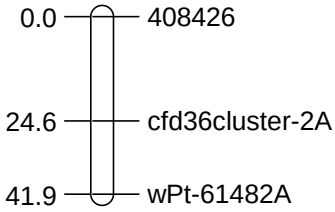
BARC229cluster-1D
375695
wPt-04131D

## 2A1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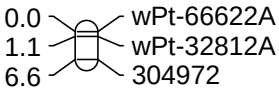
24.6
41.9
408426
cfd36cluster-2A
wPt-61482A

## 2A2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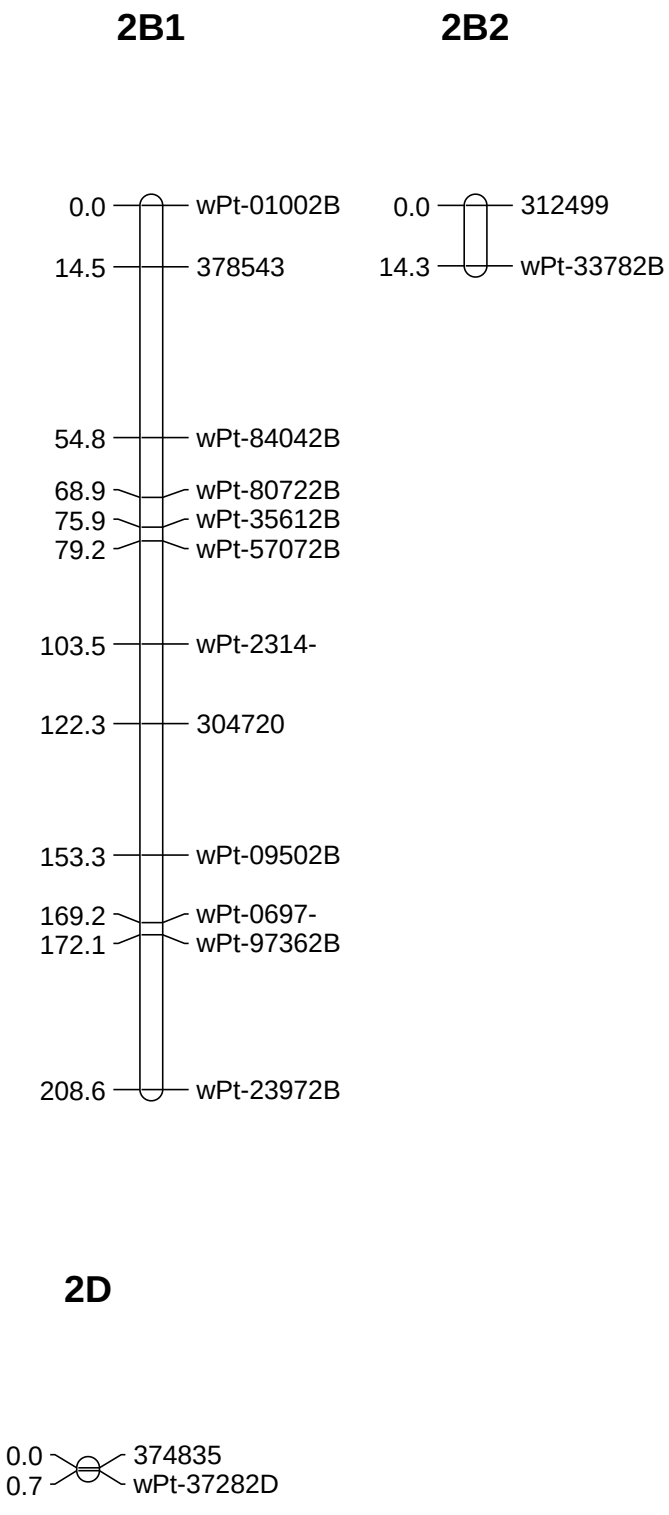
1.1
6.6
wPt-66622A
wPt-32812A
304972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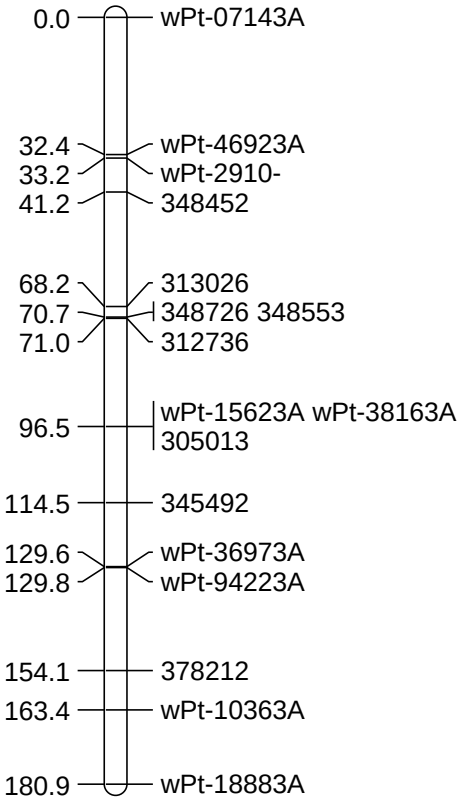
wPt-01002B
14.5
378543
54.8
wPt-84042B
68.9
wPt-80722B
75.9
wPt-35612B
79.2
wPt-57072B
103.5
wPt-2314-
122.3
304720
153.3
wPt-09502B
169.2
wPt-0697-
172.1
wPt-97362B
208.6
wPt-23972B
0.0
312499
14.3
wPt-33782B
2D
0.0
374835
0.7
wPt-37282D

**3A**


0.0
wPt-07143A
32.4
33.2
41.2
wPt-46923A
wPt-2910-
348452
68.2
70.7
71.0
313026
348726 348553
312736
96.5
wPt-15623A wPt-38163A
305013
114.5
345492
129.6
129.8
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wPt-94223A
154.1
378212
163.4
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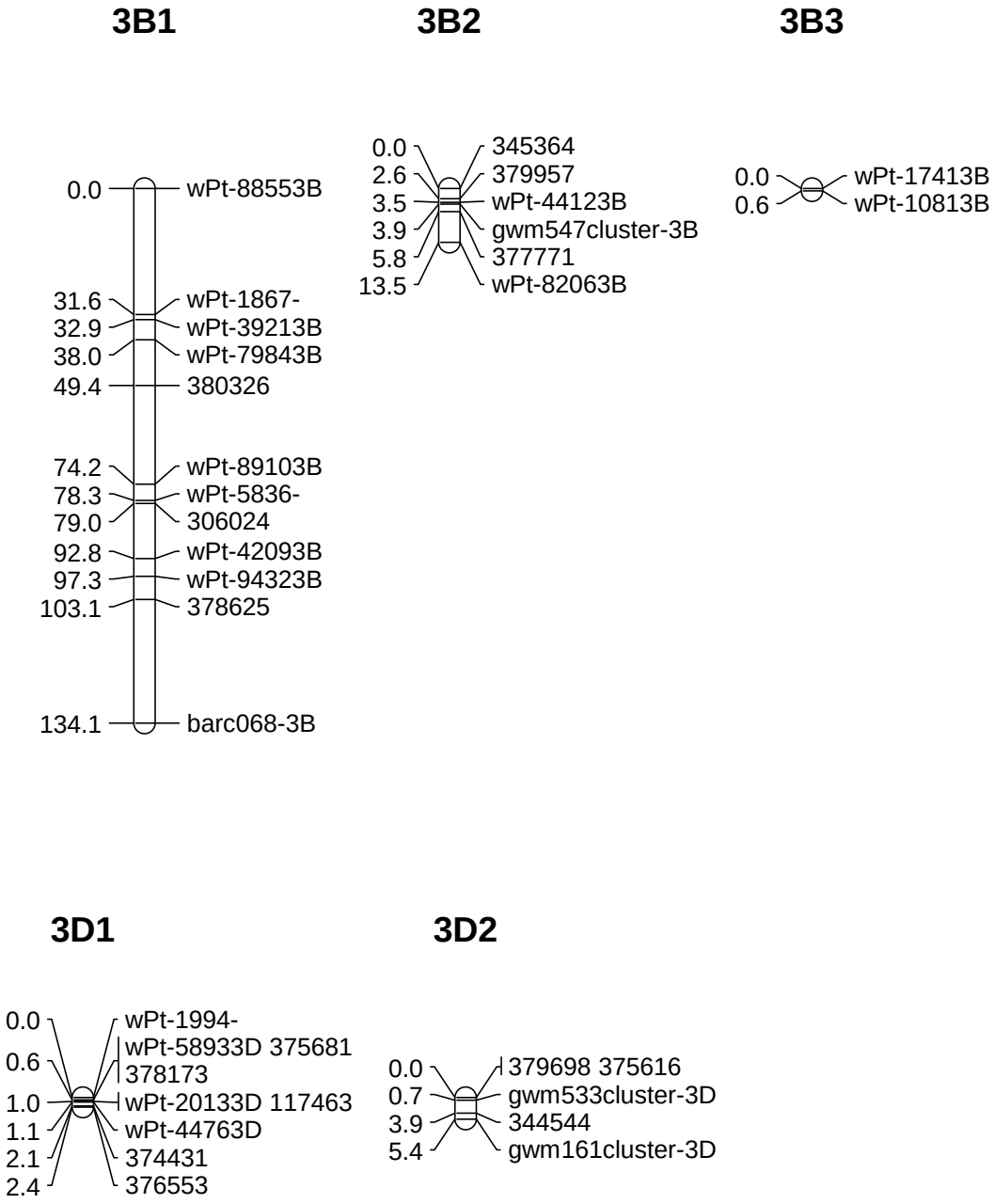
180.9
wPt-18883A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wPt-39213B
wPt-79843B
380326
74.2
78.3
79.0
92.8
97.3
103.1
wPt-89103B
wPt-5836-
306024
wPt-42093B
wPt-94323B
378625
134.1
barc068-3B
3B2
0.0
2.6
3.5
3.9
5.8
13.5
345364
379957
wPt-44123B
gwm547cluster-3B
377771
wPt-82063B
3B3
0.0
0.6
wPt-17413B
wPt-10813B
3D1
0.0
0.6
1.0
1.1
2.1
2.4
wPt-1994-
wPt-58933D 375681
378173
wPt-20133D 117463
wPt-44763D
374431
376553
3D2
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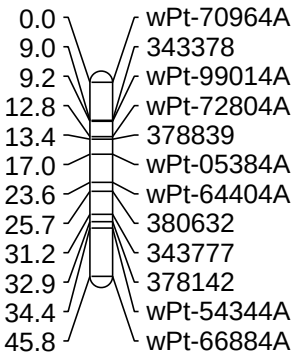
0.7
3.9
5.4
379698 375616
gwm533cluster-3D
344544
gwm161cluster-3D

**4A**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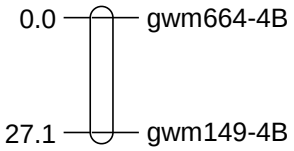
9.0 343378
9.2 wPt-99014A
12.8 wPt-72804A
13.4 378839
17.0 wPt-05384A
23.6 wPt-64404A
25.7 380632
31.2 343777
32.9 378142
34.4 wPt-54344A
45.8 wPt-66884A

**4B1**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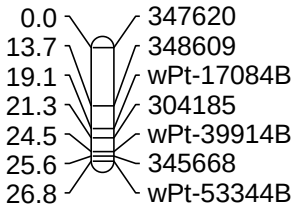
27.1 gwm149-4B

**4B2**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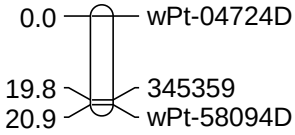
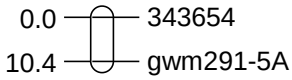
24.5 wPt-39914B
25.6 345668
26.8 wPt-53344B

**4D**


0.0 wPt-04724D
19.8 345359
20.9 wPt-58094D

**5A1**

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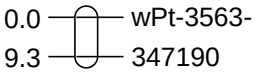


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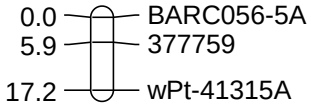
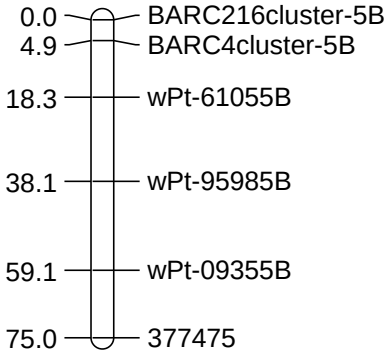



**5B**

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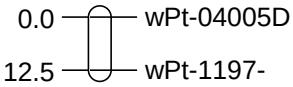

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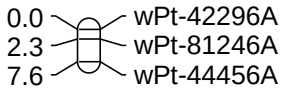


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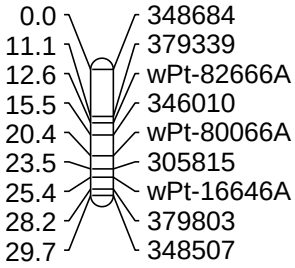


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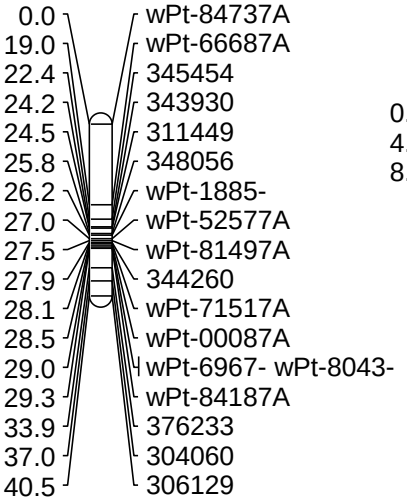


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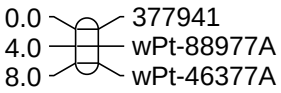


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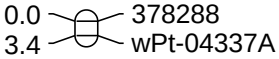
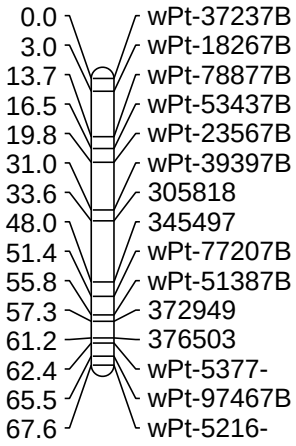


**7B**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16.5
19.8
31.0
33.6
48.0
51.4
55.8
57.3
61.2
62.4
65.5
67.6
wPt-37237B
wPt-18267B
wPt-78877B
wPt-53437B
wPt-23567B
wPt-39397B
305818
345497
wPt-77207B
wPt-51387B
372949
376503
wPt-5377-
wPt-97467B
wPt-5216-

# APPENDIX 3

# QTL VALIDATION FOR DISEASE RESISTANCE TO STRIPE RUST AND CEPHALOSPORIUM STRIPE BY ASOCIATION MAPPING IN A COLLECTION OF SEVENTY-TWO WINTER WHEAT LINES


## Abstract

Stripe rust (caused by *Puccinia striiformis* f.sp. *tritici*) and Cephalosporium stripe (caused by *Cephalosporium gramineum*) can result in severe yield and grain quality loss of wheat (*Triticum aestivum* L.) in the Pacific Northwest, USA. Favorable weather conditions and the appearance of new races are becoming important factors for annual development of stripe rust in the region. Cephalosporium stripe can be a limiting factor in the adoption of conservation tillage practices and little is known about the inheritance of resistance. Association-mapping analysis using 72 wheat cultivars genotyped by single nucleotide polymorphisms (SNP) was performed for stripe rust and Cephalosporium stripe resistance. A linear mixed-effects model (MLM) was used to detect marker-trait associations incorporating covariance of population structure and relative kinship with the objective to compare and validate quantitative trait loci (QTL) results previously identified by linkage mapping using bi-parental populations. Preliminary results showed seven SNP markers associated with stripe rust ($\alpha c$=0.05) and five SNPs associated with Cephalosporium stripe in chromosome locations where QTL for Cephalosporium stripe and stripe rust were identified in previous studies. Alignment of the sequences to known sequences, using the Basic Local Alignment Search Tool (BLAST), resulted in three SNPs: wsnp_Ex_c1246_2393249, wsnp_Ex_c17294_25964947 and wsnp_Ex_c650605-6395264 associated with the biological functions of a leucine-rich-repeat family protein,

Rp1-like protein pseudogene, and a kinase, respectively. These preliminary results hold promise for association analysis as an effective approach for identifying and validating quantitative trait locus (QTL) for disease resistance in wheat.

## Introduction

Association mapping is an approach to genetically dissect complex traits. It is based on the non-random association between alleles at a locus and the phenotypic traits of interest across a diverse germplasm set (Weir, 1996). Recently, association mapping has been used to identify associations between molecular marker loci and complex traits of interest in wheat, including disease resistance (Adhikari et al., 2012; Breseghello and Sorrells, 2006; Kollers et al., 2013; Yu et al., 2012). Association mapping does not require the development of bi-parental progeny and has potentially higher resolution power for mapping QTL and greater capacity for detecting additional alleles than traditional QTL mapping procedures based on bi-parental populations (Cavanagh et al., 2013; Zhu et al., 2008). Single nucleotide polymorphism (SNP) markers have increased the amount of sequence information available for determination of gene function in cereals (Trick et al., 2012). A SNP is a DNA sequence variation occurring when a single nucleotide in the genome differs between members of a biological species or paired chromosomes in an individual. Although the genomic distribution of SNPs is not homogenous, SNPs usually occur in non-coding regions more frequently than in coding regions (Gupta et al. 2008).

The purpose of this study was threefold: 1) To use SNP markers and association mapping to identify loci associated with resistance in wheat (*Triticum aestivum* L.) to stripe rust (caused by *Puccinia striiformis Westend* f.sp. *tritici* Erikss) and Cephalopsorium stripe (caused by *Cephalosporium gramineum* Nisikado & Ikata). 2) To use association mapping to validate loci for resistance to these two diseases that were previously identified from QTL analysis of biparental mapping populations. 3) To

determine if SNP markers associated with disease reaction align with sequences known to be associated with plant disease defense genes.

## Materials and methods

Plant material and phenotypic data

A panel of 72 diverse wheat (*Triticum aestivum* L.) lines was selected from the wheat breeding program in the Department of Crop and Soil Science at Oregon State University, Corvallis, Oregon (Table 4.1). The diversity panel consisted of lines known to be resistant to stripe rust and Cephalosporium stripe, such as NSLWW441 (cv. Einstein), as well as susceptible genotypes, such as Tubbs. Experiments were conducted at the Columbia Basin Agricultural Research Center field station near Pendleton in 2008, 2010 and 2011 for Cephalosporium stripe and 2010 and 2011 for stripe rust. Plots consisted of two rows 2.5 m long that were later trimmed to 1.8 m long post-heading and prior to collecting phenotypic data. Fertilization and weed control were appropriate for commercial winter wheat production in eastern Oregon.

Before planting, oat kernels infested with *C. gramineum* (Mathre and Johnston, 1975) were added to the seed envelopes in an amount equal to the volume of wheat seed. Stripe rust resulted from natural inoculation. Planting dates were in early September to increase chances of high disease incidence. One disease reading was taken for each plot in each year. Disease readings occurred during the last week of June, at the time of dough development Zadoks 80 (Zadoks, et al., 1974). Disease severity was assessed for Cephalosporium stripe by visual estimation of the percentage of tillers that were ripening prematurely (whiteheads) (Mathre and Johnston, 1975; Quincke et al., 2011) in each plot.

Percent stripe rust severity was estimated visually on a whole-plot basis according to the modified Cobb Scale (Roelfs et al. 1992).

SNP genotyping

For genomic DNA extraction, seed of the 72 lines were sent to Dr. Deven See at the USDA-ARS Wheat Genetics, Quality Physiology and Disease Research Unit, Pullman, WA. The seed were planted in the greenhouse. Freshly collected tissue from young leaves were immediately flash-frozen in liquid nitrogen and ground to a fine powder with mortar and pestle. Total DNA was isolated using the DNAeasy Plant Mini Kit (Qiagen, Maryland USA). Genomic DNA was genotyped with 9,000 Infinium iSelect SNPs using the BeadStation and iScan instruments according to the manufacturer's protocols (Illumina, Inc. San Diego California, USA). SNP clustering and genotype calling were performed using the GenomeStudio v2011.1 software (Illumina, Inc. San Diego California, USA).

Population structure and association analysis

A total of 5,232 polymorphic SNPs were included in the analysis. Substructure within the wheat accessions was investigated using principal component analysis (PCA) using SAS PROC PRINCOMP (SAS Institute, NC) on the SNP data for lines and the covariance matrix (Q matrix) was obtained. To display results, line scores from the first principal component were plotted against the second principal component. For the association analysis, the missing data were imputed using the data imputation function in TASSEL (http://www.maizegenetics.net/tassel/). The trimmed marker data sets were used to generate a marker similarity matrix containing all lines (Kinship or K matrix) using TASSEL. Both Q and K matrices were used in the mixed linear model (MLM) to

correct for both population and family structure Yu et al. (2005). A false discovery rate (FDR) of 0.05 was used as a threshold for significant association (Benjamini and Hochberg, 2000). The disease resistance values for Cephalosporium stripe and stripe rust measured in Pendleton 2008, 2011 and 2012 were used for marker-trait association analysis.

**Preliminary Results**

Several analyses were run using different parameters to better account for the variability in the germplasm set. Given the complex nature of the study, more runs are needed to present final results. Results for the association mapping analysis presented here, although preliminary, are promising given that some of the identified SNPs associated with disease resistance are located in the same regions where QTL for diseases resistance were identified.

Population structure

Histograms for both diseases suggest quantitative variation (Fig 4.1 and Fig 4.2). The first three principal components explain about 22% of the variation that is captured. Plotting the first two principal components shows no perceptible population structure. Small substructure is visible among lines that are closely related, as is seen in a cluster around Tubbs and around NSLWW41 (cultivar Einstein) (Fig 4.3).

Association mapping

Associations between SNPs marker mean phenotypic values were tested by MLM models, where the Q-matrix and the K-matrix were incorporated. The seven top SNPs

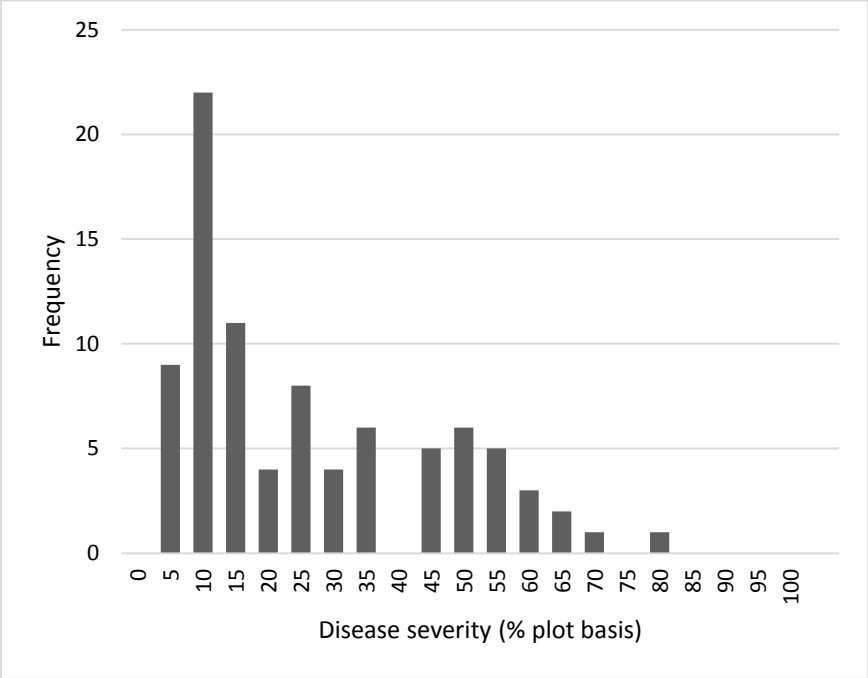


Figure 4.1 Histogram for stripe rust disease severity of the diverse wheat collection.

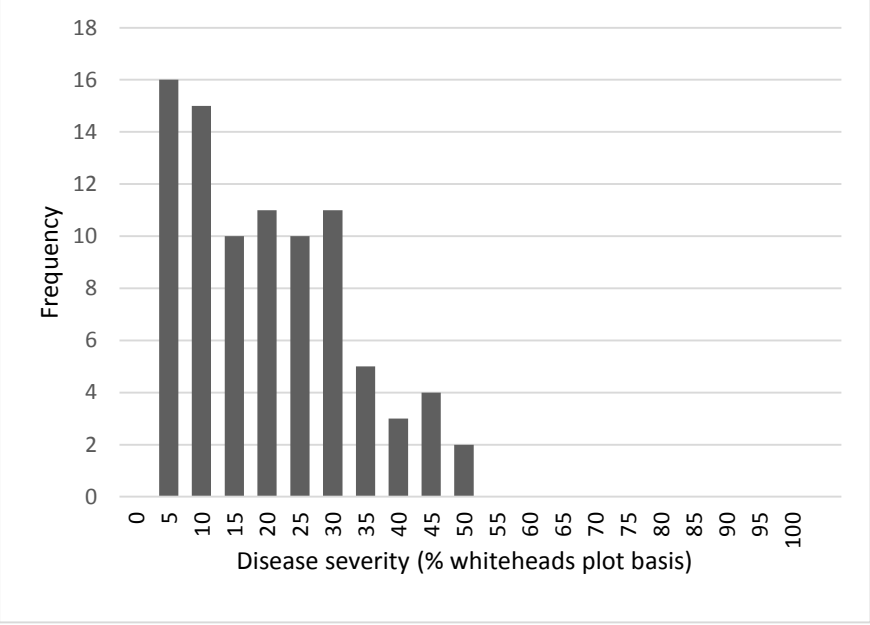


Figure 4.2 Histogram for Cephalosporium stripe disease severity of the diverse wheat collection.

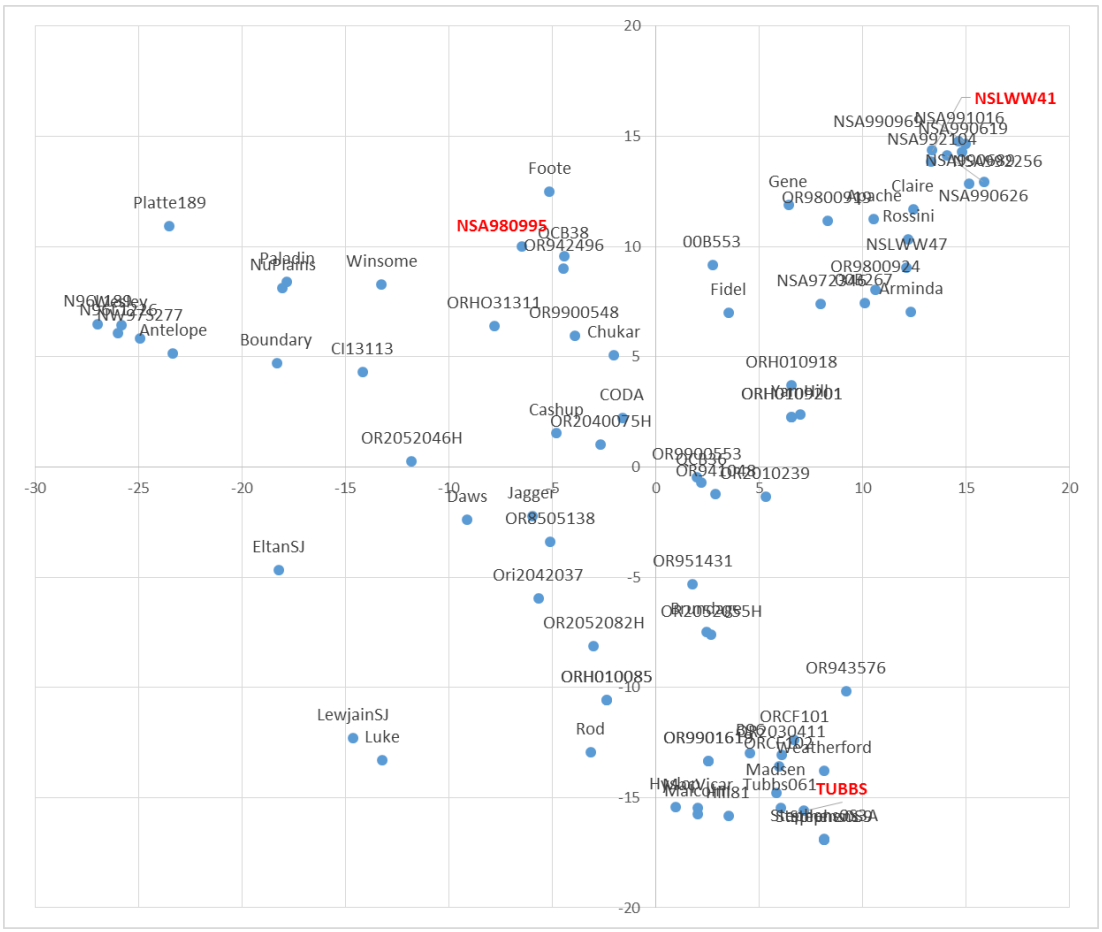


Figure 4.3 Principal component plot showing genetic structure of 84 wheat lines, including the 72 lines used for the association mapping study. In red are cultivars used as parents for the QTL studies described in Chapters 2 and 3. NSLWW41 is cultivar Einstein.

associated with Cephalosporium stripe and the five top SNPs associated with stripe rust were used to search for a match of their alignments in the Basic Local Alignment Search Tool (BLAST) database and their location in the chromosome identified (Figure 4.4 and Figure 4.5).

The following SNPs are those to which a location in the chromosome was found and a biological function associated with it: the SNPs wsnp_BE494474A_Ta_2_3 located in chromosome 3A; wsnp_Ex_c1246_2393249, wsnp_Ex_c17294_25964947 in

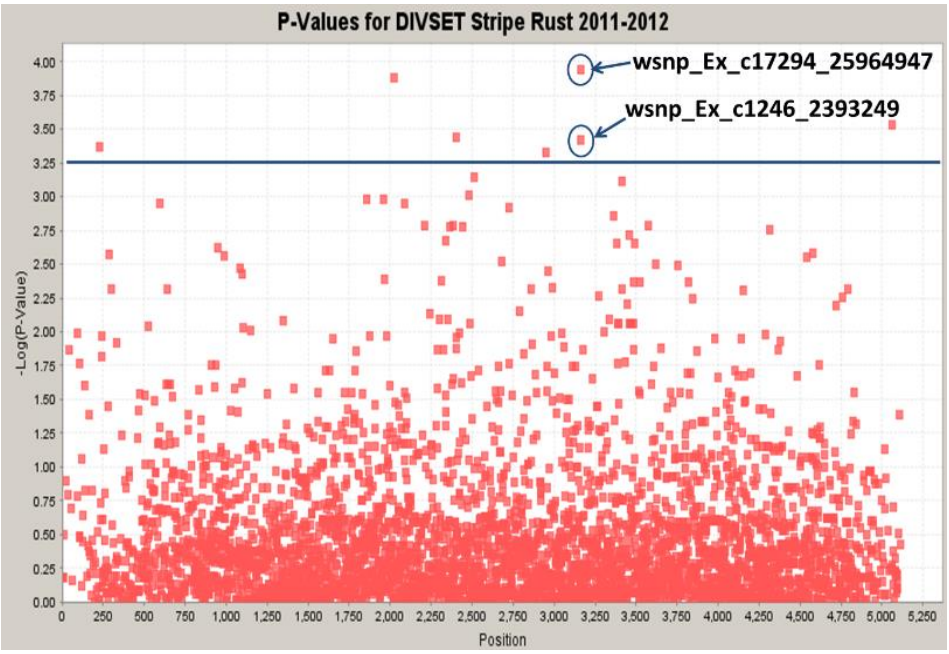


Figure 4.4. Manhattan plot showing the seven top significant SNP associated with stripe rust. The SNPs encircled are those to which a location in the chromosome was found and a biological function was associated to it via BLAST.

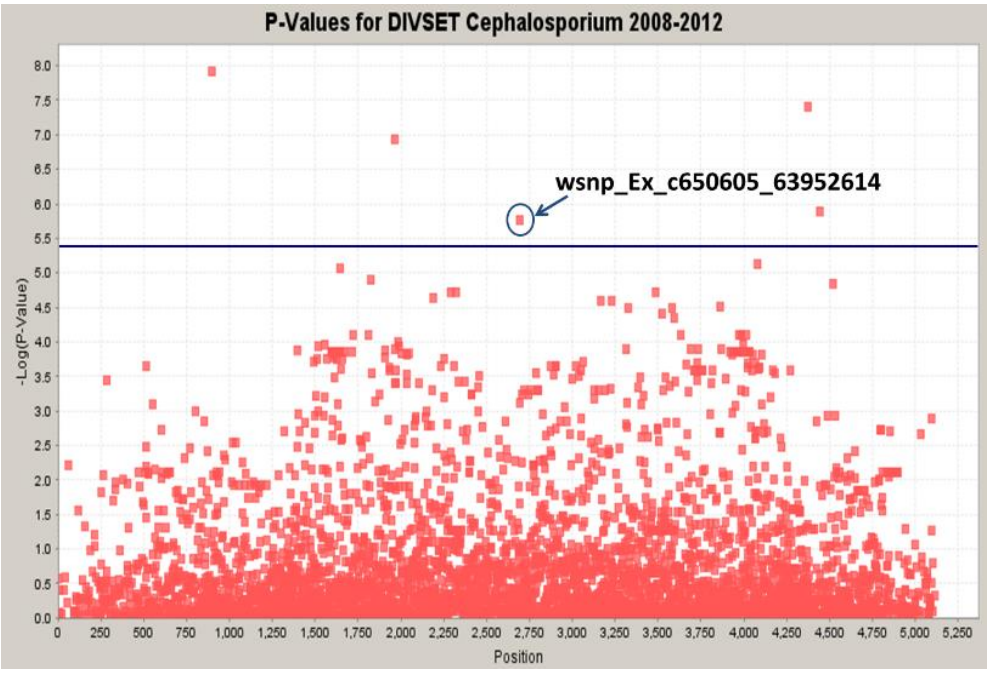


Figure 4.5. Manhattan plot showing the five top significant SNP associated with Cephalosporium stripe. The SNPs encircled are these to which a location in the chromosome was found and a biological function was associated to it via BLAST.

Table 4.1 Chromosome location and probable biological function of six SNPs out of 12 most strongly associated with stripe rust and Cephalosporium stripe.

| Disease | Chromosome location | SNP Marker | Probable biological function |
|---|---|---|---|
| Stripe rust | 3A | wsnp_BE494474A_Ta_2_3 | katanin p60 ATPase-containing subunit A-like 2-like |
| Stripe rust | 4AL | wsnp_Ex_c1246_2393249 | Leucine Rich Repeat family protein |
| Stripe rust | 4AL | wsnp_Ex_c17294_25964947 | Rp1-like protein pseudogene |
| Stripe rust | 2BL | wsnp_Ex_rep_c72569_70908990 | vacuolar protein sorting-associated protein 41 homolog |
| Cephalosporium stripe | 5B | wsnp_Ex_c65605_63952614 | phosphatidylinositol 3- and 4-kinase family protein |
| Cephalosporium stripe | 6A | wsnp_Ex_rep_c69627_68580121 | auxin response factor 16 |

chromosome 4AL; and wsnp_Ex_rep-c72569_70908990 in 2BL were all associated with resistance response to stripe rust. The SNPs wsnp_Ex_c650605-6395264 was located in chromosome translocation 5B:7B, region stated here as C5BL; SNP wsnp_Ex_rep_c69627_6880121 was located in chromosome 6A; and wsnp_BE494482b_Ta_2_1 was located in chromosome 6A are all associated with Cephalosporium stripe (Table 4.1)

## Discussion

In this study, association mapping was used to dissect the genetic basis of disease resistance to stripe rust and Cephalosporium stripe in a collection of 72 wheat accessions that correspond to elite material and, or frequent cultivars used for the development of new varieties, were evaluated in two and three environments, respectively (Table 4.7). The divergent position of each line in the principal component analysis suggests a

complex genetic background of the winter wheat germplasm set used, which is favorable in this study given the small size of the population for this type of analysis and where existing population structure and relatedness among the lines could lead to spurious results.

In the BLAST analysis, SNP markers wsnp_Ex_c1246_2393249 and wsnp_Ex_c17294_25964947 associated with stripe rust resistance aligned to a nucleotide binding site-leucine rich repeat (NBS-LRR) family protein and an Rp1-like protein pseudogene, respectively. Both proteins have previously been associated with resistance mechanisms against fungi. NBS-LRR is a resistance protein that triggers the hypersensitivity response by interaction with avirulence genes (Jones and Dangl 2006). The Rp1 gene is associated with resistance to rust disease (Sun et al., 2001). Both SNPs were located in chromosome 4AL for resistance to stripe rust. A QTL in chromosome 4AL for stripe rust resistance was reported in the mapping populations Stephens x Platte (Vazquez et al., 2012) and Tubbs x NSA-98005 (Chapter 3).

Associations found for Cephalosporium stripe resistance resulted in identification of SNP wsnp_Ex_c650605-6395264, mapped on chromosome 5B and associated with a kinase protein in similar region where the QTL on C5BL (designated C5BL because QTL is locate in the centromere-long arm 5B:7B translocation) for resistance to Cephalosporium stripe was mapped in the Einstein x Tubbs and Tubbs x xNSA-98005 populations (Chapter 2). This SNP was also mapped with a QTL for stripe rust resistance detected in this region for the Einstein x Tubbs population (Chapter 3). There are several structures related to kinases in disease resistance, such as the mitogen-activated protein kinase (MAPK), which plays an important role in disease susceptibility to necrotrophic

pathogens (Rudd et al., 2008) or receptor like kinase (RLK), such as the wall associated-kinase (WAK1) (Brutus et al., 2010). It is important to note that the SNP and the QTL identified in C5BL is located in the known translocation 5B:7B. Translocations affect the ability to determine accurate marker orders and distances. It is known that translocation 5B:7B is present in germplasm from Western Europe, an origin of many of the lines used in this study (Badaeva et al., 2007) (Table 4.6). Though this translocation complicates mapping analyses, this translocation is considered to have adaptive value, and Western European germplasm is known for its good resistance to diseases (Paillard et al., 2012; Mallard et al. 2005).

Several studies to genetically dissect resistance to biotrophic and necrotrophic pathogens have been done using association mapping (Adhikari et al., 2012; Breseghello and Sorrells, 2006; Kollers et al., 2013; Maccaferri et al., 2010 and Yu et al., 2012). The pathogen that causes stripe rust (*Puccinia striiformis tritici*) and the pathogen that causes Cephalosporium stripe (*Cephalosporium gramineum*) each has a different pathogenic biology. While biotrophic pathogens, such as *P. striiformis*, require a living host to complete their infection process, necrotrophic pathogens, such as *C. gramineum*, complete their infection cycle in dead or dying tissue. Avirulence factors associated with biotrophic pathogens and small proteinaceous molecules that act as toxins for necrotrophic pathogens may trigger the same host cell resistance signaling pathways, with the difference that necrotrophic pathogens utilize host programmed cell death for its own benefit (Wolpert, 2002). Lorang et al. (2007) identified a gene encoding a NBS-LRR 'resistance' protein that triggers host susceptibility toward the necrotrophic pathogen *Cochliobolus victoriae* via the interaction of the resistance-like protein NBS-LRR and the

fungal host-specific toxin victorin. In addition to the resistance mechanism involving a gene-for-gene interaction, there are other mechanisms that the host may deploy for resistance against necrotrophic and biotrophic pathogens. Although not entirely known, it is believed that these mechanisms include changes in the levels of reactive oxygen species and changes in the level of phytohormone auxin, among other mechanisms (Mengiste, 2013).

Additional objectives of this study were to compare and validate quantitative trait loci (QTL) results previously identified by linkage mapping using bi-parental populations and to determine associations in common for resistance between Cephalosporium stripe and stripe rust using different methodological analysis. To do so, QTL identified in previous studies for Cephalosporium stripe and stripe rust were summarized (Table 4.2 and Figure 4.6) for the four populations Tubbs/ NSA980095 (TxN) (Chapters 2 and 3), Einstein/Tubbs (ExT) (Chapters 2 and 3), Stephens/Platte (SxP) (Vazquez et al, 2012), and Coda/Brundage (CxB) (Quincke et al., 2011). The latter two studies were included because they report QTL analyses for stripe rust and Cephalosporium stripe, respectively done within the Oregon State University Wheat Breeding Program and/or were evaluated in similar environments.

The QTL in chromosome 5AL.1 was the most commonly detected QTL across populations/diseases. This QTL showed a significant effect for both Cephalosporium stripe and stripe rust in this research, and for Cephalsoprium stripe by Quincke et al. (2011). The QTL in chromosome 4AL and the C5BL QTL located in the translocation 5B:7B are in regions where SNPs were identified associated with stripe rust and Cephalosporium stripe, respectively. Both of these QTL were associated with stripe rust

Table 4.2 Four populations and two diseases are presented with the prevalence of each QTL across populations and diseases.

| | QTL | 5AL[1] | C5BL | 2AS | 4AL[1] | 4BS | 5AL[2] | 6AL | 2BL | 3BS | 4AL[2] | 5BL | 6BS[1] | 7BS | 7BL | 1BL | 2AL | 2BL[2] | 3BL | 4DS | 5DL | 6BS[2] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Disease** | **Population** | | | | | | | | | | | | | | | | | | | | | |
| Cephalosporium stripe | Tubbs/NSA98005 | * | * | | * | | * | | | * | | | * | * | | | | | | | | |
| | Einsten/Tubbs | * | * | * | | * | * | | | * | | | | | * | * | | * | | * | | * |
| | Coda/Brundage (Quincke et al., 2011) | * | * | | | * | * | | | | | | | | | | | | | | | |
| | Diversity Set | | * | | | | | * | | | | | | | | | | | | | | |
| Stripe rust | Tubbs/NSA98005 | * | | | | | | | * | | | * | * | | | | * | | * | | * | |
| | Einsten/Tubbs | | | * | | | | * | | | * | * | | | * | | | | | | | |
| | Stephens/Platte (Vazquez et al., 2012) | | | * | * | * | | * | | | | | | * | | | | | | | | |
| | Diversity Set | | | | * | | | | * | | * | | | | | | | | | | | |

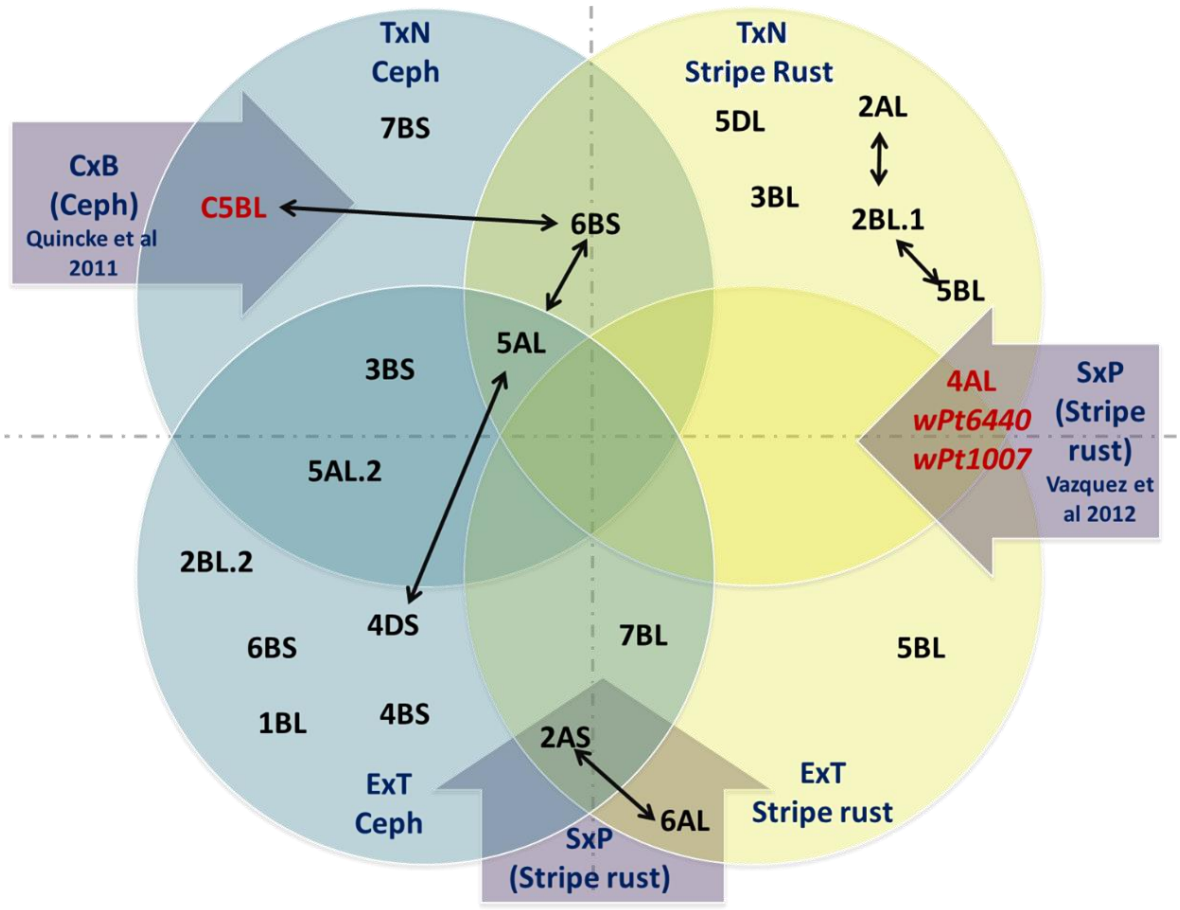


Figure 4.6 Schematic summarizing the QTL identified for Cephalosporium stripe and stripe rust as well those in common with other studies. Dotted line separates the figure in quadrats that represent the specific population and its respective disease. QTL are designated with their chromosomal location. Arrows at the edges of the images represent the other studies with population CodaxBrundage (CxB) (Quinke et al., 2011) and Stephens x Platte (SxP) with an indication of the disease study. (Vazquez et al., 2012). Chromosome locations in color red denote the QTL where SNPs are identified in a similar chromosomal region and associated to known resistance mechanism. Arrows indicate epistatic interactions detected among QTLs.

resistance by Vazquez et al. (2012) and with Cephalosporium stripe by Quincke et al. (2011) respectively. One SNP was located in chromosome 6A and was associated with Cephalosporium stripe, although it is unknown at this time in which arm of the chromosome it is located. The QTL in 6AL was had an explained a high and stable phenotypic variance response during the time the ExT population was assessed for stripe rust resistance for this study. Another SNP was identified associated with stripe rust in chromosome 2BL, while a QTL in a similar location was identified for both Cephalosporium stripe and stripe rust. Other QTL were identified only in only one or two population/disease combinations.

The preliminary results presented in this chapter show promise for association analysis as an effective approach for identifying and validating quantitative trait locus (QTL) for disease resistance in wheat. Moreover, the ability to design SSR markers from known SNP sequences would aid screening for the presence of desirable chromosomal regions/alleles that could speed the process of breeding for resistance to specific diseases. The validation of common QTL and SNPs across diseases and studies suggests the potential for use of marker-assisted selection across diverse population. To confirm these results, additional evaluation is needed. This additional work would involve: 1) adding available lines phenotyped in the field for disease response to increase the number of lines in the analysis from 72 to 95, 2) utilize data from different genotyping platforms such as the 90,000 Infinium iSelect SNPs (Illumina, Inc. San Diego California, USA), DArT (Triticarte, Australia) and PCR base SSR markers (Oregon State University) to expand on the 9,000 SNP Infinium iSelect SNPs (Illumina, Inc. San Diego California, USA) platform used for the 72 lines in this study and 3) once the data sets are merged,

redo the analysis required with different approaches to confirm and solidify the results and conclusions from this study.

Table 4.3 List of the 72 lines included in the dataset for association mapping with their market class (if known) and pedigree (if publically available).

| **Wheat accession name** | **Market class** | **Pedigree** |
|---|---|---|
| Antelope | HWW | PRONGHORN/ARLIN |
| Apache | HRW | AXIAL/NRPB-84-4233 |
| Arminda | SRW? | (CAPPELLE-DESPREZ/CARSTENS-VIII)/IBIS |
| Boundary | HRW | NORIN-10/BREVOR//2*CENTANA(IDO-34)/3/CENTANA*2/CI-14106/II-60-155/CI-14106//MCCALL/4/KIOWA/UT-222-A-437-2//DELMAR/3/PI-476212/MT-6619 |
| Brundage | SWW | NORD-DESPREZ/PULLMAN-101/GENEVA |
| CI13113 | . | Chinese*2/Agropyron elongatum//Pawnee |
| CODA | SWW | TRES//MADSEN/TRES |
| Daws | SWW | CI14484/2/CI13645/PI178383 |
| Eltan | SWW | PI-178383/2*BURT//PULLMAN-101/4/BR-70443-3(PI-167822)/3/PULLMAN-101 |
| Fidel | . | HORIZON/FRONTANA//CAPITOLE/3/MAJOR |
| Foote | SWW | HEIMA//KALYANSONA/BLUEBIRD/3/WWP-7147, F1/4/D-6301/HEINES VII//ERA/3/BUCKBUCK |

Continued Table 4.6

| **Wheat accession name** | **Market class** | **Pedigree** |
|---|---|---|
| Gene | SWW | CLEOPATRA-74/PICHON//ZENZONTLI |
| Hill81 | SWW | HEINES-VII/REDMOND/NORD-DESPREZ/2*PULLMAN-101 |
| Hyslop | SWW | NORD-DESPREZ/2*PULLMAN-101 |
| Jagger | HRW | KS-82-W-418/NORD-DESPREZ/PULLMAN-101 |
| Lewjain | SWW | LUKE/3/SUPER HELVIA//SUWON 92/CI13645 |
| Luke | SWW | PI-178383/2*BURT//PULLMAN-101 |
| MacVicar | SWW | HEINES-VII/REDMOND/MCDERMID/2/Triticum spelta var. ALBA/3/SUWON-92/ROEDEL /4/ NB 68513/ NORD-DESPREZ/2*PULLMAN-101/5/BACKA |
| Malcolm | SWW | NORD-DESPREZ/PULLMAN-101//63-189-66-7/BEZOSTAYA-1 |
| N96L1226 | HRW | |
| N96L189 | . | KS831024/4/Aurora/NE701154/3/NE7060/2/Rannaya 12/Bezostaya 4 |
| NSA972346 | . | |
| NSA980995 | HRW | |

Continued Table 4.6

| **Wheat accession name** | **Market class** | **Pedigree** |
|---|---|---|
| NSA990626 | . | |
| NSA990969 | . | |
| NSLWW41 | HRW | NHC 49/UK Yield Bulk/3/Haven//Moulin/Galahad |
| NSLWW47 | . | |
| NuPlains | HWW | OK-711252-A/W-76-1226/Plainsman V//Newton/Arthur 71 |
| NW97S277 | . | Pronghorn/Arlin |
| OR2010239 | . | CASHUP//5/VPM/MOS951//HILL/3/SPN/4/SPN |
| OR2030411 | SWW | SPN/MADSEN//ELTAN/4/TJB842-12919/SPN//SPN*2/HH/3/ELTAN |
| OR2040075H | HWW | WI88-052-13/Tomahawk//OR943576 |
| OR2052046H | HWW | OR943576//OR943576/N97S277 |
| OR2052055H | HWW | OR943576//OR943576/N97S277 |
| OR2052082H | HWW | OR943576//OR943576/N97S277 |
| OR8505138 | HWW | |

Continued Table 4.6

| **Wheat accession name** | **Market class** | **Pedigree** |
|---|---|---|
| OR941048 | HWW | ID 80-628/3/CER/YMH/HYS/4/CER/YMH/HYS |
| OR942496 | HWW | CEBECO 148//CNO/INIA//LFN/3/K//PET/RAF/4/ND/P101//AZT |
| OR943576 | HWW | MRS/CI14482//YMH/HYS/3/RONDEZVOUS |
| OR951431 | HWW | HILL/3/CER/YMH/HYS/4/CER/YMH/HYS |
| OR9800919 | SWW | ROSSINI/YSATIS//ORACLE |
| OR9800924 | SWW | ROSSINI/YSATIS//ORACLE |

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