QTL analysis of wheat leaf rust resistance in the ‘Lakin’ x ‘Roelfs F2007’ backcross population

by

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Abstract

Leaf rust, caused by the fungus *Puccinia triticina*, is a major disease of wheat in North America. Breeding efforts to achieve resistance have historically been plagued by a cycle in which an all-stage resistance gene is discovered, deployed, and rapidly defeated, thus wasting valuable sources of resistance. Adult-plant resistance genes, however, can potentially contribute to race-nonspecific resistance to the pathogen which is durable for many years. In this study, the moderately susceptible Kansas winter wheat cultivar, 'Lakin', was backcrossed to the CIMMYT-developed spring wheat cultivar, 'Roelfs F2007', which is known to possess very good adult-plant resistance (APR) to leaf rust. The 297 BC_1F_6-derived progeny of this cross were screened for resistance in five location-years at field nurseries in Kansas and Texas, as well as in two greenhouse studies. Genotyping-by-Sequencing (GBS) generated 2,541 high-quality genome-wide markers used for the construction of a linkage map covering all 21 chromosomes. Quantitative trait locus (QTL) analysis identified reproducible APR QTLs on chromosome arms 3BS, 5DS, 7BS, and 7BL. Three QTLs contributed by Roelfs F2007 appeared to correspond to locations of known APR genes *Lr74* (3BS), *Lr78* (5DS), and *Lr68* (7BL). The APR QTL on 7BS contributed by Lakin appeared to be novel. Together, the four QTLs accounted for 45% and 46% of the variation in least-squares means for disease severity and infection type, respectively. These results confirm that both Roelfs F2007 and Lakin contain APR loci which may be used in breeding for resistance to infections by *P. triticina*.

Table of Contents

List of Figures

List of Tables

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Dedication

I dedicate all of this effort to my incredible wife, my beautiful children, and to the Lord of All Creation.

Ut in omnibus glorificetur Deus.

When he was at table with them, he took the bread and blessed, and broke it, and gave it to them. And their eyes were opened and they recognized him…

Luke 24:29-31

Chapter 1 - Literature Review

Introduction

The rust fungi of wheat (*Triticum aestivum* L.), namely leaf rust (*Puccinia triticina Eriks*.), stripe rust (*Puccinia striiformis* Westend f. sp. *tritici*), and stem rust (*Puccinia graminis* Pers.: Pers. f. sp. *tritici Eriks.*) are present in all the wheat growing regions of the world. Together these pathogens comprise the most common and devastating diseases of wheat. Of the wheat rusts, leaf rust has the widest global distribution and is likely responsible for the greatest cumulative yield loss (Savary, Willocquet et al. 2019). Like the other *Puccinia spp*., *P. triticina* is a stress-tolerant parasite which is highly variable in natural populations, exhibits frequent race changes, and produces abundant spores. Aiding in the fight against leaf rust, the International Center for the Improvement of Maize and Wheat (CIMMYT) helps provide researchers with new germplasm for testing against diseases like leaf rust, as well as high yielding resistant cultivars. 'Roelfs F2007' is a CIMMYT-developed cultivar which demonstrates moderate resistance to leaf rust while having strong agronomic qualities. An exploration of the underlying cause of the resistance in Roelfs F2007 may aid in the further development of improved germplasm. The identification of unique sources of race-nonspecific resistance and understanding their performance in combination will provide new genetic resources for use in marker-assisted selection (MAS). This study is part of the much wider effort being undertaken by researchers globally to combat the effects of the rust pathogens. It is my hope that the research presented herein may contribute to this effort, provide new genetic tools for wheat development, and ultimately help improve food stability for people everywhere.

History of Wheat Cultivation

Wheat is a cultural, nutritional, agricultural, and economic pillar for much of the world's population. Cultivated in a wide band around the world stretching from 67 degrees N in Russia to 45 degrees S in Argentina, wheat supplies approximately twenty percent of the calories and protein consumed by humans worldwide (Feldman 1995). At any time, all the various stages of wheat development may be found in fields around the world. Current rates suggest a worldwide growth in wheat demand through 2030 at an annual rate of 1.4 percent, however the current rate of annual yield gain hovers around 1 percent (CIMMYT 2021). This divergence illustrates the need for continual efforts to improve wheat yields. If the needs of a growing world are to be met, these yield improvements will need to come in large part from genetic gain. When annual yields are significantly limited by rusts, improved genetic resistance has the potential to narrow this gap.

The consumption and eventual domestication of wheat coincided with the rise of the first non-nomadic human societies during the Neolithic Revolution 10,000 years ago. Prior to this time, humans are understood to have relied exclusively upon a nomadic hunter-gatherer existence. Emerging from this revolution were the civilization of Mesopotamia and the earliest evidence of farming villages occurring within or near the Fertile Crescent around 9,500 years ago (Nesbitt 2001). The origin and center of diversity of wheat is in this region of southwest Asia. This region is considered the cradle of human civilization and now comprises the territories of Syria, Iraq, and Iran. Wheat has been cultivated in this region for thousands of years and from this region, it has been established around the world. Alongside wheat domestication, barley (*Hordeum vulgare* L.) lentil (*Lens culinaris* Medikus) and chickpea (*Cicer arietinum* L.) were

also domesticated in this region around the same time, which combined with animal domestication, precipitated a fundamental change for human civilization (Faris 2014).

Prior to domestication, wheat had evolved characteristics favoring its survival under wild conditions. Domestication began with the imposition of selections ultimately favoring the survival of human populations. Early selections created novel forms of wheat designed to be successful under cultivation. These changes rendered wheat cultivars wholly dependent upon farmers for propagation which were henceforth planted, shared, sold, traded, and promoted within regions of adaptation. For the last 9,000 years, the needs of farmers, processors, and consumers have continued to advance its domestication via artificial selection (Feldman 2001). This process was central to the breeding of modern wheat. Among the earliest traits selected-for was likely the non-shattering rachis, which prevents the pre-harvest dispersal of seed and the resulting loss of grain. Another was the free-threshing trait in which glumes no longer adhere to the naked grains. (Shrewry 2001, Simons, Fellers et al. 2006). Other early traits selected-for included larger seeds, loss of seed dormancy, spring growth habit, and improved grain quality (Harlan, De Wet et al. 1973). The self-pollinated progeny of plants which survived both natural and artificial selection for hundreds of years may now be considered landraces, of which there are now thousands representing all the world's wheat growing areas and their cultural heritage (Zeven 1998). Among landraces, perhaps the most significant to Great Plains production were 'Turkey Red' brought to the region by Mennonite Germans via Russia in 1874 and the similar 'Kharkof' introduced by the agronomist M. A. Carleton in 1900. Both are estimated to be in pedigrees of thousands of commercialized crosses (Quisenberry and Reitz 1974).

The period of wheat development from domestication to the mid-nineteenth century has been referred to as the 'pre-research period', largely characterized by non-systematic trial and

error approaches to improvement. What followed has become known as the ‘modern research era’- the result of lessons learned from the fledgling study of genetic inheritance as found in Darwin’s *The Origin of Species* in 1859 and from the inheritance work done by Augustinian monk, Gregor Mendel (1822-1884), which had been rediscovered in the year 1900. This era included experimentation with pure line selection, hybridization, and backcrossing of wheat performed in the late 1800s and the early decades of the 1900s (Salmon, Mathews et al. 1953, Quisenberry and Reitz 1974). In the 1950s, the American agronomist and later Nobel laureate, Norman Borlaug (1914-2009) led what has become known as the ‘Green Revolution’- a sudden widespread adoption of several burgeoning agricultural technologies in the developing world. This revolution was in large part focused on the breeding of regionally adapted, semi-dwarf, lodging-resistant, disease resistant, high yielding wheat varieties. It has been credited with saving countless lives by lifting millions in the developing world from extreme poverty and malnourishment (Evenson and Gollin 2003).

The latter half of the 20th century and the early part of the 21st have seen profound changes in wheat development and cultivation. In the years from 1964 to 2018, global wheat production grew rather steadily from 268.7 to over 735 million metric tons (t) of grain harvested- an increase of over 173.5%. Despite this dramatic increase in production, the land area of wheat harvested fell by 1.7 million hectares (ha) to 215 million ha over that same time. The annual total area of wheat production has been on a somewhat steady downward trend since its 1981 peak of 239 million ha. Average yield from 1964-2018 nearly tripled from 1.2 - 3.4 t/ha (FAOSTAT 2020). The tremendous yield gains over the last half century illustrate the progress that has been brought forth by the Green Revolution, improvements in agricultural technology, and molecular approaches to wheat improvement (Edgerton 2009). Despite these improvements, an average of

21.5% of the global wheat yield is still lost to disease every year (Savary, Willocquet et al. 2019).

In 2018, the top five wheat growing countries in the world were China, India, Russia, The United States of America (U.S.), and France, producing approximately 131, 100, 72, 51, and 36 million t, respectively. The next five countries by production- Canada, Pakistan, Ukraine, Australia, and Germany produced a cumulative 123 million t of wheat in 2018. In North America, the U.S. is the leading wheat producer in terms of both production and area harvested with 51.2 million t produced on 16 million ha in 2018. Canada in the same year had 31.7 million t produced on 9.8 million ha and Mexico had 2.9 million t produced on roughly 541 thousand ha. (FAOSTAT 2020)

In the U.S. there are four classes of winter wheat produced: hard red, soft red, hard white, and soft white. Of spring wheat, the classes are hard red, hard white, soft white, and durum. In the area of the Great Plains from southern Texas to the Missouri River valley in South Dakota, lies the primary production corridor in the U.S. for hard red winter wheat. In the Great Plains north of the Missouri River, hard red spring wheat is primarily grown. The diverse microclimates of the Pacific Northwest in eastern Washington, Idaho, and Oregon produce seven of the eight classes of U.S. wheat. In this region, soft white winter wheat is the predominant class grown with dryland yields commonly exceeding 150 bushels/acre. East of the Mississippi river in the U.S, soft red wheat is commonly grown only in rotation with maize and soybeans as the principal crop. Durum production is concentrated in eastern Montana and western North Dakota. In 2018, the U.S. produced 1.89 billion bu of wheat, approximately 63% of which was winter type. 35% of all U.S. wheat produced was hard red winter class. The average yield for winter and spring wheat in 2018 was 47.9 and 48.3 bu/acre, respectively and the commodity price for all

classes of wheat averaged $5.14/bu. The top wheat producing states in 2018 were North Dakota (363.4 million bu), Kansas (277.4 million bu), Montana (197.6 million bu), and Washington (153.2 million bu) (USDA 2018).

Genetic Structure of Wheat

The wild progenitors of modern wheat were diploid einkorn wheat also known as *Triticum urartu* Thumanjan ex Gandilyan (2n = 14, AA), the edible goatgrass, *Aegilops speltoides* Tausch (2n = 14, BB), and the prolific goatgrass, *Aegilops tauschii* Cosson (2n = 14, DD). These three diploid species diverged 2.5- 4.5 million years ago and through two distinct natural hybridization events, combined to become hexaploid wheat (2n = 42, AABBDD). The spontaneous cross of the first two resulted in tetraploid emmer wheat (*Triticum turgidum* L. (Desf.) Husn., 2n = 28, AABB), of which durum is a subspecies. Modern bread wheat resulted from the spontaneous cross between emmer wheat and *Aegilops tauschii* Coss. (Huang, Börner et al. 2002). The first appearance of hexaploid wheat is thought to have occurred 9000 years ago (Feldman 2001).

The wheat genome is difficult to study due to its sheer size, complexity, and polyploid nature. It has a high level of gene duplication and homology among gene copies due to the relatively recent evolution of hexaploid wheat (Akhunov, Nicolet et al. 2009). The genome of bread wheat contains approximately 17 billion base nucleotide pairs, 107,000 genes, and over 132,000 polymorphisms. (Appels, Eversole et al. 2018). For comparison, the human genome contains only three billion base pairs, the maize (*Zea mays*) genome contains 2.6 billion base pairs, and the rice (*Oryza sativa* spp. *japonica*) genome contains 430 million base pairs. The bread wheat genome is understood to be more than 85% repetitive, adding to its complexity.

These attributes make the development of markers for use in marker-assisted breeding difficult as well (Wanjugi, Coleman-Derr et al. 2009).

History of Leaf Rust

For thousands of years, rusts have been described as a threat to wheat production and until the late 19th century, the different species of rust had not yet been distinguished (Chester 1946). Its perpetuity and significance are evidenced by mentions as old as agriculture itself. On several occasions, The Old Testament of the Bible describes plagues of "smut and rust" on crops (Stakman, Levine et al. 1919). Aristotle described rust symptoms and attributed variability in epidemics to moisture (Eriksson and Henning 1896). Ancient Roman folklore explains the origin of rust as divine comeuppance for a farm boy's mistreatment of a fox which had been harassing his chicken yard (Ovid, 43 B.C.-17 A.D.). It has been mentioned by Pliny II (23-79 AD), William Shakespeare (1564-1616), and students of Carl Linnaeus (1707-1778). The understanding of the cause of the disease changed from a deity in ancient times to unseen insects blown by the wind during the Renaissance until it was first identified as a parasitic fungus by Felice Fontana in 1767 (Chester 1946). Notably, in 1844, when the feasibility of widespread Kansas wheat cultivation was still in an uncertain experimental phase, the Sac and Fox homestead in Doniphan County reported an almost total loss due to a rust epidemic (Quisenberry and Reitz 1974). These accounts represent only a small sampling of the witness to rust throughout the history of agricultural societies. While rust has always been present wherever wheat is planted, it is likely that modern production practices using genetically homogeneous cultivars over large areas has worsened its effect (Roelfs 1985).

Leaf Rust Characterization

Rust pathogens are of the genus *Puccinia* and the family *Pucciniaceae*. They belong to the order *Uredinales* and the class *Basidiomycetes*. They produce several types of spores in their life cycle and require an alternate host for sexual recombination. Leaf rust caused by the fungus *Puccinia triticina* Erikss. is the most common of the rust diseases of wheat. While not the most damaging at the local level, it is the most widely distributed around the world and is responsible for the most cumulative damage (Singh 1992, Savary, Willocquet et al. 2019). It was first distinguished from other rust fungi by de Candolle in 1815. Leaf rust of wheat and rye was described as *Puccinia dispersa* in 1894 by Ericksson and Henning. The *forma specialis* of wheat leaf rust was classified as *tritici* by Eriksson in 1899 and the name, leaf rust, was first used by Carleton in 1899. In 1956, it was alternately termed *Puccinia recondita* by Cummins and Caldwell. Anikster et al. reaffirmed the suitability of *Puccinia triticina* Erikss. in 1997, though it may still be termed *recondita* in some publications. It is also commonly referred to as brown or orange rust, although brown is used more often. The alternate hosts of leaf rust were first described as such in 1921 by Jackson and Mains. They are *Isopyrum fumaroides* and *Thalictrum speciosissimum*, or meadow rue. Both alternate hosts are native to the Fertile Crescent and share a common center of diversity with modern wheat (Chester 1946, d'Oliveira and Samborski 1966).

Recent studies have generated draft genomes of *P. triticina* 135 Mb in length, containing over 14,800 genes and 52% repetitive DNA elements (Fellers, Sakthikumar et al. 2021). The diversity of *P. triticina* pathotypes is categorized into races, each of which define a distinct virulence/avirulence spectrum. When they were developed, it was thought that since the morphology of spores between rust *formae speciales* is nearly indistinguishable, specialization to

differential hosts offers a uniform means of defining races. (Jackson and Mains 1921). Originally, a differential set of eleven wheat hosts was used to distinguish different races (Johnston and Mains 1932). These were eventually augmented with other differential cultivars. Eventually it was determined that a cultivar set could not keep pace with gains in understanding the diversity of *P. triticina* (Young Jr and Browder 1965). Twenty Thatcher lines that are each near-isogenic for a single *Lr* gene comprise the modern differential set. These are grouped into sets of four and resistance combinations are assigned a letter from B to T sans vowels. Races are commonly assigned with this 3 to 5-letter code (Long and Kolmer 1989, Huerta-Espino, Singh et al. 2011, Kolmer, Herman et al. 2019).

Leaf Rust Life Cycle

The complete life cycle of leaf rust relies on the wheat plant and an alternate host of the *Thalictrum* genus. It will however, in absence of the alternate host and under the necessary conditions, readily reproduce asexually and cause widespread damage. It survives under the same environmental conditions as the wheat leaf and epidemics are most severe when temperatures are mild from jointing to flowering accompanied by frequent dews. It thrives at temperatures near 20° C, though it can achieve infection anywhere between 10°C and 30°C (Roelfs 1992, Kumar 2014). Yield losses tend to be worse if the onset of symptoms occurs prior to anthesis (Samborski and Peturson 1960).

In the first step of this macrocyclic life cycle, dikaryotic urediniospores on the leaf surface germinate within 1-3 hours of contact with free moisture, without which, germination cannot occur. A germ tube or hypha grows along the leaf surface until contact is made with a stomatal guard cell. At the stomatal opening, the hypha forms a swollen appressorium from which a vesicle is formed in the sub-stomatal cavity. Multiple hyphae emanate from the sub-

stomatal vesicle and a haustorial mother cell is formed upon cellular contact. From the mother cell, secondary hyphae penetrate the tissue cell and haustoria are formed within. The haustoria secrete enzymes for nutrient acquisition and effectors for the suppression of host defenses.

As the infection progresses with the extension of more hyphae and haustoria, a uredinium, or pustule, forms beneath the epidermal cells and eventually ruptures the epidermis. Each uredinium produces urediniospores at a rate of about 3,000 per day (Stubbs, Prescott et al. 1986). Temperature, humidity, and wind may all affect the rate of sporulation. The oval shape and reddish-brown color of the pustule are the visible characteristics of active infection by *P. triticina*. These urediniospores are carried by the wind and though they may be carried for several kilometers, 90% are no longer airborne after 100m (Roelfs 1972). They may survive away from a host for several weeks. Each clonal generation from infection to sporulation takes about 10 days, creating an exponential growth in inoculum while favorable conditions exist (Bolton, Kolmer et al. 2008).

Near the end of the vegetative period, conditions become unfavorable for continued urediniospore production. As this occurs, uredinia mature into telia and begin producing two-celled dikaryotic spores called teliospores. As teliospores mature, the two nuclei in each cell undergo karyogamy, forming a diploid nucleus. After a period of winter vernalization and when free moisture is available, teliospores germinate and produce an elongated, segmented appendage called the basidium. The diploid nuclei undergo meiosis and genetic recombination. Each basidium produces four haploid basidiospores. The haploid nucleus of each basidiospore undergoes mitosis, rendering two identical haploid nuclei per spore. These are carried by wind to the leaf surface of alternate hosts nearby.

Basidiospores land on the alternate host and infect the epidermis of leaf tissue. 7-10 days after infection, pycnia emerge on the upper leaf surface of the alternate host. The haploid spores produced by pycnia are called pycniospores. These spores are gametes capable of fertilizing nearby receptive hyphae. Fertilization is commonly mediated by insects, leaf movement, wind, or rain. Following the fertilization, dikaryotic mycelium grows through the leaf. Tubular aecia emerge approximately 7-10 days later upon the lower leaf surface. Aecia produce chains of aeciospores, which are released and carried by the wind to the leaf surface of nearby wheat plants. These aeciospores infect the leaf surface and produce uredinia, which continue the asexual reproductive cycle (Bolton, Kolmer et al. 2008).

Symptoms most commonly occur on the leaf blades, though may extend to the sheaths in severe infections. Yield reduction occurs due to reduced floret set, incomplete kernel formation, and the shriveling of grain. In the most severe epidemics, losses can reach as high as 40%, although losses of 5-10% are most common. Severe epidemics are caused by a combination of favorable conditions for the flourishing of the pathogen and the widespread planting of susceptible cultivars (Roelfs 1992). The alternate hosts are often considered insignificant to the development of *P. triticina* inoculum in North America (Saari, Young et al. 1968, Kolmer 2005).

The development of leaf rust epidemics in North America follows a seasonal pattern that has become known as the Puccinia Pathway. This annual epidemic originates in northeastern Mexico and southern Texas near the Gulf of Mexico. The continuous production of spring and winter wheat in this region permits the development of a “hot spot” of high genetic diversity among rust populations. In this area, summer volunteer wheat also provides a perpetual host for infection year-round. The Great Plains region is a largely unbroken corridor of host wheat north to the Canadian provinces of Saskatchewan and Manitoba. Along this route, asexual

reproduction of urediniospores sustains the inoculum. Atmospheric patterns drive prevailing winds northward during the winter wheat growing season. The epidemic traces the ideal pathogen conditions northward throughout the Great Plains (Kolmer 2001).

Races of Leaf Rust

Recent race diversity studies have shown that the evolution of *Puccinia* spp. populations has coincided with the evolution and cultivation of wheat (Feldman 2001, Kolmer, Herman et al. 2019). Globally, races tend to be related by geographic proximity, indicating that windblown clonal urediniospores are important to the diversity of regional populations. The *Puccinia* Pathway in North America is an example of this pattern. Epidemics of leaf rust in North America are exceptionally diverse, owing this diversity to a steady churn of extant races, which in turn adds to the complexity of genetic control. Genetic mutation, not recombination, is considered the major cause of changes in virulence in North America (Statler and Jones 1981). These virulence changes are driven in large part by the common deployment of race-specific *Lr* genes in commercial cultivars (Kolmer and Hughes 2018). To help researchers understand the prevalence and distribution of virulence phenotypes in the U.S., a survey of races of *P. triticina* in the U.S. has been undertaken annually since 1926 (Mains and Jackson 1926). Annually, there are 50-70 known races of leaf rust detected in North America (Kolmer, Ordonez et al. 2009). In 2017, 65 unique races were detected across the U.S., yet no single isolate was found to be present in all regions. Of these, the most prevalent were phenotypes MBTNB, TFTSB, and MCTNB, representing 11.3%, 10.9%, and 7.0% of the overall population, respectively. These annual surveys have revealed how the diversity of *P. triticina* races affect genetic resistance. This is exemplified in the 2017 annual survey, wherein virulence to *Lr39* was found to be present in 51.3-87.5% of isolates collected from Texas to North Dakota (Kolmer 2019).

Cultural and Chemical Control of Leaf Rust

The economic control of pests in many agricultural settings relies on the use of a multi-faceted approach including proactive agronomic practices, chemical control, and genetic resistance (Roelfs 1985). Such a strategy is known as integrated pest management. In many cases, a single method of pest control is insufficient to make the cost of cultivation worthwhile. In most cases, the costs of the strategy must be considered in relation to the attainable yield of a particular field (Cook and Vaseth 1991).

One of the most effective cultural practices against leaf rust is the destruction of volunteer plants which serve as a reservoir for uredinia in warmer climates. The avoidance of planting both spring and winter wheat in the same area can work toward the same effect as removing volunteers. Delayed planting of winter wheat can help to avoid fall infections. Unfortunately, leaf rust in the Great Plains attacks the plant during the vegetative period and through senescence. Consequently, the cultural practice of planting earlier-maturing cultivars is not as effective as with stem rust, which prefers higher daytime temperatures (25-30°C) than leaf rust and therefore tends to onset at the later growing stages. The elimination of the alternate host as in barberry for stem rust is also considered ineffectual since infections of *Thalictrum* and *Isopyrum spp*. native to North America are exceedingly rare (Saari, Young et al. 1968).

Chemical control of leaf rust may be attained by foliar fungicide applications. Among the most effective are the systemic strobilurin class of fungicides, which mimic the action of naturally occurring fungicidal derivatives of β-methoxyacrylic acid. Strobilurins (e.g., azoxystrobin) inhibit mitochondrial respiration by specific binding to cytochrome b (Bartlett, Clough et al. 2002). Strobilurins provide the benefit of protection which can last for 3-5 weeks depending on growing conditions and have been effective against all three wheat-affecting rust

pathogens since they were commercialized over 25 years ago. Triazole seed treatments (e.g., tebuconazole) may be effective in suppression of seedling infections on both spring and winter wheat. Typically, epidemics are not severe enough to economically justify chemical control of leaf rust alone. In the Great Plains of North America, if fungicides are used, it is more common to use a foliar spray upon the first identification of infection to control both stripe and leaf rust at the flag leaf stage (Zadoks 38-49) (Zadoks, Chang et al. 1974).

Despite its effectiveness, chemical control has several drawbacks. In many wheat-growing regions around the world, the availability and cost of product and application equipment may be prohibitive. In climates where favorable infection conditions persist for months as in the Pacific Northwest, several applications may be required in a single growing season, increasing production costs beyond feasibility. The pervasive adoption of a certain mode of action across geographies as a broad management strategy will ultimately lead to fungicide resistance in the pathogen population. As new fungicide modes of action are rare and expensive to develop, growers may be left without an alternative if fungicide resistance is widespread. Additionally, chemical fungicides are of major concern for their possible direct and indirect effects to ecosystems and public health, and thus are subject to regular scrutiny and regulation from political bodies. Government regulators also may prohibit certain active ingredients or application timings necessary for chemical control. For these reasons, chemical control alone is a sub-optimal strategy for controlling leaf rust epidemics.

Genetic Resistance to Leaf Rust

When available, genetic resistance is the most economical and sustainable means of reducing losses due to fungal pathogens (McIntosh, Wellings et al. 1995, Bolton, Kolmer et al.

2008) It is an essential component of any variety or breeding line suited for the developing world. Even in technologically advanced agricultural regions where the use of fungicides is commonplace, it is nevertheless a fundamental characteristic in the evaluation of any variety and remains highly sought after by breeders and growers. Due to the limitations of cultural and chemical control, the most essential component of an integrated pest management strategy against *P. triticina* is genetic resistance.

Typical wheat resistance genes (*R*-genes) function in a reciprocal relationship with avirulence (*Avr*) genes in *P. triticina*. This is known as the gene-for-gene theory and helps to explain the inheritance of race-specificity in rust fungi (Flor 1971, Samborski and Dyck 1976). The gene-for-gene theory states that in a resistant or incompatible interaction between host and pathogen, single pathogen *Avr* gene products are recognized by single host *R*-gene products to trigger a defense response. In the absence of a suitable corresponding *Avr*-*R* interaction, the disease may develop. In a compatible interaction between the wheat plant and the pathogen, haustoria will form inside host cells. In the case of an incompatible interaction, the host cell dies, and the invading haustorium along with it. If enough cells are involved, a visible hypersensitive response may be observed in the form of necrotic flecks on the leaf (Rowell 1981). In most cases, avirulence in leaf rust and resistance in wheat are dominantly inherited traits. The theory, as originally described by H. H. Flor, helps to explain many host-parasite interactions and inheritance phenomena (Ellingboe 1982). However, it does not necessarily explain all compatible/incompatible responses, as inhibitors or interactions between *Avr* genes may play a greater role than originally thought (Bolton, Kolmer et al. 2008).

PAMP-Triggered and Effector-Triggered Immunity

Most modes of resistance may be generally divided into two categories: pathogen/ microbe associated molecular pattern or PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). At a basic level, both systems act as a molecular switch for the host defense cascade. PTI involves the host cell detection of essential molecules in the pathogen such as lipids, carbohydrates, proteins, and ATP (Boller and He 2009). These extracellular molecules are detected by pattern recognition receptors (PRRs) in the plasma membrane and trigger intracellular defenses. The result is typically restricted pathogen growth in the host (Miller, Costa Alves et al. 2017). To succeed, pathogens must overcome PTI through the deployment of highly diverse effector proteins which interfere with host defenses.

To contrast with PTI, ETI occurs via intracellular recognition of pathogen Avr effector proteins by host resistance (R) protein receptors. ETI frequently results in pathogen quarantine at the site of attempted infection via programmed host cell death (hypersensitivity) or by encasement of haustoria by the deposition of callose. (Dodds and Rathjen 2010). Within the ETI category, several *R* gene families have been defined based upon their protein structure and function. The most common of ETI gene families encode proteins which trigger nucleotide binding site-leucine rich repeat (NB-LRR) type resistance (Miller, Costa Alves et al. 2017). NB-LRR resistance can occur directly by recognition of pathogen effectors. It may also occur indirectly by recognition of accessory proteins produced by the effector in response to contact with host 'bait' proteins. NB-LRR proteins have even shown to work in tandem against a single effector (Narusaka, Shirasu et al. 2009, Dodds and Rathjen 2010). NB-LRR type resistance is particularly subject to intense diversifying selection which ultimately drives the co-evolutionary arms race between hosts and pathogens (Dodds and Rathjen 2010).

Seedling and Adult-Plant Resistance

Another way to categorize resistance is by the plant stage at the initiation of infection. Seedling resistance is detectable at the seedling stage and typically confers complete control at all stages of plant development. Seedling resistance is associated with major genes and has historically been utilized because of their intense effects and relative ease of manipulation in breeding programs (Poland, Balint-Kurti et al. 2009). It is also race-specific and may confer near-immunity to a limited set of pathotypes. As a result, it exerts a maximum degree of selection pressure on the enormous populations of *P. triticina.*

In contrast, adult-plant resistance (APR), non-recognition-triggered resistance, or minor gene resistance commonly pertains to quantitative genes of relatively small effect that are only expressed by adult plants. While comparatively little is known about the specific molecular mechanisms of APR, it is understood that these quantitative genes do not induce ETI, and are generally not subject to the gene-for-gene interactions that characterize seedling genes (Poland, Balint-Kurti et al. 2009). Although their individual effects are small, they often function in an additive relationship with each other, resulting in greater levels of control than by a single APR gene. APR genes have also been observed to be pleiotropic, meaning that they may be effective against multiple related pathogens. APR generally has little effect on leaf rust infection type, it is however characterized by slower disease progress in field epidemics and is thus referred to as slow rusting. APR often manifests in one or more of the following symptoms: decreased receptivity, decreased spore production, and decreased pustule size. It is also commonly associated with longer latent period (Singh, Huerta-Espino et al. 2005).

When considering the terms, “seedling”, “APR”, “major”, “minor”, and other common descriptors for resistance, it remains important to consider their attributes generally, as

contradictions do exist. An example of these is in the race-specificity of the APR gene *Lr12*, which is in contrast to the other race-nonspecific APR genes (Singh and Bowden 2011).

Durability

By itself, a high degree of control does not necessarily depict the ideal form of genetic resistance. Breeders and researchers must also consider durability of resistance when developing new cultivars and donors. Resistance is considered durable when it remains effective under wide and prolonged deployment amid conditions favorable for the disease (Johnson 1984). Given that winter wheat cultivars frequently take eight to twelve years to develop, durable genetic resistance is a key factor in making such an investment worthwhile.

The lone deployment of seedling resistance heavily selects for virulence from the naturally occurring heterogeneity in the leaf rust population and is thus not considered to be durable (Browning and Frey 1969). Despite this, in a few rare circumstances, genes conferring seedling resistance have proven effective for several years (Van der Plank 1969).

APR, however, does not operate via pathogen effector recognition. In the absence of effectors and receptors, it is difficult for the pathogen to overcome host defenses. APR is generally not race-specific and APR genes do not individually apply tremendous selection pressure on pathogen populations. For these reasons, APR is widely associated with durability. When multiple APR genes are present, durable levels of additive resistance or tolerance may be achieved which last for many years. Combining several different genes affecting the same trait- a technique known as ‘pyramiding’- has the ability to reduce the probability of a virulent pathogen race suddenly breaking genetic resistance across an adaptation zone (Van der Plank 1969, Johnson 1984). Pyramiding slow-rusting, non-race-specific genes can likely confer durable resistance for leaf rust.

While desirable, pyramiding does have some drawbacks. Leaf rust resistance is only one of several traits which breeders must consider when designing crossing blocks and making selections. It is a common concern that the time and cost of pyramiding *Lr* genes can impede breeding for other important traits. Linkage drag is also a concern if the pyramid contains genes tightly linked to detrimental alleles. If an effective pyramid requires too many genes, the requisite cycles of crossing, evaluation, and selection can be cost- and time-prohibitive. When foliar fungicides are available and affordable in a geographic region, breeding resistance pyramids for that geography requires a particularly long-term focus. It is therefore very important that a suitable pyramid of genes be developed which durably confer acceptable resistance while still maintaining the plant characteristics necessary for commercial success.

Due to the high evolutionary potential of *P. triticina*, the constant fluctuation of rust races, and a limited supply of *R* genes, genetic resistance requires stewardship and curation to remain effective. The careless deployment of resistance genes among germplasms and geographies has led to the failure and ruination of many sources of native resistance. Once virulence to a particular gene becomes prevalent, its usefulness is highly limited. Efforts therefore must be taken to preserve newly discovered genes if genetic resistance is to remain effective and deliver its true potential to growers around the world.

The Current State of Breeding for Leaf Rust APR

Successful resistance breeding requires four criteria to be met: effective resistance sources, desirable cultivars in other attributes, wide planting, and durability of resistance. Despite these challenges, breeders have successfully developed cultivars with highly effective genetic resistance, beginning with the first hard red winter wheat cultivars with resistance to *P. triticina* in the mid-1940s (Browder 1980, Bockus, De Wolf et al. 2011). Leaf rust resistance was later

observed in the South American cultivars ‘Frontana’ and ‘Klein 33’, the latter of which became a parent to many Russia-adapted cultivars (Roelfs 1985). The North American spring wheats Alsen, Norm, and Era possess pyramids of seedling and APR genes including *Lr13* and *Lr34* and have been used extensively as parents in breeding new cultivars (Oelke and Kolmer 2005). Hard red winter wheats, ‘Santa Fe’ and ‘Duster’, are important cultivars adapted to the southern Great Plains and are known to also possess pyramids of APR genes *Lr34, Lr46,* and *Lr77* (Kolmer 2017).

There are currently ~80 (*Lr1- Lr80*) documented genes for resistance to leaf rust, the vast majority of which confer race-specific seedling resistance. Allelism among these also accounts for variants of individual genes found upon further in-depth study, as seen in *Lr14a* and *Lr14b*. The actual number of resistance QTLs found is likely much higher than the *Lr80* designation would suggest. A recent meta-QTL analysis, by integrating the results of 20 previous bi-parental QTL mapping studies, projected as many as 144 unique leaf rust QTLs (Soriano and Royo 2015). Some genes which are common in wheat germplasm no longer provide effective resistance. Examples of such genes are *Lr1, Lr3, Lr10, Lr13, Lr14a, Lr17a* and *Lr37*. Their continued presence in breeding programs effectively selects for virulence in annual epidemics (Oelke and Kolmer 2005). Several formerly successful cultivars became obsolete at least in part due to virulence changes in *P. triticina*. A key example of this is in the formerly widely planted North American hard red winter wheat cultivar, ‘Jagger’, which was released in 1994 and carries the seedling resistance gene, *Lr37*. Upon release, Jagger was rated as ‘resistant’ in Kansas observation trials. By 1997, virulence to *Lr37* in isolates of *P. triticina* were widespread in the Great Plains and Jagger was rated as ‘intermediate’. By 2000, Jagger had completely succumbed to leaf rust in the Great Plains and was rated as ‘susceptible’. Despite its many very desirable

attributes, it rapidly fell out of favor due to this susceptibility (Long, Leonard et al. 2000, Bockus, De Wolf et al. 2011, Kolmer and Hughes 2018, Xue, Kolmer et al. 2018). A similar example is in the cultivar 'Karl'. Resistant when released in 1988, Karl possesses a pyramid of R genes *Lr1*, *Lr10,* and *Lr14a* yet is nevertheless susceptible to prevalent races (USDA 2010). In the cases of Karl and Jagger, selection for virulence to these genes persisted for several years because growers continued to plant them despite their susceptibility. This was due to the otherwise desirable attributes of these lines (Bockus, De Wolf et al. 2011). Other well-known cultivars with ineffective resistance alleles include 'Overley' with *Lr39* and the North American spring wheat cultivar, 'Faller' with *Lr21*. Virulent races to *Lr9*, *Lr17a*, *Lr24*, *Lr26*, *Lr37*, and *Lr39* have recently been found to be nearly ubiquitous in the Great Plains and with increasing frequency in Canada. This condition is not necessarily permanent however, as virulence has decreased on *Lr2a*, *Lr2c*, and *Lr16* in Canada, presumably due to decreased deployment in commercial cultivars (Huerta-Espino, Singh et al. 2011). In addition to selecting for virulence, seedling genes may mask unknown APR genes in populations. Over the past few decades, some breeding programs have come to reject seedling genes from crossing blocks in favor of APR (Landeo, Gastelo et al. 1995). While a few were described decades ago, it is notable that several of the most recently characterized *Lr* genes confer race-nonspecific APR. Some of these important APR genes are *Lr34*, *Lr46*, *Lr67*, *Lr68*, *Lr74*, *Lr75*, *Lr77*, *Lr78*, and *Lr79*, described below.

Lr34 is an important and widespread APR gene originally identified in the Brazilian cultivar 'Frontana' and located on the short arm of chromosome 7D (Dyck, Samborski et al. 1966). Though it has been widely deployed for over 60 years, no leaf rust isolate with complete virulence has yet been discovered (Lagudah, Krattinger et al. 2009). One of the first

leaf rust APR genes to be cloned, it is desirable in breeding for durable resistance since it has been repeatedly shown to interact well with other quantitative resistance genes in successful cultivars (Krattinger, Lagudah et al. 2009). It is also dominantly inherited, allowing for easier and faster introgression. (Nelson, Singh et al. 1997). It is characterized as a putative ATP-binding cassette (ABC) transporter, which facilitates the movement of molecules across cellular membranes via active transport. The specific pathways involved in the resistance conferred by *Lr34* remain a subject of interest to researchers, though it has been observed that movement of the phytohormome, abscisic acid (ABA), regulates a physiological response leading to dehydration tolerance, transpiration, and seedling growth (Krattinger, Kang et al. 2019). The same study identified ABA as a substrate of the ABC transporter of *Lr34*. Resistance from *Lr34* is characterized by a reduction in the growth rate, size, and quantity of uredinia with little or no hypersensitivity or callose deposition. Low temperatures have been demonstrated to enhance this response (Rubiales and Niks 1995, Risk, Selter et al. 2012). *Lr34* is pleiotropic and is also known as stripe rust resistance gene *Yr18*, stem rust resistance gene *Sr57*, powdery mildew (*Blumeria graminis f. sp. tritici*) gene *Pm38*, leaf tip necrosis gene *Ltn1*, and barley yellow dwarf resistance gene *Bdv1* (McIntosh 1992, Lagudah, McFadden et al. 2006, Pinto da Silva, Zanella et al. 2018).

Another important gene for APR to leaf rust is *Lr46*, located on the long arm of chromosome 1B and originally identified in the cultivar ‘Pavon 76’. As in *Lr34*, however less pronounced, it causes a reduction in the size and quantity of uredinia, non-hypersensitivity, and is dominantly inherited. It also confers a chlorotic response in infected leaves and is tightly linked to leaf-tip necrosis gene *Ltn2* (Singh, Mujeeb-Kazi et al. 1998, Martinez, Niks et al. 2001). It is pleiotropic and known as stripe rust resistance gene *Yr29*, stem rust resistance gene

Sr58, and powdery mildew resistance gene *Pm39* (William, Singh et al. 2003, Singh, Huerta-Espino et al. 2005).

The APR gene, *Lr67*, is a recently characterized and cloned hexose transporter located on the long arm of chromosome 4D (Hiebert, Thomas et al. 2010). It was identified first in the Pakistani accession 'PI250413' and later crossed with the cultivar 'Thatcher' (Dyck and Samborski 1979, Pinto da Silva, Zanella et al. 2018). It too does not induce a hypersensitive response in infected leaves. It is pleiotropic with stripe rust resistance gene *Yr46*, stem rust resistance gene *Sr55*, powdery mildew resistance gene *Pm46*, and leaf-tip-necrosis gene *Ltn3* (Herrera-Foessel, Lagudah et al. 2011, Herrera-Foessel, Singh et al. 2012, Moore, Herrera-Foessel et al. 2015).

Lr68 also confers race-nonspecific APR to leaf rust and is currently of great interest to breeding programs. Although it likely originated in Frontana, it was first identified in 2012 in the cultivar 'Parula', which contains Frontana in its pedigree. It is located on the long arm of chromosome 7B (Herrera-Foessel, Singh et al. 2012). It too is associated with Ltn, though to a lesser extent than *Lr34*, and is tightly linked to several other known leaf rust seedling resistance genes. (Juliana, Rutkoski et al. 2015).

Lr74, Lr75, Lr77, Lr78, and Lr79 were all recently described and confer APR to leaf rust. *Lr74* was found in the cultivar 'Spark' and mapped to the short arm of chromosome 3B. *Lr75* was identified in the cultivar 'Forno' on the short arm of chromosome 1B (Singla, Lüthi et al. 2017). *Lr77* was mapped to the long arm of 3B in the cultivar 'Santa Fe' (Kolmer, Su et al. 2018). *Lr78* was found on the short arm of chromosome 5D in the Brazilian cultivar 'Toropi' (Kolmer, Bernardo et al. 2018, Pinto da Silva, Zanella et al. 2018) (Kolmer 2018c), (Pinto da

Silva 2018). *Lr79* was found in the durum cultivar 'Aus26582' on the long arm of chromosome 3B (Qureshi, Bariana et al. 2018).

Nearly all the deployed resistance genes were originally discovered in hexaploid bread wheat. Efforts to test wild relatives of wheat for resistance genes have succeeded in identifying new sources of resistance. These wild relatives are often highly diverse genetically and in their geographic dispersal, decreasing the likelihood of virulence in pathogen populations. When a wild source is identified, it can potentially be introgressed via hybridization with bread wheat or by the creation of synthetic cultivars. The high rate of homology between *A. tauschii* Coss. and the D genome of bread wheat makes gene transfer between them feasible. (Gill, Raupp et al. 1986). Several seedling *R* genes found in bread wheat can trace their ancestry to *A. tauschii* Coss. A recent study which evaluated both greenhouse seedling and field adult-plant responses to leaf rust infection identified 50 accessions of *A. tauschii* Coss. to possess resistance genes to leaf rust; 41 of which expressed APR (Kalia, Wilson et al. 2017).

Current Genotyping and Sequencing Approaches

Whether by natural processes or targeted crossing, incremental phenotypic variation in a trait is often the result of recombination between QTLs. Eukaryotic genomes all possess genetic sequence variation in the form of single nucleotide polymorphisms (SNPs). While several marker technologies have been developed, SNPs are the smallest unit of inheritance and can be used as highly versatile genetic markers. When these markers are associated with traits of interest in populations, the method known as marker-assisted selection (MAS) can be used to make breeding decisions. MAS allows for breeding advancement solely based on genetic enrichment for traits of interest. Once markers have been properly associated with a trait, MAS is often a faster and less expensive alternative to phenotyping when working with QTLs.

Given the enormous task of deciphering the wheat genome, advanced techniques must often be used to make gains in our understanding of traits and inheritance patterns. The recent unveiling of a fully annotated reference wheat genome sequence will undoubtedly disrupt many of the established genome research methods. This sequence DNA from Chinese Spring revealed information on more than 107,000 genes along with their regulatory sequences across 94% of the wheat genome, constituting a major breakthrough in the ability to use advanced methods for breeding wheat (Appels, Eversole et al. 2018). Among the many advantages this provides, perhaps the most significant is that large scale analyses of sequence-level data can now be used with confidence by breeders and researchers. It will also help improve our understanding of complex gene interactions for quantitative traits. A drawback to the reference sequence, however, is that it is limited to the genomic information provided by the sequence DNA of the individual(s) under study. A pan-genome incorporates the variation of several individuals to identify the common genes across an entire species as well as characteristics specific to regions of adaptation. The sequencing of 18 wheat cultivars in 2017 predicted an average pan-genome size of over 128,000 genes across the cultivars studied (Montenegro, Golicz et al. 2017). A pan-genome can be used to characterize the diversity in resistance genes, identify lost sequences due to genetic bottlenecks in the form of genes or modifiers, and distinguish between core and variable genes (Bayer, Golicz et al. 2020). It is likely that the use of the wheat reference sequence and pan-genome will drive wheat genomics for the foreseeable future.

GBS is a restriction-enzyme fragmented sequence-based genotyping system. It is aimed at reducing the complexity of the wheat genome by sequencing only a sampling of the genome-avoiding the cost of sequencing the entire genome when only a fraction is necessary to discover tens of thousands of markers for a population. GBS generates large numbers of genome-wide

markers so the likelihood of identifying causative polymorphisms closely linked to the gene is relatively high. GBS is also unique because it allows for genotyping and marker discovery to occur at the same time. Like many sequencing approaches, GBS uses one or more restriction enzymes to digest multiple samples of genomic DNA into fragments of less than 1,000 base-pairs. It uses methylation-sensitive restriction enzymes to filter out the vast repetitive segments of the genome. Tags are then ligated to the ends of the fragments. These fragments are subsequently sequenced using a suitable Next Generation Sequencing (NGS) platform. NGS is an approach to population genomics which is used to identify, sequence, and genotype thousands of polymorphic markers across a genome. With such high marker density, breaks in recombination linkage can be identified in linkage or QTL mapping studies among other uses (Poland, Brown et al. 2012, Rife, Wu et al. 2015). Using a linkage map or another anchoring tool, the newly discovered markers can be used to produce a high-density genetic map and to infer the location of recombination breakpoints. In addition to a large number of markers generated, GBS as a low-density, methylation-sensitive sequencing method, is also well known for the generation of widespread, stochastic missing genotype data. When appropriate, imputation algorithms are used to infer the nucleotide sequence with a relatively high level of accuracy.

Rationale for this Study

Growers have been plagued by leaf rust epidemics since the domestication of wheat. Genetic resistance is the most economical and sustainable means of defense against these epidemics. Despite the progress made in the last century, the discovery of new sources of resistance is in high demand in order to develop new cultivars and improve the durability of

resistance. This study aims to identify race-nonspecific resistance to leaf rust in the moderately resistant cultivar, Roelfs F2007 -a known source of resistance with strong agronomic qualities. An understanding of the underlying genetic cause of the resistance seen in Roelfs F2007 will hopefully provide breeders with new genetic resources to be used in marker-assisted breeding for durable resistance. Additionally, this study aims to identify uniquely resistant progeny from a Lakin*2/Roelfs F2007 BC1F6:8 population.

Chapter 2 - QTL Analysis of Wheat Leaf Rust in the Lakin × Roelfs F2007 Backcross Population

Abstract

Leaf rust, caused by the fungus *Puccinia triticina*, is a major disease of wheat in North America. Breeding efforts to achieve resistance have historically been plagued by a cycle in which an all-stage resistance gene is discovered, deployed, and rapidly defeated, thus wasting valuable sources of resistance. Adult-plant resistance genes, however, can potentially contribute to race-nonspecific resistance to the pathogen that is durable for many years. In this study, the moderately susceptible Kansas winter wheat cultivar, 'Lakin', was backcrossed to the CIMMYT-developed spring wheat, 'Roelfs F2007', which is known to possess very good adult-plant resistance (APR) to leaf rust. The 297 BC_1F_6-derived progeny of this cross were screened for resistance in five location-years at field nurseries in Kansas and Texas, as well as in two greenhouse studies. Genotyping-by-Sequencing (GBS) generated 2,541 high-quality genome-wide markers used for the construction of a linkage map covering all 21 chromosomes. Quantitative trait locus (QTL) analysis identified reproducible APR QTLs on chromosome arms 3BS, 5DS, 7BS, and 7BL. Three QTLs contributed by Roelfs F2007 appeared to correspond to locations of known APR genes *Lr74* (3BS), *Lr78* (5DS), and *Lr68* (7BL). The APR QTL on 7BS contributed by Lakin appeared to be novel. Together, the four QTLs accounted for 45% and 46% of the variation in least-squares means for disease severity and infection type, respectively. These results confirm that both Roelfs F2007 and Lakin contain multiple APR loci which may be used in breeding for resistance to infections by *P. triticina*.

Introduction

The rust fungi of wheat (*Triticum aestivum* L.), namely leaf rust (*Puccinia triticina Eriks*.), stripe rust (*Puccinia striiformis* Westend f. sp. *tritici*), and stem rust (*Puccinia graminis* Pers.: Pers. f. sp. *tritici Eriks.*) are present in all the wheat growing regions of the world. Together these comprise the most common and devastating diseases of wheat. Of these, leaf rust has the widest global distribution and is likely responsible for the greatest cumulative yield loss. Like the other species of genus, *Puccinia, P. triticina* is a parasite which is highly adaptive to variable conditions, produces abundant spores and exhibits frequent race changes. To date, as many as 80 resistance (R) genes have been identified which help protect against epidemics of leaf rust. Many of these genes, however, confer race-specific resistance- a form which has demonstrated to select for virulence among the extant races of *P. triticina*. A superior approach is the use of multiple race-nonspecific genes which individually exert limited defense against infection, yet also apply minimal selection pressure on pathogen populations. The result is a commercially viable resistance to *P. triticina*, comprised of cumulative minor effects and which is race-nonspecific and lasting for many years under cultivation.

Aiding in the development of resistant cultivars, the International Center for the Improvement of Maize and Wheat (CIMMYT) helps provide researchers with new germplasm for testing against rust diseases and for further crop improvement. ‘Roelfs F2007’ is a CIMMYT-developed cultivar which demonstrates moderate resistance to leaf rust while having strong agronomic qualities. An exploration of the underlying cause of the resistance in Roelfs F2007 may aid in the further development of improved germplasm. ‘Lakin’ is a winter wheat cultivar adapted to the Great Plains of the United States and known to be moderately susceptible to leaf rust. A backcross population of Lakin and Roelfs F2007 serves to reveal the resistance

mechanisms in the resistant cultivar while simultaneously producing adapted, resistant progeny. The identification of unique sources of race-nonspecific resistance and understanding their performance in combination will provide new genetic resources for use in marker-assisted selection (MAS). This study is part of the much wider effort being undertaken by researchers globally to combat the effects of the rust pathogens. It is my hope that the research presented herein may contribute to this effort, provide new genetic tools for wheat development, and ultimately help improve food stability for people everywhere.

Materials and Methods

Parentage and Progeny of the U6225 Population

'Roelfs F2007' is a semidwarf spring wheat cultivar named in honor of distinguished USDA-ARS Cereal Disease Laboratory pathologist, Alan P. Roelfs. It was adapted for the bread wheat growing regions of Mexico, most specifically, Sonora state. Bred by CIMMYT partly for its high milling and baking quality, it is a selection from the backcross Kambara-1*2/Kukuna. Roelfs F2007 is susceptible at the seedling stage, but has a moderately resistant field reaction to leaf rust and stripe rust at the adult stage. (Figueroa-López, Fuentes-Dávila et al. 2011).

The recurrent female parent of Roelfs F2007, Tacupeto F2001, is a hard, white, semi-dwarf spring wheat also released as "Kambara" or "Xinchun 23". Bred by CIMMYT, Tacupeto F2001 was among the most widely-planted cultivars in Mexico in the early 2000's. Despite expressing moderate adult-plant susceptibility to leaf rust with a severity of 20% in pre-release trials, it does nevertheless possess the seedling gene *Lr27* and the adult-plant, race specific gene *Lr31*, which is identical to *Lr12* and effective only in combination with *Lr27* (Figueroa-López, Fuentes-Dávila et al. 2011, Singh and Bowden 2011). It is a high-yielding, early maturing variety in its region of

adaptation. The male parent, Kukuna, is a Mexican spring wheat containing *Lr34* and the high-yielding 1B/1R translocation (Figueroa-López, Fuentes-Dávila et al. 2011).

'Lakin' is a hard, white winter wheat variety adapted to Kansas and bred by the Kansas Agricultural Experiment Station with USDA-ARS. First planted commercially in 2001, it is a selection from the F1 cross between Arlin and KS89H130. It was selected for low polyphenol oxidase levels in the grain, making it more suitable for Asian noodles. It is considered susceptible to leaf rust and moderately susceptible to stem rust and stripe rust (Martin 2001, Jardine 2006).

In the original F_1 cross in this study, Lakin was the female parent and Roelfs F2007 was the male parent. In the backcross, the F_1 was used as the male parent and was crossed onto Lakin, the female parent. From the BC_1F_2 generation, the progeny were advanced individually through a single-seed descent process until B_1F_6 RILs were ready for evaluation. Upon initial field evaluation, any spring-type RILs at the BC_1F_6:F_8 generation were culled from the population resulting in the final population size of 294 RILs.

Evaluation

The BC_1F_6 to BC_1F_6:F_8 generation RILs were evaluated in seven adult-plant experiments from 2013-2020. A modified McNeal Scale (McNeal, Konzak et al. 1971) was used to characterize infection type (IT) on a 0-9 scale. A rating of 0 = No symptoms (immune); 1 = Small chlorotic or necrotic flecks with no sporulation; 2 = Small to medium chlorotic or necrotic flecks with no sporulation; 3 = Small pustules with chlorosis or necrosis and trace of sporulation; 4 = Small pustules with chlorosis or necrosis and light sporulation; 5 = Small to medium pustules with chlorosis or necrosis with intermediate sporulation; 6 = Small to medium pustules with chlorosis or necrosis with moderately high sporulation; 7 = Medium to large pustules with

chlorosis or necrosis with abundant sporulation; 8 = Medium to large pustules with chlorosis and abundant sporulation; 9 = Medium to large pustules with little or no chlorosis and abundant sporulation. Leaf rust disease severity (SV) percentage (0-100%) on the flag leaf upper surface was determined using the modified Cobb Scale (Peterson, Campbell et al. 1948, Roelfs 1992). Approximately 20 flag leaves were examined at the soft dough stage, approximately Zadoks 85 (Zadoks, Chang et al. 1974), and a mean value was estimated for IT and SV for each entry. In environments where plant-to-plant variation was very high within a plot, such as when barley yellow dwarf was prevalent, the estimated 95th percentiles for IT and SV were used for evaluation. To produce the highest quality reproducible data, a visual guide for IT and SV evaluation was used to calibrate the eye of the rater in any dataset (Roelfs 1992). An instance of the population planted in a particular environment in a particular year is hereafter referred to as an environment. A single type of evaluation (IT, SV) performed in any environment is hereafter referred to as a dataset.

Two experiments were conducted in the greenhouses of Kansas State University in Manhattan, Kansas from 2014 to 2015. After six weeks of vernalization in low light (12-hr photoperiod) at 5 ± 2 °C, single 25 ml plugs containing 4-5 seedlings of each RIL were transplanted into 425 ml pots in a two-replication randomized complete block in a 16-hr supplemental photoperiod greenhouse at 21 ± 3 °C day and 18 ± 2 °C night. The inoculum consisted of vacuum-harvested urediniospores of race MFPSC (isolate PRTUS54). These collections were multiplied on susceptible hosts and stored at -80°C until ready to use, at which time they were heat-shocked at 42°C for 6 minutes. A 1% (v/v) urediniospore suspension in Soltrol 170 isoparaffin oil (Chevron Phillips, The Woodlands, TX) was evenly sprayed on batches of flowering plants using a rust inoculation atomizer (Tallgrass Solutions, Inc.,

Manhattan, KS). After spraying, inoculated plant batches were dried for 30 minutes and then placed in Percival Model I-36DL dew chambers (Percival Scientific, Perry, IA) for 16 hours in dark conditions at 18-20°C. The plants were removed the following morning and returned to the greenhouse at 21 ± 3 C day and 18 ± 2 C night, where they were rated approximately three weeks after inoculation. The datasets from the two greenhouse environments are hereafter referred to with the following convention: GH14IT (greenhouse 2014 infection type), GH14SV (greenhouse 2014 % severity), GH15IT (greenhouse 2015 infection type), and GH15SV (greenhouse 2015 % severity).

Five single-replication experiments were conducted in dryland field environments. These were evaluated at Kansas State University's Ashland Bottoms Research Farm near Manhattan, Kansas in 2013, 2014, 2015, and 2020; and near Castroville, Texas in 2014. Approximately 1-2 grams of each RIL were planted in headrows measuring 1.2 meters in length. These were arrayed in inline ranges of 4 parallel rows each spaced 25 centimeters apart. Ranges of four rows were spaced 60 centimeters apart, lengthwise. 20 ranges constituted the aggregate headrow tray measuring 37 meters in length. Susceptible checks of Lakin, Jagger, and Overley were planted in the first range of each tray. Roelfs F2007 was not included as a check because it is a spring wheat and therefore not winter hardy. A 1.5-meter-wide seed drill was used in planting the trays. A blend of leaf rust-susceptible cultivars was planted in a border around the experiment and every fourth drill pass to facilitate spread of the epidemic. At Castroville, TX, natural inoculum was sufficient to create an epidemic of leaf rust. At Ashland Bottoms, KS, a composite inoculum of approximately ten recent Kansas leaf rust isolates suspended in Soltrol 170 at a rate of 2 ml dried urediniospores per liter, was applied as an aerosol on multiple occasions from jointing until about Zadoks growth stage 40 using an Ulva+ ultralow volume sprayer (Micron

Group, Herefordshire, UK). Plants were scored at the soft dough stage of grain development (Zadoks 81-85). At Ashland Bottoms in 2020, a one-time note on green leaf (GL) area was taken concurrently with the infection type and severity data. In this note, each RIL was rated for percentage of remaining green flag leaf surface area. The datasets from each field environment are hereafter referred to with the following convention: AB13IT (Ashland Bottoms 2013 infection type), AB13SV (Ashland Bottoms 2013 severity), AB14IT (Ashland Bottoms 2014 infection type), AB14SV (Ashland Bottoms 2014 severity), TX14IT (Texas 2014 infection type), TX14SV (Texas 2014 severity), AB15IT (Ashland Bottoms 2015 infection type), AB15SV (Ashland Bottoms 2015 severity), AB20IT (Ashland Bottoms 2020 infection type), AB20SV (Ashland Bottoms 2020 severity), AB20GL (Ashland Bottoms 2020 % remaining green leaf area). Testing environments may be hereafter generally referred to by location and year with the following convention: AB14 (Ashland Bottoms 2014), and so forth.

Seedling Test

To determine whether resistance phenotypes seen at the adult stage are indicative of APR, Roelfs F2007, Lakin, the susceptible check 'Morocco', and 308 progenies of the U6225 population were evaluated for leaf rust response at the seedling stage. This evaluation took place in the facilities of the USDA-ARS Hard Winter Wheat Genetics Research Unit at Kansas State University in Manhattan, KS. In a three-replication experiment, seedlings were inoculated with isolates of *P. triticina* race BBBDB, which is avirulent to all known resistance genes except for *Lr14a.* Seedlings were grown in a 16-hour supplemental photoperiod greenhouse for 10 days at 21±3℃ during the day and 18±2 ℃ at night. When primary leaves were at full emergence, seedlings were lightly sprayed using a rust inoculation atomizer with a 1% (v/v) urediniospore suspension in Soltrol 170 isoparaffin oil. The inoculated seedlings were transferred to a dark,

18℃ I-36DL dew formation chamber at 100% humidity for 16 hours. Seedlings were then transferred back to the greenhouse. Notes were taken after 11-14 days of incubation. Infection type (IT) was recorded using the method described by Long and Kolmer as modified by Kolmer and Hughes (Long and Kolmer 1989, Kolmer and Hughes 2016). The seedling datasets are hereafter referred to as STR1, STR2, and STR3 for Seedling Test Rep 1,2, and 3, respectively.

BLUPS, Least-Squares Means, and Heritability

To summarize the effects across all adult-plant datasets, BLUPs (Best Linear Unbiased Predictions) and LSMeans (Least-Squares Means) were calculated for each RIL using PROC MIXED parameter estimates and PROC GLM in SAS version 9.4 (SAS, Cary, NC), respectively. This was repeated to summarize the seedling datasets as well. The broad-sense heritability (Table 2.1) of the U6225 population was calculated wherein σ_g^2 = covariance due to genotype, σ^2_e = the residual covariance, and *r* = the number of environments for each category.

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r}}$$

SNP Genotyping

At the BC_1F_6 generation, seedling leaf tissue was collected from a bulk of 3-5 seedlings for each of the 294 RILs. After lyophilization and grinding, DNA was extracted using Biosprint 96 DNA Plant kits (QIAGEN) with the BioSprint 96 Workstation (QIAGEN). Purified stock DNA aliquots were diluted in 1× TE buffer and normalized to a 50 ng/µL concentration and increased to a final volume of 20 µL with the PicoGreen dsDNA assay kit. GBS was the genotyping method used in this study. Key Gene N.V. owns patents and patent applications protecting its GBS and related Sequence-Based Genotyping technologies. The GBS libraries were constructed with two restriction enzymes, *PstI* (CTGCAG) and *MspI* (CCGG). Single

nucleotide polymorphisms (SNPs) were analyzed from the GBS reads with the UNEAK pipeline following the 2012 Poland et al. protocol (Poland, Brown et al. 2012). Libraries were processed at the USDA Central Small Grain Genotyping Lab, Manhattan, Kansas. A total output of 19,089 polymorphic SNP markers was identified. These were filtered for ≤50% missing genotype data resulting in 12,245 candidate markers for mapping and analysis. Imputation of missing data was performed using Beagle v.4.1 (Browning and Browning 2016).

Diagnostic Markers

In the first of two gene-specific marker tests, the extracted DNA was used to screen Roelfs F2007 and Lakin for known genes of interest along with check, 'Pavon 76'. The presence of genes *Lr34/Yr18/Sr57/Pm38*, *Lr46/Yr29/Sr58/Pm39*, *Lr68*, and *Sr2/Yr30* were determined using sequence tagged site markers *csLV34*, *csLV46*, *Lr68csGS*, and *csSr2R*, respectively (Lagudah, McFadden et al. 2006), (McNeil, Kota et al. 2008),(Herrera-Foessel, Singh et al. 2012)(E. Lagudah, unpublished data, 2012).

In the second gene-specific marker screening performed after the MQM analysis, the presence of genes *Lr34/Yr18/Sr57/Pm38*, *Lr46/Yr29/Sr58/Pm39*, *Lr78*, and *Sr2/Yr30* were determined by the Kompetitive Allele Specific PCR (KASP) method using *Lr34Exon11*, *Lr46_JF2-2A*, *IWA6289*,and *Sr2_ger9_3p KASP* markers, respectively (Fu, Szűcs et al. 2005, Beales, Turner et al. 2007, Lagudah, Krattinger et al. 2009, Mago, Brown-Guedira et al. 2011, Milec, Tomková et al. 2012, Rasheed, Wen et al. 2016). *Lr68* was screened using the sequence tagged site marker, *Lr68csGS* (Herrera-Foessel, Singh et al. 2012).

For these, Touchdown (TD-PCR) was used for primer amplification and. SNP calling was performed with GeneMarker Version 1.97 (SoftGenetics).

Linkage Map Construction

In preparation for constructing a linkage map, the set of 12,245 markers were further reduced using R version 3.4.4 and RStudio software by excluding duplicate markers, distorted segregation patterns, and those contributing to inflation of the genetic map positions. This exclusion resulted in a set of 2,541 high-quality markers with less than 20% missing genotype data with which to construct a map. Kyazama JoinMap 4.1 software (Kyazma B.V., Wageningen, Netherlands) was used to generate a linkage map with the maximum likelihood independence (LOD) algorithm and the Kosambi mapping function (Van Oojien, Kyazmay et al. 2006, Van Ooijen 2011). The linkage groups were assembled and oriented to the GBS tag position from the RefSeqV1 BLAST database from the International Wheat Genome Sequencing Consortium (IWGSC) on the Wheat@URGI portal (https://urgi.versailles.inrae.fr/blast/?dbgroup=wheat_iwgsc_refseq_v1_chromosomes&program=blastn). This yielded a map of twenty-one linkage groups with coverage of all twenty-one chromosomes of the hexaploid wheat genome. The linkage groups identified here were used for QTL analysis.

QTL Analysis

In all adult-plant experiments, disease infection type (IT) and % severity (SV) were treated independently. The addition of a green leaf area note in AB20 resulted in fifteen adult-plant datasets taken from seven independent experiments for QTL analysis. The three seedling replications were also analyzed independently for disease infection type only. In addition, BLUPS and least-squares means for infection type, severity, and seedling IT were used to summarize the results across multiple datasets.

The QTL analysis was performed with R statistical computing software v.3.4.4 with the R/qtl package v.1.47-9 and Haley-Knott Regression (Broman and Sen 2009). Using the R/qtl package, composite interval mapping (CIM) was performed for the initial genome scan for QTLs for each dataset. For each dataset, the strongest putative QTL was then used as a covariate to unmask the effects of less significant QTLs. The CIM results were compiled and used as a general guide in the initial development of models for multiple QTL mapping (MQM), which simultaneously models both the additive and epistatic effects of multiple QTLs. For all QTL mapping functions, Bayesian 95% confidence intervals were calculated. Significant QTLs were determined using the genome-wide logarithm of odds (LOD) thresholds with 1000 permutations. The type I error was set to 0.05 and 0.10 for calculating LOD thresholds for each phenotypic data set. A standard was imposed wherein any QTL that met the 0.05 error threshold from CIM in any dataset was at least initially included in the MQM model. After having generated an initial model for each dataset independently, each model was then tested for improvement by testing all QTLs found in other datasets in a maximum QTL model. In this process, any QTL which was significant at the .10 error threshold was removed one-at-a-time, after which the model was re-run to test whether significance increased across the model with its exclusion. If the exclusion of the QTL was found to be detrimental to the remaining model, the QTL would be brought back into the revised model and the script was run again. This process was repeated until all possible combinations with the maximum overall significance was found between the available QTLs meeting the minimum significance criteria.

When searching for physical positions of QTLs, the BLAST database tool for RefSeqV1 and V2 from the IWGSC on the Wheat@URGI portal BLAST tools for were used (https://urgi.versailles.inrae.fr/blast_iwgsc/?dbgroup=wheat_iwgsc_refseq_v1_chromosomes&pr

ogram=blastn). Occasionally, CIM and MQM indicated a QTL cM position between available GBS markers. In these cases, the position was interpolated using the known physical positions of flanking GBS markers from the BLAST tool and aligning them with the cM positions provided by the R script. With this information, a relative physical position of the unknown QTL could be deduced with a basic formula which assigned a proportional physical position for each cM.

Results

Linkage Mapping

The linkage groups of the population are as follows: 1A, 1B, 1D, 2A, 2B, 2D, 3AS, 3AL, 3B, 3D, 4A, 4B, 4DS, 5A, 5B, 5D, 6A, 6B, 6DS, 6DL, 7A, 7B, 7DS, and 7D. The long and short arms of linkage groups 4D, 6D, and 7D originally remained separate and were consolidated based upon their RefSeqV1 physical position. Ultimately, our map was constructed of 21 chromosomes incorporating 2,541 GBS markers (Figure 2.2) which were assembled for comparison with the RefSeqV2 physical map for validation (Figure 2.3). The D genome consistently had the lowest GBS marker coverage, especially on chromosomes 3D and 4D. The low coverage is attributable to the well-documented low frequency of polymorphisms on the D genome.

QTL Validation by Diagnostic Markers

In the first parental screening, using markers *csLV34*, *csLV46*, and *csSr2R* revealed that neither Lakin nor Roelfs F2007 possess *Lr34*, *Lr46*, and *Sr2*, respectively. Marker *Lr68csGS* indicated that Roelfs F2007 possesses *Lr68* while Lakin is negative for *Lr68*. (Table 2.2)

In the second screening, *Lr34Exon11* and *Sr2_ger9_3p* confirmed the absence of *Lr34* and *Sr2*, respectively in both Lakin and Roelfs F2007. In contrast to the prior result from *Lr46*

marker, *csLV46*, Roelfs F2007 tested positive and Lakin tested negative for *Lr46* marker, *Lr46Yr29JF-2*. Toropi and the other 18 RILs included in the second screening segregated for *Lr46Yr29JF-2*. *Lr68csGS* confirmed the prior result for *Lr68*, attributing the gene to Roelfs F2007 while Lakin was negative. SNP marker, *IWA6289* indicated that both Roelfs F2007 and check, Toropi were positive for *Lr78* while Lakin was negative (Table 2.3).

Phenotypic Response

The adult-plant broad-sense heritability for IT and SV were 0.876 and 0.790, respectively. Seedling broad-sense heritability was 0.484 (Table 2.1). Analysis of variance (ANOVA) indicated significant genotypic effects in each individual experiment and across experiments with the exception of GH14SV (Tables 2.5, 2.6 and 2.8). Under the composite inoculum and natural infections, a wide range of phenotypes were observed (Table 2.7).

The disease severity observed in the population ranged from 0 to 100% across all datasets combined. Infection type ratings ranged from 2 to 9 across all datasets. Using LSMeans IT and SV data as a benchmark, the field datasets with the highest correlation were AB13, AB15, and AB20. (Figure 2.1). These three field seasons at Ashland Bottoms were observed at the time of rating to have the most uniform plant quality and distribution of infection. Correlation for AB13IT, AB15IT, and AB20IT with LSM_IT were 0.83, 0.84, and 0.84, respectively. Conversely, both greenhouse IT datasets had a much lower correlation with LSM_IT of 0.5. LSM_IT was correlated with TX14ITat 0.69 and AB14IT at 0.66. Correlation with LSM_SV was similar to LSM_IT. AB13SV, AB15SV, and AB20SV correlate with LSM_SV at 0.83, 0.83, and 0.86, respectively. Both greenhouse severity datasets had the lowest correlation with LSM_SV at 0.53 and 0.38. TX14SV and AB14SV correlated with LSM_SV at 0.68 and 0.61, respectively. TX14 and AB14 nurseries were less informative due to extensive stress from a

combination of flooding and drought. The mean ratings for AB14 were the lowest of all datasets at 5.5 for IT and 14.7 for SV. At AB20, the additional note for green leaf area (GLA) inversely correlated with LSM_IT and LSM_SV at -0.51 and -0.58, respectively. As expected, GLA was inversely correlated with all datasets to at least some degree, ranging from -0.08 in GH15SV to -0.67 in AB20SV (Figure 2.1). AB20GL ranged from 0 to 90% green leaf remaining with a mean of 25.47%.

AB13IT ranged from a low score of 3 to a high of 8 and a mean of 6. Three significant QTLs on 5DS, 7BS, and 7BL were discovered above the 5% LOD threshold of 4.34. An additional QTL on 3BL was observed at the 10% LOD threshold of 4.0. These four QTLs were responsible for 45% of the variance observed. AB13SV ranged from a score of 1 to 100 with a mean of 53%. The same four QTLs were observed above the 5% LOD threshold of 4.2 and explained 36% of the total variance in SV. At AB13, the 7BL QTL was highly significant with LOD scores of 19.56 and 21.18 for IT and SV, respectively (Table 2.4).

AB14IT scores ranged from 3-8 with a mean of 5.5. Two QTLs were found: 6BL at 144 cM and 7BL at 249 cM. The 6BL QTL was only found above the 10% LOD threshold while 7BL was found above the 5% LOD threshold. Together, they explained 23% of the variability. This small MQM model and R^2 value is explained by the relatively poor field conditions at AB14. AB14SV identified the same two QTLs and explained the lowest variability (34.5%) of any SV dataset in which any significant loci were found. The 6BL QTL was only found in AB14 (Table 2.4).

AB15IT scores ranged from 2 to 9 with a mean of 6. Four QTLs on 3BS, 5DS,7BS, and 7BL were found. The MQM model explained 48.86% of the variability, which was the highest of any IT dataset. AB15SV scores ranged from 0 to 95% with a mean of 37.5%. The AB15SV

model included the same four QTLs with the addition of a QTL on 7BS at position 35 cM attributed to Roelfs F2007 (Table 2.4).

AB20IT scores ranged from 2 to 9 with a mean of 6. The four most common QTLs from other adult-plant datasets comprised the MQM model: 3B at 19, 5D at 29, 7B at 54, and 7B at 249. Together these explained 49% of the variability. AB20SV ranged from 0 to 100% with a mean of 56%. The model for AB20SV was highly similar to IT as it included 3BS at 21, 5DS at 30, 7BS at 55, and 7BL at 255. The SV model explained 55.5% of the variability. Remaining green leaf area was only recorded at AB20. The GL note ranged from 0 to 90% with a mean of 25% green flag leaf area remaining. Composite interval mapping identified the 7BL QTL at position 250, explaining 41% of the variability. No other QTL mapped above the 5% LOD threshold. MQM was unable to detect any additional significant QTLs (Table 2.4).

TX14IT scores ranged from 3 to 8 with a mean of 6. The MQM model included four QTLs: 2DS at position 37, 3BS at 14, 5BL at 159, and 7BS at 39, together responsible for 35% of the variance. Except for 3BS, the QTLs in the model for TX14IT were atypical in their cM position relative to other datasets with QTLs on the same chromosomes. TX14SV scores ranged from 0.1 to 100% with a mean of 38% The model for TX14SV included 7 QTLs- two more than the next largest model. The QTLs and cM positions are as follows: 3BS at 12, 5BL at 159, 5DL at 108, 7BS at 33, 7BS at 56, 7BL at 249, and 7DS at 26. Together they explained 61% of the variability. As with TX14IT, many of the cM positions of these QTLs were not seen in other datasets, except the QTLs on 7BS at 56 and 7BL at 249 (Table 2.4).

GH14IT and GH15IT were similar in several attributes as were GH14SV and GH15SV. Although these two greenhouse IT datasets had low correlation to most field datasets, these correlated relatively well to each other (.53) (Figure 2.1). IT scores ranged from 3 to 8 with in

GH14. In GH15IT, scores ranged from 2 to 9. Mean IT scores were the highest in both greenhouse environments at 8 and 7.5 for GH14IT and GH15IT, respectively. In GH14IT The MQM model included only two QTLs from 3BS and 5DS, at positions 19 and 4, respectively. Together these explained 29% of the variance. The GH15IT model included the same two QTLs at positions 22 and 30 for 3BS and 5DS, respectively. These explained 33.5% of the variance in GH15IT. The greenhouse SV datasets had a relatively low correlation of 0.25. In GH14SV, scores ranged from 0.1 to 90% and in GH15SV scores ranged from 0 to 97%. In GH14IT The MQM model included only two QTLs from 3BS and 5DS, together explaining 29% of the variance. GH14SV scores ranged from 0.1 to 100% with a mean of 46% and did not identify any QTLs above the 5% LOD threshold. The 7BL QTL was not identified in any model from the greenhouse, though it was found in every other environment (Table 2.4).

Seedling Tests

Seedling infections of Roelfs F2007, Lakin and the RILs rated for IT performed in the 7-9 range- similar to the highly susceptible check, Morocco. When analyzed using SIM and CIM with the R/qtl package, there were no LOD peaks above the 5% significance threshold for any of the three seedling repetitions. The correlation between LSM_IT and STR1, STR2, and STR3 were 0.19, 0.21, and 0.26, respectively (Figure 2.1). There was also very little correlation between any of the seedling repetitions. Despite this, the seedling repetitions did faintly register the inverse correlation of AB20GL as in the adult-plant datasets. The race used in the inoculum was BBBDB, which is avirulent for all known genes except *Lr14a*. The population and parents showed no significant resistance detectable via CIM or MQM in any seedling dataset (Table 2.4).

QTL Identification and Naming

The postulated QTLs from the different datasets were collated in Table 2.7 and sorted by chromosome and position to assess which ones are reproducible. QTLs that appeared to be reproducible in more than two datasets were given designations of the form *QLr.hwwg-3BS.2* where "*Lr*" is for the leaf rust resistance trait, "hwwg" is the designation for the USDA-ARS Hard Winter Wheat Genetics Research Unit in Manhattan, KS, "3BS" is the wheat chromosome arm, and ".2" would indicate that it is the second such QTL described from the 'hwwg' research group on that arm.

QTL Validation by Additivity of Phenotypic Effects

Each QTL behaved strictly additive in combination (Figures 2.7 and 2.9). The resulting IT and SV were directly dependent upon the number and specific QTLs in combination (Figures 2.6 and 2.8). No significant QTL interactions were observed in any dataset as detectable by MQM. The comprehensive adult-plant LSMeans data validated the four-QTL model in MQM with all four QTLs above the 5 and 10 percent LOD thresholds for both IT and SV (Figures 2.4 and 2.5). When using the LSMeans data from the three most correlative datasets, AB13IT, AB15IT, and AB20 IT, a steady improvement in IT response was observed as the number of QTLs increase (Figure 2.6). The same pattern was observed in SV when using LSMeans for AB13SV, AB15SV, and AB20SV (Figure 2.8). The combination resulting in the lowest IT and SV included all of the four highly reproducible QTLs, though this was only confirmed by three RILs. The combination of three genes resulting in the lowest IT and SV included *Qlr.hwwg-5DS, QLr.hwwg-7BS, QLr.hwwg-7BL*. Of the combinations of two QTLs, the combination including *QLr.hwwg-7BS* and *QLr.hwwg-7BL* resulted in the lowest IT and SV. *QLr.hwwg-7BL* alone had

the greatest single-gene effect with a median IT of 5.3 and a median SV of 20% (Figures 2.6 and 2.8).

Discussion

The experiments in this study have yielded evidence of multiple APR loci which can be attributed to the resistant cultivar, Roelfs F2007 and, unexpectedly, one to the moderately susceptible cultivar, Lakin. From the RIL progeny of the Roelfs F2007 x Lakin cross, four QTLs were identified to be replicable and significant. These were found on chromosomes 3BS, 5DS, 7BS, and 7BL. From gene-specific marker data, *QLr.hwwg-7BL* is co-located with the *Lr68* marker, *Lr68csGS*, and *QLr.hwwg-5DS* is co-located with the *Lr78* marker, *IWA6289*. Additional gene-specific markers indicated the presence of *Lr46* in Roelfs F2007, however our study found no evidence of a QTL on 1BL where *Lr46* resides. Based on the findings of this study as well as previous studies, there are no known leaf rust resistance loci shared by Roelfs F2007 and Lakin.

QTL on Chromosome Arm 3BS

A significant QTL for leaf rust resistance attributed to Roelfs F2007 was located on the short arm of chromosome 3B. Named *QLr.hwwg-3BS.2* in this study, this allele was detected within the 5% LOD significance threshold at the adult-plant stage in six of the seven adult-plant experiments for either infection type or severity and in multiple instances, both. It conferred a reduction in SV ranging from 9.65 to 11.04%. The IT was reduced by a range of 0.49 to 0.71. This QTL was responsible for anywhere between 4.5% and 24.5% of the variability observed in the datasets where it was detected (Table 2.7). It was not detected in the seedling tests, and therefore appears to be an APR gene.

The short arm of chromosome 3B is a region containing several loci of interest to wheat breeding, including *Lr27*, *Sr2*, *Wsm2* and *Fhb1*. Gene *Lr27* is on 3BS and its expression requires the additional presence of *Lr12/Lr31* on chromosome 4BS. When combined with *Lr12/Lr31*, *Lr27* produces a very low IT in seedling plants to specific isolates of *P. triticina*. (McIntosh, Wellings et al. 1995, Singh and Bowden 2011). *Lr27* is not considered a candidate for this QTL for several reasons. First, *Lr27* did not map on any of the three seedling tests in this study though it is known to be a seedling gene. No significant reduction in IT was attributed to any QTL in the seedling tests. Second, *Lr27* is also known to be tightly linked to stem rust resistance gene *Sr2*, which confers the pseudo black chaff (PBC) phenotype (Mago, Tabe et al. 2011). Singh et al. (1984) indicated rare exceptions to this linkage, though later study utilizing induced mutation on 3BS could not separate the effects of *Sr2* and *Lr12/Lr31* (Mago, Tabe et al. 2011). KASP marker *Sr2_ger9_3p* (Mago, Brown-Guedira et al. 2011) was used to screen both parents for *Sr2* in this study, which confirmed the absence of *Sr2* in Lakin and Roelfs F2007. Additionally, the PBC phenotype was not observed in this population. Lastly, no QTL was observed on 4BS in any dataset, which could rule out the presence of *Lr12/Lr31* unless both parents carry the gene. Therefore, *QLr.hwwg-3BS.2* is not *Lr27*.

The physical position of *QLr.hwwg-3BS.2* aligns generally with the reported position of *Lr74*, which was the first designated leaf rust gene on 3BS and provisionally reported in cultivars, ‘Spark ‘, and ‘Caldwell’(Temesgen 2015, Kolmer, Chao et al. 2018). In those studies, the 3BS QTL was noted having an intermediate effect. In another study evaluating cultivar ‘Clark’ the *Lr74* locus *QLr.hwwg-3B.1* on 3BS was responsible for a reduction in severity of 14 to 18% and was additive in combination with other APR loci (Li, Wang et al. 2017). Both the Kolmer et al. and Li et al. studies concluded that the putative QTL on 3BS conferred APR. The

Li et al. study used markers *IWA4654* and *IWA1702* to refine the position adjacent to *Xgwm533*. RefSeqV1 and V2 BLAST place *IWA4654* at 13.96 Mb and 19.29 Mb respectively, each with an 86% percent sequence similarity to the marker. RefSeqV2 BLAST of GBS marker, M_02985 places *QLr.hwwg-3BS.2* at 5.4 Mb at 89% sequence similarity. The difference between these physical positions is relatively small and represents a relatively short genetic distance as well. We conclude that *QLr.hwwg-3BS.2* is consistent with the location and phenotype of APR gene *Lr74*.

It should be noted that in a previous study of a DH population of cross CI13227 x Lakin, a QTL was observed at 0.4 cM on 3BS in a single field experiment (Lu, Bowden et al. 2017). This QTL named *QLr.hwwg-3B* was attributed to Lakin, the susceptible parent, and conferred a reduction in final severity of 6.26%. Based upon the relative locations of KASP markers *IWB5899* and *IWB35536* on the PopSeq Chinese Spring assembly, the study likened the QTL to loci in cultivars, 'Capo' and "Sinvalocho MA" observed in other studies (Ingala, López et al. 2012, Buerstmayr, Matiasch et al. 2014). Given the repeated attribution of the *QLr.hwwg-3BS.2* to Roelfs F2007 in the current study and a much higher LOD threshold relative to the Lu et al. study, the result from Lu et al. not only remains unconfirmed but is likely in error. A possible explanation for this is that two alleles of differing effect may exist for *Lr74*- the stronger of which is carried by Roelfs F2007. However, this is not plausible because we repeatedly observed 100% severity in multiple datasets, which would not occur if both parents were contributing a resistance locus at 3BS. Additionally, the Roelfs F2007 allele is also not a particularly strong allele and to yet find a weaker allele simultaneously in Lakin is improbable.

This QTL is an interesting possibility for use in breeding given the other useful loci on 3BS, namely *Sr2*, *Wsm2*, and *Fhb1*. Unfortunately, *Lr74* is likely in repulsion phase with all of

them. As was suggested by Li et al., the prospect of coupling *Sr2, Wsm2, Fhb1* and *Lr74* is of great interest for future breeding work.

QTL on Chromosome Arm 5DS

The significant QTL on the short arm of 5D was found via QTL mapping between positions 4 and 32 cM and is named *QLr.hwwg-5DS.* It was significant at the 5% LOD threshold in four of the seven experiments. In two of the experiments, it significantly reduced both infection type and severity. When it was found, it explained approximately 6-8% of the variability observed. This QTL reduced infection type by approximately 0.4 to 0.7. The additive effect on severity was approximately 9 to 10.5% but was observed to be significant in only two of the experiments (Table 2.7).

Known leaf rust resistance loci previously found on this chromosome include *Lr1*, *Lr57/Yr40*, *Lr70*, *Lr76/Yr70,* and *Lr78*. *Lr1* is located at the distal end of the long arm of 5D and therefore is not in consideration for the QTL on 5DS (Feuillet, Messmer et al. 1995). *Lr57/Yr40* may be excluded since it is shown to confer all-stage resistance, whereas the current study found no evidence of seedling resistance. Additionally, *Lr57/Yr40*, originating in the ovate goat grass *Aegilops geniculata* Roth (syn. *Aegilops ovata* L.), was only introgressed after the development of both Roelfs F2007 and Lakin and therefore can be excluded (Kuraparthy, Chhuneja et al. 2007). *Lr76/Yr70* is similarly an alien segment only recently introgressed from *Aegilops umbellulata* (syn. *Triticum umbellulatum* (Zhuk.) and can also be excluded on the same grounds (Bansal, Kaur et al. 2017). In a recent seedling study, *Lr70* mapped to position 5.6 cM and an IT of 1–22+ whereas susceptible individuals showed an IT of 33+–4 on the Stakman scale (Stakman, Levine et al. 1919). Despite the similarity of effect and location, *Lr70* may be excluded since it is a seedling gene and induces hypersensitivity upon infection (Hiebert,

McCallum et al. 2014) , which was not observed in the U6225 population. Another gene on 5D which may affect the response to leaf rust infection is *Vrn-D1*. A segregating vernalization allele may induce variable maturity in the population, thus leading to possible avoidance of natural infection. However, *Vrn-D1* has been cloned and is well-characterized on the long arm of 5D (Yan, Loukoianov et al. 2003) and may be excluded based on its position.

Lr78 is an APR gene located on 5DS. Kolmer et al. 2018 mapped *Lr78* 2.2 cM distal to KASP marker *IWA6289*. In that study, RILs homozygous for the *IWA6289* allele inherited from parent Tc*3/Toropi 4A21A had an average leaf rust severity of 35.8%, which was 27% lower than the homozygous negative allele. URGI RefSeqV2 BLAST located *IWA6289* at 33 Mb on the physical map. By referencing the most consistent and significant GBS marker for the 5DS QTL, *M_06148*, with RefSeqV2, we estimated that *QLr.hwwg-5DS* is at 60 Mb. However, we had relatively few markers on 5DS and the position of the QTL was not well defined. Nevertheless, Roelfs F2007 and many of the most resistant progeny from the U6225 population including RIL U6225-214 screened positive for the *Lr78*-associated allele of IWA6289 in the second gene-specific marker test (Table 2.3). For this reason, and the general position of the QTL from the MQM model, we conclude that *QLr.hwwg-5DS* is likely *Lr78*. Future work to identify the precise location of this QTL should involve further fine mapping of the 5DS chromosome arm.

Novel QTL on Chromosome Arm 7BS

An APR QTL between cM positions 54 and 63 on 7BS on the linkage map was attributed to Lakin. Here named *QLr.hwwg-7BS*, it significantly reduced IT and/or SV in four field tests although it was not significant for either in AB14IT or the greenhouse studies. The reduction in IT ranged from 0.30 to 0.58. The reduction in severity ranged from 8% to 32%. Across all

experimental datasets, it contributed between 4.5 and 11% of the variability observed in the model (Table 2.7).

A recent study identified a durum wheat gene on 7B that while ineffective against races of *P. triticina* affecting over 80% of the CIMMYT durum wheat germplasm, did confer all-stage resistance against races affecting bread wheat (Herrera-Foessel, Huerta-Espino et al. 2014). The authors identified this QTL as novel resistance gene *Lr72* and found it 5.0 cM distal to SSR marker *wmc606* and *wmc303.* This places *Lr72* at the extreme distal end of 7BS, and thus too far away to reasonably be considered the same locus as *QLr.hwwg-7BS*. This is the only named leaf rust resistance locus on 7BS (Soriano and Royo 2015). Maccaferri et al.(Maccaferri, Sanguineti et al. 2010) reported an otherwise unnamed QTL on 7BS near 60cM, however, it was only found in durum wheat and was effective at the seedling stage. Therefore, it does not appear to match *QLr.hwwg-7BS*, which is ineffective at the seedling stage. The possibility of pleiotropy with a resistance gene from stem or stripe rust was considered and rejected based upon the absence of known APR genes in this region of 7BS. Lakin had previously been observed as moderately susceptible to leaf rust. Despite confirming this observation, Lakin did repeatedly contribute a relatively small yet significant level of resistance in the population. It is likely that this QTL is responsible for the transgressive segregation demonstrated by levels of susceptibility greater than Lakin in the current study. *QLr.hwwg-7BS* is strong enough to have potential value in breeding programs. Figures 2.6 and 2.8 both demonstrate that *QLr.hwwg-7BS* together with *Lr68* produced the strongest 2-gene reduction in IT and SV from the QTLs in this study. While the single-gene resistance conferred by *QLr.hwwg-7BS* is not especially powerful, its compatibility and shared chromosome with *Lr68* could make it uniquely valuable for pyramiding APR. For these reasons, we propose that *QLr.hwwg-7BS* should eventually be given a leaf rust resistance

gene designation. That will likely require fine mapping of 7BS to validate a diagnostic marker and tightly linked flanking markers as well.

QTL on Chromosome Arm 7BL

The most statistically significant QTL observed in this study was found on 7BL and is named *QLr.hwwg-7BL.2*. It was also the most consistent and mapped tightly to refined positions on the linkage map. This QTL is located between cM positions 246 and 250 on the linkage map close to GBS marker *M_12103* and using the RefSeqV2 BLAST, was found between 744.9 Mb and 751,5 Mb. The locus reduced severity in all five field studies anywhere between 6% and 26% with a mean of 14.99%. Infection type was reduced by 0.57 to 0.99 in four of the field studies with a mean reduction of 0.73. Across all datasets, the QTL explained between 5.2and 41.26% of the variability observed. It was frequently the QTL with the strongest signal in field datasets by LOD score (Table 2.7) and was often used as a covariate for composite interval mapping. It was not observed in the seedling tests, suggesting that it is an APR gene.

Other previously described leaf rust QTLs on the long arm of chromosome 7B are *Lr2*, *Lr14a*, *Lr14b*, and *Lr68*. *Lr68* and *Lr14b* are known to be tightly linked, and though *Lr68* is thought to have originated in Frontana, it is possible that both were transferred from cultivar 'Maria Escobar' (Herrera-Foessel, Singh et al. 2012). *Lr2*, *Lr14a*, and *Lr14b* are all known seedling genes. If *Lr2* or *Lr14b* were present, either would have been revealed in the seedling test. *Lr14a* would be otherwise undetectable in the seedling test due to the use of *Lr14a*-virulent race BBBDB as the inoculum. Originally considered alleles of the same gene, Dyck and Samborski confirmed that *Lr14a* and *Lr14b* are located at distinct loci on 7B (Dyck and Samborski 1970). Regardless, virulence to *Lr14a* and *Lr14b* is ubiquitous in the Central Plains (Kolmer, Jin et al. 2007) and it is thus very unlikely that it contributed the resistance observed,

given the consistency with which the QTL was significant across all field tests. Of the potential candidates, only *Lr68* is a known APR gene. Two separate gene-specific marker screens indicated the presence of *Lr68* in Roelfs F2007. In the first, using marker *Lr68-csGS*, Roelfs F2007 and check Pavon 76 both scored positive for *Lr68* while Lakin was negative (Table 2.2). The second test indicated Roelfs F2007 and check, 'Toropi' as positive for *Lr68* while Lakin was again negative (Table 2.3). In 2012 Herrera-Fossel et al. found that *Lr68* consistently conferred a reduction in leaf rust severity across several field experiments of 30 to 60% in positive cultivars, 'Arula1' and 'Arula2' compared to susceptible checks, however this reduction was typically less than that of *Lr34* and *Lr46*. In combination, these demonstrated a strong level of APR, demonstrating that *Lr68* is suitable for pyramiding with other APR genes. This is supported by the performance of *QLr.hwwg-7BL.2* in other combination as demonstrated in Figure 2.8.

It is of note that *Lr68*, despite its strength in field experiments, did not map in any greenhouse experiment in the current study. Additionally, LSMeans and BLUP analyses which included greenhouse data did not map *Lr68* in the most resistant progeny (Table 2.6). The same analyses, with greenhouse data omitted, indicated *Lr68* as the greatest contributor of resistance in the population. This is most clearly demonstrated by the MQM analyses of LSMeans data omitting greenhouse environments when looking at individual QTLs (Figure 2.6 and 2.8). These analyses revealed that *Lr68* was also essential to any of the most resistant combinations of two or more QTLs. The Lu et al. study did however identify *Lr68* in the greenhouse in CI13227, however at a much weaker LOD score. The diminished effect or absence of evidence in greenhouse data is perhaps due to UV light filtration that is known to occur in the greenhouse environment. Greenhouse temperature dynamics may also play a role in limiting or enhancing

trait expression. Variation between greenhouse environments may also be a contributor. This observation supports the indispensability of field testing for quantitative traits.

A "Missing" QTL on 1BL

The absence of evidence of *Lr46* in the QTL analysis was unexpected based on the results of the second diagnostic marker screening (Table 2.3). In the first diagnostic marker screening, Lakin and Roelfs F2007 tested negative for Lr46 with marker *csLV46* while check Pavon 76 tested negative (Table 2.2). In the second parental screen, Lakin and Toropi screened negative while Roelfs F2007 screened positive using KASP marker *Lr46Yr29JF-2*. A recent study examining stripe rust resistance with GWAS mapped *Lr46* near *IWA3892*, which is at 668.1 Mb on RefSeqV1 (Maccaferri, Zhang et al. 2015). RefSeqV2 BLAST places *IWA3892* nearby at 676.7 Mb on 1B with an 81% sequence similarity. Another recent study used microsatellite marker *Xwmc44* to verify the presence of *Lr46* in Pavon 76 as a control. URGI RefSeqV2 BLAST locates *Lr46* marker *Xwmc44* at 670.7 Mb (Skowrońska, Kwiatek et al. 2019). These physical positions between 668 and 670.7 Mb translate to cM positions 216 to 233 on the linkage map- where no QTL was found.

There are a few possibilities that could explain this. It is possible that a previously unreported ineffective allele of *Lr46* is present in Roelfs F2007. Such variability as to be considered ineffective has been demonstrated to occur in the multiple alleles of *Lr34*. In a recent study, the ineffective allele of *Lr34a* in Jagger was found to be the result of a loss-of-function mutation and subsequent deficiency in the *Lr34* protein product. (Fang, Lei et al. 2020) It is possible that a similar mutation has occurred for *Lr46*. It is also of note that the most resistant RIL in the U6225 population lacks the positive marker allele for *Lr46* using marker *Lr46Yr29JF-2* (Table 2.3). Another possibility is that the markers used to detect *Lr46* are not as tightly linked

to the gene as previously thought. If, after the original F1 cross in this study, crossing-over did occur between the marker and the gene, the segregation and effect of *Lr46* would likely still be seen in the progeny. It is also possible that crossing-over could have occurred many generations prior and that Roelfs F2007 never carried the allele. Another possibility is that both parents possess *Lr46*, yet that would require Lakin to have a false negative result for both gene-specific markers. Our CIM plots seem to indicate a slightly higher LOD score around 1BS, however no dataset, LSMeans, or BLUP analysis indicated anything near a significant response even at the 10% LOD significance threshold (Table 2.7).

Other Loci

Other QTLs found above the 5% LOD significance threshold yet are not considered reproducible resistance loci were found on 2DS, 3BL, 5BL, 5DL, 6B, 7B, and 7D.

The QTL on 2DS is located at 36 cM on our map and was found only in TX14IT (Table 2.7). This QTL was contributed by Roelfs F2007, explained 5.9% of the phenotypic variability, and caused a 0.53 reduction in IT. This QTL is relatively weak compared to the other loci observed at TX14. Previously, APR genes *Lr22a* and *Lr22b* have been reported on 2DS. These alleles are well-characterized at position 2.9 cM, at the distal end of 2DS (Dyck 1979, Hiebert, Thomas et al. 2007). Also, virulence to *Lr22b* is very common in U.S epidemics (Kolmer 1996). Since the donor for this QTL is spring-type and the trait was observed in a latitude close to its region of adaptation, it is plausible that the effect was the result of *Ppd-D1* expression. The *Ppd-D1a* allele confers photoperiod insensitivity in which plants progress in maturity regardless of daylength. This can mimic disease resistance by inducing a plant development rate that outpaces the progression of seasonal epidemics, resulting in infection avoidance. *Ppd-D1a* effects can also be manifested by experimental limitations wherein majority RILs lacking the QTL mature rather

uniformly and thus dominate the overall perception of growth stage, meaningfully at decision points for disease rating. Those RILs with the insensitive allele are observed at a growth stage less ideal for infection. This is unavoidable if disease notes are only taken on one occasion, as was the case in TX14. Using marker sequence *TraesCS2D01G079600* and RefSeqV2 BLAST, *Ppd-D1* is found at 36.2 Mb on chromosome 2D. While GBS marker coverage is relatively low in this area of our map, consecutive markers at 33 and 62 cM collocate to RefSeqV2 physical positions 21.3 Mb and 40.2 Mb, respectively. Using regression of our genetic map and the physical map, we interpolate the QTL position of 36 cM to approximately 28.9 Mb. This position lies near the RefSeqV2 BLAST hit and between the two closest markers to position 36, therefore, it is possible that the 2DS QTL is *Ppd-D1*.

The expression of QTLs on 5BL and 5DL may be explained by *Vrn-B1* and *Vrn-D1* for similar reasons to those described above for *Ppd-1D*. *Vrn-B1* and *Vrn-D1* are homeologous major genes which control the winter/spring growth habit. They have previously been mapped to colinear regions on the long arms of 5B and 5D. *Vrn-B1* is located between physical positions 573.802 Mb and 573.816 Mb according to T3 (https://triticeaetoolbox.org/wheat). In support of this hypothesis, the QTL on 5B maps in a wide segment between cM positions 117 and 159. It was attributed to Lakin in three of the four datasets where it was significant at the 5% LOD significance threshold. Two of the Lakin attributions were from TX14IT and TX14SV, where the QTL was found at cM position 159. The lone Roelfs F2007 attribution was from GH15SV and was located at cM position 143 (Table 2.7). Positions 143 and 159 on our map interpolate to the RefSeqV2 physical position at 563 and 580 Mb, respectively. *Vrn-B1*, at approximately 573 Mb, would map within this window. The Lakin-attributed locus at 117 cM was from AB15SV and only slightly above the 5% LOD threshold. It was also the weakest QTL from that dataset by

both significance and percent variability explained. It is far enough away from the other three that it may not be considered the same locus and could be credited to experimental noise. The Roelfs F2007 attribution of the 143 cM locus may be explained by greenhouse conditions affecting maturity, resulting in avoidance. Otherwise, attribution by Lakin can be explained by Lakin being the winter-type parent. The QTL on 5DL was found it TX14SV and GH15SV at cM positions 108 and 110, respectively. Consistent with the QTL on 5BL, this QTL was attributed to Lakin for TX14SV and Roelfs F2007 for GH15SV. RefSeqV1 places *Vrn-D1* at 467 Mb (https://triticeaetoolbox.org/wheat). Map positions 108 and 110 interpolate to 471 and 474 Mb, respectively. This, along with the consistency in the donor with 5BL, lends support to the *Vrn-D1* hypothesis. Leaf rust genes *Lr18* and *Lr1* are located on 5BL and 5DL, respectively. They are also the only other Lr genes described in these regions. These, however, are both seedling genes and given the homeologous nature of *Vrn1* alleles, the dependency of QTL expression upon growing environment, the general inconsistency of expression, and the lack of a seedling effect, the *Vrn1* hypotheses seem more plausible. It is also plausible, however that the indication of two homeologous loci may simply be the result of incorrect marker assignment during map creation and that the population has either a QTL at 5BL or 5DL but not both.

The QTL on 3BL was conferred by Lakin and indicated in datasets AB13IT and AB13SV at cM position 162. The SV locus was found above the 5% LOD threshold, yet the IT locus was only significant slightly above the 10% LOD threshold (Table 2.7). AB13 reported no QTLs on 3BS, though the *QLr.hwwg-3BS.2* was discoverable for at least IT or SV in every other environment except AB14. The interval estimates for these QTLs are reported by MQM to be between cM position 22 and 179 in AB13IT and between 149 and 178 in AB13SV. *Lr77* was recently found on 3BL and is traced to cultivar ‘Santa Fe’ (Kolmer, Su et al. 2018). It, however,

is an unlikely candidate considering it was originally reported by Kolmer et al. in field and greenhouse studies as a major APR gene, reproducing a reduction in SV of approximately 20%. The SV reduction in AB13 was only 9.75%. The same study reported a reduction in IT of as much as five points compared to a less-than-one-point IT reduction observed in AB13. *Lr79* also mapped to 3BL, however it is an unlikely candidate as it originates in durum wheat and is a seedling gene (Qureshi, Bariana et al. 2018). Given the enormous interval estimate for AB13IT, low statistical significance, lack of verification from other experiments, and the small effect of the QTL, it is either spurious or of little breeding value.

The QTL conferred by Roelfs F2007 at 144 cM on the long arm of 6B was only significant above the 10% LOD threshold in the AB14SV dataset (Table 2.7). Possible leaf rust resistance genes for this locus include one of the four *Lr3* variants and *Lr9*. The *Lr3* variants (*Lr3a*, *Lr3b*, *Lr3c*, and *Lr3d*) and *Lr9* are unlikely candidates as they produce a very low infection type at the seedling stage (Sacco, Suarez et al. 1998, Ingala, López et al. 2012). *Lr9* is also an unlikely candidate as it originates in wild relative *Aegilops umbellulata* (Sears 1956), which is not in the pedigree of Roelfs F2007.

On the short arm of 7B, two datasets indicated QTLs contributed by Roelfs F2007. This is in contradiction to *QLr.hwwg-7BS.2*, which is contributed by Lakin. AB15SV at cM position 11 and TX14SV at cM position 33 conferred a reduction in SV of 7.9 and 29%, respectively (Table 2.7). These loci are far enough apart to not be considered the same QTL. As stated earlier, the only other known QTL on 7BS is *Lr72*, located 5.0 cM distal to SSR markers *wmc606* and *wmc303*, which are located at 6.3 Mb according to RefSeqV2 BLAST. The cM position 11 on our linkage map is proximal to 27 Mb on the RefSeqV2 map. Therefore, these two loci are not *Lr72* or the putative novel QTL, *QLr.hwwg-7BS.2*.

The last non-reproducible locus was found on 7DS in dataset TX14SV and was contributed by Lakin. This QTL was located at position 26 with a LOD score of 6.38, which is above the 5% significance threshold of 4.20 (Table 2.7). *Lr34* is located on 7DS, however is unlikely given the major effect of *Lr34* and its association with Ltn. The marker for *Lr34* was also negative. There are no other designated *Lr* genes on 7DS.

Conclusions

Through this study, the identification and characterization of *Lr74*, *Lr78*, *Lr68,* and the novel gene *QLr.hwwg-7BS* were reproducible in different environments. Together, these resistance genes explained the high level of APR to leaf rust in Roelfs F2007 and the low level of APR in Lakin. The experiments revealed Roelfs F2007 is perhaps more susceptible to leaf rust than previously thought while Lakin is somewhat more resistant than previously thought. Roelfs F2007 could reasonably be characterized as MR to leaf rust while Lakin could be considered S to MS. The QTL characterization we performed provides evidence for breeders about how the QTLs perform in combination and individually, such as the QTL effect via LS Means analysis. The MQM analysis provides interval estimates for these genes as well (Table 2.7). Validated markers are already available for *Lr68*, *Lr74*, and *Lr78*. Additional work is needed to develop a validated marker and tightly linked flanking markers for *QLr.hwwg-7BS*.

Reasonable next steps in research include the analysis of stripe and stem rust phenotypic data in the U6225 population to further examine the effects of these QTLs for pleiotropy and to search for additional QTLs for the other rust diseases. Another important step is the further fine mapping of each QTL region, particularly for 5DS and 7BS, where some ambiguity lies in the physical position of the QTLs. This could begin with the recreation of the linkage map anchored solely using new KASP markers, likely yielding a more precise genetic map. The saturation of

ambiguous or low coverage areas of the map with KASP markers of known location will also help refine the map. *QLr.hwwg-7BS* should be further considered for an *Lr* gene designation. Also, the APR genes identified here have demonstrated effectiveness in combination and could be used as donors for crossing with germplasm resistant to other rust pathogens. This would be another step toward a "universal donor" for rust resistance breeding.

The data supporting (and refuting, in the case of *Lr46*) the leaf rust resistance QTLs found in Roelfs F2007 and Lakin are new tools for breeders to use in improving resistance to leaf rust. Researchers may build on this work to lessen the effects of leaf rust epidemics globally, helping to bring prosperity to growers, and to help meet the nutritional needs of people everywhere.

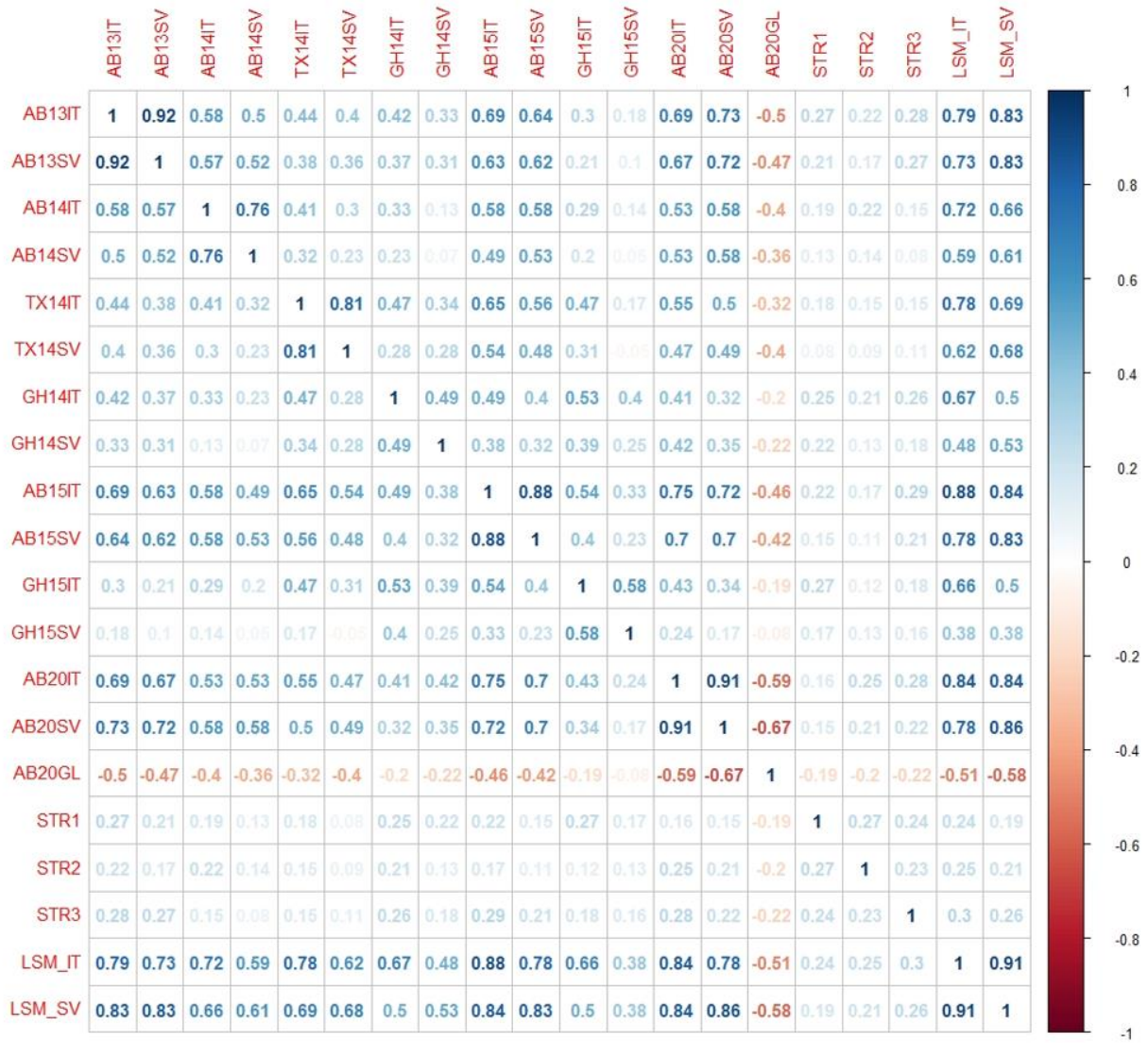

Figure 2.1 Phenotypic data correlation matrix

Phenotypic correlation between datasets. Adult-plant datasets are ordered chronologically from AB13IT to AB20GL. Seedling datasets follow and are indicated by STR1, STR2, and STR3. Phenotypic data from AB13, AB15, and AB20 for IT and SV are summarized by least-squares means. These are shown in LSM_IT and LSM_SV. Deep blue values indicate a large, positive correlation between datasets. Deep red values indicate a large inverse correlation between datasets. Faint colored values indicate no correlation

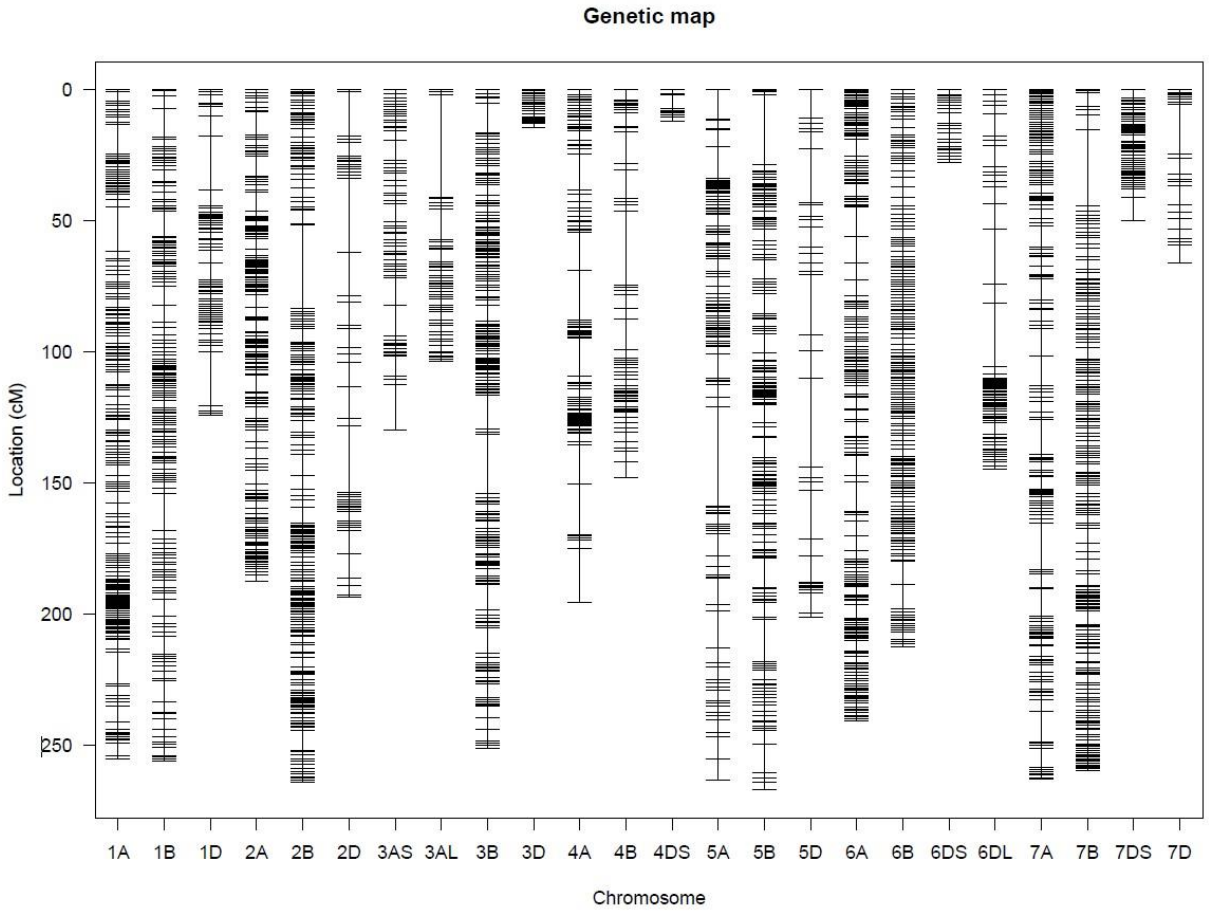

Figure 2.2 Genetic map

Genetic map indicating the distribution of the 2,541 GBS markers across all linkage groups.

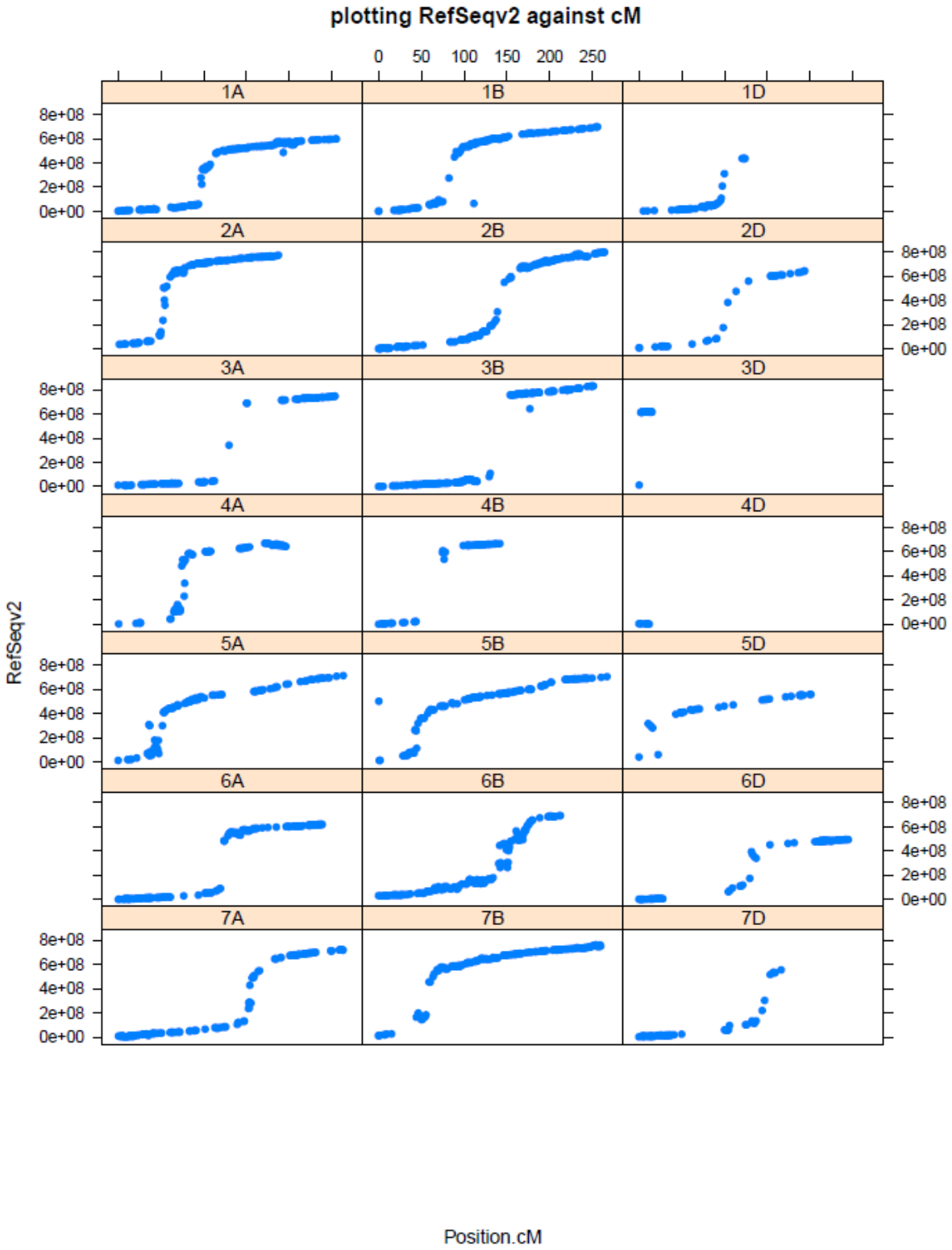

Figure 2.3 Correlation of the genetic map with RefSeqV2 physical positions

Each blue point indicates a GBS marker within a specific linkage group that was also identified on the physical map. Data not shown if top BLAST hit chromosome did not match linkage group chromosome assignment.

Table 2.1 Heritability estimates

	covariance due to genotype	residual covariance	number of environments		
SV[a]	**σ^2_g**	**σ^2_e**	**replicates, r**	**broad sense heritability H^2**	**N**
	305.07	566.67	7	0.790	294
IT[b]	**σ^2_g**	**σ^2_e**	**replicates, r**	**broad sense heritability H^2**	**N**
	0.9399	0.9304	7	0.876	294
Seedling IT[c]	**σ^2_g**	**σ^2_e**	**replicates, r**	**broad sense heritability H^2**	**N**
	0.325	1.0384	3	0.484	293

[a]Adult-plant % severity (SV) BLUP data.

[b]Adult-plant infection type (IT) BLUP data.

[c]Seedling plant infection type (IT) BLUP data.

σ_g^2 = covariance due to genotype, σ^2_e = the residual covariance, and *r* = the number of environments.

N= number of recombinant inbred lines.

Table 2.2 Results of gene-specific marker screening no. 1

Marker[a]	Gene[b]	**Lakin**	**Roelfs F2007**	**Pavon 76**
Lr68csGS	***Lr68***	-	+[c]	+
csSr2R	***Sr2***	-	-	+
csLV46	***Lr46***	-	-	+
csLV34	***Lr34***	-	-	-

Results of first gene-specific marker screening indicate the presence of *Lr68* in Roelfs F2007. Cultivar, 'Pavon 76' was used as a check.

[a] Marker name.

[b] *Lr* gene designation corresponding to the marker.

[c] "+" indicates the cultivar is positive for the marker. "-"indicates negative for the marker.

Table 2.3 Results of gene-specific marker screening no. 2

Marker[a]	Gene[b]	**Lakin**	**Roelfs F2007**	**Toropi**	**RIL-214**
Lr68csGS	***Lr68***	-	+[c]	+	+
Sr2_ger9_3p	***Sr2***	-	-	-	-
Lr46Yr29JF-2	***Lr46***	-	+	-	-
Lr34Exon11	***Lr34***	-	-	-	-
IWA6289	***Lr78***	-	+	+	+

Results of second gene-specific marker screening indicate the presence of *Lr46*, *Lr68,* and *Lr78* in Roelfs F2007. Cultivar, 'Toropi' was used as a check. RIL-214 was screened along with other RILs of interest.

[a] Marker name.

[b] *Lr* gene designation corresponding to the marker.

[c] "+" indicates the line/cultivar is positive for the marker. "-"indicates negative for the marker.

Table 2.4 MQM model by dataset

Environment	Trait[a]	Significance Threshold	Chr	Pos. cM[b]	RefSeqV2 estimate (Mb)[c]	LOD	Variance (R^2)[d]	Additive effect[e]
Ashland Bottoms 2013	IT	5%: 4.34 10%: 4.0	3BL	162	762.496	4.62	5.55	0.40
			5DS	4	187.033	4.91	5.92	-0.69
			7BS	63	422.732	5.16	6.23	0.31
			7BL	249	750.143	19.55	27.60	-0.77
Ashland Bottoms 2013	SV	5%: 4.20 10%: 3.86	3BL	178	772.258	4.77	5.52	9.98
			5DS	32	370.145	4.66	5.39	-9.53
			7BS	63	422.732	5.02	5.82	8.87
			7BL	250	751.865	21.18	29.27	-21.58
Ashland Bottoms 2014	IT	5%: 4.49 10%: 4.18	6BL	144	353.304	4.28	7.56	-0.28
			7BL	249	750.143	11.25	21.65	-0.57
Ashland Bottoms 2014	SV	5%: 8.85 10%: 7.61	6BL	144	353.304	9.66	17.07	-2.54
			7BL	249	750.143	8.97	15.7	-6.09
Ashland Bottoms 2015	IT	5%: 4.42 10%: 4.06	3BS	19	7.113	6.71	9.74	-0.56
			5DS	20	328.757	5.57	7.27	-0.40
			7BS	54	237.533	6.6	9.57	0.57
			7BL	246	744.978	7.63	11.17	-0.56
Ashland Bottoms 2015	SV	5%: 4.23 10%: 3.90	3BS	25	9.359	9.52	11.58	-11.04
			5BS	117	535.755	5.006	5.829	8.548

Environment	Trait[a]	Significance Threshold	Chr	Pos. cM[b]	RefSeqV2 estimate (Mb)[c]	LOD	Variance (R^2)[d]	Additive effect[e]
			7BS	35	57.016	5.30	6.19	-28.15
			7BS	54	237.533	8.50	10.24	31.39
			7BL	249	750.143	10.08	12.33	-11.81
Greenhouse 2014	IT	5%: 7.07 10%: 6.14	3BS	19	7.113	12.60	18.97	-0.55
			5DS	4	187.033	6.20	7.96	-0.58
Greenhouse 2014	SV	5%: 4.22 10%: 3.90	No significant QTLs above the LOD threshold					
Greenhouse 2015	IT	5%: 5.8 10%: 5.2	3BS	22	8.236	16.16	24.48	-0.70
			5DS	30	364.462	6.51	8.96	-0.41
Greenhouse 2015	SV	5%: 4.25 10%: 3.86	3BS	22	8.236	7.60	10.14	-10.03
			5BL	140	560.02	11.16	15.42	-11.87
			5DL	110	474.171	8.12	10.89	-10.81
Castroville 2014	IT	5%: 4.18 10%: 3.89	2DS	37	28.971	4.20	6.153	-0.51
			3BS	14	5.241	8.52	13.06	-0.70
			5BL	159	580.065	4.77	7.01	0.46
			7BS	39	76.9	5.30	7.84	0.29
Castroville 2014	SV	5%: 4.20 10%: 3.89	3BS	12	4.492	5.13	5.76	-10.80
			5BL	159	580.065	12.25	14.88	16.85
			5DL	108	471.602	8.44	9.83	17.72
			7BS	33	49.715	5.89	6.67	-28.61
			7BS	56	272.003	9.28	10.91	30.30
			7BL	249	750.143	4.72	5.27	-8.96

Environment	Trait[a]	Significance Threshold	Chr	Pos. cM[b]	RefSeqV2 estimate (Mb)[c]	LOD	Variance (R^2)[d]	Additive effect[e]
			7DS	26	103.173	6.04	6.85	8.17
Ashland Bottoms 2020	IT	5%: 4.8 10%: 4.34	3BS	19	7.113	7.29	6.59	-0.48
			5DS	29	361.476	7.65	6.94	-0.48
			7BS	54.5	245.817	5.99	5.35	0.36
			7BL	249.7	751.348	27.77	29.95	-0.98
Ashland Bottoms 2020	SV	5%: 4.54 10%: 4.19	3BS	21	7.862	5.55	4.49	-9.65
			5DS	30	364.462	7.91	6.52	-10.43
			7BS	55	254.322	5.53	4.47	8.14
			7BL	249	750.143	35.06	36.62	-26.09
Ashland Bottoms 2020	GL	5%: 5.10 10%: 4.61	7BL	250	751.865	32.54	41.19	18.54

All QTLs found via Multiple QTL Mapping (MQM) grouped by dataset. 5% genome-wide LOD thresholds ranged from 4.18 - 8.85. QTL groupings can be seen in Table 2.1.

[a] SV=Severity, IT=Infection Type, GL=% Green Leaf Area Remaining.

[b] cM position based off linkage map constructed in JoinMap 4.1.

[c] % Estimate of physical position based upon linkage and RefSeqV2 BLAST positions.

[d] Percent phenotypic variance attributed to the QTL.

[e] Additive effect of QTL; For IT and SV: if negative, QTL is attributed to Roelfs F2007, if positive, QTL is attributed to Lakin. For GL: if negative, QTL is attributed to Lakin; if positive, QTL is attributed to Roelfs F2007.

*QTLs that are not significant based off of 5% genome wide LOD thresholds.

Table 2.5 MQM model for BLUPs and least-squares means

Analysis	Trait[a]	LOD Threshold	Chr	Pos. N (cM)[b]	cM[c]	RefSeq V2 estimate (Mb)[d]	Pos. S (cM)[e]	LOD	Variance (R^2)[f]	Additive Effect[g]
BLUP	IT	5%: 4.92 10%: 4.35	3BS	15	**19**	7.113	25	18.91	19.10	-0.46
			5DS	2	**5**	206.683	30	8.41	7.81	-0.37
			7BS	54	**55**	254.322	60	8.74	8.13	0.28
			7BL	249	**250**	751.865	251	15.37	15.09	-0.38
BLUP	SV	5%: 4.6 10%: 4.2	3BS	15	**21**	7.113	25	11.63	11.10	-6.06
			5DS	2	**30**	364.462	37	7.16	6.52	-4.42
			7BS	54	**55**	254.322	67	6.95	6.28	4.40
			7BL	249	**250**	751.865	253	20.79	21.46	-7.81
LSMeans	IT	5%: 4.95 10%: 4.42	3BS	15	**21**	7.862	22	20.08	20.03	-0.54
			5DS	2	**5**	206.683	20	9.70	8.89	-0.64
			7BS	54	**56**	272.003	61	8.77	7.98	0.33
			7BL	249	**250**	751.865	254	14.25	13.53	-0.43
LSMeans	SV	5%: 4.45 10%: 4.01	3BS	14	**18**	7.113	25	13.49	12.27	-8.25
			5DS	4	**19**	324.240	32	7.56	6.87	-7.10
			7BS	46	**55**	254.322	64	7.87	7.17	6.18
			7BL	249	**249**	750.143	252	22.03	22.00	-10.62

All QTLs found via BLUP and LSMeans analysis of APR data.

[a]SV=Severity, IT=Infection Type.

[b] 95% Bayesian confidence interval position north of the QTL.

[c]cM position based off linkage map constructed in JoinMap 4.1.

[d] % Estimate of physical position based upon linkage and RefSeqV2 BLAST positions.

[e]95% Bayesian confidence interval position south of the QTL.

[f] Percent phenotypic variance attributed to the QTL.

[g] Additive effect of QTL; For IT and SV: if negative, QTL is attributed to Roelfs F2007, if positive, QTL is attributed to Lakin.

Table 2.6 Phenotypic and genotypic summary by dataset

Dataset	Low[a]	High[b]	Mean[c]	# of QTLs[d]	% Variance[e]	5% LOD[f]	10% LOD[g]
TX14SV	0.1	100	38.43	7	60.58	4.2	3.87
AB13SV	1	100	52.91	4	48.88	4.2	3.86
AB14SV	0.001	80	14.73	2	34.52	8.72	7.54
GH14SV	0.1	90	45.55	0	0	4.22	3.9
GH15SV	0	97	50.82	3	36.4	4.31	3.83
AB15SV	0	95	37.45	5	46.39	4.21	3.87
AB20SV	0	100	55.99	4	55.402	4.38	4.11
TX14IT	3	8	6.06	4	34.88	4.09	3.85
AB13IT	3	8	6.07	4	44.86	4.34	4
AB14IT	3	8	5.45	1	22.95	4.52	4.16
GH14IT	3	9	7.82	2	29.12	7.19	6.22
GH15IT	2	9	7.42	2	33.35	5.94	5.05
AB15IT	2	9	5.87	4	40.69	4.26	3.95
AB20IT	2	9	6.04	4	48.86	4.88	4.47
AB20GL	0	90	25.47	1	41.26	5.05	4.55
STR1	1.33	9	7.10	0	0	6.96	6.09
STR2	1.33	9	7.74	0	0	10.35	9.42
STR3	4.67	9	8.11	0	0	11.7	10.6
BLUP_IT	-2.99	1.51	0.00	4	52.71	4.81	4.36
BLUP_SV	-33.19	31.09	0.00	4	45.25	4.6	4.2
BLUP_SIT	-1.96	0.44	0.00	0	0	7.44	6.51
LSM_IT	3	8.17	6.42	4	51.81	8.82	8.52
LSM_SV	-1.14	85.46	42.83	4	45.10	4.38	4.08
LSM_SIT	2.55	8.56	7.64	0	0	4.95	4.42

Summary findings by phenotype and MQM results for each dataset.

[a] Lowest phenotype score observed.

[b] Highest phenotype score observed.

[c] Mean of phenotypic scores observed.

[d] # of QTLs in the final MQM model for the dataset.

[e] % of phenotypic variance explained by the MQM model.

[f] 5% Logarithm of odds (LOD) significance threshold in the MQM model.

[g] 10% Logarithm of odds (LOD) significance threshold in the MQM model.

Table 2.7 MQM summary by QTL

Chromosome arm and/or QTL name	Environment	Trait[a]	Position cM[b]	RefSeqV2 estimate (Mb)[c]	LOD	Variance (R^2)[d]	Additive effect[e]
2DS	TX14	IT	37	28.971	4.21	6.15	-0.51
3BS *QLr.hwwg-3BS.2*	TX14	SV	12	4.492	5.14	5.77	-10.81
	TX14	IT	14	5.241	8.53	13.07	-0.71
	AB15	IT	19	7.113	6.71	9.74	-0.57
	GH14	IT	19	7.113	12.61	18.97	-0.56
	AB20	IT	19	7.113	7.29	6.59	-0.49
	AB20	SV	21	7.862	5.56	4.49	-9.66
	GH15	IT	22	8.236	16.17	24.48	-0.70
	GH15	SV	22	8.236	7.61	10.14	-10.03
	AB15	SV	25	9.359	9.53	11.59	-11.04
3BL	AB13	IT	162	762.496	4.63	5.56	0.40
3BL	AB13	SV	178	772.258	4.77	5.52	9.99
5BL	AB15	SV	117	535.755	5.01	5.83	8.55
5BL	GH15	SV	140	560.02	11.16	15.42	-11.87

Chromosome arm and/or QTL name	Environment	Trait[a]	Position cM[b]	RefSeqV2 estimate (Mb)[c]	LOD	Variance (R^2)[d]	Additive effect[e]
5BL	TX14	IT	159	580.065	4.77	7.02	0.47
5BL	TX14	SV	159	580.065	12.25	14.88	16.86
5DS *QLr.hwwg-5DS*	AB13	IT	4	187.033	4.92	5.93	-0.69
	GH14	IT	4	187.033	6.20	7.97	-0.59
	AB15	IT	20	328.757	5.58	7.28	-0.40
	AB20	IT	29	361.476	7.66	6.94	-0.48
	AB20	SV	30	364.462	7.91	6.52	-10.43
	GH15	IT	30	364.462	6.52	8.96	-0.41
	AB13	SV	32	370.145	4.67	5.39	-9.53
5DL	TX14	SV	108	471.602	8.45	9.84	17.72
5DL	GH15	SV	110	474.171	8.12	10.89	-10.82
6BL	AB14	SV	144	353.304	9.67	17.07	-2.55
7BS	TX14	SV	33	49.715	5.90	6.68	-28.62
	AB15	SV	11	57.016	5.31	6.19	-28.16
7BS	TX14	IT	39	76.9	5.31	7.85	0.29
7BS *QLr.hwwg-7BS*	AB15	IT	54	237.533	6.60	9.57	0.57
	AB15	SV	54	237.533	8.51	10.24	31.39
	AB20	IT	54.5	245.817	5.99	5.36	0.37

Chromosome arm and/or QTL name	Environment	Trait[a]	Position cM[b]	RefSeqV2 estimate (Mb)[c]	LOD	Variance (R^2)[d]	Additive effect[e]
	AB20	SV	55	254.322	5.54	4.48	8.15
	TX14	SV	56	272.003	9.29	10.92	30.31
	AB13	IT	63	422.732	5.16	6.24	0.32
	AB13	SV	63	422.732	5.02	5.83	8.87
7BL *QLr.hwwg-7BL.2*	AB15	IT	246	744.978	7.63	11.18	-0.57
	AB13	IT	249	750.143	19.56	27.61	-0.77
	AB14	IT	249	750.143	11.26	21.65	-0.57
	AB14	SV	249	750.143	8.97	15.70	-6.10
	AB15	SV	249	750.143	10.09	12.34	-11.81
	TX14	SV	249	750.143	4.72	5.28	-8.96
	AB20	SV	249	750.143	35.06	36.63	-26.09
	AB20	IT	249.7	751.348	27.78	29.95	-0.99
	AB13	SV	250	751.865	21.18	29.28	-21.58
	AB20	GL	250	751.865	32.35	41.26	18.44
7DS	TX14	SV	26	103.173	6.04	6.85	8.17

All QTLs found via Multiple QTL Mapping (MQM) analysis of the phenotypic data. Groupings are based on chromosome, relative cM position, LOD score, R^2 value, and additive effect. 5% genome-wide LOD thresholds ranged from 4.18 - 8.85 as can be seen by dataset in table 2.2.

[a] SV=Severity, IT=Infection Type, GL=% Green Leaf Area Remaining

[b] cM position based on linkage map

[c] Estimate of physical position based upon linkage and RefSeqV2 BLAST positions.

[d] % phenotypic variance attributed to the QTL.

[e] Additive effect of QTL; For IT and SV: if negative, QTL is attributed to Roelfs F2007, if positive, QTL is attributed to Lakin. For GL: if negative, QTL is attributed to Lakin; if positive, QTL is attributed to Roelfs F2007.
*QTLs that are not significant based off of 5% genome wide LOD thresholds.

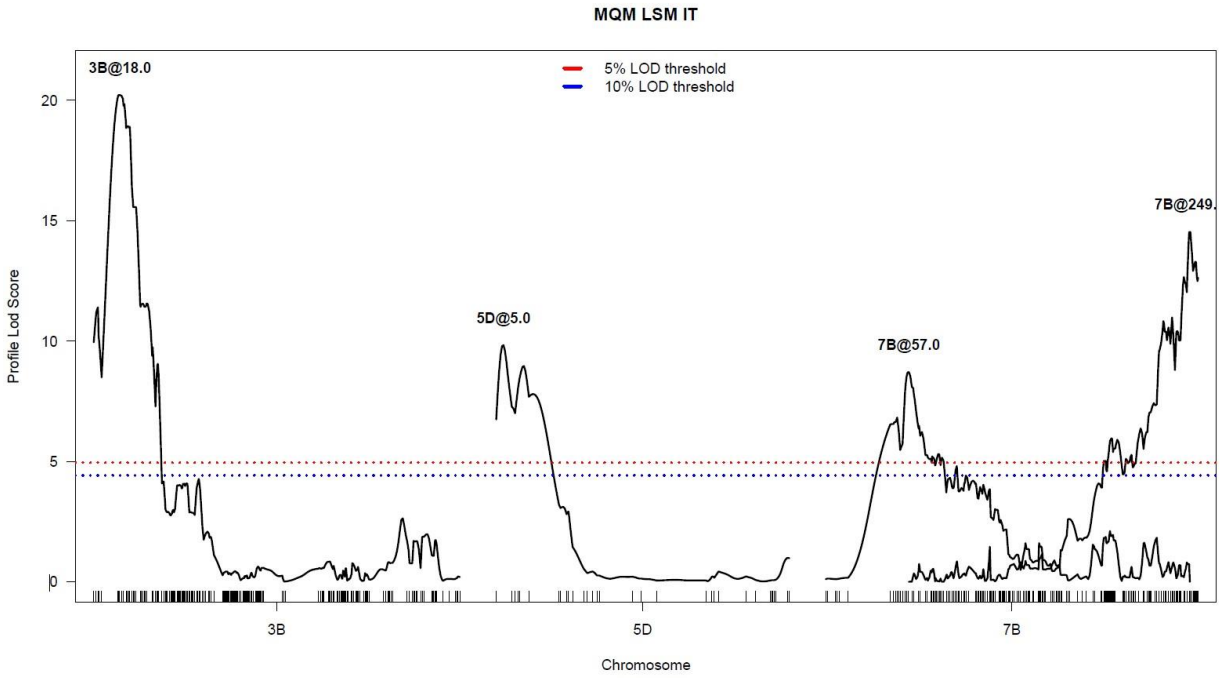

Figure 2.4 MQM plot for least-squares means IT

LSMeans IT incorporated IT data from AB13, AB15, and AB20. Model indicates QTL peaks above the 5% LOD threshold (red) and the 10% LOD threshold (blue).

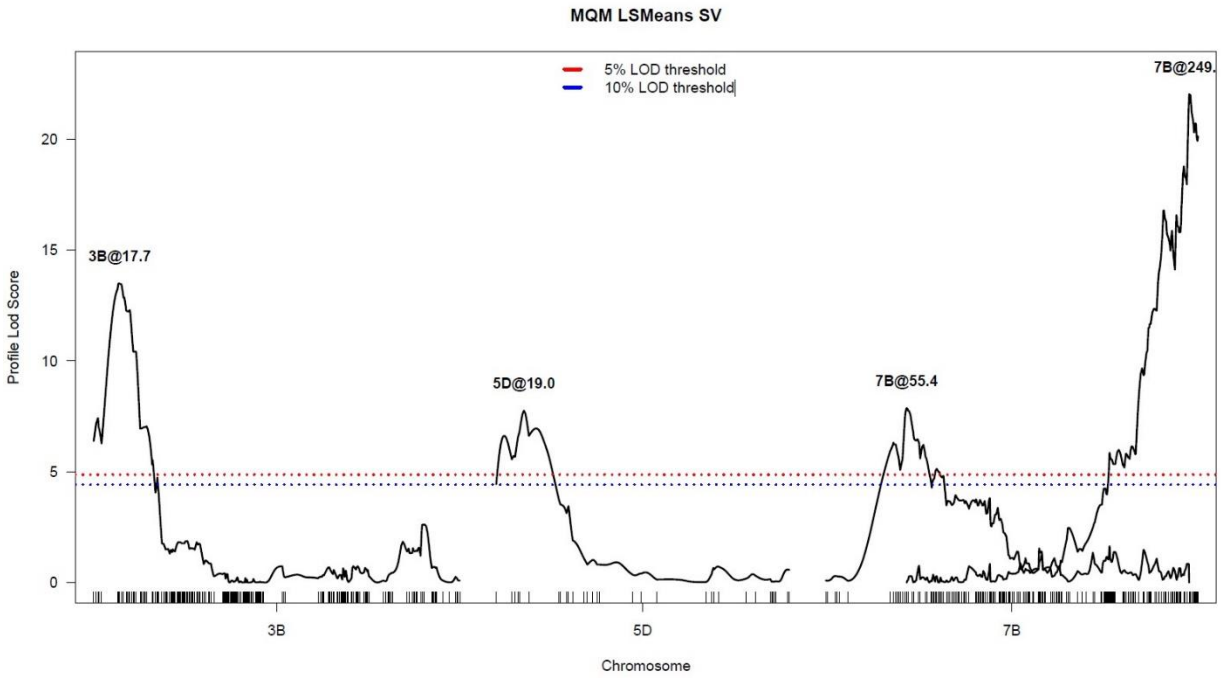

Figure 2.5 MQM plot for least-squares means SV

LSMeans SV incorporated SV data from AB13, AB15, and AB20. Model indicates QTL peaks above the 5% LOD threshold (red) and the 10% LOD threshold (blue).

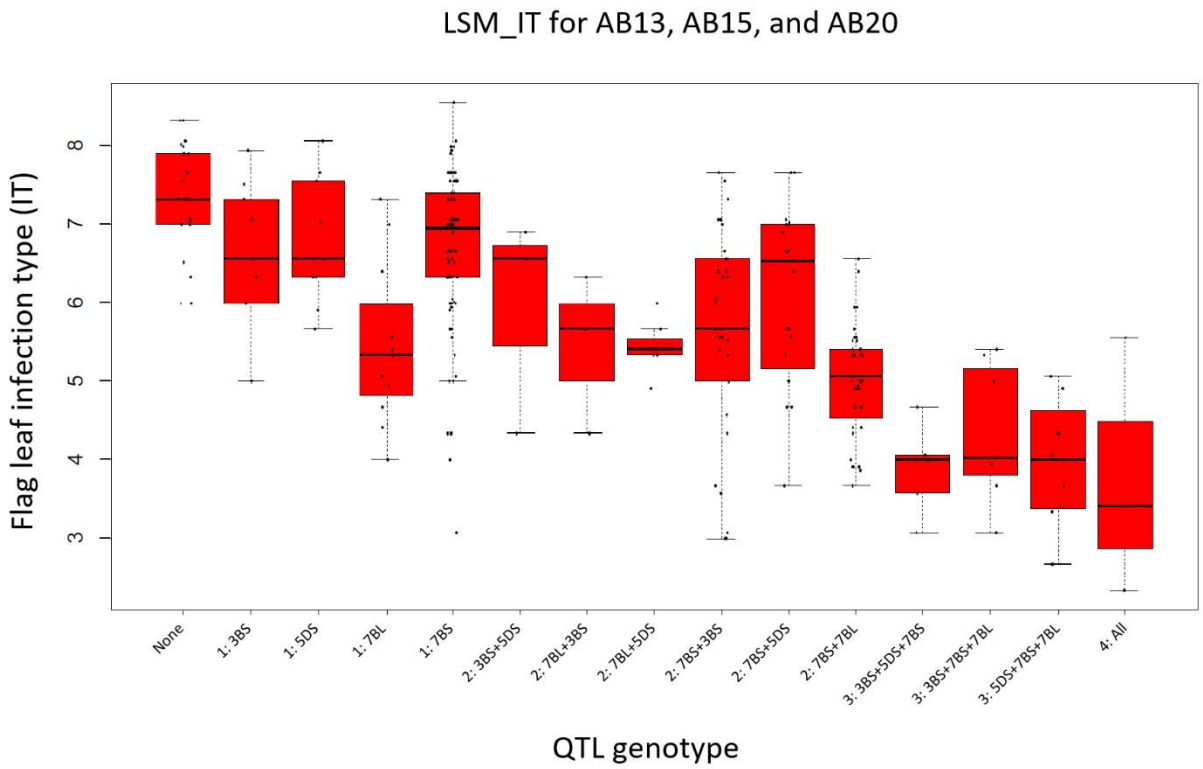

Figure 2.6 Least-squares means IT by QTL combination

LSMeans plot of AB13IT, AB15IT, and AB20IT. Indicates effect of specific QTLs in observed combinations on leaf rust infection type. Black points indicate the performance of individual RILs with the described genotype.

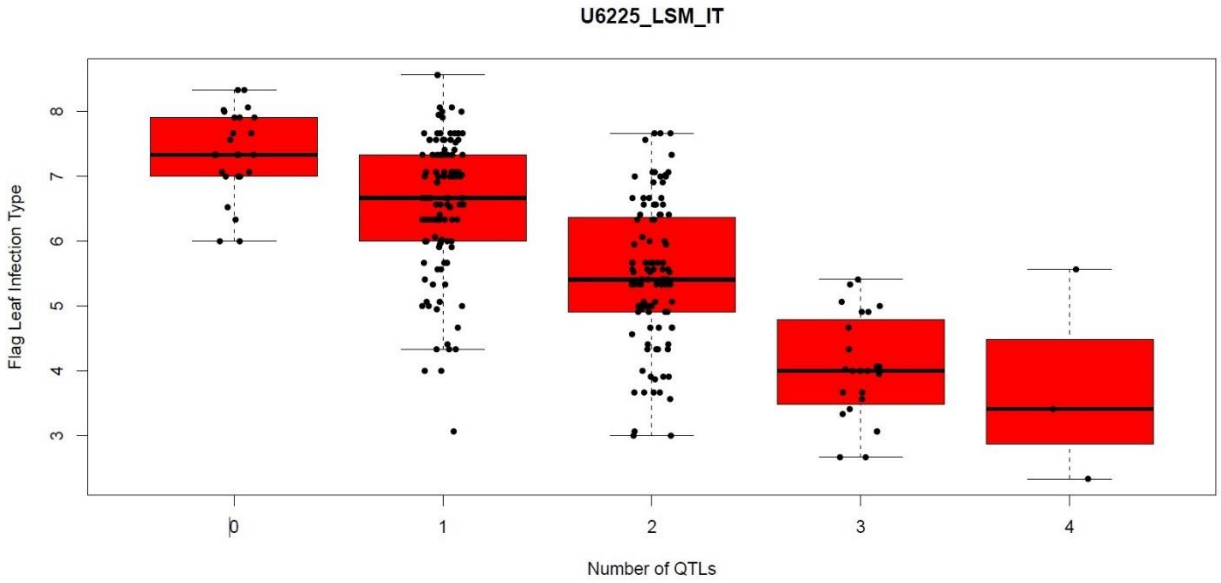

Figure 2.7 Least-squares means IT by number of QTLs

LSMeans plot of AB13IT, AB15IT, and AB20IT. Indicates the additive effect on leaf rust infection type as determined by the number of QTLs in our MQM model. Black points indicate the performance of individual RILs with the described genotype.

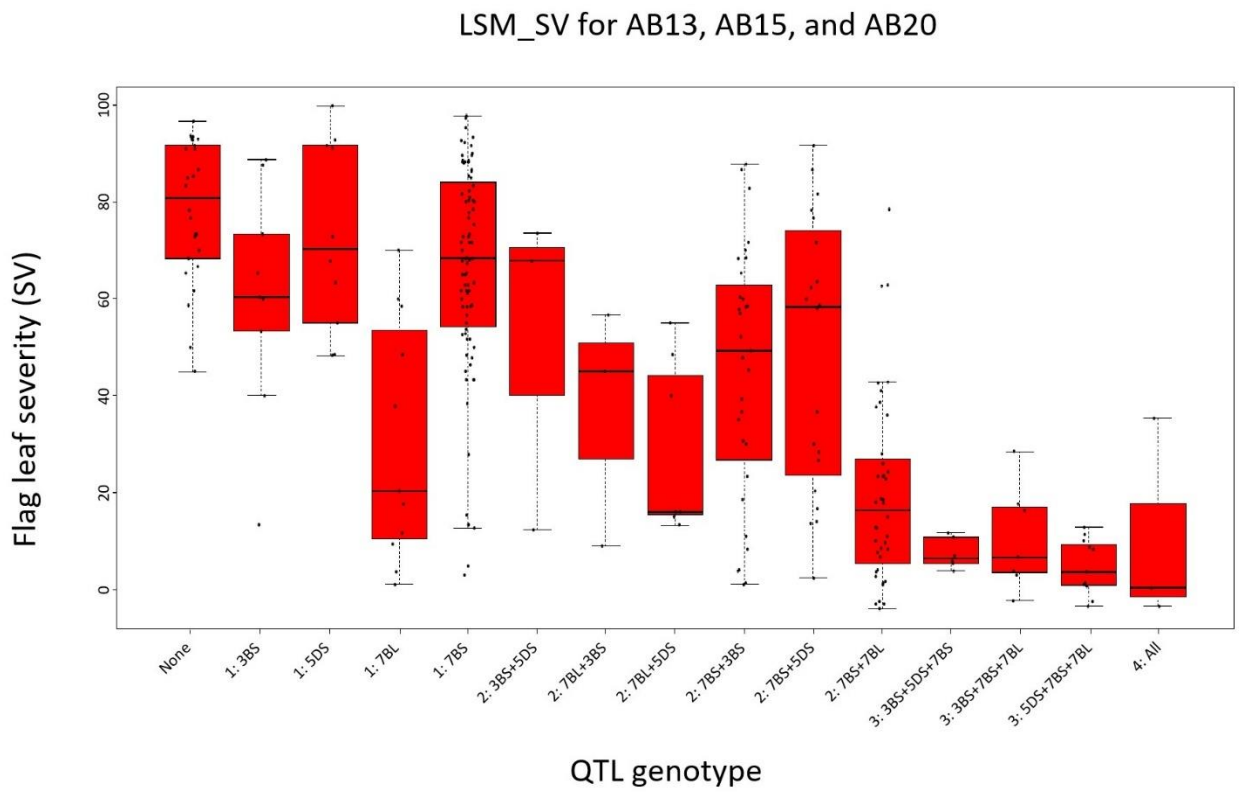

Figure 2.8 Least-squares means SV by QTL combination

LSMeans plot of AB13SV, AB15SV, and AB20SV. Indicates effect of specific QTLs in observed combinations on leaf rust severity. Black points indicate the performance of individual RILs with the described genotype.

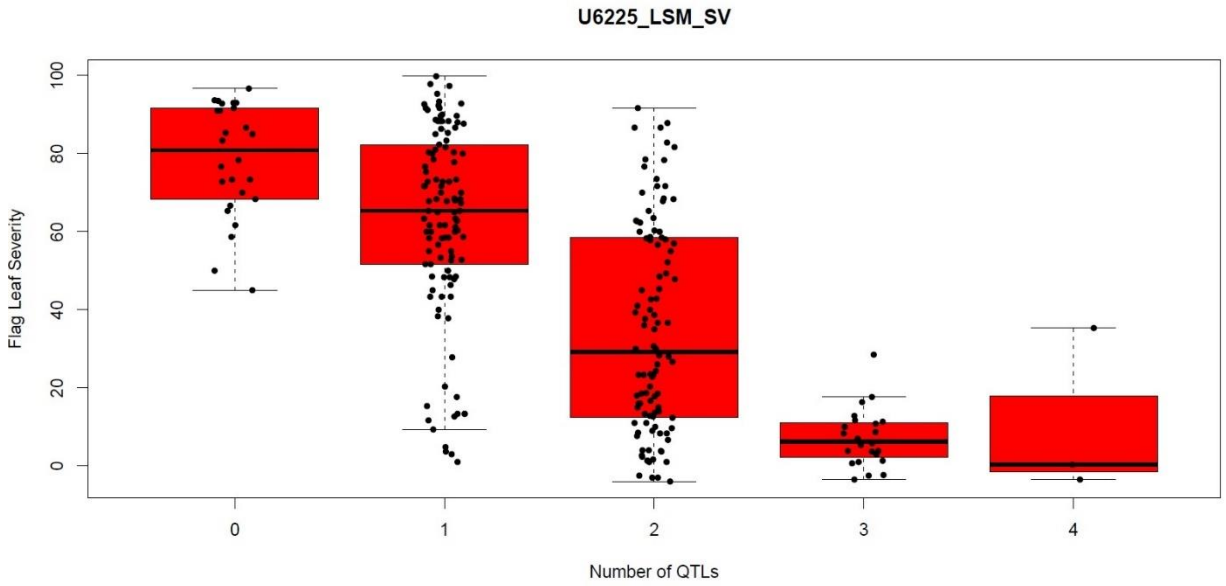

Figure 2.9 Least-squares means SV by number of QTLs

LSMeans plot of AB13SV, AB15SV, and AB20SV. Indicates the additive effect on leaf rust severity as determined by the number of QTLs in our MQM model. Black points indicate the performance of individual RILs with the described genotype.

Chapter 2 Supplemental Figures

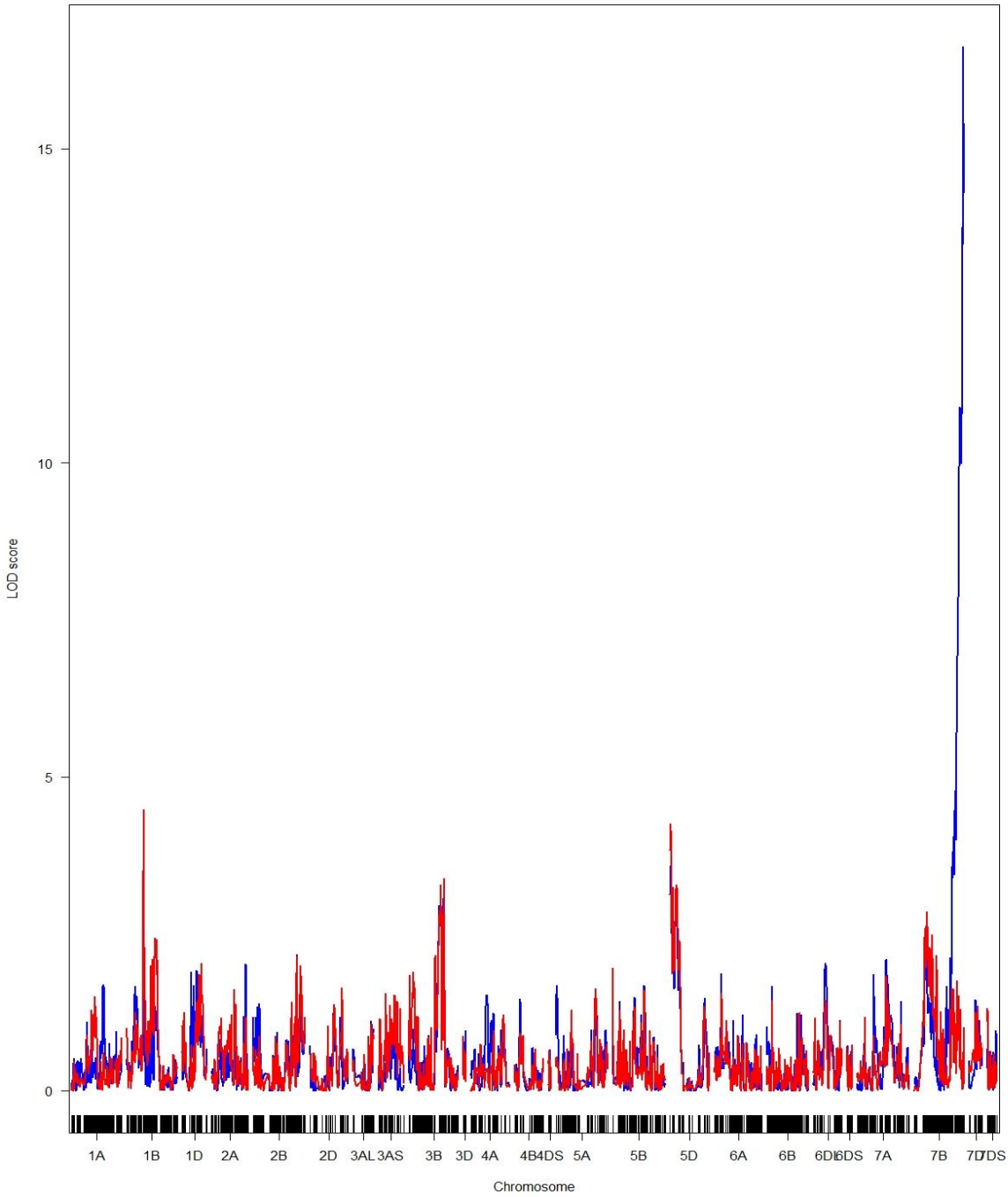

Figure 2.10 AB13IT CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in AB13IT was 7B at position 249. The 5% threshold was 4.34. The 10% threshold was 4.00.

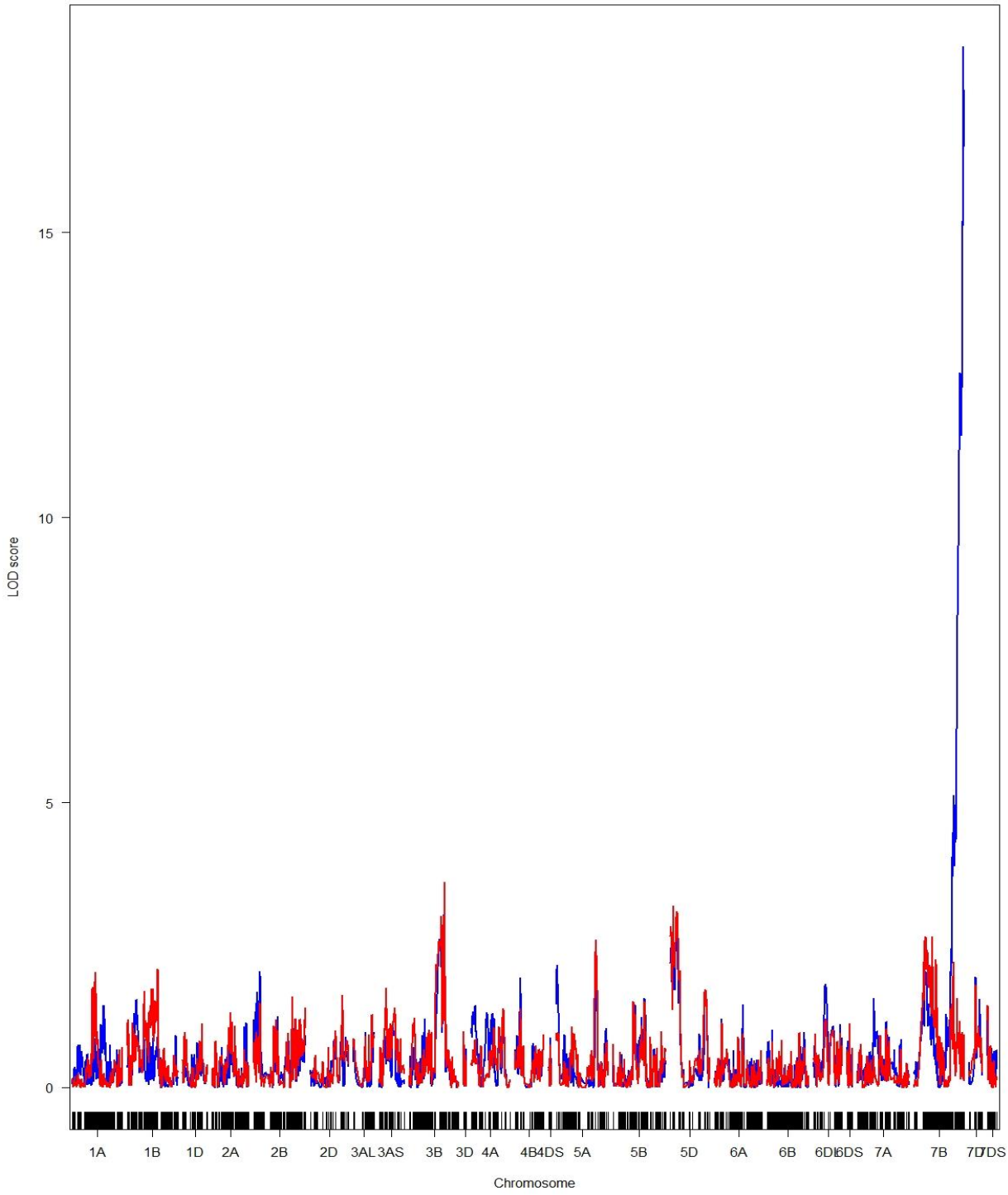

Figure 2.11 AB13SV CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in AB13SV was 7B at position 250. The 5% threshold was 4.20. The 10% threshold was 3.86.

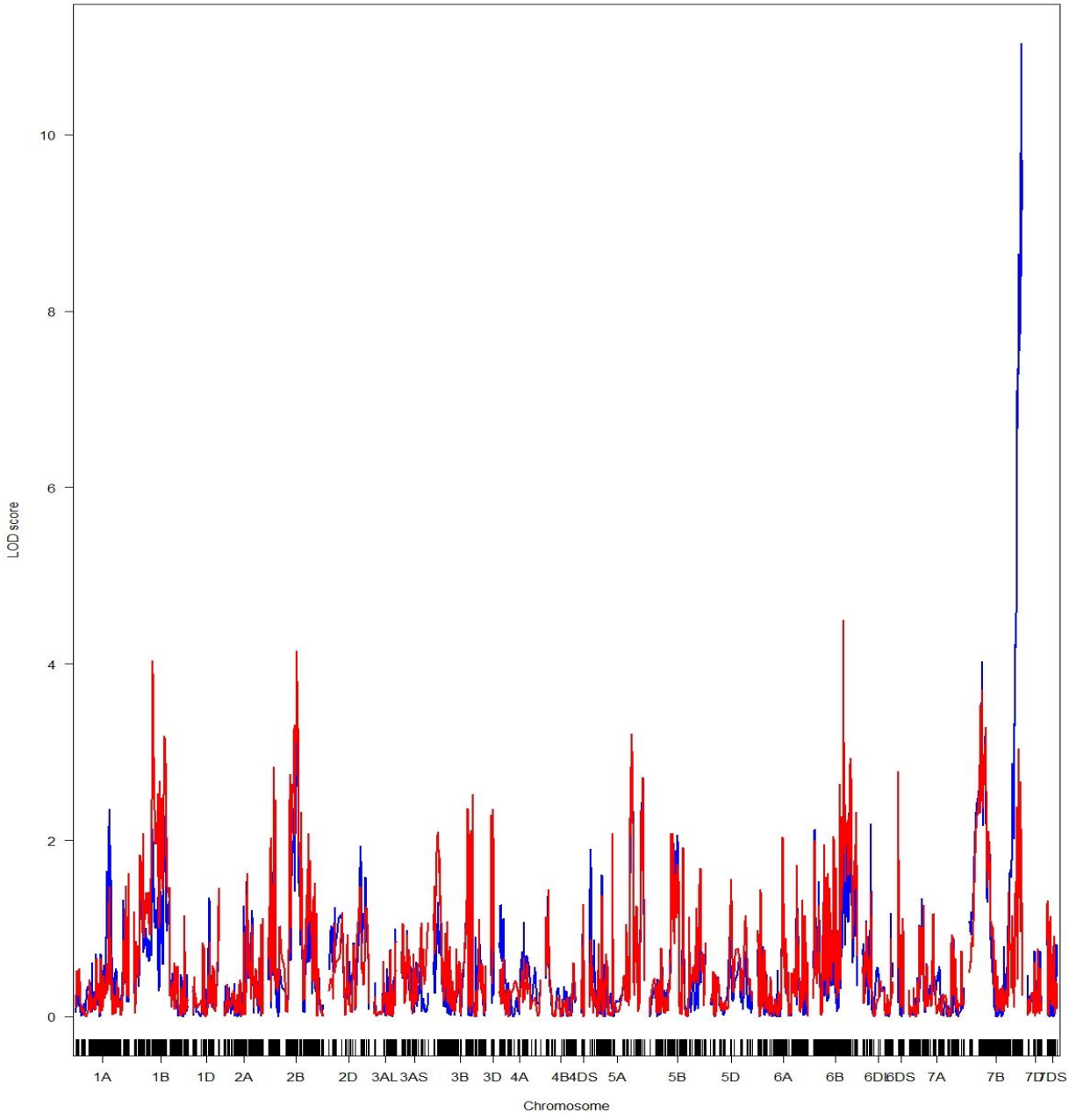

Figure 2.12 AB14IT CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in AB14IT was 7B at position 249. The 5% threshold was 4.52. The 10% threshold was 4.16.

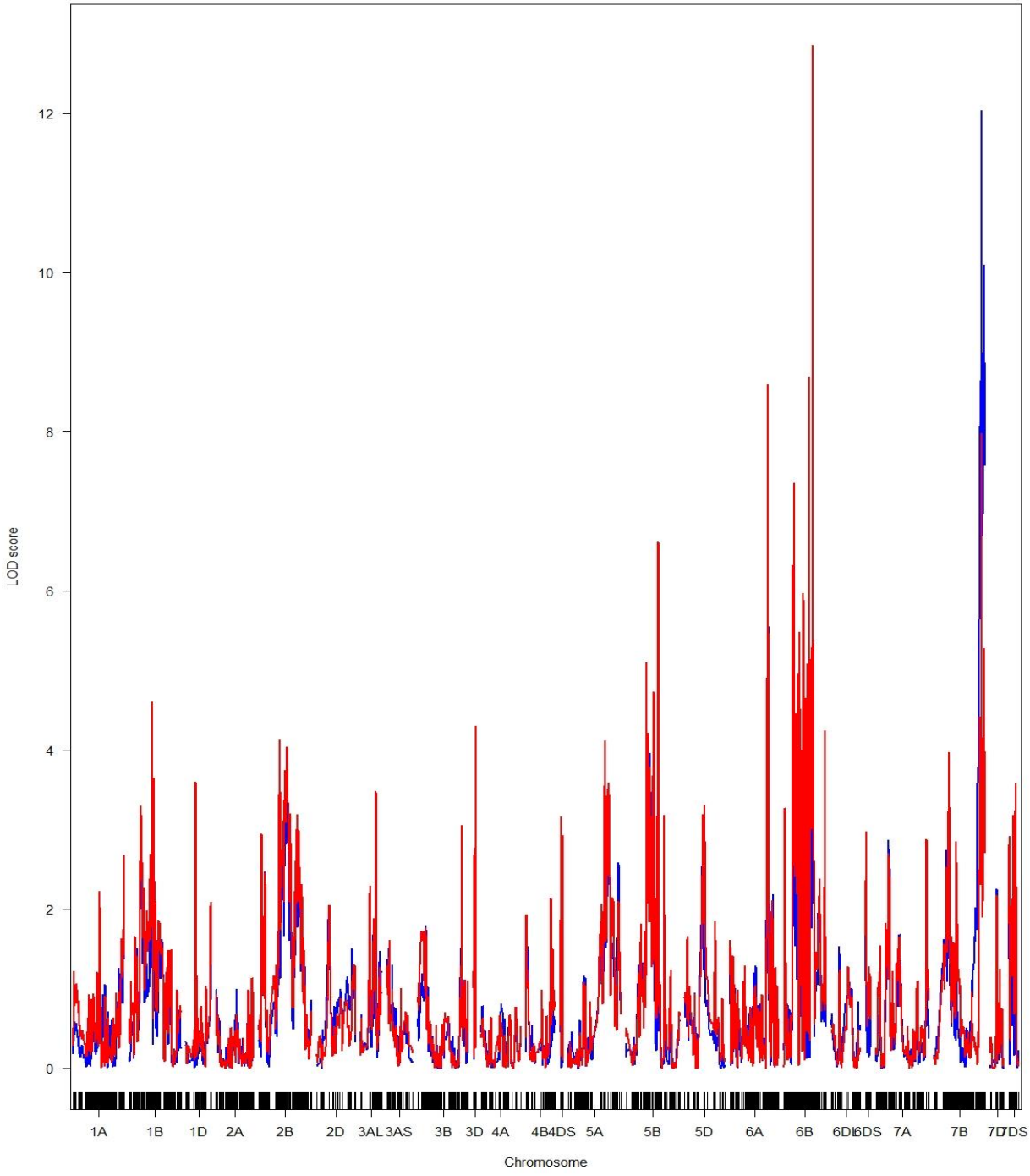

Figure 2.13 AB14SV CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in AB14SV was 7B at position 250. The 5% threshold was 4.21. The 10% threshold was 3.87.

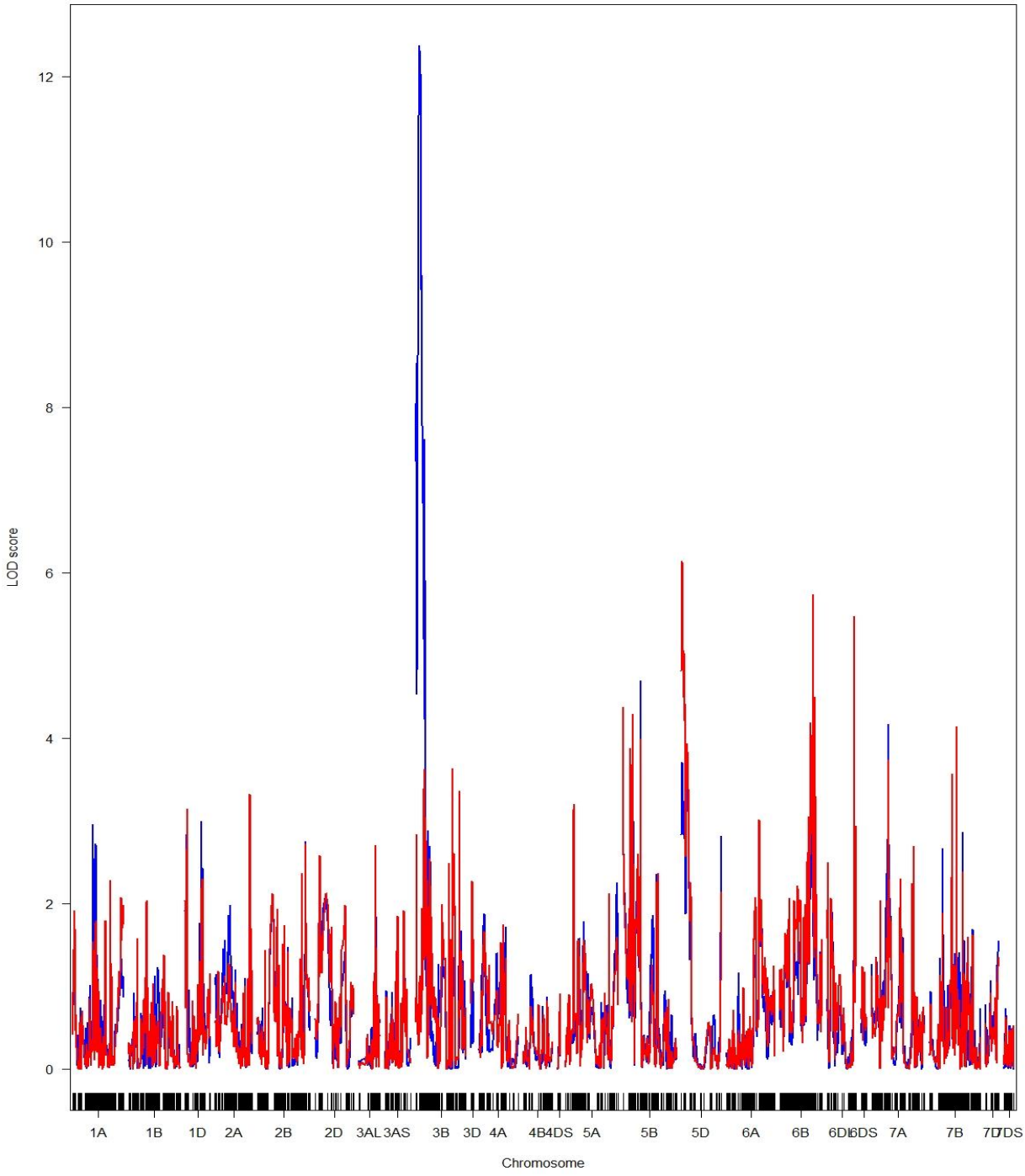

Figure 2.14 GH14IT CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in GH14IT was 3B at position 19. The 5% threshold was 7.19. The 10% threshold was 6.22.

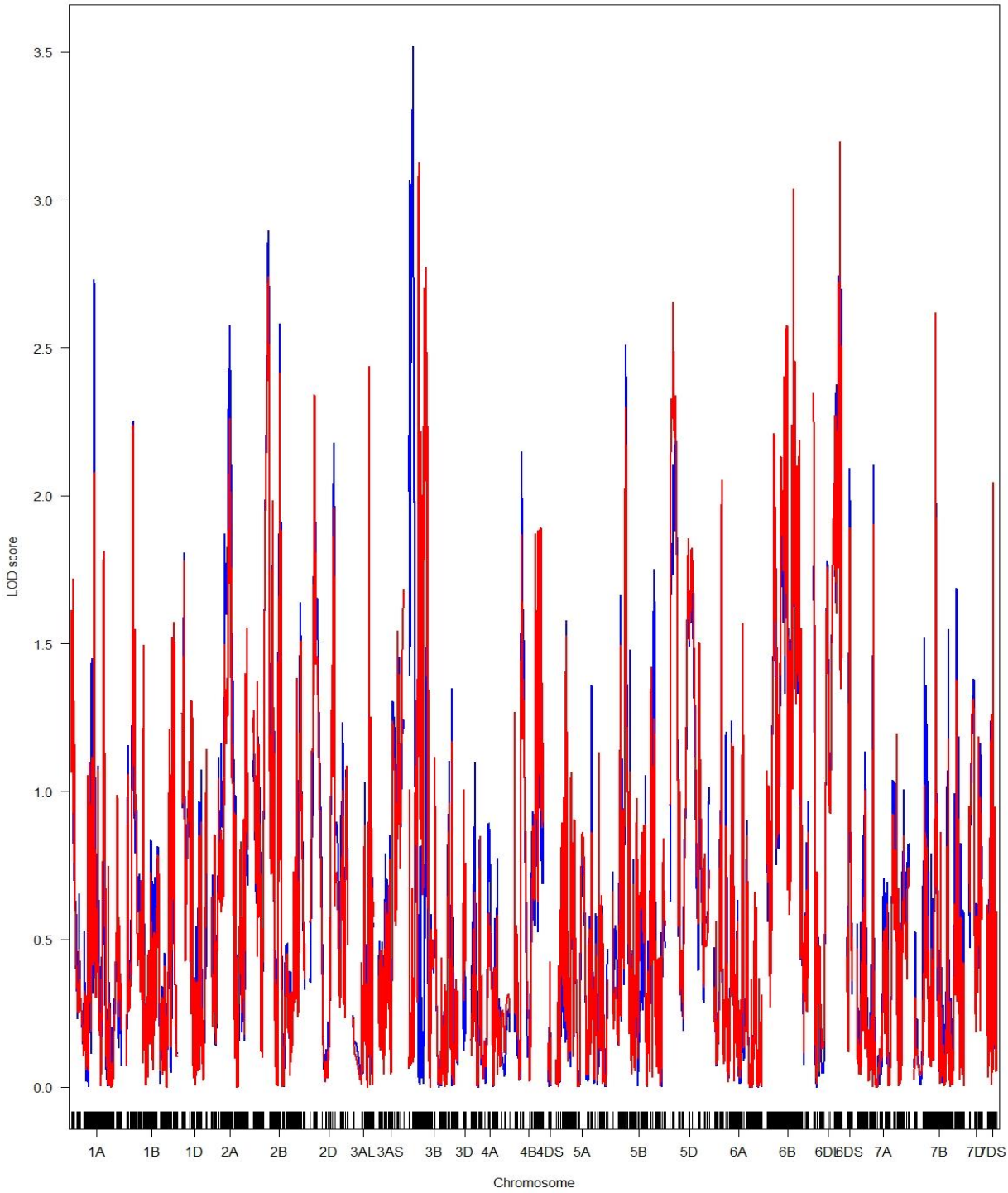

Figure 2.15 GH14SV CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in GH14SV was 3B at position 20. The 5% threshold was 4.22. The 10% threshold was 3.90. There were no LOD peaks above the threshold.

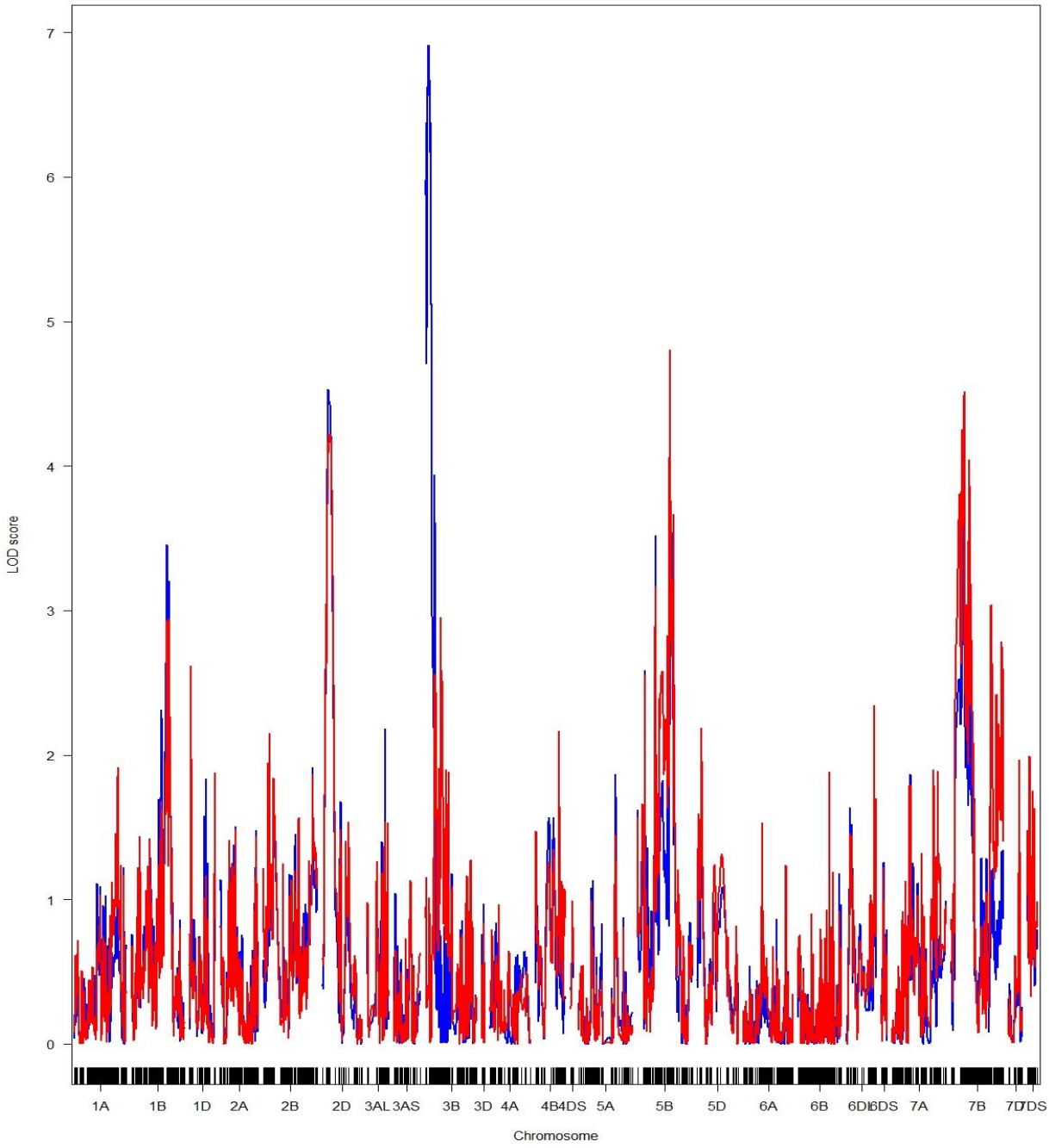

Figure 2.16 TX14IT CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in TX14IT was 3B at position 19. The 5% threshold was 4.09. The 10% threshold was 3.85.

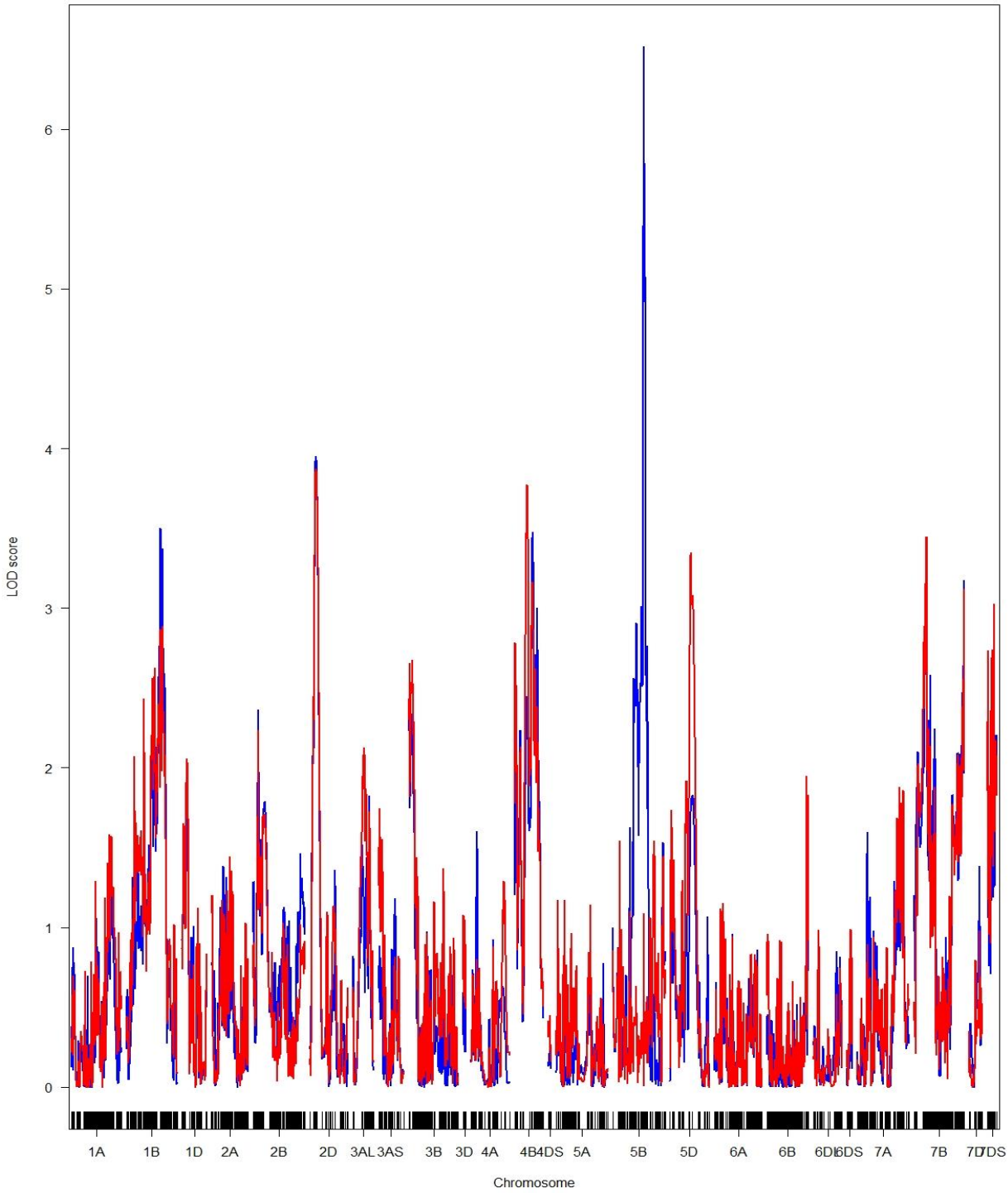

Figure 2.17 TX14SV CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in TX14SV was 5B at position 159. The 5% threshold was 4.20. The 10% threshold was 3.87.

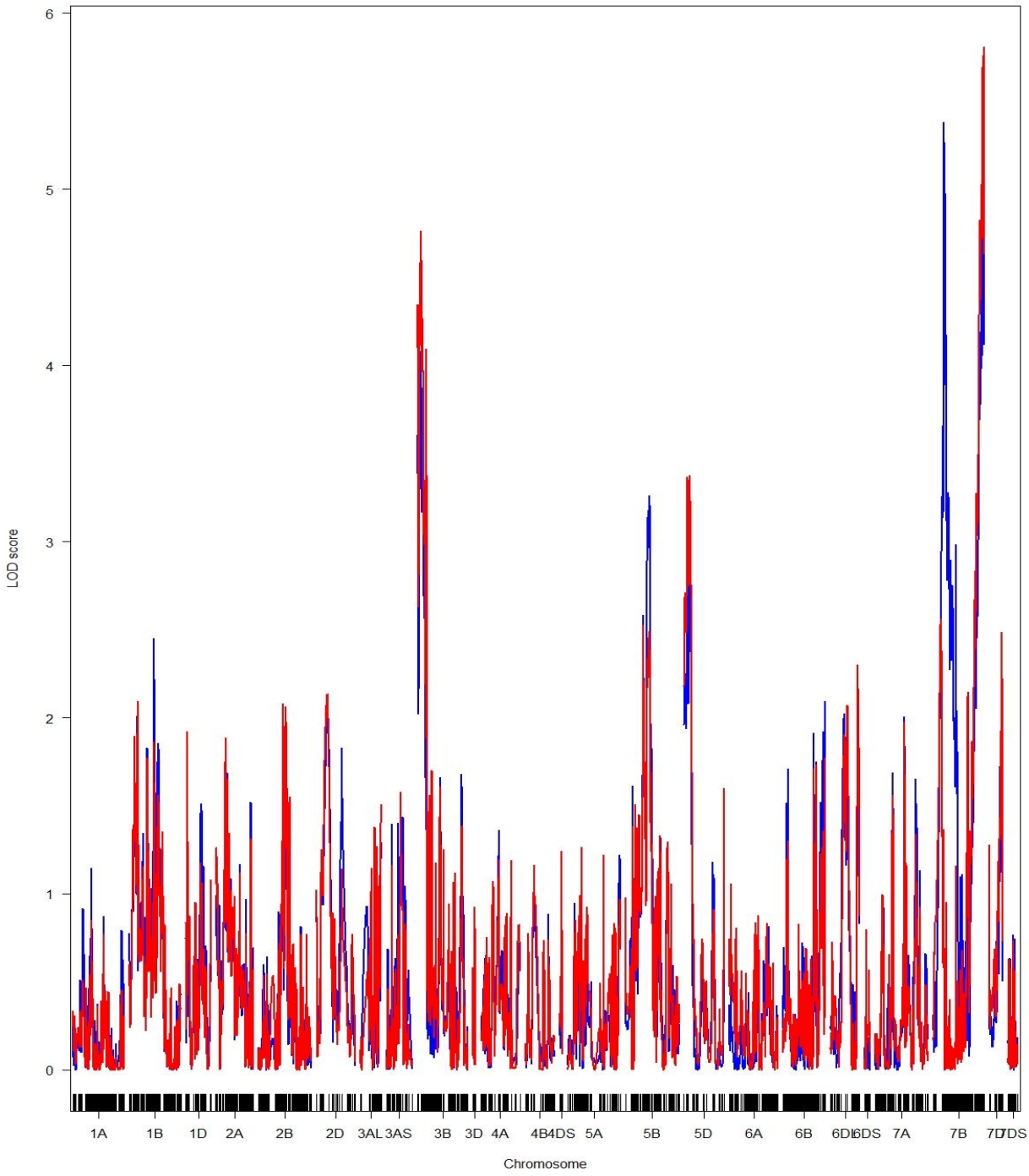

Figure 2.18 AB15IT CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in AB15IT was 7B at position 249. The 5% threshold was 4.26. The 10% threshold was 3.95.

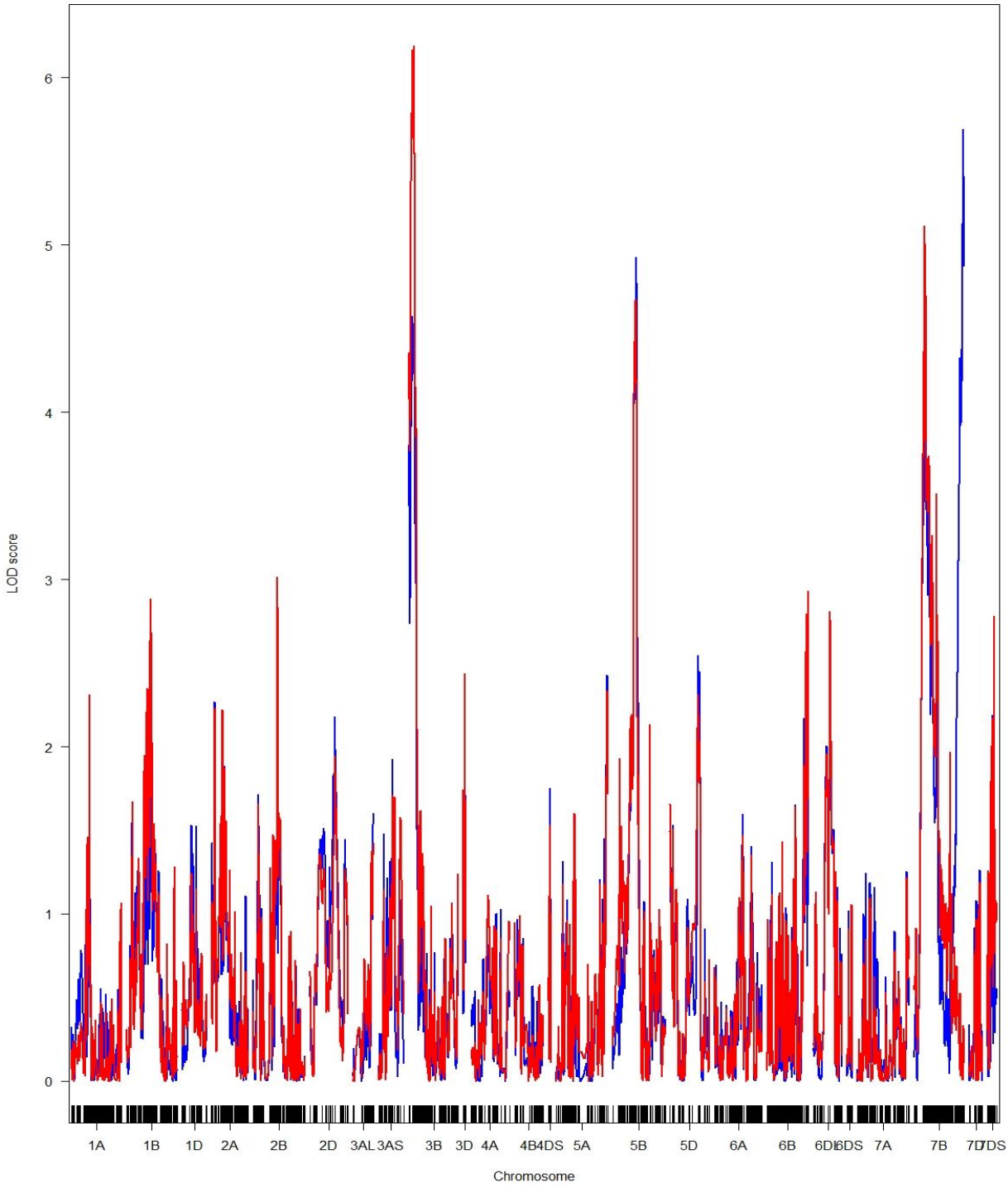

Figure 2.19 AB15SV CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in AB15SV was 7B at position 250. The 5% threshold was 4.21. The 10% threshold was 3.87.

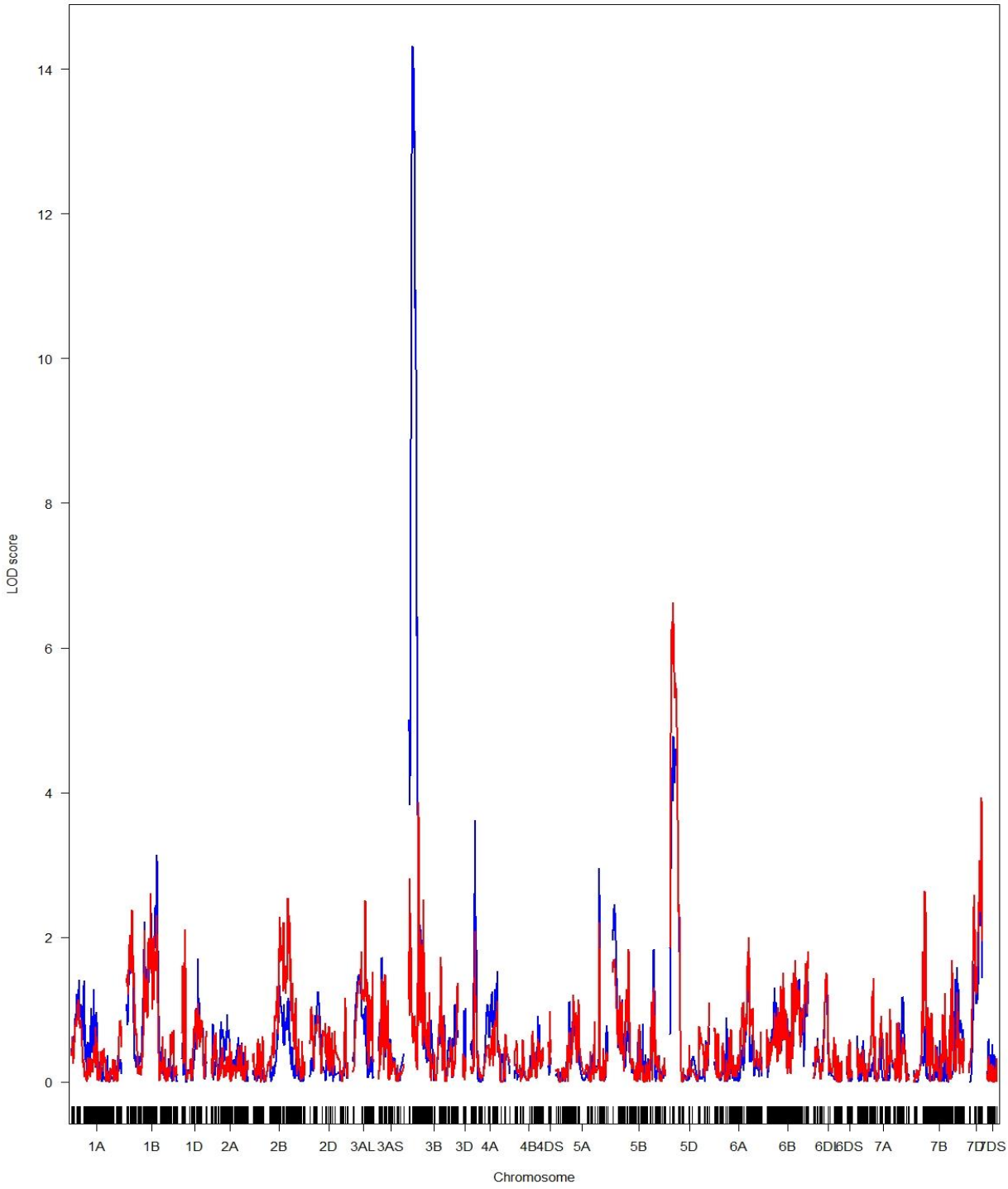

Figure 2.20 GH15IT CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in GH15IT was 3B at position 22. The 5% threshold was 5.94. The 10% threshold was 5.05.

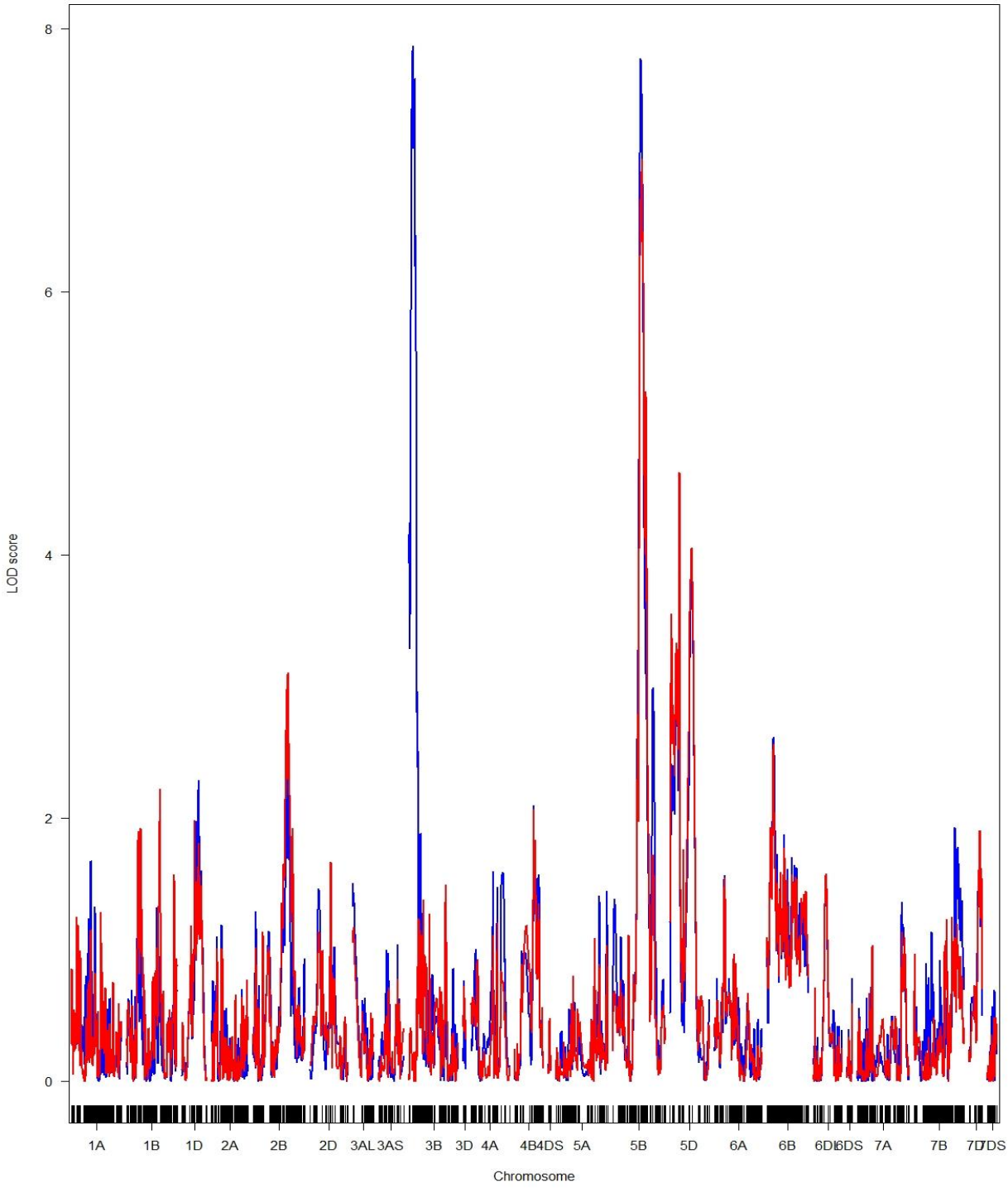

Figure 2.21 GH15SV CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in GH15SV was 3B at position 19. The 5% threshold was 4.31. The 10% threshold was 3.83.

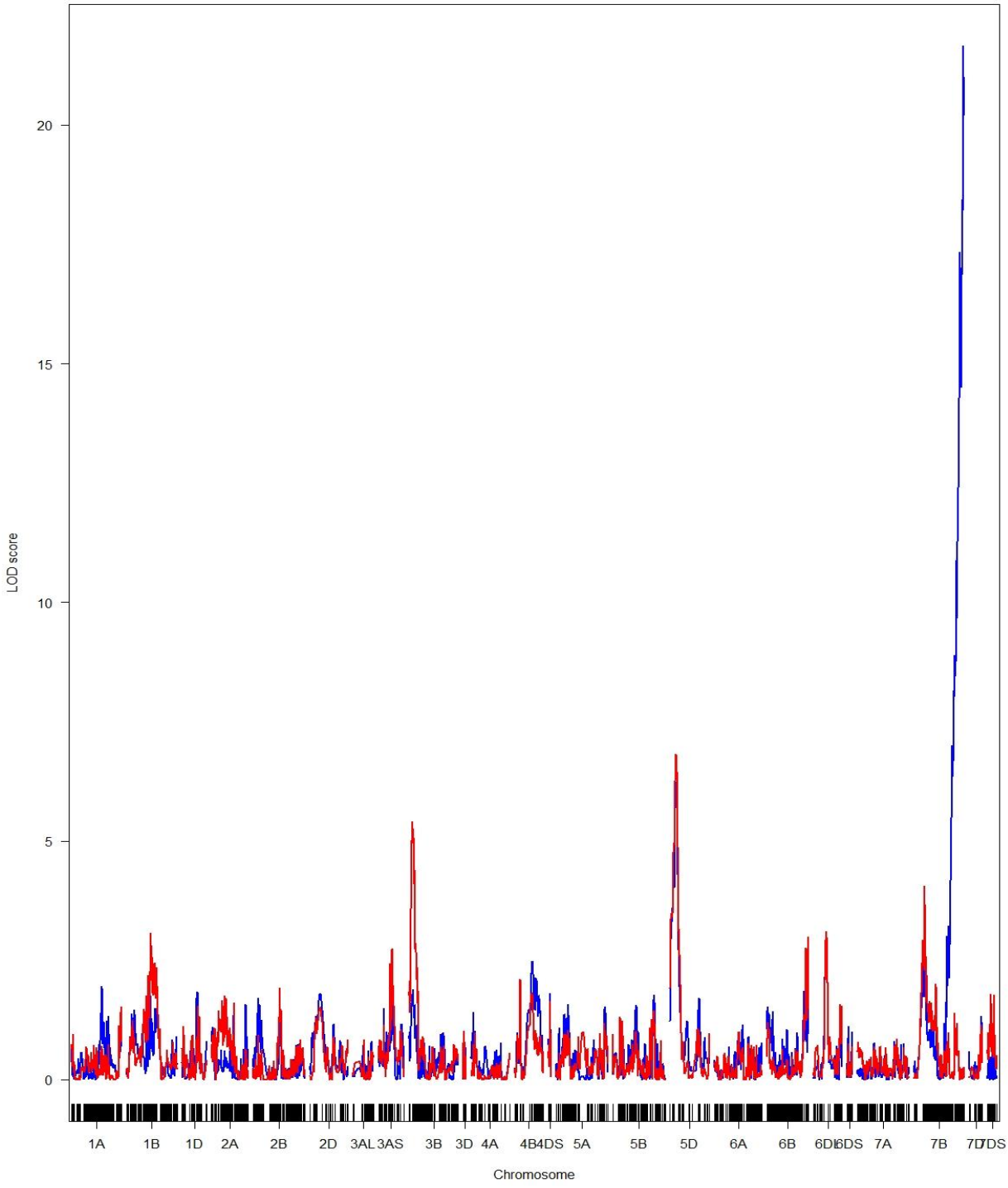

Figure 2.22 AB20IT CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in AB20IT was 7B at position 249. The 5% threshold was 4.88. The 10% threshold was 4.47.

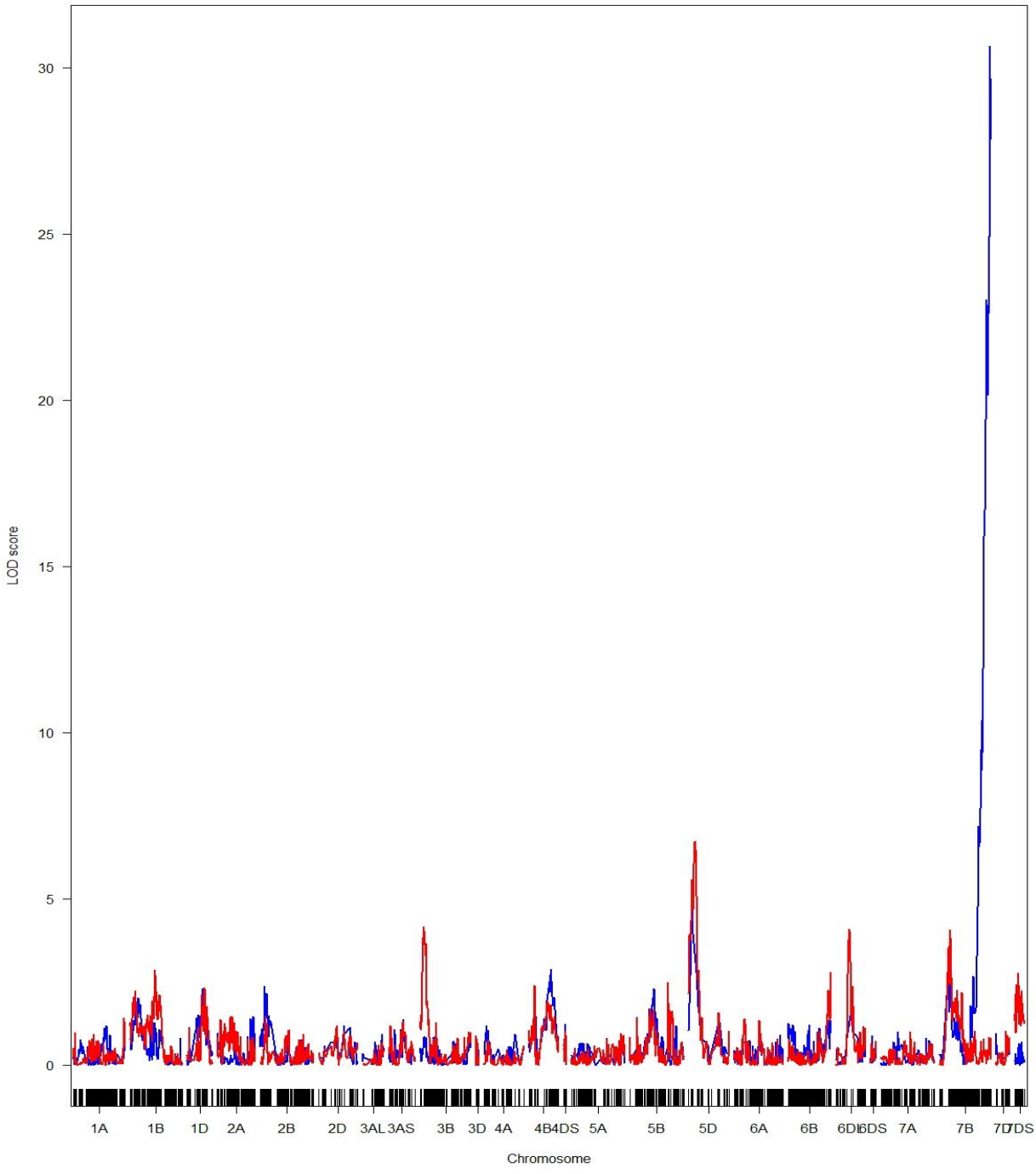

Figure 2.23 AB20SV CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in AB20SV was 7B at position 250. The 5% threshold was 4.38. The 10% threshold was 4.11.

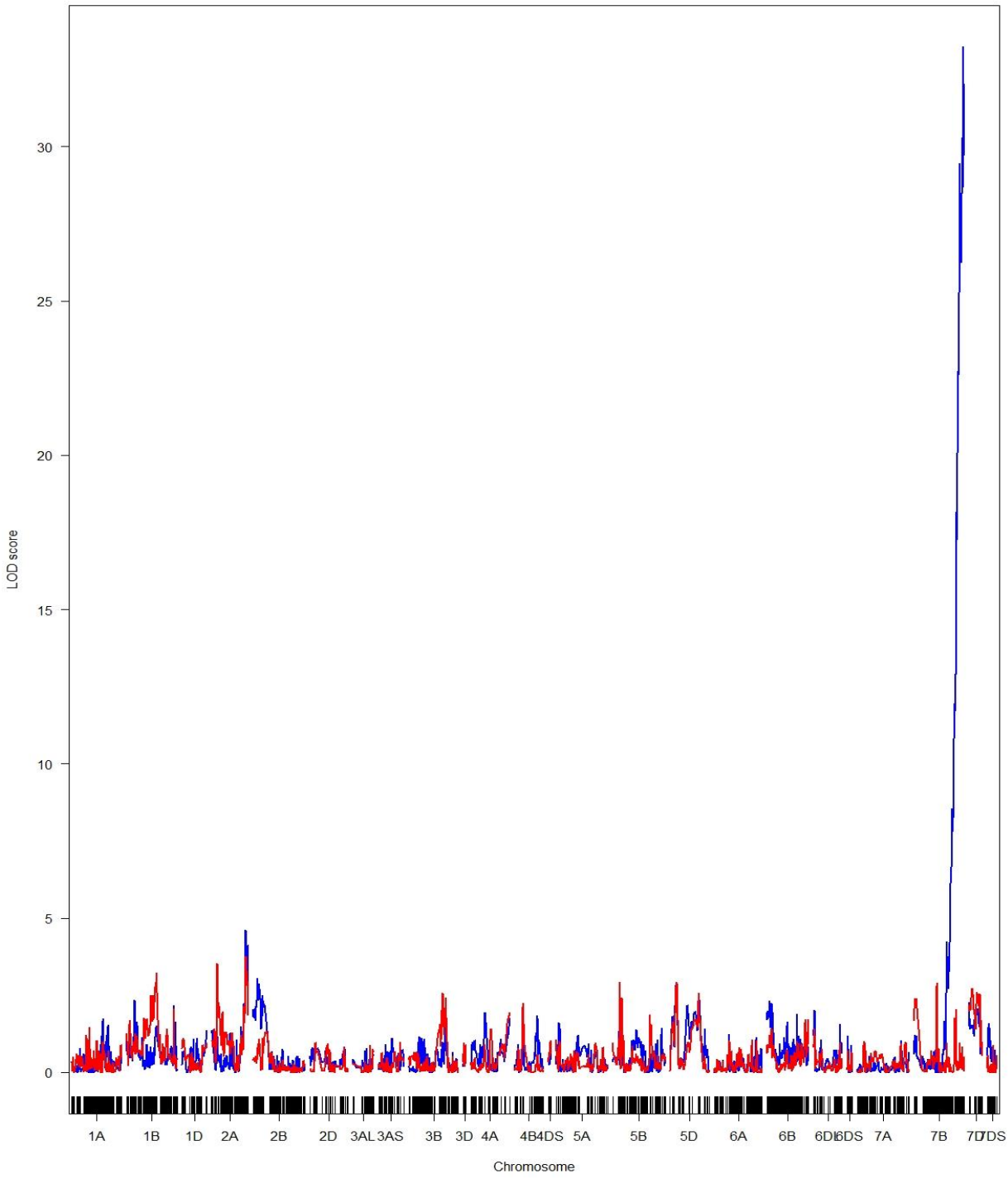

Figure 2.24 AB20GL CIM plot

Blue and red lines indicate the LOD significance across the genome before and after the removal of the covariate, respectively. The covariate in AB20GL was 7B at position 249. The 5% threshold was 5.05. The 10% threshold was 4.55.

Appendix A - 1

Beagle v.4.1 command line

```
java -Xss1g -Xmx16g -jar beagle.08Jun17.d8b.jar nthreads=2 err=0.1 niterations=10
gtgl=hapFile.filtered.vcf out=beagle_hapFile.filtered
```

Appendix B - 1

R/qtl script

```
library(qtl)
##Import genotype and phenotype data files.##
LR <- read.cross("csvsr", ".", "genotypes.csv", "phenotypes.csv")
LR <- jittermap(LR)
plotMap(LR)
LR<-drop.nullmarkers(LR)
totmar(LR)
LR<- calc.genoprob(LR, step=1)
LR<-fill.geno(LR)
geno.image()
LR<-shiftmap(LR)
summary(LR)

##Estimate the recombination fractions between all pairs of markers and plot them.##
LR<- est.rf(LR)
LR
checkAlleles(LR)
plotRF(LR, what=c("lod"), alternate.chrid=TRUE,zmax=75, mark.diagonal=FALSE,
col.scheme="redblue")



##SIM##
## In place of "DATASET", insert the row name from the phenotype file which corresponds to
the dataset being run. Also enter the row # of the dataset from the phenotype file into
"pheno,col=  ".##
out.hk_DATASET <- scanone(LR, pheno.col=2, method="hk")
plot(out.hk_DATASET, ylab="LOD score")
summary(out.hk_DATASET)

##SIM permutations. Enter desired number after "n.perm=  ".##
operm_DATASET <- scanone(LR, pheno.col=2, method="hk", n.perm=1000)
summary(operm_DATASET, alpha=c(0.05, 0.1))
summary(out.hk_DATASET, perms=operm_DATASET, alpha=0.2, pvalues=TRUE)
```

```
##CIM##
LR<-calc.genoprob(LR, step=1, error.prob=0.001)

##Enter linkage group name and cM position of the covariate QTL here.##
mar<-find.marker(LR, "7B", 250)
g<-pull.geno(LR)[,mar]
sum(is.na(g))
g<-pull.geno(fill.geno(LR))[,mar]
sum(is.na(g))
g
out.ag_DATASET<-scanone(LR, pheno.col=2, method="hk", addcovar=g)
plot(out.hk_DATASET, out.ag_DATASET, col=c("blue", "red"), ylab="LOD score")
operm.agDATASET <- scanone(LR, addcovar=g,pheno.col=2, method="hk", chr="-7B",

##CIM permutations.##
                    n.perm=1000)
summary(out.ag_DATASET, perms=operm.agDATASET, alpha=0.2, pvalues=TRUE)
plot(operm.agDATASET)
plot(operm.agDATASET, ylab="LOD score")
summary(operm.agDATASET,alpha=c(0.05,0.1))

##Two-QTL scan.##
LR<-calc.genoprob(LR, step=2.5, err=0.001)

##Haley knott regression.##
out3_DATASET <- scantwo(LR, pheno.col=2, method="hk", verbose=FALSE)



##MQM##

LR<-sim.geno(LR, step=2, n.draws=1000, err=0.001)

##Enter the names of each putative QTL in the model. Then add their respective cM positions in
the next line in the same order.##
qtl<-makeqtl(LR, what="prob", chr=c("3B","5D","7B","7B"),
          pos=c(22,31,63,250))
qtl
plot(qtl)

##Out.fq provides the drop-one ANOVA table. Adjust the string of "Q1+Q2+ etc." for the
number of QTLs in the model.##
out.fq<-fitqtl(LR, pheno.col=2, method="hk", qtl=qtl, formula=y~Q1+Q2+Q3+Q4)
```

```
summary(out.fq)

##Search for improved estimates of QTL locations.##
rqtl<-refineqtl(LR, method="hk", qtl=qtl, pheno.col=2, formula=y~Q1+Q2+Q3+Q4,
        verbose=FALSE)
rqtl

##Assess fit of refineqtl. IF % variability changes drastically from above, plug in new locations
into the model above and re-run.##
out.fq3<-fitqtl(LR, pheno.col=2, method="hk",qtl=rqtl, formula=y~Q1+Q2+Q3+Q4,
        dropone=FALSE)
summary(out.fq3)
plotLodProfile(rqtl, ylab="Profile LOD SCORE")

##Search for additional QTLs.##
out.aq<-addqtl(LR, method="hk", pheno.col=2, qtl=rqtl, formula =y~Q1+Q2+Q3+Q4)
max(out.aq)
plot(out.aq, ylab="LOD score")

##Test for all possible QTL x QTL interactions that are not already included in the model.##
addint(LR, qtl=rqtl, method="hk", pheno.col=2, formula=y~Q1+Q2+Q3+Q4, pvalues=FALSE)

##Drop-one analysis is when each locus is dropped from the model, one at a time, and a
comparison is made btw the full model and the model with the term omitted.##
##This should be a final step for MQM model determination.##
out.fq2<-fitqtl(LR, pheno.col=2, method="hk", qtl=qtl, formula=y~Q1+Q2+Q3+Q4,
        dropone=FALSE, get.ests=TRUE)
summary(out.fq2)

# Interval Estimates. Add a line for each QTL in the MQM model.#

bayesint(rqtl, qtl.index =1)
bayesint(rqtl, qtl.index =2)
bayesint(rqtl, qtl.index =3)
bayesint(rqtl, qtl.index =4)

##Find markers associated with QTLs in the model. Enter QTL name and cM position.
Add/remove rows depending on model size.##

find.marker(LR, "3B", 22)
find.marker(LR, "5D", 31)
find.marker(LR, "7B", 63)
find.marker(LR, "7B", 250)
LR <- sim.geno(LR, n.draws=16, error.prob=0.001)

##For QTL effects, enter dataset, QTL name, and marker name.##
```

```
eff_DATASET_3B <- effectplot(LR, pheno.col=2, mname1="M10430", draw=FALSE)
eff_DATASET_3B

eff_DATASET_5B <- effectplot(LR, pheno.col=2, mname1="M8748", draw=FALSE)
eff_DATASET_5B

eff_DATASET_7B <- effectplot(LR, pheno.col=2, mname1="M263", draw=FALSE)
eff_DATASET_7B

eff_DATASET_7B<- effectplot(LR, pheno.col=2, mname1="M10232", draw=FALSE)
eff_DATASET_7B


##Plotting MQM##

pdf(file=paste("DATASET.pdf",sep=""),  width = 14, height = 8, family = "Helvetica")
plotLodProfile(rqtl, ylab="Profile Lod Score", main="DATASET PLOT")
legend('top',legend=c("5% LOD threshold", "10% LOD threshold"),
      lty=c(5,5),col=c("red","blue"),lwd=c(1,1), bty = "n")
##10% LOD threshold line. Enter value for LOD significance.##
abline(h= 4.35,lty="dotted", lwd=2, col="blue")
##5% LOD threshold line. Enter value for LOD significance.##
abline(h= 4.92,lty="dotted", lwd=2, col="red")

dev.off()
```

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