Identification and Mapping of Resistance to *Puccinia striiformis* and *Puccinia triticina* in Soft Red Winter Wheat

Neal Ryan Carpenter

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Carl A. Griffey

Antonius B. Baudoin

Jason A. Holliday

M. A. Saghai-Maroof

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ACADEMIC ABSTRACT

Disease resistance is critical in soft red winter wheat (*Triticum aestivum* L.) cultivars. Leaf rust caused by *Puccinia triticina* Eriks and stripe rust caused by *Puccinia striiformis* Westend. f.sp. *tritici* Eriks. are destructive pathogens of wheat. From 2014 to 2015 phenotypic data was collected at diverse locations for resistance to leaf rust (North Carolina, Texas, and Virginia) and stripe rust (Arkansas, North Carolina, Georgia, Texas, and Virginia) in a Pioneer '25R47' /'Jamestown' (P47/JT) population composed of 186 $F_{5:9}$ recombinant inbred lines (RILs). Analysis of the P47/JT population identified two quantitative trait loci (QTL) for leaf rust resistance on chromosome 5B and two QTL for stripe rust resistance on chromosomes 3B and 6A. Phenotypic variation (%) explained by the putative leaf rust resistance QTL of Jamestown on 5B was as high as 22.1%. Variation explained by the putative stripe rust resistance QTL of Jamestown on 3B and 6A was as high as 11.1 and 14.3%, respectively.

Jamestown is postulated to contain gene *Lr18*. Seedlings of 186 $F_{5:9}$ recombinant inbred lines from the P47/JT population and 200 F_2 seedlings from eight other crosses including Jamestown and/or the *Lr18* host differential line RL6009 (Thatcher*6/Africa 43) were screened with *P. triticina* race TNRJJ. Genetic analysis of the populations was conducted to validate the presence of *Lr18* in Jamestown. Results of linkage analysis identified SNP maker IWB41960 linked within 5 cM of gene *Lr18* in all three populations.

From 2016 to 2017 phenotypic data was collected at diverse locations for resistance to leaf rust (Illinois, North Carolina, and Virginia) in a '2013412' (PI 667644) / VA10W-21 (PI 676295) population (412/21) composed of 157 doubled haploid (DH) lines. The 412/21 DH lines were genotyped via genotyping by sequence (GBS). Analysis of the 412/21 population identified one quantitative trait loci (QTL) region associated with adult plant resistance to leaf rust on chromosome 1B. Phenotypic variation (%) explained by the putative leaf rust resistance QTL of 2013412 on 1B was as high as 40.1%. Kompetitive allele-specific (KASP) markers KASP_S1B_8414614 and KASP_S1B_8566239 were developed as markers for use in marker assisted selection.

Identification and Mapping of Resistance to *Puccinia striiformis* and *Puccinia triticina* in Soft Red Winter Wheat

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GENERAL AUDIENCE ABSTRACT

Disease resistance to leaf rust and stripe rust is important when growing soft red winter wheat. Genetic resistance can have a benefit to cost ratio of up to 27:1, considerably better than that of fungicide treatments. From 2013 to 2017 disease data was collected across multiple locations spanning the eastern United States (Arkansas, Georgia, Illinois, North Carolina, Texas, and Virginia). DNA molecular markers were used to identify specific chromosome regions containing genes associated with leaf and stripe rust resistance. DNA markers associated with genes conferring resistance to leaf rust resistance were identified in three chromosome regions, and genes in two regions were associated with stripe rust resistance. These genes and molecular markers associated with them can be used by scientists to further enhance resistance in wheat cultivars.

Another study was conducted to determine if *Lr18*, a gene for leaf rust resistance that has a large effect, is present in the Virginia Tech soft red winter wheat breeding material. This gene (*Lr18*) is known to have been introduced from an ancestral species highly related to wheat. Wheat seedlings derived from crosses between lines postulated to carry *Lr18* with susceptible lines were tested for resistance to a specific strain of leaf rust lacking virulence to *Lr18*. Genetic analysis of the ratio of resistant versus susceptible seedlings and association between DNA molecular markers and resistant seedlings were conducted to validate the presence of gene *Lr18*. A molecular marker linked tightly to gene *Lr18* was identified in the study. This gene was found to be widely distributed in soft red winter wheat breeding materials and the molecular marker associated with gene *Lr18* will be useful for scientists to further improve resistance in wheat cultivars.

DEDICATION

This dissertation is dedicated to my father, Kevin Carpenter. Without his sacrifice and support over the years, I would not have been able pursue and achieve my goals. My father has worked selflessly with the sole purpose to provide his children opportunities he never had; for that this work is dedicated to him.

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Dr. Carl Griffey was one of the co-principal investigators for the grants that supported this research. Dr. Subas Malla aided in the experimental design and data analysis for this research. Dr. Paul Murphy organized and maintained field plots in Kinston, NC. Dr. Marla Barnett collected field data in Castorville, TX. Dr. David Marshall and Myron Fountain organized and maintained plots in Plymouth, NC. Dr. Eugene Milus organized and collected field data in Arkansas. Drs. Jerry Johnson and James Buck organized and collected field data in Griffin, GA. Drs. Shiaoman Chao and Gina L. Brown-Guedira conducted the 90K iselect analysis. Emily Wright performed simple sequence repeats reactions. All authors contributed edits to the manuscript.

CHAPTER III

Dr. Carl Griffey was one of the co-principal investigators for the grants that supported this research. Dr. Subas Malla aided in the experimental design and data analysis for this research. Drs. Shiaoman Chao and Gina L. Brown-Guedira conducted the 90K iselect analysis.

CHAPTER IV

Dr. Carl Griffey was one of the co-principal investigators for the grants that supported this research. Dr. Paul Murphy organized and maintained field plots in Kinston, NC and Plymouth, NC. Dr. Mark Christopher organized and maintained field plots in Champaign, IL. Dr. Marla Barnett collected field data in Castorville, TX. Drs. Priyanka Tyagi and Gina L. Brown-Guedira performed the GBS procedure and called SNPs from the reference genome.

CHAPTER I

LITERATURE REVIEW

GENERAL INTRODUCTION

Wheat is one of the most important agricultural commodities produced in the world today. It is considered one of the "big three" cereal crops, with over 600 million tonnes of wheat harvested each year. Of the wheat grown worldwide about 95 % is hexaploid and the remainder is tetraploid (Shewry, 2009). Wheat produced by farmers in the United States is very diverse and provides many essential products used throughout the world. In 2013 there were 711 million metric tonnes (MMT) produced worldwide (Vocke and Liefert, 2013). There were 23 MMT of United States wheat exported in the 2012/2013 market year; the largest importers were Sub Saharan Africa (3.67 MMT), Japan (3.64 MMT), Mexico (2.91 MMT), the Philippines (1.85 MMT), and Egypt (1.74 MMT) (U.S. Department of Commerce, 2013; Vocke and Liefert, 2013).

Soft wheat is produced in two main regions of the United States, the eastern part of the country, i.e. east of the Mississippi River, and the Pacific Northwest, which consists of Washington, Oregon, and Idaho (Morris et al., 2005). Soft red winter (SRW) wheat cultivars are grown primarily for grain quality characteristics appropriate for the production of cakes, cookies, and crackers (Kolmer, 2010). One of the main focuses of wheat producers and end users is to minimize yield losses and reductions in grain quality. A key research priority of breeders is to focus on the development of superior cultivars using marker-assisted-selection to provide more durable resistance and thus limit yield losses and maintain high grain quality (Dubcovsky, 2004).

LEAF RUST OF WHEAT

ABSTRACT

Leaf rust (*Puccinia triticina* Eriks) is the most common type of rust of wheat (*Triticum aestivum* L.) worldwide (Bolton et al., 2008) and can be found on every continent with the exception of Antarctica (Huerta-Espino et al., 2011). Other species of *Puccinia* also infect and cause leaf rust in barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.) (Kolmer, 2013). Losses from leaf rust are typically less severe than those resulting from the other two common rust diseases, stem rust and stripe rust, but leaf rust causes greater overall losses due to its wider distribution and prevalence (Huerta-Espino et al., 2011). Leaf rust is known to overwinter frequently in the southern United States from Texas to Nebraska, and occasionally as far north as southern Minnesota and South Dakota. In the north-central spring wheat regions, infections will reach their highest severity levels by the end of July. In the southeastern soft red wheat region, severity will typically peak in Georgia around April and by the end of May as far north as Virginia (Kolmer and Hughes, 2013). Selection pressure forced on the pathogen population by the presence of only a few resistance genes deployed among the predominant wheat cultivars results in extensive genetic diversity among *P. triticina* virulence phenotypes (Kolmer, 1992). Control of leaf rust via deployment of genetic resistance in cultivars versus reliance upon fungicides, has been made known to be the most cost effective method with an estimated 27:1 benefit to cost ratio (Marasas et al., 2004). There are 78 leaf rust resistance genes that have been mapped to chromosome locations and assigned gene designations, and there are also 18 temporarily designated leaf rust resistance genes (McIntosh et al., 2013).

INTRODUCTION

Leaf rust (*Puccinia triticina* Eriks) is the most common type of rust of wheat (*Triticum aestivum* L.) worldwide. It is probable that *P. triticina* originated in the Fertile Crescent zone of the Middle East and references to *P. triticina* can be traced back as far as the bible and ancient Greek literature (Bolton et al., 2008). Other Puccinia species also infect and cause leaf rust in barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.) (Kolmer, 2013). Losses from leaf rust are typically less severe than those resulting from the other two common rust pathogens stem rust and stripe rust, but leaf rust generally causes greater losses due to its broader distribution and endemic prevalence (Huerta-Espino et al., 2011). Use of genetic resistance is the most economical solution of reducing damage due for leaf rust as opposed to fungicides. Many breeding programs throughout the world have produced cultivars with long lasting, durable leaf rust resistance (Kolmer, 1996).

Many leaf rust (*Lr*) genes operate on a race-specific gene for gene basis (Bolton et al., 2008). This indicates that each specific gene will provide resistance to only certain races of leaf rust. Genetic resistance to leaf rust can be exploited best when knowledge of resistance genes in commonly used parental germplasm and released cultivars is available and, thus, facilitates pyramiding of unique genes. Race-specific resistance typically involves a hypersensitive response of rapid cell death or a chlorotic (yellowing) response of tissue around the rust pustule (Bolton et al., 2008; Duplessis et al., 2011). Identification of diverse leaf rust resistance genes allows for effective integration and pyramiding of different genes into breeding populations, and thus helps to avoid the release of cultivars that are genetically similar for resistance (Kolmer, 1996). High levels of variation and mutation among races of *Puccinia triticina* create the need for identification , incorporation, and pyramiding of novel resistance genes (Bolton et al., 2008).

Adult plant resistance (APR) is more durable and effective against multiple races of a pathogen. Introgression of multiple seedling resistance genes and APR genes into elite cultivars will result in broad-spectrum and durable resistance (Griffey and Allan, 1988).

Development of gene pyramids requires the identification of unique genes possessing resistance to different races, and these pyramids are best implemented by the incorporation of multiple resistance genes into a high yielding cultivar (Singh et al., 1992). Development of cultivars with durable resistance requires genetically diverse sets of resistance sources, and thus requires genetic studies to determine the number, chromosome location, identity, and mode of action of genes associated with resistance (Griffey and Allan, 1988).

Life Cycle and Spread of the Leaf Rust Pathogen

In the early 17th century, leaf rust became established in North America along with wheat cultivation (Chester, 1946). Rust fungi are obligate parasites and require a living host to complete their life cycle (Kolmer, 2013). If leaf rust infections are established during the fall after seedling emergence and survive the winter, *P. triticina* has the potential to rapidly increase spores at the same time the wheat seedlings are breaking dormancy. These early infections typically lead to greater leaf rust damage, including increased yield losses (Chester, 1946). Cereal rusts are macrocylic and thus have all spore stages as opposed other fungal diseases which are demicyclic or microcylic.

There three distinct spore types of rusts on cereal hosts including production of teliospores, basidiospores, and urediniospores (Kolmer, 2013). Urediniospores can be continuously produced in multiple cycles on the cereal host plants as they have a dikaryotic nuclear condition. Teliospores develop during the uredinial infection and a diploid nucleus is formed from the

dikaryotic nuclei merger. Optimal germination of urediniospores occurs after four to eight hours at 20°C at 100% humidity and spores are typically viable for one to three days (Bolton et al., 2008). When the teliospores germinate they undergo meiosis which forms four haploid basidiospores and then eject these basidiospores into the air which subsequently infect an alternate host (Kolmer, 2013). The alternate sexual host for leaf rust is meadow rue (*Thalictrum speciosissimum* L.), although this is not native and does not grow naturally in North America. Therefore as there is no sexual cycle in North America leaf rust is initiated by uredinial spore infections in North America (Kolmer, 2013; Samborski, 1985), and the asexual production of urediniospores occurs on wheat (Ordoñez and Kolmer, 2009).

In the southeastern states and southern Great Plains, leaf rust epidemics are initiated by urediniospores in the fall months, these infected spores typically survive the winter dormancy period of wheat. Infection foci are visible in early spring in Texas and other Gulf Coast states. When winter wheat breaks dormancy, the production of urediniospores increases as temperatures rise (Chester, 1946; Kolmer, 2013). These spores are then spread by wind to infect developing wheat crops further north (Kolmer, 2013; Roelfs, 1989).

Environments having high relative humidity often favor leaf rust epidemics. Late planted varieties are also more susceptible because they have a greater chance of being exposed to higher relative humidity (Kolmer et al., 2007). Leaf rust develops rapidly at temperatures between 10°C and 30°C, while at lower temperatures longer dew or periods of high relative humidity are required for infection (Singh et al., 1992).

Studies dealing with chemical control of cereal rusts began as far back as the mid-19th century, and it was quickly concluded that chemical control was not economical with the available chemicals (Dickson, 1959). There have been times when severe epidemics occurred, such as in

the 1976-1977 season in northwestern Mexico, where chemical control was a viable control option. During the 1976-1977 growing season producer's used Bayleton (triadimefon) to control wheat leaf rust epidemics when genetic resistance became ineffective (Dubin and Torres, 1981). While genetic control of wheat leaf rust is economically ideal, when genetic resistance fails, a chemical application is necessary to save a producer's crop.

Detection and Evolution of Leaf Rust and Losses in the United States

Yield losses due to *P. triticina* can be considerable, yet the extent of loss depends upon when the initial infection occurs and the relative resistance of the wheat cultivar. The greatest yield loss associated with leaf rust occurs with early infection, this is especially true when infection occurs before the tillering stage (Kolmer et al., 2007). Leaf rust primarily affects the leaf and its major impact on yield results from infections of the flag leaf blade. Leaf rust development is most rapid when there are warm moist conditions and such conditions also favor above average wheat yields, which often causes losses in yield to be underestimated. From 2000 to 2004, losses due to leaf rust in the United States were estimated to be over 3 million tonnes and valued at over 350 million dollars (Huerta-Espino et al., 2011).

Races of *P. triticina* have been studied in existing populations in the United States since 1926 and about 70 different races are identified annually (Bolton et al., 2008). In the United States most wheat cultivars have race-specific resistance genes, so new rust races with virulence to widely deployed genes increase very quickly. Such new races occur at a high frequency in existing *P. triticina* populations throughout the United States (Bolton et al., 2008).

Even with the lack of sexual recombination, *P. triticina* is still very efficient at maintaining genetically diverse populations for virulence specificities. Mutation of a *P. triticina* race

resulting in virulence for a specific resistant gene in wheat is common, because the pathogen populations are so large. Wheat cultivars grown in a compressed area are generally considered heterogeneous for a specific leaf rust resistance gene(s), when the cultivars contain different combinations of resistance genes (Kolmer, 2001). Selection pressure on the pathogen population applied by the presence of a single or few resistance genes in different cultivars of wheat promotes genetic diversity of *P. triticina* virulence phenotypes. *P. triticina* is dikaryotic so heterozygosity is an additional source of genetic variation (Kolmer, 1992). Therefore if isolates of *P. triticina* are heterozygous at virulence loci, they would only need a single mutation at those loci to become virulent to a particular resistance gene (Kolmer, 1992; Kolmer, 2001).

In the United States, the *P. triticina* population has been observed to have heterozygosity comparative to expected levels under random mutation (Kolmer, 2013). In addition elevated levels of linkage disequilibrium between simple sequence repeat (SSR) markers and a parallel between virulence and SSR genotypes has led to the conclusion that the *P. triticina* population has characteristics of highly clonal populations (Kolmer, 2013). In North America there are six groups of *P. triticina* races based upon SSR genotype groupings (Ordoñez and Kolmer, 2009). The isolates within the groups have very similar SSR genotypes and have similar virulence spectra to specific leaf rust (*Lr*) genes, while different groups have highly distinct virulence spectrum as opposed to the other groups. There are common groups that are found in Virginia, the Great Plains, and other eastern and southern states. Two groups NA-3 and NA-5 account for the majority (95%) of the isolates that are presently found in the *P. triticina* population. Groups NA-2 and NA-6 are also found in the United States but less frequently (Kolmer, 2013). Collections of *P. triticina* populations from durum wheat (*Triticum durum* Desf.) in Europe, South America, Mexico, and the Middle East were extremely similar for SSR genotypes and

virulence spectra of North American races, which indicates a common origin of isolates (Ordoñez and Kolmer, 2007).

In 2011 the most common phenotypes for the *P. triticina* population in the United States respectively were TBBG, MLDSD, TCRKG, and TNBGJ. Races TBBG and TNGBJ are both virulent to leaf rust resistant genes *Lr39* and *Lr41* that are contained in many hard wheat cultivars. The second most common race in 2011, MLDSD is virulent to *Lr39*, *Lr41*, and *Lr17*. Race TCRKG is virulent to genes *Lr11*, *Lr18*, and *Lr26* (Kolmer and Hughes, 2013). In 2010 the most common races where MLDSD, TDBJG, and TCRKG. There are notable geographic differences in P. triticina races. For example, the most common races in areas where SRW wheat cultivars are grown are MCTNB, TBRKG, TCRKG, and TCBJG. MCTNB is virulent to *Lr11* and *Lr26*, TBRKG is virulent to *Lr11*, TCRKG is virulent to *Lr11*, *Lr18*, and *Lr26*, and TCBJG is virulent to *Lr26*. It was postulated that most SRW wheat cultivars grown contain leaf rust resistant genes *Lr11*, *Lr18*, and *Lr26* (Kolmer and Hughes, 2013). Races MFDSB, TBBGJ, TDBGG, and TNBGJ were most common in the Great Plains where most hard red winter (HRW) wheat is grown. MFDSB is virulent to *Lr24*, *Lr26*, and *Lr17*, TBBGJ is virulent to *Lr39* and *Lr41*, and TNBGJ is virulent to *Lr9*, *Lr24*, *Lr39*, and *Lr41* (Kolmer and Hughes, 2013). In tests at the USDA- ARS Cereal Disease Laboratory, it was determined that previously resistant cultivars had high infection type to leaf rust race TCRKG that is virulent to *Lr18* and *Lr26*. In 2013, virulence to *Lr26* was 44% and virulence to *Lr18* was 33% in the southeastern population. Races with virulence to both *Lr18* and *Lr26* may be further increasing in frequency or a new race(s). (Hughes, 2014)

Widespread use of cultivars having different race-specific *Lr* genes in different regions has resulted in regional differences in the populations of *P. triticina* for virulence spectra that are

vastly diverse for virulence in the United States. Since these populations are extremely large, there are frequent mutations resulting in new races in response to the leaf rust resistance genes in wheat cultivars (Kolmer and Hughes, 2013). There will always be a need to develop new cultivars with different combinations of leaf rust resistant genes to counter virulence changes in *P.triticina*.

Emergence of New Races and the Worldwide Significance of Leaf Rust

Leaf rust has been an issue for northwestern Mexico since 1976 when the race TBD/TM was first discovered (Singh et al., 2004). Leaf rust in northwestern Mexico is now primarily in durum wheat and has caused significant yield losses from 2001 to 2009. This is primarily due to the emergence of the newly virulent leaf rust race BBG/BN. Between 2001 and 2003 the estimated loss due to this leaf rust race was $32 million dollars (Singh et al., 2004). To combat this new race two resistant cultivars were released in 2001 and 2004, these both became susceptible in 2008 due to a new mutation resulting in, race BBG/BP that is virulent to both leaf rust resistance gene *Lr27* and *Lr34* in durum wheat (Huerta-Espino et al., 2011). The estimated loss for northwestern Mexico for the 2008/2009 growing season was $40 million. The only reason that bread wheat has not succumbed to the same epidemics as durum wheat is because of the utilization of slow rusting or durable resistance genes/QTL in addition to *Lr34* in cultivars, and before these new genes were used resistance lasted about three years (Huerta-Espino et al., 2011).

There are 9 million hectares of wheat planted annually in South America in five countries (Argentina, Brazil, Chile, Paraguay, and Uruguay). Changes in leaf rust races caused a $172 million loss in yield production (Huerta-Espino et al., 2011). In this southern cone of South America there are typically a large number of leaf rust races prevalent annually, and these races

have continued to adapt and become virulent to cultivars that are widely grown in the area (Germán et al., 2007). The races that are predominant in South America are virulent *to Lr1, Lr2a, Lr3a, Lr3ka, Lr10, Lr11, Lr14a, Lr14b, Lr16, Lr17a, Lr18, Lr20, Lr23, Lr24, Lr26,* and *Lr30*. The most recent races have high virulence on *Lr1, Lr3a, Lr3ka, Lr10, Lr11, Lr17a, Lr26,* and *Lr30* (Germán et al., 2007). Until 2003, races MCD, MHD, MHJ, MHK, and MHT were an issue in Brazil, Chile, Paraguay and Argentina. There has been a new shift to races MFR, MCD-10,20, MCP-10 in Brazil and Uruguay (Germán et al., 2007). In 2002 and 2003 there was extensive damage to cultivars in Argentina by the race MCP/MHP-10, and race MDR-10,20 that was identified in 2003 and has rapidly spread in Uruguay, Argentina, and Brazil, Paraguay (Huerta-Espino et al., 2011).

Leaf rust annually affects 63% of the 23.7 million hectares of wheat grown in China (Huerta-Espino et al., 2011). From 1992 to 1996 the most prevalent race of *P. triticina* in China was PHT; this race was found in 26% of samples collected in those four years (Chen et al., 1998). The most common races of *P. triticina* found between 2001 and 2007 were PHT, THT, PHK, PHS, and PHJ ranking respectively (Huerta-Espino et al., 2011). In a recent gene postulation study in China it was found that all the races tested were virulent to genes *Lr2b, Lr2c, Lr3, Lr3bg, Lr10, Lr11, Lr33*, and *LrB* (Li et al., 2009). In South Asia, specifically the countries India, Pakistan, Bangladesh, and Nepal, there are 37 million hectares of wheat grown annually and an estimated 81% of these are at risk to leaf rust losses (Huerta-Espino et al., 2011).

In Europe leaf rust caused by *P. triticina* is the most common disease of wheat. From 1960 to 1980 there were 77 different races of *P. triticina* identified in Europe (Kolmer et al., 2013). In 1995 a study was conducted surveying *P. triticina* in Western Europe (Park and Felsenstein, 1998). There were 53 different races identified in Austria, Belgium, France, Germany, northern

Italy, Switzerland, and the United Kingdom. The four most prevalent phenotypes were virulent to leaf rust resistant genes *Lr2c, Lr3a, Lr3bg, Lr3ka, Lr10, Lr11, Lr15, Lr17, Lr26, Lr30, LrH*. These four races accounted for 64% of the examined isolates. A study conducted in the United Kingdom in the early 2000's noted that the most common leaf rust resistance genes found in U.K. cultivars were *Lr13, Lr26, Lr37, Lr10, Lr17b, Lr1, Lr3a,* and *Lr20* respectively (Singh et al., 2001). From 1992 to 2002 cultivars were screened for resistance to leaf rust in northern Europe (Hysing et al., 2006). In Denmark, Finland, Norway, and Sweden, *genes Lr1, Lr2a, Lr3, Lr10, Lr13, Lr14a, Lr17, Lr23* and *Lr26* were found in 47 of the 84 cultivars screened. The most frequently occurring genes in cultivars grown in Sweden were *Lr13, Lr14a,* and *Lr26*. Gene Lr14a was the most common in cultivars grown in Norway, Lr13 was the most common gene in Denmark, and Lr10 was the most common gene in Finland. In 2002 and 2003 all leaf rust races in Germany had virulence to *Lr1, Lr2a, Lr2b, Lr15, Lr17,* and *Lr20* (Lind and Gultyaeva, 2007). In the Northern Caucasus of Russia there are 4.5 million hectares of wheat planted each year, and yield losses from leaf rust have caused losses from 18 to 25% (Volkova et al., 2009). Trends of an increase in virulence to *Lr1* and *Lr2a* observed in Germany were not observed in Russia, this may be due to significant agro-climatic differences between regions (Lind and Gultyaeva, 2007).

In Australia potential losses in wheat due to leaf rust are estimated up to 197 million dollars while actual losses typically are around 12 million dollars (Murray and Brennan, 2009). Wheat leaf rust was uncommon in western Australia in the 1970's and 1980's, but the race pt-104-2,3,(6),(7),11 which is virulent to *Lr20* was discovered in eastern Australia in 1988 and in western Australia in 1990 (Park, 1996). In the early 2000's a new races was identified with virulence to *Lr24* (Park et al., 2002). Although, Lr24 still remains effective when paired with *Lr13* and *Lr37* (Park, 2008). Most adult plant resistance in Australian wheat cultivars can be

attributed to Lr34 (Singh et al., 2007). Race Pt 76-3,5,9,10 was first isolated from Inverleigh, Victoria in July 2006, and now is present throughout southeastern Australia, this race is virulent to *Lr13* and *Lr37* (Huerta-Espino et al., 2011). This race has the possibility of spreading throughout Australia, which will call for use of new resistance genes.

In South Africa samples of leaf rust were collected from 2008 to 2010 and based on their virulence reactions on a standard set of host differential lines, eight races were identified (Terefe et al., 2014). The most dominant races were 3SA133 and 3SA145. Only one new *P. triticina* (Pt) race was reported in South Africa during 1989–2008. The two recently detected new races, 3SA145 and 3SA146, are believed to be exotic introductions. In 2009 samples collected from the Western and Eastern Cape contained race 3SA145 which is virulent to APR genes Lr12, Lr13, and Lr37. In 2010 a new race, 3SA146, was detected. This race has the same virulence of 3SA146 along with virulence to *Lr1* and *Lr23*, and is avirulent for *Lr3ka* and *Lr30*.

The emergence of new *P. triticina* races is an ever present danger in the global struggle to combat leaf rust and calls for effective and new combinations of leaf rust resistance genes implemented worldwide.

Host Resistance Genes and Evolution of Pathogen Virulence

There are 78 leaf rust resistance genes (Table 1.1) that have been mapped to chromosome locations and given gene designations, there are also 18 temporarily designated leaf rust resistance genes (McIntosh et al., 2013). Due to the highly variable nature of *P. triticina*, durable leaf rust resistance in wheat cultivars has been difficult to achieve.

In the United States many winter and spring wheat cultivars are or soon become susceptible to leaf rust due to emergence of virulent races. Most resistance genes such as *Lr1*, *Lr10*, and *Lr21*

are effective in the seedling stage, and remain effective through the adult stage (Dyck and Kerber, 1985). Wheat genotypes with combinations of adult plant resistance genes *Lr34, Lr46,* and *Lr68* have shown durable leaf rust resistance, although cultivars that have only *Lr34* do not have high levels of resistance even though no isolates with virulence to *Lr34* have been detected in bread wheat (Kolmer, 2013).

Complex loci, gene clusters, and pleiotropy at a single locus often provide effective resistance to multiple diseases (Bariana et al., 2007). This can be seen in many gene combinations (Table 1.1) such as *Sr24/Lr24* and *Sr38/Lr37/Yr17* which are known to be in many Australian cultivars and may be useful in combinations with other genes (Bariana et al., 2007). The two main breeding strategies to improve leaf rust resistance are the pyramiding of major resistance genes conferring resistance, in addition to or the accumulation of minor resistance genes that confer quantitative resistance (Messmer et al., 2000). Plant disease resistance can be classified into two categories: qualitative resistance conferred by a single resistance (*Lr*) gene or better known as seedling or race specific resistance, and quantitative resistance conferred by multiple genes or quantitative trait loci (QTLs), also known as adult plant resistance, with each QTL or additive gene providing a partial increase in resistance (Messmer et al., 2000; Vanzetti et al., 2011). The gene *Lr34* as mentioned previously offers both durable and broad-spectrum resistance, and works synergistically with other leaf rust resistance genes (Lillemo et al., 2008). Genotypes possessing *Lr34* in combinations with seedling resistant genes *Lr2a, Lr9,* or *Lr26* were highly resistant to leaf rust, while those with Lr34 combined with resistance genes *Lr10, Lr11,* or *Lr18* had moderately to low resistance (Kolmer, 2003). Gene *Lr46* also provides durable and broad spectrum resistance to leaf rust yet to a lesser degree than *Lr34* (Lagudah et al., 2009), which is the same case for *Lr67* (Herrera-Foessel et al., 2011). Gene Lr68 has an even less effect than

Lr34, Lr46, and *Lr67,* but is still an effective adult plant resistance gene used throughout Brazil, Argentina, and Mexico (Herrera-Foessel et al., 2012). In a recent study (Dakouri et al., 2013) genes *Lr1, Lr3, Lr10,* and *Lr20* were found to be the most prevalent genes around the world, while *Lr9, Lr14b, Lr3ka, Lr30,* and *Lr26* were the most rare. Genes *Lr1* and *Lr10* were the most frequent seedling resistance genes found in North America and Asia, while *Lr3, Lr10,* and *Lr20* were most frequent in South America.

Leaf Rust Resistance and Significant QTL used in SRW Wheat

There will always be a significant need to identify more novel QTL governing leaf rust resistance in wheat. Development of cultivars with durable resistance requires genetically diverse sets of resistance, and thus requires genetic studies to determine the number, chromosome location, identity, and mode of action of genes that control resistance (Griffey and Allan, 1988).

In a study conducted by Xu et al. (2005), two QTLs for APR to leaf rust, also known as "slow rusting" were mapped on chromosomes 2B and 7B. The QTL *QLr.osu-2B* was linked with microsatellite markers Xbarc18-2B and Xbarc167-2B, and *QLr.osu-7BL* was associated with microsatellite marker Xbarc182-7B (Xu et al., 2005). This result was confirmed again in 2008 along with the additional discovery of small effect QTL on chromosomes 2BS, 2BL, and 7BL (Rosewarne et al., 2008). Five QTL for APR, *QLr.fcu-3AL, QLr.fcu-3BL, QLr.fcu-5BL , QLr.fcu-6BL, QLr.fcu-4DL* were discovered after seedling screening with races MJBJ, TDBG, MFPS, followed by field tests with natural field inoculum in Fargo, North Dakota (Chu et al., 2009). Using European wheat cultivars, QTL for resistance to Australian races were identified

on chromosomes 4BS (Xbarc20-4B) and 5AS (QTLBvr5AS, Xbarc10-5A) using races virulent to *Lr13* and *Lr26* (Singh et al., 2009). In 2014 a genetic study was conducted using wheat accession KU3198 from the Kyoto collection which is highly resistant to Pt collections in Canada (Hiebert et al., 2014). In was determined that a novel resistance gene, designated as *Lr70*, resides on chromosome 5DS, since previous *Lr* genes have not been identified on 5DS. In a similar study gene *Lr73* was mapped to chromosome 2BS, but it is unlikely to be of value unless used in conjunction with other sources of leaf rust resistance due to its susceptibility to many pathotypes (Park et al., 2014).

In a study conducted to determine genetic resistance of SRW wheat to leaf rust it was reported that *Lr12* and *Lr34* confer effective resistance in the field. Wheat genotypes having genes *Lr2a*, *Lr9*, and *Lr26* combined with adult plant resistance were highly resistant to leaf rust, while genotypes with *Lr1*, *Lr10*, *Lr11*, and *Lr18* combined with adult plant resistance had low to moderate levels of resistance to leaf rust (Kolmer, 2003).

CONCLUSIONS

Importance of Leaf Rust to Virginia and growers in the Southeastern U.S.

The widespread use of cultivars that have different race-specific *Lr* genes in different regions has resulted in regional differences in virulence spectra among populations of *P. triticina*, which are vastly diverse for virulence in the United States. Since these populations are extremely large, it is postulated that frequent mutations take place and create new phenotypes in response to the leaf rust resistance genes in wheat cultivars (Kolmer and Hughes, 2013). Such mutations lead to

rapid changes in virulence spectra within the pathogen population and thus leaf rust remains a perpetual problem in Virginia.

The soft red winter wheat cultivar Jamestown (PI 653731) is productive in the southern Corn Belt, the Deep South, and throughout the mid-Atlantic region including Virginia. This can be attributed to its notable resistance to leaf rust, stripe rust, and Hessian fly (Griffey et al., 2010). Jamestown is postulated to contain both *Lr10* and *Lr18* (Griffey et al., 2010). The high yielding cultivar Shirley (PI 656753) expresses high levels of resistance to leaf rust (Griffey et al., 2010), Shirley is postulated to contain *Lr18* and *Lr26*. Shirley is a widely used cultivar throughout the United States and both Jamestown and Shirley are extensively used as parents in breeding programs throughout the eastern U.S. (Carl Griffey, personal communication, 2014). The leaf rust resistance gene *Lr18*, derived from *Triticum timopheevii*, is known to be located on the long arm of the 5B chromosome (McIntosh, 1983). Since Lr18 is part of a translocation, it is believed to likely lie somewhere between markers Xgwm499 and Xgwm1016 on chromosome 5BL (Leonova et al., 2002). Seedling resistance governed by *Lr18* is best conferred between 15 and 18°C, and as temperatures increase the gene becomes less effective, and at 25°C gene *Lr18* becomes ineffective (McIntosh, 1983). Identification, characterization, and mapping of leaf rust resistance genes allows for effective integration of different leaf rust genes into germplasm pools, and thus helps to avoid the release of cultivars that are genetically uniform (Kolmer, 1996).

In a study conducted to determine the impact of powdery mildew and leaf rust on milling and baking quality of SRW wheat, quality losses due to disease occurred both before and during grain fill (Everts et al., 2001). Leaf rust occurred later in the season than powdery mildew, and it had a relatively greater impact on quality parameters (Everts et al., 2001). A study conducted by

Green et al. (2014) in Virginia demonstrated that among 50 SRW wheat cultivars, yield losses due to leaf rust were as high as 33 percent. It was also demonstrated that leaf rust was most negatively correlated with plant biomass and harvest index (Green et al., 2014). Based on the Virginia Small Grains report in 2014 (http://www.sites.ext.vt.edu/newsletter-archive/small-grains/CSES-97-PDF.pdf), Jamestown expressed moderate seedling leaf rust resistance, while Shirley had exceptional seedling leaf rust resistance. This suggests that genes *Lr26* and *Lr18* may work synergistically with each other to provide durable seedling leaf rust resistance. Further research should be conducted to validate this relationship in SRW wheat.

STRIPE RUST OF WHEAT

ABSTRACT

Stripe rust, also known as yellow rust (*Puccinia striiformis* Westend *f.sp. tritici* Eriks) of wheat (*Triticum aestivum* L.) is considered to be one to the most widely destructive plant diseases in the world and one of the most important diseases of wheat since the 1960's (Line, 2002). Stripe rust reduces grain/forage yield and quality. Seed harvested from crops heavily infected with stripe rust exhibit low vigor and emergence (Chen, 2005). Breeding and production of wheat and barley cultivars with durable resistance is the most economical and effective approach to controlling stripe rust (Chen, 2007). Stripe rust is a major disease of wheat in the United States, especially in cooler climates. Stripe rust has been most damaging in the western regions and occurs sporadically in central regions (Wiese, 1977). In 2000, 21 new races of stripe rust were identified in the U.S.; of these new races, eight had combinations of virulence to resistance genes that were previously known to provide exceptional resistance in the United States (Chen et al., 2002). From 2000 to 2007 a total of 115 races of stripe rust had been identified (Chen et al., 2010), and by 2010 that number had grown to 146 (Wan and Chen, 2014). New isolates that are able to germinate at warmer temperatures allow for disease development later in the season. The new stripe rust population has increased adaptation and fitness yet contains many virulence alleles that are not required to overcome resistance in soft red winter (SRW) wheat cultivars east of the Rocky Mountains. There are 56 stripe rust resistance genes that have been mapped to chromosome locations and given gene designations, there are also numerous temporarily designated leaf rust resistance genes (McIntosh et al., 2013) and over 140 QTL that govern stripe rust resistance (Rosewarne et al., 2013).

INTRODUCTION

Stripe rust (*Puccinia striiformis* Westend *f.sp. tritici* Eriks) of wheat is one of the most important diseases of wheat since the 1960's (Line, 2002), and has believed to have caused epidemics as far back as in 1725 in England and in 1794 in Sweden (Chester, 1946). Breeding wheat and barley cultivars with durable resistance and their production is the most economical and effective approach to controlling stripe rust (Chen, 2007).

Stripe rust genes are classified into two categories: all stage resistance (race specific hypersensitive resistance) and high temperature adult plant resistance (HTAP) (Chen, 2007). Expression of these genes results in various amounts of chlorosis and/or necrosis depending on the level of resistance of the plant and environmental factors such as temperature. Stripe rust consumes water and nutrients from the host plant, and thus weakens the plant (Chen, 2005). The easy incorporation of race-specific resistance into commercial cultivars due to their simple inheritance, makes their use appealing in breeding programs. However, the most severe epidemics caused by stripe rust were the result of failure of a race specific resistance genes in widely grown cultivars. Adult plant resistance (APR) is more durable and effective against multiple races of a pathogen. Introgression of multiple seedling resistance genes and APR genes into elite cultivars will result in broader spectrum and more durable resistance (Griffey and Allan, 1988).

Multiline cultivars and gene pyramiding, have been successfully used to control stripe rust (Chen, 2007). Relying on single gene resistance is often considering a "ticking bomb" as resistance only remains effective until pathogens become virulent to that particular gene,

especially if that cultivar and/or gene is widely used in a region. Development of gene pyramids requires the identification of diverse genes for resistance, and their combined incorporation into a high yielding cultivar (Singh et al., 1992). The development of cultivars with durable resistance requires genetically diverse sets of resistance, and thus requires genetic studies to determine the number, chromosome location, identity, and mode of action of genes that govern resistance (Griffey and Allan, 1988)

Life Cycle and Spread of the Stripe Rust Pathogen

Stripe rust infection can start very early in the crop season and can cause more damage in some areas than leaf rust (*P. triticina*) and stem rust (*P. graminis*) because both of these diseases require a higher optimal temperature than stripe rust (Chen, 2005). Infection can occur between the one leaf stage to plant maturity (Chen, 2005). Stripe rust is a basidiomycete (Hovmøller et al., 2002), that produces and is spread by urediniospores which are yellow to orange in color when in large masses (Chen, 2005). Stripe rust is heteroecious and may have five different spore stages with both asexual and sexual reproductions. Sexual reproduction may be completed in the presence of the alternate host barberry (*Berberis vulgaris*). The uredinia are produced asexually with repeated cycles of infection. Once the infected tissue begins to senesce, telia may be produced. The telia will form many two-celled teliospores, which contain a diploid nucleus formed via karyogamy. The teliospores that germinate produce basidiospores which can infect the alternate host barberry. The infection of the basidiospores on the alternate host barberry results in pycnial infections on the upper side of the leaf, followed by aecial growth on the lower side of the leaf. (Hovmøller et al., 2011; Jin et al., 2010).

In the United States these urediniospores are blown from Texas throughout the Pacific-Northwestern region (Kolmer, 2005). The optimal temperature for initial infection is 8 to 12°C

with high relative humidity, and the optimal temperature for sporulation is from 12 to 20°C (Xianming Chen, personal communication, 2014). These temperatures are much cooler than optimal for leaf rust or stem rust. Stripe rust will rapidly develop in the field at 15°C when there is recurrent rain or dew. Numerous disease cycles may take place within one season, and the time between infection and sporulation under optimal conditions is 8 days. Mild winters, and long, cool, wet springs and falls favor stripe rust development (Maloy and Inglis, 1993).

The three essential factors required for stripe rust development and epidemics are moisture, temperature, and wind (Chen, 2005). Moisture is required for urediniospore germination, but urediniospores do not germinate very well in free water, they rather require high relative humidity near saturation levels for a minimum of 3 hours (Chen, 2005; Rapilly, 1979). Temperature affects spore germination, latent period, sporulation, spore survival, and host resistance (Chen, 2005). Stripe rust favors cooler climates. Wind affects stripe rust by drying urediniospores, which reduces germination and infection, but increases the period of spore viability. Wind is the major contributing factor to spore dispersal (Chen, 2005).

In the mid 1920's, a barberry (*Berberis vulgaris* L.) eradication program was created to eliminate common barberry as an inoculum source of stem rust in Minnesota (Roelfs, 1989). Barberry also is an alternate host of stripe rust (Jin et al., 2010). Once barberry was nearly eradicated it revealed a second major source of inoculum, windborne from the southern Great Plains. The third source of inoculum comes from overwintering mycelium that also produce urediniospores (Roelfs, 1989). There is also indirect evidence that agronomic practices of furrow planting and minimum cultivation increase the area and frequency of overwintering (Roelfs and Long, 1987).

The main tactic to control stripe rust is to control the spread of windborne urediniospores and the overwintering of mycelium that produce urediniospores. Resistant cultivars are developed and

commonly used to control stripe rust and prevent epidemic proportions (Roelfs, 1989). Stripe rust initially develops in "concentrated patches" arising from overwintering mycelia and these initial foci which spread throughout and between fields over 1000 kilometers. Stripe rust epidemics spread throughout fields with cumulative velocity over time and space (Cowger et al., 2005).

Detection and Evolution of Stripe Rust and Losses in the United States

Stripe rust has been an important disease of wheat in the United States, especially in cooler climates. Stripe rust has been most damaging in the western regions and occurs sporadically in central regions (Wiese, 1977). This disease was first recognized in the United States in 1915 (Carleton, 1915). In the 1950's and 1960's there was a severe epidemic of stripe rust in California and the Pacific Northwest. This lead to increased emphasis on breeding for resistance to stripe rust in the United States (Line and Qayoum, 1992).

This disease was only an occasional concern in wheat in the south central region of the United States from 1941 to 1999, but since 2000 stripe has steadily been a severe threat to wheat throughout much of the United States. New races of stripe rust in the United States have evolved that have shorter latent periods and can tolerate higher temperatures than previous races (Milus et al., 2006). In 2000, 21 new races of stripe rust were identified. Among these new races, eight had combinations of virulence to resistance genes that were previously known to provide exceptional resistance in the United States (Chen et al., 2002). From 2000 to 2007 there were a total of 115 identified races of stripe rust (Chen et al., 2010), and by 2010 that number had grown to 146 (Wan and Chen, 2014).

Since there is a lack of the sexual stage in the life cycle of stripe rust under most natural conditions, genetic recombination does not occur and therefore does not result in new race variations. New races of *P. striiformis* are the result of mutations of genes from avirulence to virulence in response to existing resistance genes, and this is considered to be a rapid process as many new races have evolved in a short amount of time. As compared to other pathogenic fungi of agronomic crops, genetic diversity of *P. striiformis* at a molecular level is very low, however this has not prevented new races of stripe rust from evolving virulence to genes in previously known resistant cultivars (Hovmøller et al., 2002).

In 2013 the distribution of stripe rust surpassed 2012's record distribution in the United States, but stripe rust was largely not as severe as the previous year in most locations. Dry spring conditions in the Pacific Northwest and the extensive use of fungicides limited stripe rust development in this region in 2013. Stripe rust was more severe in Louisiana, Mississippi, Georgia and Arkansas, but damage was alleviated by the application of fungicides (Hughes, 2013).

In 2013 trace amounts of stripe rust were found in eastern Virginia in late May. A single stripe rust lesion was found on one leaf in a plot of the cultivar 38158 (PI 619052) at Blackstone in southern Virginia in mid-May. Wheat stripe rust was found only in a few plots at Painter in eastern Virginia in mid-May and cultivar Tribute (PI 632689) had a few plants with severity ranging from 1 to 40%. Stripe rust in plots at Blacksburg in western Virginia had increased to 90% severity on highly susceptible lines by mid-June (Hughes, 2013).

The occurrence of stripe in 2000 was the most widespread recorded since 1957 and 1958. In 2000 there were major stripe rust induced yield reductions, and a combined loss of 244,938 tonnes (Ramburan et al.) of wheat in the states of Arkansas (123,003), Washington (45,371),

Oklahoma (40,066), California (20,621), Texas (9,117), Kansas (4,877), Oregon (2,400), and Louisiana (1,347). The highest annual yield losses accredited to stripe rust from 1990 to 1999 was only an estimated 6,532 tonnes in 1993 (Chen et al., 2002). The total estimated national yield loss in 2013 was 282,223 tonnes and a 0.7% yield loss (Hughes, 2013).

In the eastern United States there are two genetically distinct populations of *Puccinia striiformis*. The 'old' population includes isolates collected before 2000, and the 'new' population includes isolates collected since 2000 that have shorter latent periods and are virulent to more resistant genes. Using molecular markers it was postulated that the new population likely was a result of a new/foreign introduction rather than a mutation in the old population (Markell and Milus, 2008). The isolates that were collected prior 2000 had very little virulence to genes *Yr8* and *Yr9*, but isolates that were collected since 2000 and beyond had virulence to these genes. The *Yr9* gene has been widely used in SRW wheat due to its linkage with stem rust resistance gene *Sr31* and leaf rust resistance gene *Lr26* on the 1B/1R translocation. This is of major concern as very few other known stripe rust resistance gene were incorporated to SRW wheat prior to 2000 (Markell and Milus, 2008). Gene *Yr9* is present in many popular SRW wheat cultivars such as Shirley and USG 3555 (Griffey et al., 2009; Griffey et al., 2010). Many of the most widely grown cultivars were susceptible to moderately susceptible to the 'new' race of stripe rust PST-100, which is now widespread in the southeastern United States (Chen, 2007). Race PST-100 is virulent to many resistance genes including *Yr2, Yr3a, Yr4a, Yr6, Yr7, Yr8, Yr9, Yr19, Yr20, Yr21, Yr22, Yr23, YrCle, YrSte, YrYam, YrPr1, YrPr2*, and *YrHVII* (Chen et al., 2002; Chen, 2007).

From the year 2000 to 2005 there was rapid changes in the *P. striiformis* population in the United States (Table 1.4). Many previous forms of resistance were overcome by the "new" populations

(Chen, 2005; Chen et al., 2010; Chen, 2007). The top five Pst races in 2013 (Table 1.4) were recently reported (Xianming Chen, personal communication, 2014). The top three races (PSTv-52, PSTv-37, and PSTv-73) were detected in both the western and eastern United States, while PSTv-11 and PSTv-14 were not detected in the eastern U.S. in 2013.

Under optimal field conditions for stripe rust in the United States, a grower may lose up to 85% of their grain yield (French-Monar, 2010). The earlier the infection occurs, the more severe the yield loss will be. This is the reason that stripe rust can be more devastating than leaf rust or stem rust, because the infection can occur earlier than either of the other two rust diseases. When the pathogen overwinters in fields after planting, epidemics may start earlier in the growing season, as soon as the first node forms. Ongoing effort will be needed to provide continued resistance to stripe rust in cultivars across the United States.

Emergence of New Races and Expansion in Area of Adaptation

New races of stripe rust have evolved to survive in warmer climates since 2000, and thus the geographical area of the fungus has expanded. When mycelium overwinters it functions as an inoculum source for urediniospores, and the northern regions where the fungus cannot overwinter are infected by these windborne urediniospores. Mutation of *P. striiformis* allowing for survival and overwintering of new races over wider areas likely will lead to earlier infection and subsequently cause more damage than previous races. Since the pathogen has adapted to a larger range of temperature it has allowed the pathogen to spread north quicker.

The emergence of these new races since 2000 and their near complete replacement of the older races is postulated to be due to an increase in aggressiveness (Milus et al., 2006). Stripe rust spores were found to germinate at higher temperatures, and it was flourishing in areas where it

previously had not been found. This aggressiveness could be attributed to increased temperature tolerance for spore germination (18°C) and shorter latent periods. The newer races with short latent periods were projected to cause 2.5 times more disease in a typical growing season. Under field conditions, races with faster spore germination are favored because there are a greater number of short periods than long periods that are favorable for greater germination. The new races that are able to germinate at warmer temperatures also allow for disease development later in the season. The increased adaptation and fitness of the new stripe rust population has many implications and it contains many unnecessary virulence factors needed to overcome resistance genes in SRW wheat cultivars east of the Rocky Mountains. High levels of aggressiveness in races that have unnecessary virulence genes may explain why stabilizing selection does not work and indicates that some unnecessary virulence genes may be linked to genes for high aggressiveness. Normally, a race having many unnecessarily virulence factors would have a lower fitness and could be selected against, this would favor races into having only a few virulence factors that are necessary (Milus et al., 2006). Race frequency of stripe rust can be determined by two factors, virulence spectrum and cost of unneeded virulence genes. *P. striiformis* is an obligate parasite and must infect a host plant to grow and reproduce. The more virulent genes a race has, the more capable it is to infect cultivars increases. The second factor is cost of unneeded virulence genes, which are no longer required to overcome the host's resistance genes and can be detrimental to the pathogen's fitness to retain. These factors are also dependent on the diversity among wheat cultivars being grown in a particular region (Chen, 2005).

In China the two most predominant races CRY32 and CRY33, are aggressive (Chen et al., 2013). These were similar in aggressiveness, tolerance to extreme environmental conditions, and high urediniospore production as PST-100 and races found in the North America after 2000 (Chen et

al., 2013; Markell and Milus, 2008). Races present in Australia recently also were found to have spore production capabilities similar to the other new races, but temperature tolerance did not appear to be the same (Loladze et al., 2013).

The most at risk regions for stripe rust epidemics throughout the world are the USA (Pacific Northwest), East Asia (northwest and southwest China), South Asia (Nepal, Pakistan), Oceania (eastern Australia) and East Africa (Kenya). These regions have stripe rust on a regular basis with estimated losses between 1 and 10% (Wellings, 2011). In 2000 there were major stripe rust yield reductions in the Pacific Northwest such as in Washington (1,667,100), California (757,700), and Oregon (88,200). In the 2001-2002 growing season in China, a stripe rust epidemic affected over 6.6 million hectares, the amount of damage was 1.3 million metric tons of wheat. This was primarily due to a relatively warm winter and early spring, which favored overwintering and earlier development of stripe rust (Wan et al., 2004). This epidemic was primarily due to races CRY31 and CRY32, which are virulent to *Yr1, Yr2, Yr3, Yr4, Yr6, Yr7, Yr9, Yr17, Yr22, Yr23, Yr27, YrA, YrCV1, YrCV2, YrCV3, YrG, YrSD*, and *YrSO*. Race CRY32 is known as race 239E175 in other parts of the world (Wan et al., 2004). In Pakistan in 2002 there were large losses due to stripe rust overcoming resistance in the cultivar Inqilab 91. This was due to a new race 166E143A+ which is virulent to Yr27 and many other stripe rust resistance genes in Pakistani commercial cultivars (Hussain et al., 2012). Stripe rust of wheat is also becoming a major issue in other parts of south Asia such as India (Joshi et al., 2007). Stripe rust, has the highest average potential cost of 994 million dollars per year to the Australian wheat industry. Due to losses in the Northern, Southern, and Western regions of Australia (Murray and Brennan, 2009). Stripe rust of wheat was first reported in western Australia in 2002, and the identified race was virulent to *Yr6, Yr7, Yr8, Yr9, YrA* (Wellings et al., 2003).

The emergence of new *P. striiformis* races, is an ever present danger, in the global struggle to combat stripe rust is a constant struggle and calls for effective and new combinations of stripe rust resistance genes to be implemented worldwide.

Host Resistance Gene and Evolution of Pathogen Virulence

There are 56 stripe rust resistance genes (Table 1.3) that have been mapped to chromosome locations and given gene designations; there are also numerous temporarily designated stripe rust resistance genes. Resistance genes Yr3 and Yr4 both contain multiple alleles (McIntosh et al., 2013). There are over 140 QTL that govern stripe rust resistance (Rosewarne et al., 2013). Due to the highly variable nature of *P. striiformis*, durable stripe rust resistance in wheat cultivars has been difficult to achieve.

Stripe rust epidemics reported in the 1970s were associated with the failure of resistance in the cultivar 'Siete Serros' and related cultivars in North Africa, India, Middle East, Africa and China. It is presumed, although not definitively proven, that an important basis of these epidemics was the failure of gene Yr2 (McIntosh, 2009). Virulence for *Yr9* in East Africa and migration of this races through the Middle East, Pakistan, and India caused significant wheat damage in the 1990's (Singh et al., 2004). In 2000, virulence was discovered wheat genotypes containing Yr8 and Yr9 resistance genes in the United States.

The new group of races that were found in United States after 2000 were virulent to resistant genes *Yr7*, *Yr8*, and *Yr9*. Gene *Yr5* and *Yr15* are the only stripe rust resistance genes that are effective against all races in the United States (Chen, 2007; Chen, 2014). Genes *Yr5* and *Yr15* have recently been utilized in SRW breeding programs, but *Yr17* may be in multiple cultivars due to its linkage with *Lr37*. In 2010 a resistant cultivar Jagger that was widely grown in the

Great Plains and known to have *Yr17*, had heavy stripe rust infection. This indicated that a new race of stripe may have overcome *Yr17* (Christopher et al., 2013; Fang et al., 2011). Recently virulence to gene Yr17 deployed widely in SRW wheat breeding programs was confirmed in the United States (Lee, 2013).

Stripe rust was first detected in Australia in 1979, despite earlier predictions that the pathogen would not survive the summer. Stripe rust quickly adapted and has been seen every summer since, despite several droughts (Wellings, 2007). By 1990 the stripe rust races in Australia had already become virulent to genes *Yr2*, *YrA*, *Yr5*, *Yr7*, and *Yr8* (Kolmer, 2005). In 2002 a second change occurred producing strains virulent to genes *Yr6*, *Yr7*, *Yr8*, *Yr9*, and *YrA* (Wellings et al., 2003). In 2009 it was reported that resistance genes *Yr4, Yr18, and Yr30* still provided some protection, while genes *Yr17, Yr27*, and *Yr30* have been overcome by various stripe rust races in Australia (Wellings, 2010).

Gene Yr18 has provided durable slow rusting resistance, but under optimal conditions of stripe rust it becomes ineffective (Imtiaz et al., 2005). Genes *Yr2, Yr3, Yr6*, and *YrA* have become ineffective in Mexico, and this is often thought to be where stripe rust spores originate and spread annually into the United States via wind dispersal (Kolmer, 2005). Stripe rust races virulent to *Yr8* and *Yr9* were discovered in 2000 east of the Rocky Mountains (Milus et al., 2006). Gene Yr17 has been used widely in Europe, the United States, and Australia but virulence has been recently reported for *Yr17* in both the United States and Australia (Christopher et al., 2013; Lee, 2013; Wellings, 2007). Recently *Yr45* was mapped to chromosome 3DL and there have been few reports of virulence to this gene in the United States. Even though there are few virulent races it is recommended that this gene be considered as race

specific and used in combination with other genes for effective all-stage resistance or non-race specific HTAP durable resistance (Li et al., 2011).

Cultivar 'Karamu' from New Zealand was tested for the presence of the HTAP gene Yr18. It was believed that this cultivar contained both *Yr18* and *YrA*. In 1995 a novel race of stripe rust emerged in New Zealand and Karamu was significantly damaged during the stripe rust epidemic. It was postulated that the race was virulent to *Yr18*, which was ineffective under high disease pressure, or that Karamu may not contain *Yr18*. After a doubled haploid mapping study was conducted, it was determined that Karamu does not contain *Yr18* and that *Yr18* does not alone provide resistance under high disease pressure (Imtiaz et al., 2005).

Stripe rust was first observed in South Africa in 1996 and has since spread to the areas around it. Only one races was detected in 1996, 6E16A- which is virulent to *Yr2, Yr6, Yr7, Yr8*, and *Yr17*. In 1998, race 6E22A- was detected with the same virulence as 6E16A- with addition of virulence to Yr25 (Boshoff et al., 2002). In 2001 a new variant of 6E22A- was detected, 7E22A- which was also virulent to *Yr1* (Pretorius et al., 2007). Race 6E22A+, was identified in 2005 and was also virulent to *YrA* (Agenbag et al., 2012).

It is widely recognized that developing cultivars with multiple forms of resistance and use of gene pyramids is paramount. Relying on single gene resistance is often considering a "ticking time bomb" where resistance remain effective only until the pathogen becomes virulent to that particular gene, especially if that cultivar and/or gene is widely used in a region (Griffey and Allan, 1988; Singh et al., 1992). Often resistance genes are located within gene clusters or are tightly linked to other resistance genes that provide pleiotropic effects. This can be seen in the case of *Lr34/Yr18*, which has provided slow rusting resistance to leaf and stripe rusts for over 50 years (Singh et al., 2007). Although *Yr18* does not provide sufficient resistance on its own, it

may be worthwhile to add *Yr18* to a gene pyramid as the locus provides resistance to many diseases. This locus also governs resistance to Barley yellow dwarf virus (*Bdv1*) and provides adult plant resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*) (Lagudah et al., 2009). This locus also causes leaf tip necrosis which serves as a phenotypic marker (Shah et al., 2011).

The linkage block of genes of *Lr46/Yr29* located on chromosome 1BL also is associated with resistance to powdery mildew (Lillemo et al., 2008). Recent studies have shown that *Lr67/Yr46* are pleiotropic or tightly linked and provide durable slow rusting adult plant resistance (Herrera-Foessel et al., 2011). The genes *Lr37/Yr17/Sr38* are closely linked on chromosome 2AS (Bariana and McIntosh, 1993). A recent study suggests that there may be incomplete linkage between *Lr52* and *Yr47* (Bansal et al., 2011). The frequency of gene clusters indicates that multiple gene complexes confer durable resistance to multiple diseases of wheat and there are more than likely many more yet to be discovered.

There are 47 chromosome identified regions that have an effect on stripe rust, and these were found on every chromosome except 5D (Rosewarne et al., 2013). Chromosome 1A is known to contain 4 QTL regions governing stripe rust resistance, including a recently mapped novel QTL on chromosome 1AS (Christopher et al., 2013). The *Lr46/Yr29* locus is located on chromosome 1B along with 2 other QTL that may or may not contain a novel resistance gene (Yang et al., 2013). Three QTL regions located on chromosome 1DS were considered to have minor effects. The QTL located on chromosome 2AS are mostly associated with *Yr17,* although QTL discover in Pioneer '26R61' did not have the alien translocation governing *Yr17,* and it may be assumed that these lines contain potentially new major genes for resistance (Hao et al., 2011). Both 2AS and 2AL also contain QTL with minor resistance genes. There are at least four regions associated with stripe rust resistance located within chromosome 2B. *QRYr2B.1* and *QRYr2B.2*

were both located on 2BS. *QRYr2B.2* has been identified in six different studies and was associated with resistant genes such as *Yr27, Yr31, Lr23,* and *Lr13*. *QRYr2B.2* may be considered a very gene rich region (Rosewarne et al., 2012; Yang et al., 2013). *QRYr2B.3* was also identified on chromosome 2BS and has been used extensively in breeding programs. The fourth region of 2B is associated with *Yr7*. There are three regions located on chromosome 2D, which may be associated with *Yr16*. A majority of the QTL identified on the group three chromosomes are located on the short arms. *QRYr3B.1* is known to contain the location of *Yr30,* which works well with other genes such as *Yr18*. The QTL *QRYr3D.1* and *QRYr3D.2* are located on chromosome 3DL and further fine mapping is needed to differentiate the two. Chromosome 4A contains very few QTL, although, it may contain a major all stage resistance gene (Ramburan et al., 2004). There are very few QTL associated with 4BL although there was recent novel QTL mapped on chromosome 4BL (Christopher et al., 2013). Few QTL exist on 4D, but those that do contain the gene locus *Lr67/Yr46/Sr55/Pm46* (Hiebert et al., 2010). The most significant region on chromosome 5A is QTL *QRYr5A.2*. Chromosome 5B also contains QTL *QRYr5B.1* and *QRYr5B.3* which are likely inherited together (Feng et al., 2011). *QRYr6A.2* is located on chromosome 6AL and confers resistance associated with the Sr6 translocation (Rao, 1996). Chromosome 6B contains *QRYr6B.2* which is associated with HTAP gene *Yr36* (Uauy et al., 2005). Chromosome 6DL contains minor QTL. Chromosome 7A contains 5 QTL regions governing stripe rust resistance, while chromosomes, 7B and 7D contain 3 and 1 QTL regions, respectively. A recently mapped novel QTL on chromosome 7D (Christopher et al., 2013), most QTL located on 7D are associated with *Yr18* (Rosewarne et al., 2013).

Stripe Rust Resistance in SRW Wheat

In a genetic study involving the inheritance of stripe rust resistance in three SRW wheat lines 'McCormick', VA96W-270, and VA96W-270V, three recessive races specific APR genes were found in McCormick. Two genes were found in VA96W-270 and at least one gene is common between McCormick and VA96W-270. Stripe rust resistance in VA96W-270V, which is a variant of VA96W-270, was determined to be controlled by one recessive all-stage resistance gene and one dominant adult-plant gene (Markell et al., 2009). In a recent mapping study one major and one minor QTL were located on the chromosomes 2AS and 6AS of Pioneer 25R61, respectively (Hao et al., 2011). Their results concluded that QTL *YrR61* was significantly different than *Yr17*, and the minor QTL *Qyr.uga-6AS* most likely conditions HTAP. In a recent study conducted using SRW wheat cultivars USG 3555 and Neuse as parents to identify QTL governing effective adult plant resistance to stripe rust, QTL were discovered on chromosomes 1AS, 4BL, and 7D of USG 3555 (Christopher et al., 2013). A QTL on chromosome 4BL was also identified in the Virginia Tech experimental wheat line VA00W-38 and the SRW wheat cultivar Coker 9553, of which both possess adult plant resistance to stripe rust.

CONCLUSIONS

Importance of Stripe Rust to Virginia and growers in the Southeastern U.S.

Due to the exceptional rate of mutation in *P. striiformis*, development of resistant varieties is an ongoing battle to combat this disease. Epidemics of stripe rust have occurred in one or more eastern states from 2000-2006. It was severe in Virginia in 2005 and prevalent in 2013. With recent revelations of virulence to Yr17, there is a pressing need to obtain additional resistance to stripe rust before serious damage is done in Virginia and the surrounding areas (Lee, 2013).

The SRW wheat cultivars Jamestown, USG 3555, Pioneer 26R61, 72014415, and 102015123 all exhibit some levels of adult plant resistance. In studies conducted at Washington State University in multiple locations and in greenhouse tests many SRW cultivars were evaluated for stripe rust. Virginia Tech experimental line VA11W-108, derived from Pioneer25R47/Jamestown population, and Jamestown expressed moderate resistance in the field and through greenhouse experiments and were postulated to have of HTAP resistance (Chen, 2014). Little is known about the genes conferring stripe rust resistance in Jamestown and this should be further investigated to further improve cultivars such as Shirley which is highly susceptible to stripe rust (Griffey et al., 2010).

Table 1.1. Catalogued leaf rust resistance genes, source, location, linked genes and markers, remarks, and citation. Obtained from ARS USDA, (McIntosh et al., 2013), and modified by the author.

Lr Gene	Origin/Source	Genome location	Linked Genes, Molecular Markers and other Remarks	References
1	Common Wheat	5DL		(McIntosh and Baker1970)
2a	Common Wheat	2DS		(Dyck and Samborski 1968)
2b	Common Wheat	2DS		(Dyck and Samborski, 1974)
2c	Common Wheat	2DS		(Dyck and Samborski, 1974)
3a	Common Wheat	6BL	Sr11, Xmwg798	(Dyck and Samborski, 1968)
3bg	Common Wheat	6BL	Sr11	(Haggag and Dyck 1973)
3ka	Common Wheat	6BL	Sr11	(Haggag and Dyck 1973)
9	Aegilops umbellulata	6BL	Xmwg684	(Sears 1956)
10	Common Wheat	lAS	Xsfr1	(Dyck and Kerber 1971)
11	Common Wheat	2A		(Dyck and Johnson1983)
12	Common Wheat	4BS	§	(Dyck et al. 1966)
13	Common Wheat	2BS	Ne2m, Lr23, §	(Dyck et al. 1966)
14a	Yaroslav emmer	7BL		(Dyck and Samborski 1970)
14b	Common Wheat	7BL		(Dyck and Samborski 1970)
15	Common Wheat	2DS	Lr2, Sr6	(Luig and McIntosh1968)
16	Common Wheat	2BS	Sr23	(Dyck and Samborski 1968)
17a	Common Wheat	2AS	Lr37, Sr38, Yr17, Xbarc212	(Dyck and Samborski 1968)
17b	Common Wheat	2AS	Lr37, Sr38, Yr17	(Dyck and Samborski 1968)
18	Triticum timopheevi	5BL		(Dyck and Samborski 1968)
19	Thinopyrum elongatum	7DL	Sr25	(Sharma and Knott 1966)
20	Common Wheat	7AL	Pm1, S15, Sr22, cdo347-7A, Xpsr121-7A, Xpsr680-7A,	(Browder 1972)

			Xpsr687-7A, Xbzh232(Tha)-7A, Xrgc607-7A and Xsts638-7A	
21	T. tauschii	1DL	XksuD14	(Rowland 1974)
22a	T. tauschii	2DS	Tg, W2, APR+, Xgwm455	(Rowland 1974)
22b	Common Wheat	2DS	Tg, W2, §	(Dyck 1979)
23	Durum Wheat	2BS	Lr13, Sr9, Xksu904	(McIntosh 1975)
24	Thinopyrum ponticum	3DL	Sr24	(Browder 1973)
25	Secale cereale	4BS	Lr48, Pm7	(Driscoll and Anderson 1967)
26	Secale cereale	lBL	Sr31, Yr9	(Singh et al 1990)
27	Common Wheat	3BS	Sr2, Functional only with Lr31	(Singh and McIntosh 1984)
28	Ae. speltoides	4AL		(McIntosh 1982)
29	Thinopyrum ponticum	7DS		(Sears 1973)
30	Common Wheat	4BL		(Dyck and Kerber 1981)
31	Common Wheat	4BS	Functional only with Lr27, Possible commonality with Lr12	(Singh and McIntosh 1984)
32	T. tauschii	3D	Xbcd1278	(Kerber 1987)
33	Common Wheat	1BL	Lr26	(Dyck 1987)
34	Common Wheat	7D	Yr18, Bdv1, Ltn (leaf tip necrosis), Pm38, Xwg83	(Dyck 1987)
35	Ae. speltoides	2B	Sr32, §	(Kerber and Dyck 1990)
36	Ae. speltoides	6BS		(Dvorak and Knott 1990)
37	Ae. ventricosa	2AS	Sr38, Yr17, Xcmwg682	(Bariana and McIntosh 1993)
38	Thinopyrum intermedium	2AL		(Friebe et al. 1992)
39	T. tauschii	2DS	Xbarc124- 2D, Xgwm210-2D, Xgdm35-2D and Xcfd36-2D	

40	-	-	†	-
41	-	-	†	-
42	T. tauschii	1D	Xwmc432	(Cox et al 1994)
43	-	-	†	-
44	T. spelta	1BL		(Dyck and Sykes 1994)
45	Secale cereale	2AS		(McIntosh et al 1995)
46	Common Wheat	1BL	Yr29, §, Xgwm140	(Singh and Huerta-Espino 1998)
47	A. speltoides	7AS		(Dubcovsky et al 1998)
48	Common Wheat	4BL	Lr25, § with Lr34, Xwmc332	(Saini et al. 2002)
49	Common Wheat	2AS	§ with Lr34	(Saini et al. 2002)
50	T. timopheevi	2BL	Xgwm382	(Brown-Guedira et al. 2003)
51	Ae. speltoides	1BL	XAga7	(Helguera 2005)
52	Common Wheat	5BS		(Hiebert et al. 2005)
53	T. dicoccoides	6BS	Xcfd	(Marais et al. 2005)
54	A. kotschyi	2DL		(Marais et al. 2005)
55	Elymus trachycaulis	1B□		(Brown-Guedira 2005)
56	Ae. sharonensis	6A	Yr38	(Marais et al. 2006)
57	Ae. geniculata	5DS	Yr40, Gsp, Xfbb276 and Xbcd873	(Kuraparthy et al, 2007)
58	Ae. triuncialis	2BL	Xcfd50	(Marais et al. 2007)
59	Ae. peregrina	1AL		(Marais et al. 2007)
60	Common Wheat	1DS		(Hiebert 2007)
61	T. turgidum	6BS		(Herrera-Foessel et al 2008)
62	Ae. neglecta	6A	Yr42	(Marais et al 2009)
63	T. monococcum	3AS	Xbarc321/Xbarc57	(Kolmer et al. 2010)
64	T. dicoccoides	6AL		(McIntosh et al. 2009)

65	T. Spelta	2AS	Xwmc382	(Mohler, Volker, et al. 2012)
66	Ae. speltoides	3A		(McIntosh et al. 2009)
67	Common Wheat	4DL	Pleiotropic with Yr46 and Sr55, APR+, Xcfd71 and Xbarc98	(Hiebert, Colin, et al. 2010)
68	Yet to be named	7BL	§	(Herrera-Foessel, Sybil et al. 2012)
69	Yet to be named	3DL		(Barcellos Rosa S et al. 2012)
70	Common Wheat	5DS	Xgwm190	(Hiebert et al. 2014)
71	T. spelta	1B‡	Xbarc137	(Singh et al. 2012)
72	Durum Wheat	7BS	5.0 cM - Xwmc606-7B	(Herrera-Fossel et al. 2013)
73	Common Wheat	2BS	1.4 cM - wPt8235	(Park et al. 2013)
74	Unknown	3BL	GBS2256311 - 3.9 cM, §	(Bansal 2014)

Table does not include temporarily designated genes for leaf rust resistance.

† Lr40, Lr41 and Lr43 have been deleted. Lr40 was shown to be Lr21; Lr41 shown to be Lr39; and Lr43 is not a unique gene, germplasm line had Lr21 and Lr39.

‡ 1B centromere region not resolved

§ Adult Plant Resistance

Table 1.2. Catalogued stripe resistance rust genes, source, location, linked genes and markers, remarks, and citations. Obtained from ARS USDA, (McIntosh et al., 2013), and modified by the author.

Yr Gene	Origin Source	Genome location	Linked Genes, Molecular Markers and other Remarks	References
1	Chinese 166	2A	Xgwm382-2AL, Xgwm311-2AL, Xfba8a-2AL, Xstm673acag	(Lupton FCH & Macer RCF, 1962)
2	Heines Vll	7B	Recessive	(Lupton FCH & Macer RCF, 1962)
3a	Capelle-Desprez	1B	Xwmc356- 2B	(Lupton FCH & Macer RCF, 1962)
3b	Hybrid 46	1B		(Lupton FCH & Macer RCF, 1962)
3c	Minister	1B		(Lupton FCH & Macer RCF, 1962)
4a	Capelle-Desprez	6B		(Lupton FCH & Macer RCF, 1962)
4b	Hybrid 46	6B		(Lupton FCH & Macer RCF, 1962)
5	Triticum spelta album	2BL	Xwgp17-2B, Xwgp19-2B, Xwgp26-2B, Xbarc349-2B and YrSTS-7/8	(Macer RCF, 1966)
6	Heines Kolben	7BS		(Macer RCF 1966)
7	Triticum turgidum	2BL	Xgwm526-2B, Sr9g	(Macer RCF 1966)
8	T. comosa	2D	Sr34	(Riley R, Chapman V & Johnson R, 1968)
9	Secale cereale	1BL	Xgwm582-1BL, Sr31, Lr26	(Macer RCF, 1975)
10	Moro	1BS	Xpsp3000-1B, RgaYr10a	(Macer RCF, 1975)
11	Joss Chambier		§	(Priestley RH, 1978)
12	Mega		§	(Priestley RH, 1978)
13	Maris Huntsman		§	(Priestley RH, 1978)
14	Hobbit		§	(Priestley RH, 1978)

15	T. dicoccoides G-25	1BL	Xgwm33	(McIntosh RA, Silk J & The TT, 1996)
16	Capelle-Desprez	2DS	§	(Worland AJ & Law CN, 1986)
17	T. ventricos	2AS	Lr37, Sr38, Xcmwg682	(Bariana HS & McIntosh RA, 1993)
18	Frontana	7D	Lr34, Pm38, Ltn, Bdv1, Xgwm295,Xgwm120	(Singh RP, 1992)
19	Compair	5B		(Chen XM, Jones SS & Line RF, 1995)
20	Fielder	6D		(Chen XM, Jones SS & Line RF, 1995)
21	Lemhi	1B	YrRpsLem	(Chen XM, Jones SS & Line RF, 1995)
22	Lee	4D		(Chen XM, Jones SS & Line RF, 1995)
23	Lee	6D		(Chen XM, Jones SS & Line RF, 1995)
24	T. turgidum (K733 durum)	1BS	Xgwm11-1B, Yr24 is identical to Yr26	(McIntosh RA et al, 1995)
25	TP1295	1D		(Calonnec A & Johnson R, 1998)
26	Haynaldia villosa	1BS	Xgwm11, Xgwm18, Yr26 is identical to Yr24	(Yildirim, Jones, Murray & Line RF, 2000)
27	Selkirk	2BS	Xcdo152, Xcdo405	(McDonald D, McIntosh RA, Wellings CR, Singh RP & Nelson JC, 2004)
28	T. tauschii W-219	4DS	Xmwg634	(Singh RP, Nelson JC & Sorrells ME, 1998)
29	Lalbahadur	1BL	Ltn2, Lr46, §	(William M, Singh RP, Huerta-Espino J, Islas SO & Hoisington D, 2003)
30	Opata 85	3BS	Sr2, Lr2, §	(Singh RP, Personal communication, 2000)

31	Pastor	2BS		(Singh RP, William HM, Huerta-Espino J & Crosby M., 2003)
32	Carstens V	2AS	Xwmc198	(Eriksen L et al, 2004)
33	Batavia	7DL	Xgwm111, Xgwm437	(Zahravi M et al, 2003)
34	WAWHT2046	5AL	Xgwm410.2, §	(Bariana HS et al, 2006)
35	T. dicoccoides	6BS	Lr53	(Marais GF et al, 2005)
36	T. dicoccoides	6BS	Yr36 is between Xucw74-6B and Xucw77-6B, §	(Chicaiza O et al, 2005)
37	Ae. kotschyi	2DL		(Marais GF et al 2005)
38	Aegilops sharonensis	6A	Lr56	(Marais GF et al, 2006)
39	Alpowa	7BL	HTAP resistance	(Lin F & Chen XM, 2007)
40	Aegilops geniculata	5DS	Gsp, Xfbb276,Xbcd873, Lr57	(Kuraparthy V et al, 200&)
41	Yet to be named	2BS	Xgwm410	(Luo PG et al, 2008)
42	Ae. neglecta		Lr62	(Marais F et al, 2009)
43	PI 591045	2BL		(Cheng P & Chen XM, 2009)
44	Zak			(Cheng P & Chen XM, 2009)
45	PI 181434	3DL	Xbarc6, †	(Li Q et al, 2011)
46	RL6077	4DL	Sr55, Lr67, §	(Herrera-Foessel et al, 2011)
47	AUS28183	5BS	‡	(Bansal et al, 2011)
48	UC1110	5AL	Co-segregated with Vrn-2A, BE495011, Xcfa2149-5AL, Xgpw2181a-5AL, Xwmc74-5AL, and Xwmc410-5AL, §	(Lowe et al, 2011)
49	Chuanmai	3DS	§	(Spielmeyer W, et al. 2010 Personal communication)
50	Th. intermedium	4BL		(Liu J et al, 2009)

51	AUS 91456	4AL		(Bansal U et al. 2011 Personal communication)
52	PI 183527	7BL	§	(Ren RS et al, 2012)
53	PI 480148	2BL		(Xu LS et al, 2013)

†This gene is highly effective and confers resistance to all North American Pst pathotypes.

‡ This is a seedling resistance gene (IT 1CN), effective against the main Australian groups of Pst. V336 is the original source of Lr52.

§ Adult Plant Resistance

Table 1.3. Changes in *P. Striiformis* populations from 2000 to 2005			
Race	Date	Remarks	References
PST-78, PST-80	2000	The most prevalent races	(Chen, 2007)
PST-90	2001	Combination of virulence on 'Tres' and virulences of PST-78	(Chen, 2007)
PST-78	2002	The most prevalent race	(Chen, 2005; Chen, 2007)
PST-97	2002	Virulence's of PST-78 plus virulence to Stephens	(Chen, 2005; Chen, 2007)
PST-98	2002	Virulence's of PST-80 plus virulence to Stephens	(Chen, 2005; Chen, 2007)
PST-99	2002	Virulence's of PST-78 plus virulence's to Stephens and Yamhill	(Chen, 2005; Chen, 2007)
PST-98,PST-100	2003	The most prevalent races	(Chen, 2005; Chen, 2007)
PST-102	2003	Virulence to 'Tres' and the virulence's of PST-100	(Chen, 2005; Chen, 2007)
PST-100	2004	The most prevalent race	(Chen, Penman, et al., 2010; Chen, 2007)
PST-111	2004	Virulence to Paha and the virulence's of PST-100	(Chen, Penman, et al., 2010; Chen, 2007)
PST-115	2004	Virulence to Paha and the virulence's of PST-102	(Chen, Penman, et al., 2010; Chen, 2007)
PST-114	2004	Virulence to Moro and the virulence's of PST-102	(Chen, Penman, et al., 2010; Chen, 2007)
PST-115, PST-100, PST-102	2005	The most prevalent races	(Chen, Penman, et al., 2010; Chen, 2007)
PST-116	2005	Virulence of PST-115 plus virulence on Moro	(Chen, Penman, et al., 2010; Chen, 2007)

Table 1.4. Top Pst races in 2013 reported (Xianming Chen, personal communication, 2014)		
Race	Virulence	Avirulence
PSTv-52	Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr43, Yr44, YrExp2	Yr1, Yr5, Yr10, Yr15, Yr24, Yr32, YrSP, YrTr1, YrTye
PSTv-37	Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr43, Yr44, YrTr1, YrExp2	Yr1, Yr5, Yr10, Yr15, Yr24, Yr32, YrSP, YrTye
PSTv-73	Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr43, Yr44, YrExp2, YrTye	Yr1, Yr5, Yr10, Yr15, Yr24, Yr32, YrSP, YrTr1
PSTv-11	Yr1, Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr43, Yr44, YrExp2, YrTye	Yr5, Yr10, Yr15, Yr24, Yr32, YrSP, YrTr1
PSTv-14	Yr1, Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yr43, Yr44, YrTr1, YrExp2, YrTye	Yr5, Yr10, Yr15, Yr24, Yr32, YrSP

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CHAPTER II

Identification of Quantitative Resistance to *Puccinia striiformis* and *Puccinia triticina* in the Soft Red Winter Wheat Cultivar 'Jamestown'

Neal R. Carpenter, Carl A. Griffey, Subas Malla, Marla Barnett, David Marshall, Myron O. Fountain, J. Paul Murphy, Eugene Milus, Jerry Johnson, James Buck, Shiaoman Chao, Gina L. Brown-Guedira, and Emily Wright

N.R. Carpenter and C.A. Griffey, Dep. of Crop and Soil Environmental Sciences, Virginia Tech, Blacksburg, VA 24060; S. Malla, Texas A&M AgriLife Research & Extension Center, Texas A&M, Uvalde, TX 78801; M. Barnett, Limagrain Genetics, Wichita, KS 67204; D. Marshall and M.O. Fountain, USDA-ARS, Raleigh, NC 27695; J.P. Murphy, Dep. of Crop Science, North Carolina State Univ., Raleigh, NC 27695; E. Milus, Dep. of Plant Pathology, Univ. of Arkansas, Fayetteville, AR 72701; J. Johnson, Dep. of Crop and Soil Sciences, Univ. of Georgia, Griffin, GA 30223; J. Buck, Dep. of Plant Pathology, Univ. of Georgia, Griffin, GA 30223; S. Chao, USDA-ARS, Fargo, ND 58102; G.L. Brown-Guedira, USDA-ARS, North Carolina State Univ., Raleigh, NC 27695; E. Wright, Dep. of Agronomy, Iowa State Univ., Ames, IA 50010.

Corresponding author (cgriffey@vt.edu).

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ABSTRACT

Disease resistance is critical in soft red winter wheat (*Triticum aestivum* L.) cultivars. Leaf rust caused by *Puccinia triticina* Eriks and stripe rust caused by *Puccinia striiformis* Westend. f.sp. *tritici* Eriks. are destructive pathogens of wheat. Phenotypic data were collected at diverse locations for resistance to leaf rust (North Carolina, Texas, and Virginia) and stripe rust (Arkansas, North Carolina, Georgia, Texas, and Virginia) in a Pioneer '25R47' /'Jamestown' (P47/JT) population composed of 186 $F_{5:9}$ recombinant inbred lines (RILs). The P47/JT RILs were genotyped with a public 90K iSelect single-nucleotide polymorphism array. Analysis of the P47/JT population identified two quantitative trait loci (QTL) for leaf rust resistance on chromosome 5B and two QTL for stripe rust resistance on chromosomes 3B and 6A. These QTL were associated with both infection type and disease severity. Phenotypic variation (%) explained by the putative leaf rust resistance QTL of Jamestown on 5B was as high as 22.1%. Variation explained by the putative stripe rust resistance QTL of Jamestown on 3B and 6A was as high as 11.1 and 14.3%, respectively. Introgression and pyramiding of these QTL with other genes conferring resistance to leaf and stripe rusts via marker-assisted selection will facilitate development of soft red winter wheat cultivars having more durable resistance.

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INTRODUCTION

Leaf rust (*Puccinia triticina* Eriks) is the most common type of rust of wheat (*Triticum aestivum* L.) worldwide (Bolton et al., 2008) and can be found on every continent with the exception of Antarctica (Huerta-Espino et al., 2011). In the southeastern US soft red winter wheat region, disease severity will typically peak during April in Georgia and at the end of May in Virginia (Kolmer and Hughes, 2013). Losses from leaf rust are typically less severe than those resulting from the other two common rust diseases, stem rust (*Puccinia graminis* Pers.:Pers.) and stripe rust (*Puccinia striiformis* Westend. *f.sp. tritici* Eriks.), but leaf rust causes greater overall losses due to its wider distribution and occurrence (Huerta-Espino et al., 2011). Selection pressure forced on the pathogen population by the presence of only a few resistance genes deployed among the predominant wheat cultivars has resulted in extensive genetic diversity among *P. triticina* virulence phenotypes (Kolmer, 1992). Control of leaf rust through deployment of diverse and durable genetic resistance in cultivars, as opposed to reliance on fungicides, has been demonstrated to be the most cost-effective method, with an estimated 27:1 benefit to cost ratio (Marasas et al., 2003). There are >74 leaf rust resistance genes that have been mapped to chromosome locations and given gene designations, as well as numerous temporarily designated leaf rust resistance genes (McIntosh et al., 2013). Due to the highly variable nature of *P. triticina*, durable leaf rust resistance in wheat cultivars has been difficult to achieve.

Stripe rust (*P. striiformis*) of wheat is considered to be one of the most widely destructive plant diseases in the world and one of the most important diseases of wheat since the 1960s (Line, 2002). Stripe rust reduces grain and forage yield and quality. Seed harvested from crops infected with stripe rust often exhibit low vigor and emergence (Chen, 2005). Stripe rust consumes water and nutrients from the host plant, weakening the plant (Chen, 2005). In 2000, 21 new races of

stripe rust were identified in the United States, and among these new races, eight had combinations of virulence to resistance genes that were previously known to provide exceptional resistance in the United States (Chen et al., 2002). From 2000 to 2007, there were a total of 115 identified races of stripe rust (Chen et al., 2010), and by 2010, that number had grown to 146 (Wan and Chen, 2014). New races were identified that are able to germinate at warmer temperatures, allowing for disease development later in the season. This new stripe rust population has increased adaptation and fitness yet contains many virulence alleles that are not required to overcome resistance in soft red winter wheat cultivars east of the Rocky Mountains (Chen, 2005; Dracatos et al., 2016). There are >60 stripe rust resistance genes that have been mapped to chromosome locations and given gene designations, and there are also numerous temporarily designated stripe rust resistance genes (McIntosh et al., 2013). The common incorporation of race-specific resistance genes into commercial cultivars due to their simple inheritance makes use of such genes that are appealing in breeding programs. However, the most severe epidemics caused by stripe rust were the result of failure of race-specific resistance genes in widely grown cultivars. Stripe rust genes are classified into two categories: all-stage resistance (race-specific, hypersensitive resistance), or qualitative resistance, and high-temperature adult plant resistance (HTAP), or quantitative resistance (Chen, 2007). Expression of these genes results in various amounts of chlorosis and/or necrosis, depending on the level of resistance of the plant and environmental factors such as temperature. Due to the highly variable nature *of P. striiformis*, durable stripe rust resistance in wheat cultivars has been difficult to achieve.

Adult plant resistance, or quantitative resistance, is more durable and effective against multiple races of a pathogen. Introgression of multiple seedling resistance genes and adult plant resistance genes into elite cultivars will result in broader spectrum and more durable resistance (Griffey and

Allan, 1988). Multiline cultivars and gene pyramiding have been successfully used to control stripe rust (Chen, 2007). Development of gene pyramids requires the identification of diverse genes and QTL for resistance, and their combined incorporation into a high-yielding cultivar (Singh et al., 1992).

Soft red winter wheat cultivars Jamestown and Pioneer '25R47' were crossed and used to develop recombinant inbred lines to map QTL associated with resistance to leaf and stripe rust. Jamestown has been documented to have leaf rust resistance and displayed HTAP resistance to stripe rust in regional nurseries across the United States. The soft red winter wheat cultivar Jamestown (PI 653731) is productive in the southern Corn Belt, the Deep South, and throughout the mid-Atlantic region. This can be attributed to its resistance to leaf rust, stripe rust, and Hessian fly (*Mayetiola destructor* L.) (Griffey et al., 2010) The objectives of this study were to characterize QTL conferring resistance to leaf rust and stripe rust, and to identify or develop diagnostic DNA markers that can be used in marker-assisted breeding to pyramid these genes with other complementary genes to provide effective and durable resistance.

MATERIALS AND METHODS

Plant Materials

One hundred and eighty-six RILs were derived from the cross of Pioneer '25R47' (PI 631473) (Lively et al., 2004) by Jamestown (PI 653731) (Griffey et al., 2010). The cultivars are adapted to the eastern US soft red winter wheat growing region. The cultivar Jamestown was derived from the cross 'Roane'/Pioneer '2691' developed by Virginia Tech. Pioneer Hi-Bred International developed cultivar 25R47 from the cross WBE-2190-B-1 ('Frankenmuth' /Pioneer '2555' sib//Pioneer '2551' sib)/WBA-416-H-2 ('Houser' /MO-9545//W-4034-D/'Augusta')//Pioneer '2552'.

Field Assessment

The RIL population was evaluated in replicated field tests composed of 1.2-m rows as the experimental unit arranged in a randomized complete block design. Disease-resistant and susceptible checks, along with both parents of the population, were spaced throughout the design. Disease spreader strips of the leaf rust-susceptible cultivar 'Massey' (PI 17953) (Starling et al., 1984) and stripe rust-susceptible line VA10W-21 (Z00-5018/VA01W-158) bordered the RIL population at each location.

Leaf rust assessments of the RIL population were conducted at Warsaw, VA (one replication in 2013–2014 and two replications in 2014–2015), where susceptible borders were inoculated with leaf rust race TNRJ using Soltrol light mineral oil in addition to natural infection. The RIL population was also evaluated at Castroville, TX (one replication), and Plymouth, NC (two replications), under natural infection in 2014 and 2015.

Stripe rust assessments of the RIL population was conducted at Blacksburg, VA (one replication in 2013–2014 and two replications in 2014–2015), where susceptible borders were infected by transplanting inoculated seedlings of VA10W-21 infected with the race PST-100 in addition to any naturally occurring infection. Race PST-100 was classified and designated under the old US race nomenclature system and differential host set and is similar to predominant races PSTv-32 and PSTv-52, which were identified using the newer set of 18 stripe rust single-gene line differentials. The RIL population was also evaluated at Castroville, TX (one replication), and Laurel Springs, NC (two replications), under natural infection in both years. In addition, the population was evaluated 1 yr each in Fayetteville, AR (two replications), in 2014 and in Griffin, GA (two replications), in 2015, both under natural infection.

Disease ratings were assessed when the susceptible checks displayed no less than 30% severity. The population was assessed for infection type using a 0-to-9 scale (Line and Qayoum, 1992; Singh et al., 1992) and disease severity from 0 to 100% based on the modified Cobb scale (Peterson et al., 1948) for both *P. triticina* and *P. striiformis*. Each rating was recorded one to three times at each location per growing season, dependent on optimal infection levels of *P. triticina* and *P. striiformis*.

DNA Extraction

Tissue of each RIL was collected when seedlings reached the three-leaf stage and placed into 2-mL test tubes, each containing two stainless steel beads for tissue grinding. Tissue samples were frozen in an ultra-low-temperature (−80°C) freezer and then subsequently ground using a Spex CertiPrep 2000 Geno-Grinder for 15 s or until finely ground. The DNA extraction was then implemented using a modified cetrimonium bromide (CTAB) method (Saghai-Maroof et al., 1984).

Microsatellite Assay

Over 400 simple sequence repeat (SSR) markers were analyzed using bulk segregant analysis of 142 samples with 71 of each extreme phenotype. The SSR primer pairs were synthesized by Applied Biosystems (Carlsbad, CA) and Integrated DNA Technologies (Coralville, IA). Primers were directly labeled with a fluorescent dye or indirectly labelled with an M13 fluorescent tail (5′-ACGACGTTGTAAAACGAC-3′ or 5′-CACGACGTTGTAAAACGAC-3′). Simple sequence repeats were run using similar procedures to Christopher et al. (2013). The polymerase chain reaction (PCR) products of four separate M13-PCRs were combined for analysis in an Applied Biosystems 3130xl Genetic Analyzer. The PCR products were transferred to a 96-well

PCR plate with each well containing 9.9 μL of Hi-Di formamide and 0.1 μL of size standard. Samples were denatured at 95°C for 5 min. The PCR products were visualized on an Applied Biosystems 3130xl Genetic Analyzer, and the generated data were analyzed using the genotyping software Genemarker version 1.70 (SoftGenetics, 2007).

Single-Nucleotide Polymorphism Array

A 90K iSelect single-nucleotide polymorphism (SNP) genotyping assay was performed on the RIL population at the USDA-ARS Small Grains Genotyping Laboratory in Fargo, ND. Genome Studio v2.0 software (Illumina, 2016)was used to analyze the SNPs according to genotype. Procedures for calling SNP(s) were similar to the procedures used in Cavanagh et al. (2013) and Wang et al. (2014). In summary, there were multiple parental clusters called, similar to Liu et al. (2016), which were then converted to the A, B, H format (Supplemental Table 2.1). Manual scoring was assessed for each SNP cluster that could not be categorized by the default algorithm. Each SNP cluster was manually authenticated by visually assessing each cluster. KASPar oligos were ordered from Integrated DNA Technologies, with primers carrying standard FAM or VIC compatible tails (FAM tail: 5′-GAAGGTGACCAAGTTCATGCT-3′, VIC tail: 5′-GAAGGTCGGAGTCAACGGATT3′) and the target SNP in the 3′ end. Primer mix was set up as recommended by LGC Genomics (46 μL distilled H2O, 30 μL common primer [100 μM], and 12 μL of each tailed primer [100 μM]). The KASPar primers were used for functional validation of results found in the QTL analysis.

Linkage Map Construction and QTL Analysis

Linkage maps were constructed using JoinMap 4.0 (Van Ooijen, 2006), the Kosambi mapping function (Kosambi, 1943) was used to estimate map distance, and linkage groups were

constructed based on a minimum logarithm of odds (LOD) threshold value of 3.0. Windows Cartographer (WinQTLCart version 2.5) (Wang et al., 2007), R/QTL (Broman et al., 2003), and IciMapping 4.1.0 (Wang et al., 2012) were used to identify QTL via interval mapping and composite interval mapping. The critical LOD value of 3.0 to declare a QTL significant (p = 0.05) was based on 1000 permutations (Doerge and Churchill, 1996) for all traits and linkage groups. MapChart 2.2 (Voorrips, 2002) was used to draw linkage maps.

RESULTS

Linkage Maps

Markers were placed on all 21 chromosomes, but there was low coverage on the D genome due to a majority of the markers being in complete correlation. The SSRs found to be polymorphic among the bulk segregant analysis were genotyped for the entire population. A total of 1760 unique SNP (1685) and SSR markers (75) were polymorphic between Jamestown and Pioneer '25R47'. Among these markers, 1682 were mapped in the final linkage analysis use to segregation distortion or poor quality of some SNPs and SSRs.

QTL Analysis

Four QTL were identified in Jamestown including two associated with leaf rust resistance on chromosome 5B and two for stripe rust resistance residing on chromosomes 3B and 6A. Markers linked to each QTL with the highest LOD scores in each year for each trait are presented in Tables 2.1 and 2.2. The QTL on chromosome 5B conferring leaf rust resistance has the largest effect and was identified in each year for both infection type and disease severity. The smaller-effect QTL on 3B and 6A for stripe rust infection type were identified in each year.

Leaf Rust Resistance QTL on Chromosome 5B

The LOD peak of the QTL located on chromosome 5B, designated QLr.vt-5B.1 (Fig. 2.1), and was located at SNP markers IWB7835 and IWB24418 between positions 22 and 25 cM, respectively. The second QTL, designated QLr.vt-5B.2 on 5B, was located between positions 38 and 39 cM at SNP markers IWB32871 and IWB26068 (Fig. 2.1). The QTL *QLr.vt-5B.1* and *QLr.vt-5B.2* are located at separate physical positions on the current survey sequence (Supplemental Table 2.2) (IWGSC RefSeq v1.0) and multiple other genetic maps (International Wheat Genome Sequencing Consortium, 2014; Chapman et al., 2015). Phenotypic variation in infection type explained by QLr.vt-5B.1 was highest (22.1%) in the 2015 Virginia test, 3.7% in the 2014 Virginia test, and 1.7% in the 2014 North Carolina test (Table 2.1). The LOD scores were 4.6, 7.9, and 4.2, respectively. Phenotypic variation in leaf rust severity explained by *QLr.vt-5B.1* also was highest (16.3%) in the 2015 Virginia test, 4.2% in the 2014 Virginia test, 10.7% in the 2015 Texas test, and 1.6% in the 2014 North Carolina test. The LOD scores were 9.1, 3.7, 4.2, and 3.1, respectively. Phenotypic variation in infection type explained by QLr.vt-5B.2 was highest for Virginia tests in 2015 (5.5%) and 2014 (4.0%), 3.3% in the 2015 Texas test, and 2.2% in the 2014 North Carolina test (Table 2.1). The LOD scores were 9.0, 9.5, 8.0 and 4.2, respectively. Phenotypic variation in disease severity explained by *QLr.vt-5B.2* was highest (8.1%) in the 2015 North Carolina test, 5.1 and 3.5% in the 2015 and 2014 Virginia tests, and 2.4% in the 2015 Texas test. The LOD scores were 4.2, 6.8, 5.9, and 3.9, respectively. The variation of the phenotypic variation of QTL *QLr.vt-5B.1* and *QLr.vt-5B.2* can be explained by the difference in the infection type and severity from multiple environments, and subsequently the environment × genotype interactions (Fig. 2.2).

Stripe Rust Resistance QTL on Chromosome 3B

The LOD peak of the QTL located on chromosome 3B, designated *QYr.vt-3B*, was located at SNP markers IWB60584 and IWB23272 between positions 61 and 65 (Fig. 2.3). This QTL explained 8.9% of the phenotypic variation for infection type in the 2014 Virginia test, 6.9% in the Texas test, and 8.7 (2014) and 7.4% (2015) in the North Carolina tests (Table 2.2). The LOD scores were 7.1, 3.3, 7.0, and 4.4, respectively. For disease severity, QYr.vt-3B explained 11.1% of the variation in the 2015 North Carolina test and 8% in the 2014 Virginia test with LOD scores of 10.2 and 4.4, respectively. The slight variation of the of the phenotypic variation of QTL *QYr.vt-3B* can be described by the variance in the infection type and severity from Laurel Springs was more severe than in Blacksburg in both 2014 and 2015 (Fig. 2.4).

Stripe Rust Resistance QTL on Chromosome 6A

The LOD peak of the QTL located on the long arm of chromosome 6A between positions 83 and 87.5, designated *QYr.vt-6A* (Fig. 2.5), was located at SNP markers IWB5971and IWB63000. The phenotypic variation in infection type explained by this QTL was highest for the Virginia test in 2015 (14.3%) and 2014 (12.2%), and 6.3% for the 2014 North Carolina test (Table 2.2). The LOD scores were 13.1, 3.7, and 4.0, respectively (Table 2.2). For severity, *QYr.vt-6A* accounted for 7.7 and 1.9% of the variation in the Virginia and North Carolina tests, respectively, with an LOD score of 3.9 for both tests. The variation of the of the phenotypic variation of QTL *QYr.vt-6A* can be explained by the difference in the infection type and severity from Laurel Springs in 2014 was less severe than in Blacksburg in 2014 and 2015 (Fig. 2.6).

Phenotypic Effects of QTL Marker Loci on Leaf and Stripe Rust Resistance

To predict the effect of individual and pyramided QTL on leaf rust and stripe rust resistance, individuals (RILs) of the mapping population containing different combinations of the QTL were delineated into separate groups (Tables 2.3 and 2.4). The means of each group of RILs associated with disease infection type and severity were compared using a Students t test. Lines containing both *QLr.vt-5B.1* and *QLr.vt-5B.2* were similar to lines having only *QLr.vt-5B.2* but had significantly lower infection type and severity than the lines containing only *QLr.vt-5B.1*. Lines containing both *QLr.vt-5B.1* and *QLr.vt-5B.2* were similar to lines having only *QLr.vt-5B.2* but had significantly lower infection type and severity than the lines containing only *QLr.vt-5B.1*. This indicates that there may be some interaction between *QLr.vt-5B.1* and *QLr.vt-5B.2* (Table 2.3). Lines possessing a combination of *QYr.vt-3B* and *QYr.vt-6A*, *QYr.vt-3B* alone, or *QYr.vt-6A* alone were not statistically different, which indicates that these two QTL may not have major additive or epistatic effects (Table 2.4).

DISCUSSION

Leaf Rust Resistance QTL on Chromosome 5B

Two known leaf rust genes, *Lr18* and *Lr52*, have previously been reported to reside on chromosome 5B (McIntosh, 1983; Hiebert et al., 2005). It is unlikely that the source of resistance in Jamestown is *Lr52*, as this gene provided high resistance in seedling tests conducted by Hiebert et al. (2005), whereas seedlings of Jamestown and RILs in the Pioneer '25R47'/Jamestown population expressed only moderate resistance (N.R. Carpenter, C. Griffey, S. Malla, Shiaoman Chao, G.Brown-Guedira, unpublished data, 2017). The QTL on 5B most likely are flanking *Lr18* and conceivably working epistatically or additively with *Lr18* (N.R. Carpenter, C. Griffey, S. Malla, Shiaoman Chao, G.Brown-Guedira, unpublished data, 2017). Two other regions on 5B have been reported to be a cluster of QTL associated with leaf rust

resistance (Soriano and Royo, 2015). The QTL located on 5B by Prins et al. (2011) (barc4) and Zhou et al. (2014) (barc128) were on the short arm of the chromosome, which is distant from *QLr.vt-5B.1* and *QLr.vt-5B.2* based on consensus maps (Somers et al., 2004; Wang et al., 2014; Yu et al., 2014; Maccaferri et al., 2015). A QTL detected in cultivar Capo (wPt-7006) was located close to the same position as *QLr.vt-5B.2* (Buerstmayr et al., 2014) indicating these may be the same QTL (Supplemental Table 2.3). *QLr.vt-5B.2* on chromosome 5B contains SNP markers that are higher throughput and less costly to evaluate than the diversity arrays technology (DArT) marker located on Capo. The QTL *QLr.cdl-5BL* (Xfcp) and *QLr.fcu-5BL* (wPt-0837) are located on the more distal end of chromosome 5B (Chu et al., 2009; Kolmer, 2015) and 5 to 13 cM away from *QLr.vt-5B.2* (Maccaferri et al., 2015). This indicates that QLr.vt-5B.2 is likely not in the same region as the QTL reported by Kolmer (2015) and Chu et al. (2009) (Supplemental Table 2.3). Further future research, requiring a larger fine mapping population would be necessary to definitively prove *QLr.vt-5B.1* and *QLr.vt-5B.2* are separate QTL and differ previously identified QTL.

Stripe Rust Resistance QTL on Chromosome 3B

Although numerous QTL for stripe rust have been reported on chromosome 3B, they are located a significant distance from *QYr.vt-3B* (Somers et al., 2004; Wang et al., 2014; Yu et al., 2014; Maccaferri et al., 2015). These include *QYr-3B.1-Pavon76, QYr.cim-BS_Chapio_Yr30, QYr.tam-3B_Quaiu, QYr.cim-3BS.2_Franklin, QYr-3B_Oligoculm, QYr-3B_Alturas, QYr.inra-3BS_Renan, QYr.ucw-3BS_UC1110, QYr.ucw-3BS.2*, and *QYr.uga_AGS2000*. All of these QTL were mapped to the proximal end of the short arm of chromosome 3B, which is a significant distance from *QYr.vt-3B* (Somers et al., 2004; Wang et al., 2014; Yu et al., 2014; Maccaferri et al., 2015). The QTL *QYrpi.vt-3BL_VA00W-38* (wPt-0267) was originally mapped to the long

arm of chromosome 3B; however, its location based on newer consensus maps may actually reside on the short arm of chromosome 3B like the QTL described above (Somers et al., 2004; Wang et al., 2014; Yu et al., 2014; Maccaferri et al., 2015). The *QTL QYR.sun-3B_Wollaroi* (wPt-9577), *QYrid.vi.ui-3B_Rio Blanco* (gwm299), and *QYrex.wgp-3BL_Express* (gwm299) map to the long arm of chromosome 3B. However, these QTL are significantly closer to the distal end of the long arm of chromosome 3B than *QYr.vt-3B* (Supplemental Table 2.3).

Stripe Rust Resistance QTL on Chromosome 6A

QYr.vt-6A is located on the long arm of chromosome 6A. The QTL *QYr.uga-6AS-26R61* (wPt-671561), *QYr.wgp-6AS_Express* (gwm334), and *QYr.cim-6A_Avocet* (wPt-2573) are located on the short arm (Somers et al., 2004; Wang et al., 2014; Yu et al., 2014; Maccaferri et al., 2015). *QYr.cim-6AL_Francolin* (wPt-733679) is located closer to the proximal end of chromosome 6AL, whereas *QYr.vt-6A* is located closer to the distal end of 6AL. The *QTL QYr.orr-6AL_Stephens* (wPt-1642), *QYr-6A_Saar* (wPt-7063), and *QYr.ufs-6A_Kariega* (wPt-7181) are located at the same position on the consensus map as *QYr.vt-6A* (Supplemental Table 2.3). Although this indicates that *QYr.vt-6A* likely is not novel, it does confirm that Jamestown has at least one QTL in common with the HTAP-resistant cultivar Stephens.

Breeding Applications

Two QTL were detected on chromosome 5B associated with leaf rust resistance (Table 2.1) and two other QTL associated with stripe rust resistance were located on 3B and 6A (Table 2.2). Once markers closely linked to these QTL are validated, they can be used in marker-assisted selection (MAS) to incorporate and pyramid these QTL with other effective resistance genes. Stripe rust resistance conferred by *QYr.vt-3B*, *QYr.vt-6A*, or the combination of *QYr.vt-3B* and

QYr.vt-6A was not statistically different; therefore, these QTL may not have major additive or epistatic effects with one another. Therefore, MAS for *QYr.vt-3B* and/or *QYr.vt-6A* may be equally effective in reducing stripe rust susceptibility. Lines containing both *QLr.vt-5B.1* and *QLr.vt-5B.2* had significantly lower leaf rust infection type and severity than lines containing only *QLr.vt-5B.1*. In addition, lines having *QLr.vt-5B.2* also had significantly lower infection type than lines having *QLr.vt-5B.1*. Therefore, it likely will be beneficial to implement MAS for both *QLr.vt-5B.1* and *QLr.vt-5B.2* to reduce leaf rust susceptibility. Future work will include the development of kompetitive allele-specific markers to validate the QTL in cultivars with Jamestown in the genetic background for use and validation in the USDA Small Grains Genotyping Labs.

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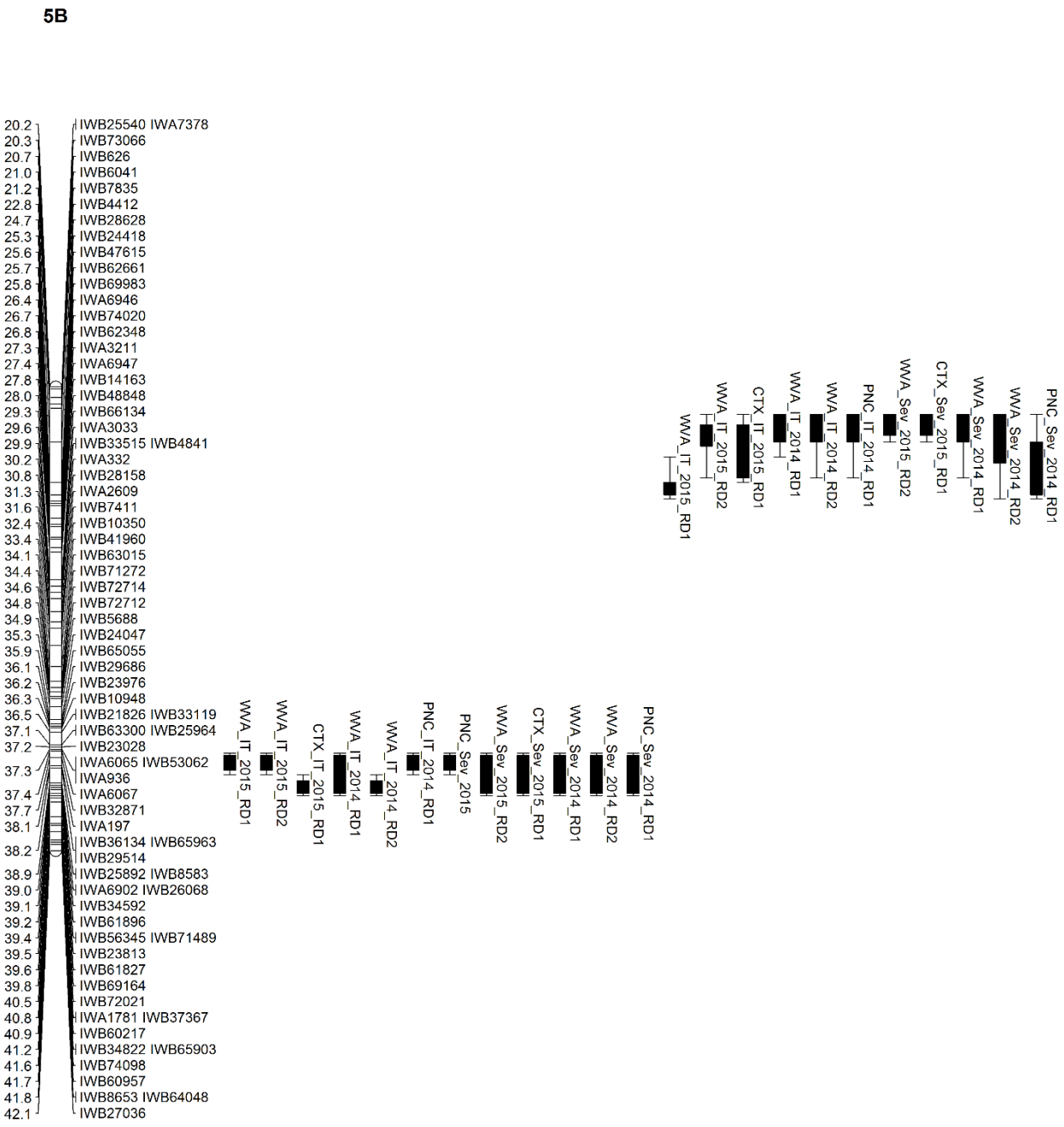

Fig. 2.1. Partial linkage map of chromosome 5B indicating location of traits associated with QLr.vt-5B.1 (above) and QLr.vt-5B.2 (below).

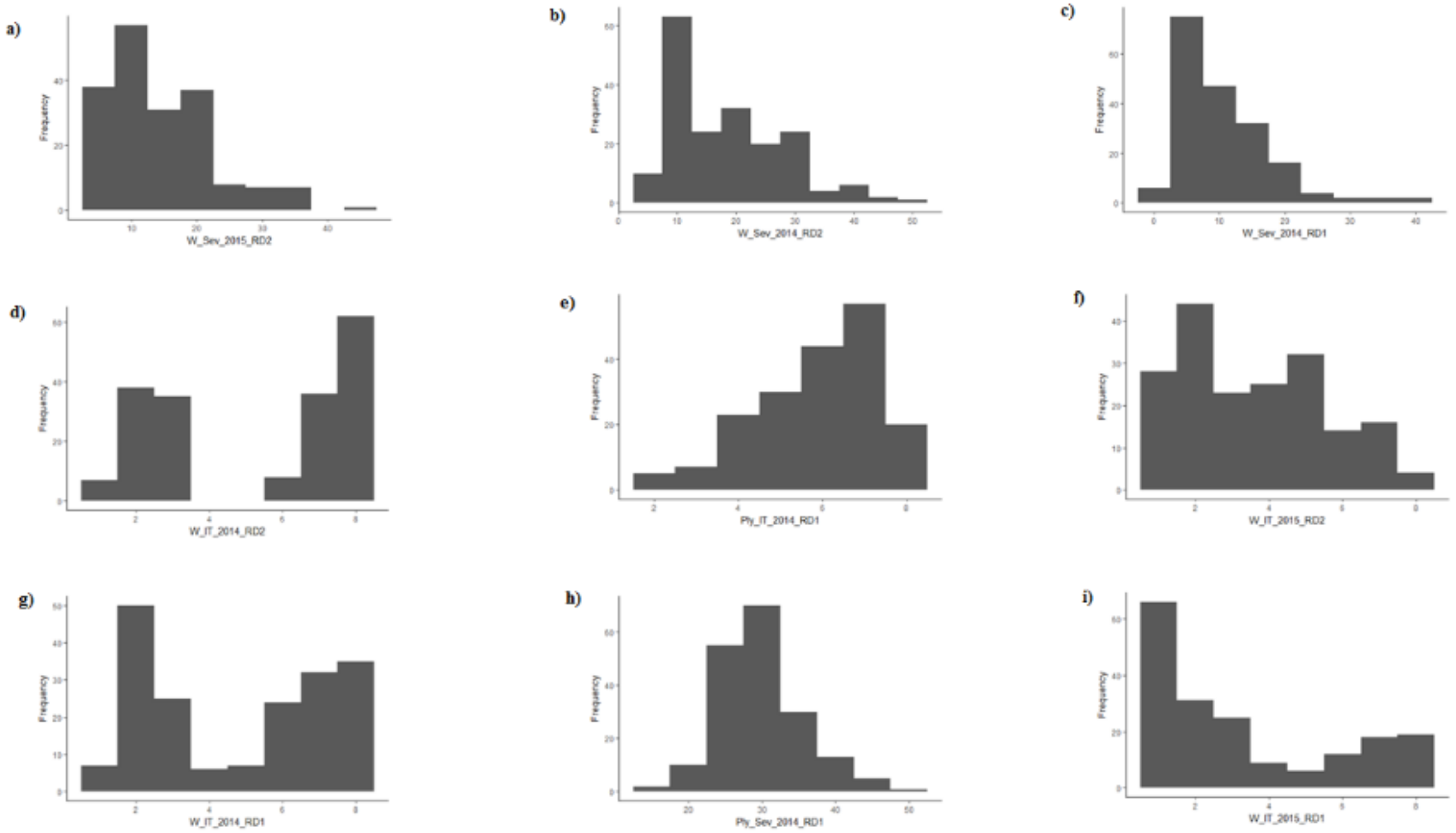

Fig. 2.2. Histograms of infection type and severity for significant locations associated with chromosome 5B.

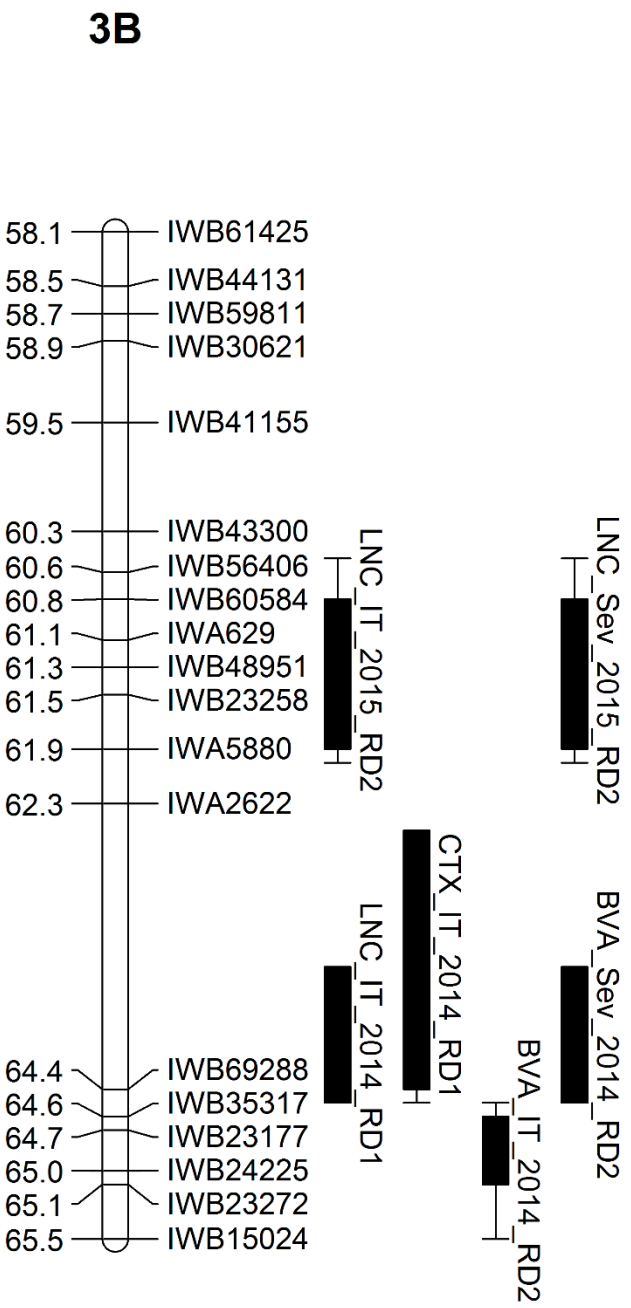

Fig. 2.3. Partial linkage map of chromosome 3B indicating location of traits associated with QYr.vt-3B.

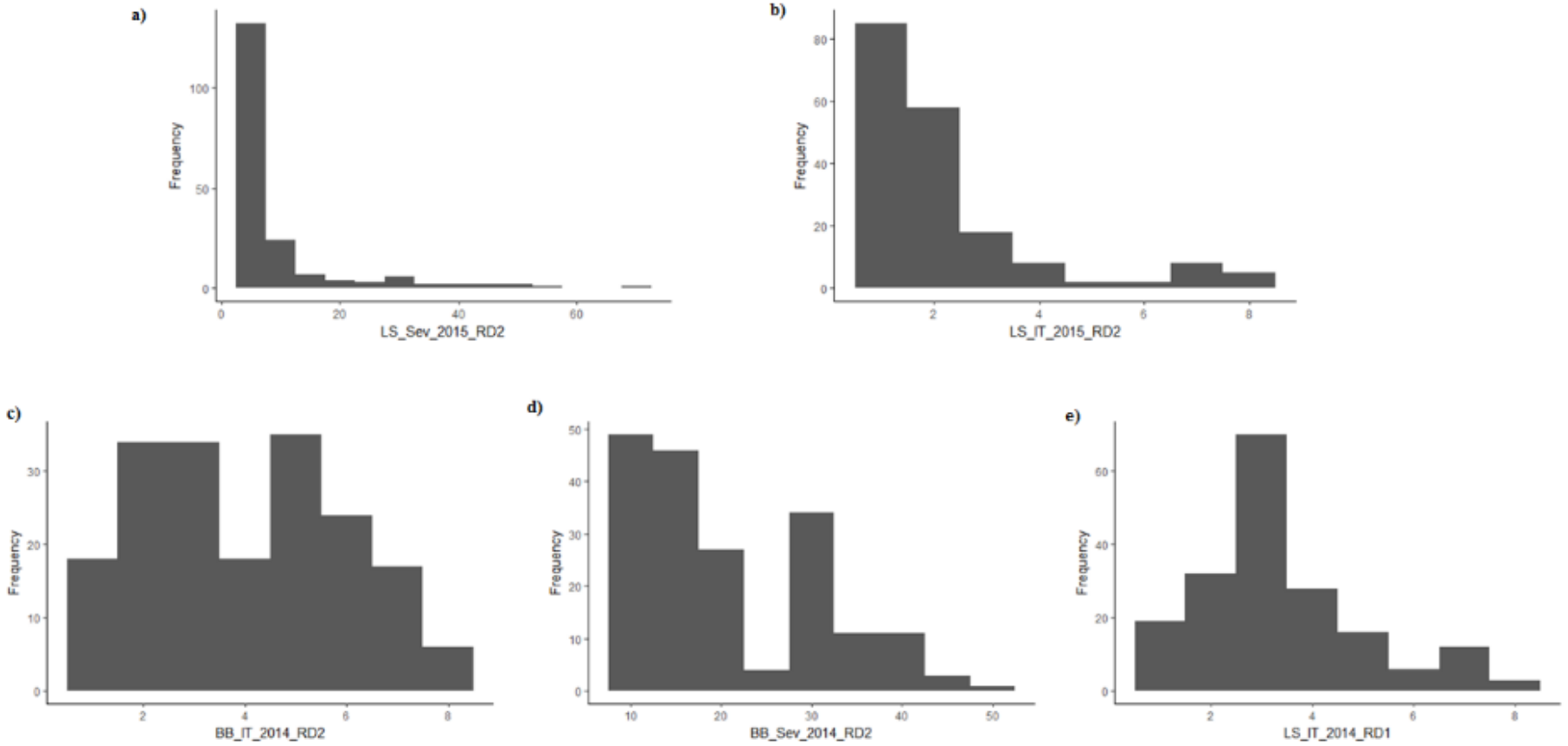

Fig. 2.4. Histograms of infection type and severity for significant locations associated with chromosome 3B.

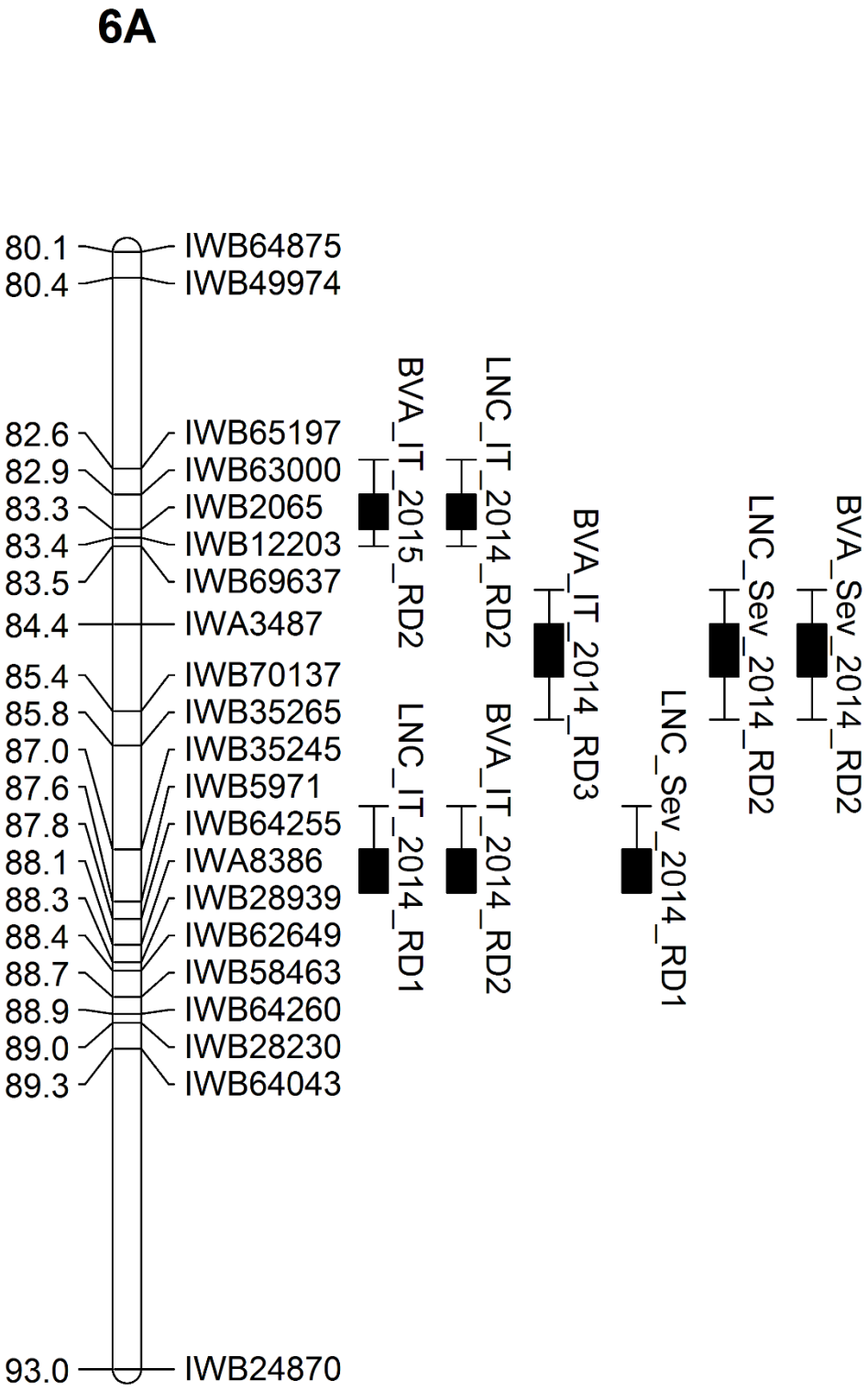

Fig. 2.5. Partial linkage map of chromosome 6A indicating location of traits associated with QYr.vt-6A.

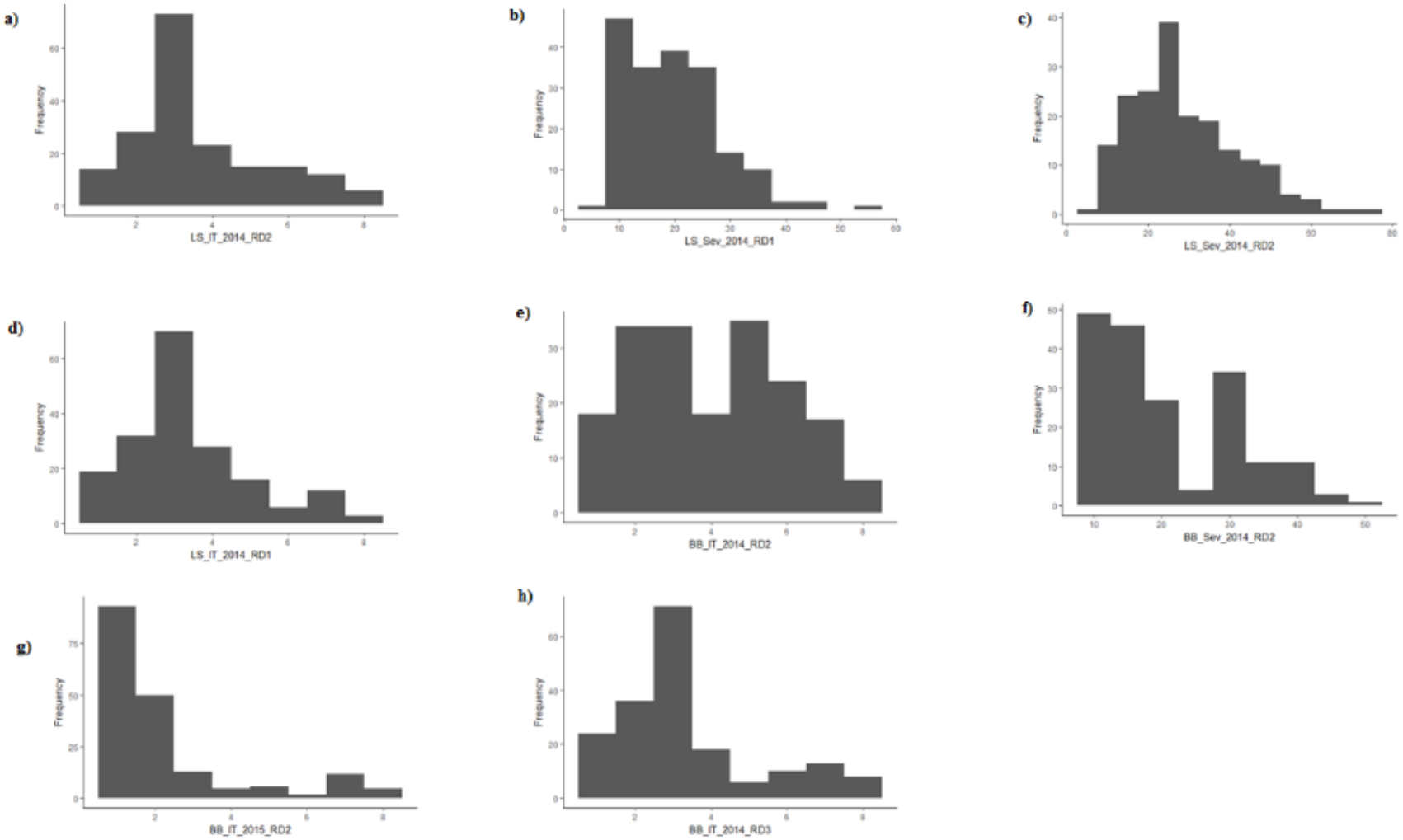

Fig. 2.6. Histograms of infection type and severity for significant locations associated with chromosome 6A.

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Table 2.1. Quantitative trait loci (QTL) associated with leaf rust infection type and severity in Pioneer '25R47'/Jamestown recombinant inbred lines (RILs) evaluated in 2014 and 2015 seasons.

Trait name†	Chr‡	Position	Confidence interval	Left marker	Right marker	LOD§	PVE¶	Add#
WVA_IT_2015_RD1	5B	25	23.5–25.5	IWB28628	IWB24418	3.4	5.0	-0.8
WVA_IT_2015_RD2	5B	22	21.5–24.5	IWB7835	IWB4412	4.6	22.1	-0.6
CTX_IT_2015_RD1	5B	23	21.5–24.5	IWB4412	IWB28628	6.0	2.6	-0.6
WVA_IT_2014_RD1	5B	22	21.5–23.5	IWB7835	IWB4412	7.9	3.7	-1.1
WVA_IT_2014_RD2	5B	22	21.5–24.5	IWB7835	IWB4412	8.2	3.3	-1.2
PNC_IT_2014_RD1	5B	22	21.5–24.5	IWB7835	IWB4412	4.2	1.7	-0.5
WVA_Sev_2015_RD2	5B	22	21.5–22.5	IWB7835	IWB4412	9.1	16.3	-3.6
CTX_Sev_2015_RD1	5B	22	21.5–22.5	IWB7835	IWB4412	4.2	10.7	-7.2
WVA_Sev_2014_RD1	5B	22	21.5–23.5	IWB7835	IWB4412	3.7	4.2	-2.2
WVA_Sev_2014_RD2	5B	22	21.5–24.5	IWB7835	IWB4412	4.6	3.0	-3.3
PNC_Sev_2014_RD1	5B	24	21.5–25.5	IWB4412	IWB28628	3.1	1.6	-1.7
WVA_IT_2015_RD1	5B	38	37.5–38.5	IWB32871	IWA197	4.2	5.5	-0.8
WVA_IT_2015_RD2	5B	38	37.5–38.5	IWB32871	IWA197	9.0	5.5	-1.0
CTX_IT_2015_RD1	5B	39	38.5–39.5	IWA6902	IWB26068	8.0	3.3	-0.8
WVA_IT_2014_RD1	5B	38	37.5–39.5	IWB32871	IWA197	9.5	4.0	-1.2
WVA_IT_2014_RD2	5B	39	38.5–39.5	IWA6902	IWB26068	8.9	3.3	-1.3
PNC_IT_2014_RD1	5B	38	37.5–38.5	IWB32871	IWA197	5.9	2.2	-0.5
PNC_Sev_2015	5B	38	37.5–38.5	IWB32871	IWA197	4.2	8.1	-6.0
WVA_Sev_2015_RD2	5B	38	37.5–39.5	IWB32871	IWA197	6.8	5.1	-3.3
CTX_Sev_2015_RD1	5B	38	37.5–39.5	IWB32871	IWA197	3.9	2.4	-7.1
WVA_Sev_2014_RD1	5B	39	37.5–39.5	IWA6902	IWB26068	3.5	3.6	-2.3
WVA_Sev_2014_RD2	5B	39	37.5–39.5	IWA6902	IWB26068	5.9	3.5	-3.9
PNC_Sev_2014_RD1	5B	38	37.5–38.5	IWB32871	IWA197	3.6	1.6	-1.8

† First two letters indicate states (WVA = Warsaw, VA; CTX = Castorville, TX; PNC = Plymouth, NC); letters following underscore represent traits (IT = infection type; SEV = severity); the last two digits indicate rating date (RD, each rating date was analyzed separately for each location).
‡ Chromosome.
§ Logarithm of odds value.
¶ Plant variation.
# Level of additivity.

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Table 2.2. Quantitative trait loci (QTL) associated with stripe rust infection type and severity in Pioneer '25R47'/Jamestown recombinant inbred lines (RILs) evaluated in 2014 and 2015 seasons.

Trait name†	Chr‡	Position	Confidence interval	Left marker	Right marker	LOD§	PVE¶	Add#
LNC_IT_2015_RD2	3B	61	60.5–62.0	IWB60584	IWA629	4.4	7.4	-0.5
LNC_IT_2014_RD1	3B	64	63.5–64.5	IWA2622	IWB69288	7.0	8.7	-0.6
CTX_IT_2014_RD1	3B	64	62.5–64.5	IWA2622	IWB69288	3.3	6.9	-0.3
BVA_IT_2014_RD2	3B	65	64.5–65.5	IWB24225	IWB23272	7.1	8.9	-0.7
LNC_Sev_2015_RD2	3B	61	60.5–62.0	IWB60584	IWA629	10.2	11.1	-4.6
BVA_Sev_2014_RD2	3B	64	63.5–64.5	IWA2622	IWB69288	4.4	8.0	-3.1
BVA_IT_2015_RD2	6A	83	82.5–83.5	IWB63000	IWB2065	13.1	14.3	-1.0
LNC_IT_2014_RD1	6A	87	86.5–87.5	IWB35245	IWB5971	4.4	2.2	-0.6
LNC_IT_2014_RD2	6A	84	82.5–83.5	IWB63000	IWB2065	4.0	6.3	-0.5
BVA_IT_2014_RD2	6A	85	86.5–87.5	IWB35245	IWB5971	3.1	3.4	-0.6
BVA_IT_2014_RD3	6A	84	84.0–85.5	IWA3487	IWB70137	3.7	12.2	-0.5
LNC_Sev_2014_RD1	6A	87	86.5–87.5	IWB35245	IWB5971	3.2	1.2	-2.6
LNC_Sev_2014_RD2	6A	84	84.0–85.5	IWA3487	IWB70137	3.9	1.9	-4.3
BVA_Sev_2014_RD2	6A	84	84.0–85.5	IWA3487	IWB70137	3.9	7.7	-3.0

† First two letters indicate states (WVA = Warsaw, VA; CTX = Castorville, TX; LNC = Laurel Springs, NC); letters following underscore represent traits (IT = infection type; SEV = severity); the last two digits indicate rating date (RD, each rating date was analyzed separately for each location).
‡ Chromosome.
§ Logarithm of odds value.
¶ Plant variation.
# Level of additivity.

Table 2.3. Mean leaf rust infection type and severities of recombinant inbred lines (RILs) and parent Jamestown with combinations of quantitative trait loci (QTL).

QTL combination†	Infection type	Severity	Individuals‡
Jamestown	3.05a	17.3a	–
QLr.vt-5B.1, QLr.vt-5B.2	3.57a	18a	48
QLr.vt-5B.2	3.65a	18.5ab	10
QLr.vt-5B.1	5.33b	25.6b	36
None	6.7c	32.2c	92

† Mean phenotype of QTL combination of RILs. Those with the same letter are not significantly different ($p < 0.05$). All markers within the respective QTL were used.

‡ Denotes number of RILs in each QTL combination.

Table 2.4. Mean stripe rust infection type and severities of recombinant inbred lines (RILs) and parent Jamestown with combinations of quantitative trait loci (QTL).

QTL combination†	Infection type	Severity	Individuals‡
Jamestown	3.00a	16.2a	–
QYr.vt-3B, QYr.vt-6A	3.03a	16.4a	24
QYr.vt-3B	3.11a	16.8a	57
QYr.vt-6A	3.19a	17.4a	48
None	5.23b	29.4b	57

† Mean phenotype of QTL combination of RILs. Those with the same letter are not significantly different ($p < 0.05$). All markers within the respective QTL were used.

‡ Denotes number of RILs in each QTL combination.

Supplemental Table 2.1. Types of polymorphic markers in the mapping population

Type of Polymorphism†	Female Parental SNP	Male Parental SNP	Number of SNPs
I	AA	AB	339
II	AA	BB	147
III	AB	AA	255
IV	AB	BB	420
V	BB	AA	97
VI	BB	AB	427
Total	-	-	1685

†Types of SNP polymorphisms based on the SNP calling of the female and male parents with random combinations of AA, AB, and BB.

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Supplemental Table 2.2. Physical and Genetic positions of QTL *QLr.vt-5B.1* and *QLr.vt-5B.2*

QTL	SNP	Sequence[†]	IWGSC RefSeq v1.0[‡]		Chr[§]	(%)[¶]	IWGSC_Survery_Sequence_Chromosome_V2[#]	(%)[¶]	POPSEQ (cM)[††]	WGS w7984_ Scaffolds[‡‡]	Chr[§]	WGS w7984 (cM) [‡‡]	(%)[¶]
QLr.vt-5B.1	IWB7835	aggtatatcaaacaaatactctatagtctatctctccatcccacagggaRtatatatgtagaattaaactactctataagcaacagacgactgcaaaatc	701189782	701189682	5B	99	IWGSC_chr5BL_ab_k71_contigs_longerthan_200_10846141	99	171.68	Scaffold5147272	5B	145.90	98
QLr.vt-5B.1	IWB24418	atcctcactgactgacttgtctactctacttggaaactacatgctgcaacRcaacttcagtgagcacaacaaattccgacaaatatctgctgacctattac	701155563	701155463	5B	99	IWGSC_chr5BL_ab_k71_contigs_longerthan_200_10791774	99	171.68	Scaffold2596642	5B	145.90	98
QLr.vt-5B.2	IWB32871	cagttggcaacctatcatactatcaaagaggggaaaacatgggttyRtgttttatatttacctgccagtatgtgagaaacacgactgatttgattat	671094291	671094195	5B	98	IWGSC_chr5BL_ab_k71_contigs_longerthan_200_10896979	98	150.60	Scaffold598336	5B	129.98	97
QLr.vt-5B.2	IWB26068	gtaatcactgctaatgctagccaaaccatccaagccactagatgtctgggRtaactgttttttcagaactagggatagcaccagcatgataatcgttggaag	656255230	656255154	5B	99	IWGSC_chr5BL_ab_k71_contigs_longerthan_200_10863380	99	140.98	Scaffold7247	5B	113.89	98

† Sequence associated with SNP.

‡ Physical Position from IWGSC RefSeq v1.0.

§ Chromosome from IWGSC RefSeq v1.0, POPSEQ, and WGS w7984 maps.

¶ Percentage aligned from IWGSC RefSeq v1.0, IWGSC_Survery_Sequence_Chromosome_V2, POPSEQ, and WGS w7984 maps.

Physical position from IWGSC_Survery_Sequence_Chromosome_V2.

†† Genetic (cM) positions from POPSEQ map (Consortium, I. W. G. S., 2014).

‡‡ Physical (Scaffolds) and genetic (cM) positions from WGS w7984 map (Chapman, J. A. et al., 2015).

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Supplemental Table 2.3. Locations of *QLr.vt-5B.1, QLr.vt-5B.2, QYr.vt-3B, QYr.vt-6A* and various QTL in proximity.

Source	Expected Chr	Marker	Marker Type	IWGSC_Survery_Sequence_Chromosome_V2[†]	IWGSC (%)[‡]	Chr[§]	POPSEQ_cM[¶]	WGS w7984_Scaffolds[#]	WGS w7984 (%)[‡]	Chr[§]	WGS w7984_(cM)[#]
Prins	5B	barc4	SSR	IWGSC_chr5BS_ab_k71_contigs_longerthan_200_2252884	100	5BS	45.99	Scaffold319037	99	6D	50.77
Zhou	5B	barc128	SSR	IWGSC_chr5BL_ab_k71_contigs_longerthan_200_10838825	88	5BL	48.76	Scaffold1091319	97	5B	38.77
Buerstmayr	5B	wPt-7006	DArT	IWGSC_chr5BL_ab_k71_contigs_longerthan_200_4925465	97	5BL	150.60	Scaffold8212	96	5B	129.986
Kolmer	5BL	wPt-0837	DArT	-	-	-	-	-	-	-	-
Chu	5BL	Xfcp	SSR	-	-	-	-	-	-	-	-
QLr.vt-5B.1	5BL	IWB7835	SNP	IWGSC_chr5BL_ab_k71_contigs_longerthan_200_10846141	99	5BL	171.68	Scaffold5147272	98	5B	145.902
QLr.vt-5B.1	5BL	IWB24418	SNP	IWGSC_chr5BL_ab_k71_contigs_longerthan_200_10791774	99	5BL	171.68	Scaffold2596642	98	5B	145.902
QLr.vt-5B.2	5BL	IWB32871	SNP	IWGSC_chr5BL_ab_k71_contigs_longerthan_200_10896979	98	5BL	150.60	Scaffold598336	97	5B	129.986
QLr.vt-5B.2	5BL	IWB26068	SNP	IWGSC_chr5BL_ab_k71_contigs_longerthan_200_10863380	99	5BL	140.98	Scaffold7247	98	5B	113.896
QYr.vt-3B	3B	IWA629	SNP	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10638690	99	3B	-	Scaffold1417841	99	3B	48.94
QYr.vt-3B	3B	IWA2622	SNP	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10588096	99	3B	62.23	Scaffold2697982	99	3B	48.94
Yrns-B1	3B	wPt-1612	DArT	IWGSC_chr3B_ab_k71_contigs_longerthan_200_10762332	85	3B	105.18	Scaffold1616625	92	3B	42.14

QYrpi.vt-3BL_VA00W-38	3B	wPT-0267	Proprietary	-	-	-	-	-	-	-	-
QYR.sun-3B_Wollaroi	3B	wPT-9577	Proprietary	-	-	-	-	-	-	-	-
3B_Rio Blanco	3B	gwm299	Proprietary	-	-	-	-	-	-	-	-
QYrex.wgp-3BL_Express	3B	gwm299	Proprietary	-	-	-	-	-	-	-	-
QYR.vt-6A	6A	IWB63000	SNP	IWGSC_chr6AL_ab_k71_contigs_longerthan_200_5817978	99	6AL	118.54	Scaffold567898	99	6A	90.29
QYR.vt-6A	6A	IWB35245	SNP	IWGSC_chr6AL_ab_k71_contigs_longerthan_200_5771383	99	6AL	-	Scaffold1822818	99	6A	98.25
QYr.orr-6AL_Stephens	6AL	wPt-1642	DArT	IWGSC_chr6AL_ab_k71_contigs_longerthan_200_5821574	87	6AL	-	Scaffold1558553	96	6A	99.39
QYr-6A_Saar	6A	wPt-7063	Proprietary	-	-	-	-	-	-	-	-
QYr.ufs-6A_Kariega	6A	wPt-7181	Proprietary	-	-	-	-	-	-	-	-
QYr.uga-6AS-26R61	6A	wPt-671561	Proprietary	-	-	-	-	-	-	-	-
QYr.wgp-6AS_Express	6A	gwm334	Proprietary	-	-	-	-	-	-	-	-
QYr.cim-6A_Avocet	6A	wPT-2573	Proprietary	-	-	-	-	-	-	-	-
QYr.cim-6AL_Francolin	6A	wPt-733679	Proprietary	-	-	-	-	-	-	-	-

† Physical position from IWGSC_Survery_Sequence_Chromosome_V2.

‡ Percentage aligned from IWGSC_Survery_Sequence_Chromosome_V2 and WGS w7984 maps.

§ Chromosome from IWGSC_Survery_Sequence_Chromosome_V2, POPSEQ, and WGS w7984 maps.

¶ Genetic (cM) positions from POPSEQ map (Consortium, I. W. G. S., 2014).

Physical (Scaffolds) and genetic (cM) positions from WGS w7984 map (Chapman, J. A. et al., 2015).

CHAPTER III

Mapping *Lr18*: a Leaf Rust Resistance Gene Widely Deployed in Soft Red Winter Wheat

Neal R. Carpenter, Carl A. Griffey, Subas Malla, Shiaoman Chao, and Gina L. Brown-Guedira

N.R. Carpenter, C.A. Griffey, Dept. of Crop and Soil Environmental Sciences, Virginia Tech, Blacksburg, VA 24060; S. Malla, Texas A&M AgriLife Research, Texas A&M AgriLife Research & Extension Center, Uvalde, TX 78801; S. Chao, USDA-ARS, Fargo, ND 58102; G.L. Brown-Guedira, USDA-ARS, North Carolina State University, Raleigh, NC 27695

ABSTRACT

Leaf rust caused by *Puccinia triticina* is a destructive pathogen of wheat (*Triticum aestivum)* in a majority of the world's wheat producing areas. Host resistance is the most economical solution for providing full season control and reducing damage due to leaf rust as opposed to use of multiple fungicide applications. The soft red winter wheat cultivar Jamestown is productive in the Deep South, southern Corn Belt, and mid-Atlantic regions, and this can be attributed in part to its resistance to multiple diseases including leaf rust. Jamestown is postulated to contain gene *Lr18*. Seedlings of 186 $F_{5:9}$ recombinant inbred lines (RILs) from a cross of Pioneer '25R47' / Jamestown and 200 F_2 seedlings from eight other crosses including Jamestown and/or the *Lr18* host differential line RL6009 (Thatcher*6/Africa 43) were screened with *P. triticina* race TNRJJ. Genetic analysis of the populations was conducted to validate the presence of *Lr18* in Jamestown. Linkage analysis conducted with SNP markers in the Pioneer 25R47 / Jamestown population identified markers that were tightly linked with *Lr18*, and these were validated in Jamestown / VA10W-21 and RL6009 / VA10W21 F_2 populations. Results of linkage analysis identified SNP maker IWB41960 linked within 5 cM of gene *Lr18* in all three populations.

INTRODUCTION

Leaf rust (*Puccinia triticina* Eriks) is the most common type of rust of wheat (*Triticum aestivum* L.) worldwide (Bolton et al., 2008) and can be found on every continent with the exception of Antarctica (Huerta-Espino et al., 2011). In the southeastern soft red winter (SRW) wheat region of the United States, leaf rust epidemics will typically peak during April in Georgia and at the end of May in Virginia (Kolmer and Hughes, 2013). Losses from leaf rust are typically less severe than those resulting from the other two common rust diseases, stem rust (*Puccinia graminis f. sp. tritici*) and stripe rust (*Puccinia striiformis* Westend. f.sp. *tritici Eriks*); however, leaf rust causes greater overall losses due to its wider distribution and occurrence (Huerta-Espino et al., 2011). Control of leaf rust through deployment of diverse and durable genetic resistance in cultivars as opposed to reliance upon fungicides, has been shown to be the most cost effective method with an estimated 27:1 benefit to cost ratio (Marasas et al., 2003). There are more than 74 leaf rust resistance genes that have been mapped to chromosome locations and given gene designations, and numerous temporarily designated leaf rust resistance genes (McIntosh et al., 2013).

Most leaf rust (*Lr*) resistance genes operate on a gene for gene basis (Bolton et al., 2008) and their effectiveness is race-specific. Genetic resistance to leaf rust can be exploited best when knowledge of the specific resistance genes present in commonly used parental germplasm and cultivars is available. Identification of diverse leaf rust resistance genes and effective combinations allows for effective integration and pyramiding of different and complementary genes into breeding populations, and thus aids in the release and deployment of cultivars that are not genetically uniform (Kolmer, 1996). High levels of variation and mutation within *P. triticina*

populations highlights the need for identification, incorporation, and pyramiding of novel and complementary resistance genes (Bolton et al., 2008).

The leaf rust resistance gene *Lr18*, derived from *Triticum timopheevii* Zhuk., is known to be located on the long arm of the 5B chromosome (McIntosh, 1983). It is the only documented leaf rust resistance gene derived from *Triticum timopheevii*. Seedling resistance governed by *Lr18* is most effective between 15 and 18°C, and as temperatures increase the gene becomes less effective, and at 25°C it becomes ineffective (McIntosh, 1983). While the initial source of *Lr18* in North American wheat is unknown (McIntosh et al., 1995), it has been prevalent and maintained in SRW wheat varieties for more than half a century. Leaf rust genes postulated from seedling tests using differential races of *P. triticina* at the Cereal Disease Lab were first reported for SRW wheat lines in the 1987-88 USDA-ARS Uniform Southern and Eastern SRW Wheat Nurseries (Harold Bockelman, personal communication, 2017). In the 1999 – 2016 nurseries (https://www.ars.usda.gov/pacific-west-area/aberdeen-id/small-grains-and-potato-germplasm-research/docs/uniform-nurseries/), 44 lines in the Uniform Southern and 34 lines in the Uniform Eastern nurseries were postulated to carry *Lr18*. One or more lines in all but five of the 18 years tested in the nurseries were postulated to carry *Lr18*.

The SRW wheat cultivars Bledsoe (CItr 13238) and GA1123 (CItr 13292) developed at the University of Georgia in 1956 and 1961, both have *Triticum timopheevii* in their pedigrees. Cultivar Holley (CItr 14579) having both of the former cultivars in its pedigree has been postulated to carry *Lr18* based on the presence of this gene in subsequent lines (VA87-54-558

and VA88-52-69) derived from the cross 'Massey' (CItr 17953) / Holley. Massey is very susceptible to leaf rust and does not carry gene *Lr18*. Subsequent cultivars postulated to carry gene *Lr18* on the basis of ancestry and/or via seedling reaction to differential races of *P. triticina* conducted at the USDA-ARS Cereal Disease Lab are listed in Table 3.1. In the current study, gene *Lr18* was mapped in the SRW wheat cultivar Jamestown, which was postulated to contain *Lr18* (Griffey et al., 2010).

MATERIALS AND METHODS

Pathogen material

Puccinia triticina race TNRJJ was used throughout this study. The original four letter code used to denote race identity is based on reaction of a given race to a set of wheat host differential lines having known resistance genes (Roelfs and Martens, 1987). Race TNRJJ is avirulent to gene *Lr18*, but contains virulence for genes *Lr1, Lr2a, Lr2c, Lr3, Lr9, Lr24, Lr3ka, Lr11, Lr30, Lr10, Lr14a, Lr28, Lr39*.

Host material

One hundred and eighty six recombinant inbred lines (RILs) were derived from a cross of Pioneer '25R47' (PI 631473) (Lively et al., 2004) by Jamestown (PI 653731) (Griffey et al., 2010). The varieties are adapted to the soft red winter wheat growing regions of the eastern United States. The cultivar Jamestown is postulated to possess *Lr18*. In addition to the initial mapping population, 1600 individuals from eight other F_2 populations (Table 3.2) (200

individuals per population) were evaluated to validate the presence of *Lr18* in Jamestown. These validation populations included postulated resistant by susceptible lines Jamestown / 'MCIA Venus' (PI 669575), Jamestown / VA10W-21 (PI 676295), RL6009 / VA10W-21, Jamestown / P0537A1-12 (IN0411/2754 // IN0412 / 98134), and VA08MAS-369 (McCormick / GA881130LE5) / Jamestown. VA10W-21 and MCIA Venus are susceptible to TNRJJ. P0537A1-12 and VA08MAS-369 were postulated to lack *Lr18*. The postulated resistant by resistant lines included RL6009 (Thatcher*6/Africa43) / Jamestown, RL6009 / VA10W-119 ((KY97C-0540-04 / GF951079-2E31 (PI 644020)), RL6009 / Shirley (PI 656753), and RL6009 / VA09W-110 (GA931241E16 / VA01W-303). Near isogenic line RL6009 (CItr 15242) is the host differential with gene *Lr18*. VA09W-110, VA10W-119, and Shirley were postulated to contain *Lr18*.

Inoculation and *P. triticina* evaluation

Race TNRJJ was maintained and increased on the cultivar Tribute (PI 632689) (Griffey et al., 2005). The Pioneer 25R47 / Jamestown population (186 RIL), eight F_2 populations, experimental lines, two parental lines, and a set of leaf rust host differentials consisting of 24 'Thatcher' wheat near isogenic lines having different *Lr* genes (*Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17, Lr30, LrB, Lr10, Lr14a, Lr18, Lr21, Lr28, Lr41, Lr42, Lr3bg, Lr14b, Lr20, Lr23*) were evaluated for reaction to race TNRJJ. Seedlings were inoculated with urediniospores of TNRJJ using a light mineral oil Soltrol 170 (Phillips Petroleum Co. Itex Plant, Borger, TX). The inoculated seedlings were then allowed to air dry for ten minutes and then were placed in a Percival Scientific (Perry, Iowa 50220) dew chamber (Model No. I-36DLC8) held at 99% relative humidity and 18-20°C for approximately 16 hours. The seedlings were then

transferred and incubated in a Conviron (Winnipeg, Manitoba, Canada R3H 0R9) growth chamber (Model CMP5000) at 18°C, 55% relative humidity, and 16 hours of light (227 μMol). Disease assessments were made at 10 to 14 days after inoculation, using a 0-4 rating system described by Roelfs et al (1992). In summary 0 was scored as no visible spores, 1 as small spores with hypersensitive responses, 2 as small to medium sized spores that are bordered by chlorosis of leaf tissue, 3 as medium sized spores without chlorosis, and 4 as large spores with no chlorosis. Those scores with a ; included indicates a hypersensitive response often referred to as a "fleck" response. Infection types 0–2 were classified as resistant and infection types 3–4 were classified as susceptible.

DNA extraction

Tissue of each RIL (Pioneer '25R47' / Jamestown), F_2 (Jamestown / VA10W-21, RL6009 / VA10W-21), and experimental lines were collected when seedlings reached the three-leaf stage and placed into 2 ml test tubes, each containing two stainless steel beads for tissue grinding. Tissue samples were frozen in an ultra-low temperature (−80°C) freezer and then subsequently ground using a Spex CertiPrep 2000 Geno-Grinder (Metuchen, NJ, USA) for 15 s or until finely ground. DNA was extracted using a modified CTAB method (Saghai-Maroof et al., 1984).

Microsatellite assay

Over 400 Simple Sequence Repeat (SSR) markers were analyzed similar to Carpenter et al. (2017) using bulk segregant analysis (Michelmore et al., 1991) of 142 samples including 71 of each extreme phenotype (most resistant and most susceptible) from the Pioneer 25R47 /

Jamestown population. Simple sequence repeat (SSR) primer were either directly labeled with a fluorescent dye or indirectly labelled with an M13 tail sequence (5' – ACGACGTTGTAAAACGAC – 3' or 5' – CACGACGTTGTAAAACGAC – 3'). Fluorescent dye labels included FAM (blue), NED (yellow/black), VIC (green), HEX (green) and PET (red). A single Polymerase Chain Reaction (PCR) with four direct-labeled fluorescent SSR primers was conducted in a volume of 14.0 µl containing 4 µl of DNA (50 ng), 1.5 mmol mL^{-1} $MgCl_2$, 0.20 mmol mL^{-1} dNTP, 0.20 µmol mL^{-1} forward primer and 0.20 µmol mL^{-1} reverse primer of direct-labeled primers (10 µl), and 0.03 units *Taq* polymerase, and 8.37 µl of molecular grade water. A single PCR for each M13-labelled tailed primer pair was used in a volume of 14.0 µl that contained 4.0 µl of DNA (50 ng), 1.5 mmol mL^{-1} $MgCl_2$, 0.20 mmol mL^{-1} dNTP, 0.20 µmol mL^{-1} forward primer and 0.20 µmol mL^{-1}0.3 µl of M13-tailed primer , 0.03 units of *Taq* polymerase and 3.47 µl of molecular grade water. The PCR products of four separate M13-PCRs were combined for analysis in an Applied Biosystems 3130xl Genetic Analyzer (Foster City, CA, USA). The PCR products were transferred to a 96-well PCR plate with each well containing 9.9 µl of Hi-Di formamide and 0.1 µl of size standard. Samples were denatured at 95°C for 5 min. PCR products were visualized on an Applied Biosystems 3130xl Genetic Analyzer, and the generated data were analyzed using the genotyping software Genemarker version 1.70 designed by SoftGenetics (SoftGenetics, 2007).

Single Nucleotide Polymorphism (SNP) array

A 90K iSelect SNP genotyping assay was performed on the Pioneer 25R47/Jamestown population at the USDA-ARS Small Grains Genotyping Laboratory in Fargo, ND. Genome Studio v2.0 software (Illumina, 2016) was used to cluster the SNPs based on genotypes.

Procedures for calling SNP(s) were similar to the procedures used in Cavanagh et al. (2013), Wang et al. (2014), and Carpenter et al. (2017). In summary, there were multiple parental clusters called, similar to Liu et al. (2016), which were then converted to the A, B, H format. Manual scoring was performed for each SNP cluster that could not be categorized by the default algorithm. Each SNP cluster was manually authenticated by visually assessing each cluster. The data was filtered for a minor allele frequency less than 5%, and heterozygosity levels above 10%. Each SNP was then calculated for percentage of missing data, any SNP above 20% missing data was dropped due to the poor quality of the SNP. Oligos for Kompetitive allele specific (KASP) PCR assays were developed from source sequences of iSelect SNP(s) linked to *Lr18* in the Pioneer 25R47/Jamestown population, with primers carrying standard FAM or VIC compatible tails (Table 3.3). Reaction were performed based upon upon manufacturer's instructions (LGC Genomics). KASP primers were used for validation of results found in the initial linkage analysis.

Linkage map construction and data analysis

Chi squared (χ^2) analysis was performed on the data collected from the rust screening tests to confirm the goodness-of-fit of observed ratios to theoretical expectations. Linkage maps were constructed using JoinMap 4.0 (Van Ooijen, 2006), the Kosambi mapping function (Kosambi, 1943) was used to estimate map distance, and linkage groups were constructed based upon a minimum logarithm of odds (LOD) threshold value of 3.0. MapChart 2.2 (Voorrips, 2002) was used to draw linkage maps.

RESULTS

The *Lr18* host differential RL6009 (IT = 12;) / Jamestown (IT = 12;) population showed no segregation in reaction to *P. triticina* race TNRJJ, e.g. all progeny were resistant (IT = 12;). This indicates that both RL6009 and Jamestown contain the resistance gene *Lr18*, and Jamestown has a single dominant resistance gene.

Progeny derived from populations Jamestown / MCIA Venus ($\chi^2_{3:1}$ = 0.04, p = 0.84), Jamestown / VA10W-21 ($\chi^2_{3:1}$ = 2.47, p = 0.12), RL6009 / VA10W-21 ($\chi^2_{3:1}$ = 2.19, p = 0.14), Jamestown / P0537A1-12 ($\chi^2_{3:1}$ = 0.79, p = 0.37), VA08MAS-369 / Jamestown ($\chi^2_{3:1}$ = 2.32, p = 0.13), and Pioneer '25R47'/Jamestown ($\chi^2_{3:1}$ = 2.59, p = 0.11) segregated in reaction to TNRJJ and fit a 3:1 single gene segregation pattern, thus validating that P0537A1-12, VA08MAS-369, VA10W-21, Pioneer '25R47', and MCIA Venus lack *Lr18*.

Progeny (F_2) derived from populations RL6009 / VA09W-110 ($\chi^2_{3:1}$ = 2.47, p = 0.12) and RL6009 / VA10W-119 ($\chi^2_{3:1}$ = 1.18, p = 0.28), also segregated and fit a single gene segregation pattern indicating that VA09W-110 and VA10W-119 do not possess *Lr18*. The RL6009 / Shirley population segregated 180 resistant to 15 susceptible plants and fit a 15:1 segregation pattern ($\chi^2_{15:1}$ = 0.69, p = 0.40) indicating that two genes were segregating. Shirley is known to possess gene *Lr26* residing on the 1BL.1RS translocation, which also confers resistance to race TNRJJ (Griffey et al., 2010). These data indicate that Shirley does not possess *Lr18* or another gene for seedling resistance to TNRJJ.

In the Pioneer 25R47/Jamestown population *Lr18* mapped to the distal end of chromosome 5BL using the 90K SNP array data. A set of 20 SNP markers residing near *Lr18* in the Pioneer 25R47/Jamestown population mapped to within 10 cM and flanked *Lr18* in the Jamestown/VA10W-21 and RL6009/VA10W-21 populations (Fig. 3.1). Three markers were polymorphic (Fig. 3.1) among the Jamestown/Pioneer 25R47, Jamestown/VA10W-21, and RL6009/VA10W-21 populations (Table 3.2). The SNP maker IWB41960 mapped 3 to 5 cM proximal to *Lr18* in all three populations (Fig. 3.1). Marker IWB41960 was used to screen on multiple parental lines in the Virginia Tech small grains breeding program and to test for the presence of *Lr18* throughout the germplasm (Table 3.4). The lines in Table 3.4 were also screened with race TNRJJ using similar methods to the RIL and F_2 populations.

DISCUSSION

Data from greenhouse seedling tests conducted at the Cereal Disease Lab and Virginia Tech, previously indicated that Jamestown possesses *Lr18* (Carl A. Griffey, personal communication, 2017). The other leaf rust resistance genes reported to reside on chromosome 5B, *Lr52* and *LrK1*, were mapped to the short arm (Hiebert et al., 2005; Hiebert et al., 2014), while the current study mapped gene *Lr18* to the distal end of 5BL (McIntosh, 1983). It also is unlikely that the source of resistance in Jamestown is *Lr52* as this gene provides higher levels of resistance than *Lr18* in seedling tests (Hiebert et al., 2005). Gene *Lr18* does not provide effective resistance when used as the sole source of leaf rust resistance (McIntosh et al., 1995), but has contributed to effective resistance when combined with other complementary resistance genes (Carl A. Griffey, personal communication, 2017). As Jamestown possesses adult plant leaf rust resistance at higher temperatures (Griffey et al., 2010), it is conceivable that *Lr18 is* working in an epistatic or additive manner with *QLr.vt-5B.1* and *QLr.vt.5B.2* which were identified in Jamestown

(Carpenter et al., 2017). While *Lr18* mapped in a region close to *QLr.vt-5B.1* and *QLr.vt.5B.2*, *Lr18* did not map to the same region as *QLr.vt-5B.1* and *QLr.vt.5B.2* (Fig. 3.2). This is likely since *QLr.vt-5B.1* and *QLr.vt.5B.2* were discovered in field conditions where races of *P. triticina* were virulent to *Lr18*. *P. triticina* races virulent to *Lr18,* TCRKG and TBRKG were detected in 2014 and 2015 in Plymouth, NC and Warsaw, VA. Significant QTL for leaf rust resistance was detected in 2014 and 2015, in both Plymouth, NC and Warsaw, VA (Carpenter et al. 2017).

Gene *Lr18* was linked tightly (within 5 cM) to SNP marker IWB41960 in the $F_{5:9}$ population of Pioneer 25R47 / Jamestown, and in the F_2 populations Jamestown / VA10W-21 and RL6009 / VA10W-21 (Fig. 1). Allelism tests validated that Jamestown possesses *Lr18*, while Shirley, P0537A1-12, VA08MAS-369, VA10W-21, VA09W-110, VA10W-119, and MCIA Venus lack *Lr18*. Marker IWB41960 validated results of the allelism tests using Jamestown as the positive control. Marker IWB41960 was also screened on multiple parental lines in the Virginia Tech small grains breeding program and indicated that *Lr18* is widely distributed throughout the germplasm (Table 3.4) and, thus, is still contributing to overall leaf rust resistance in current cultivars. Therefore, it is likely that selection for leaf rust resistance per se has maintained *Lr18* in breeding programs since it was first introduced into SRW wheat germplasm more than 50 years ago.

The DNA markers identified as being linked to *Lr18* in this study are useful for understanding the relationship with other leaf rust resistance genes. In this study IWB41960 proved diagnostic for identifying *Lr18* in both Jamestown and RL6009. However, these markers are not perfect for

marker assisted genotyping or selection because linkage between *Lr18* and the closest marker IWB41960 is not extremely tight (3.1 cM), and there are no distal flanking markers that were polymorphic among all populations tightly linked to *Lr18*. This is evident in Table 3.4 as a few susceptible lines (IT = 3) were identified as possessing *Lr18*, although this could also be due to suppressor genes which have been identified for leaf rust seedling resistance genes such as *Lr23* (Nelson et al., 1997). Future work on *Lr18* should include the cloning or further identification of more tightly linked markers via fine mapping that would allow for more reliable selection in breeding programs. In a future study, most of the cultivars and/or ancestral parents listed in Table 3.1, postulated to possess *Lr18*, and other cultivars known to lack this gene, will be evaluated for seedling reaction to race TNRJJ and screened using marker IWB41960 to determine whether they have or lack *Lr18*.

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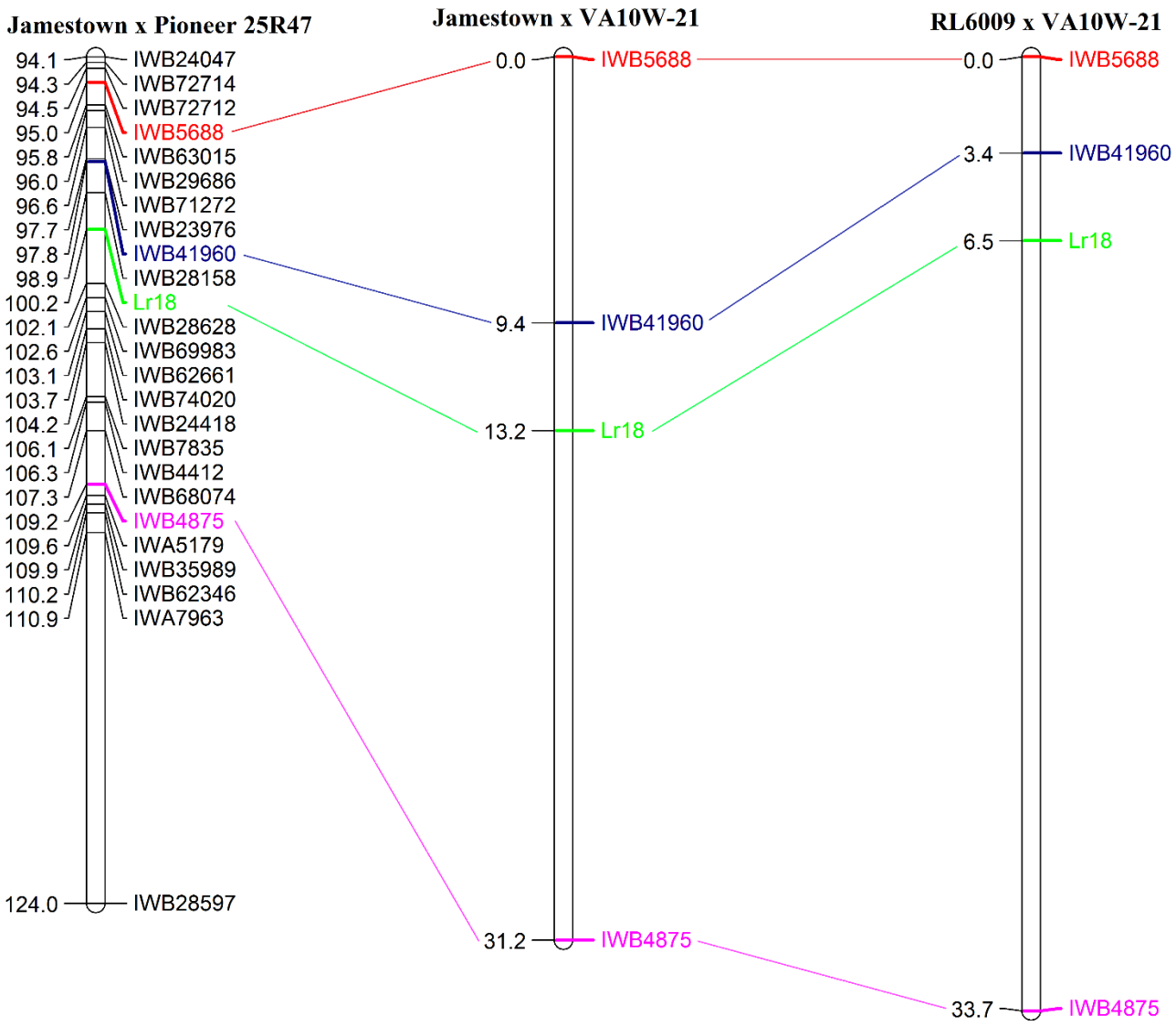

Figure 3.1. Partial linkage map of chromosome 5B indicating location of SNPs associated with gene *Lr18* in populations P47/JT, JT/VA10W-21, and RL6009/VA10W-21.

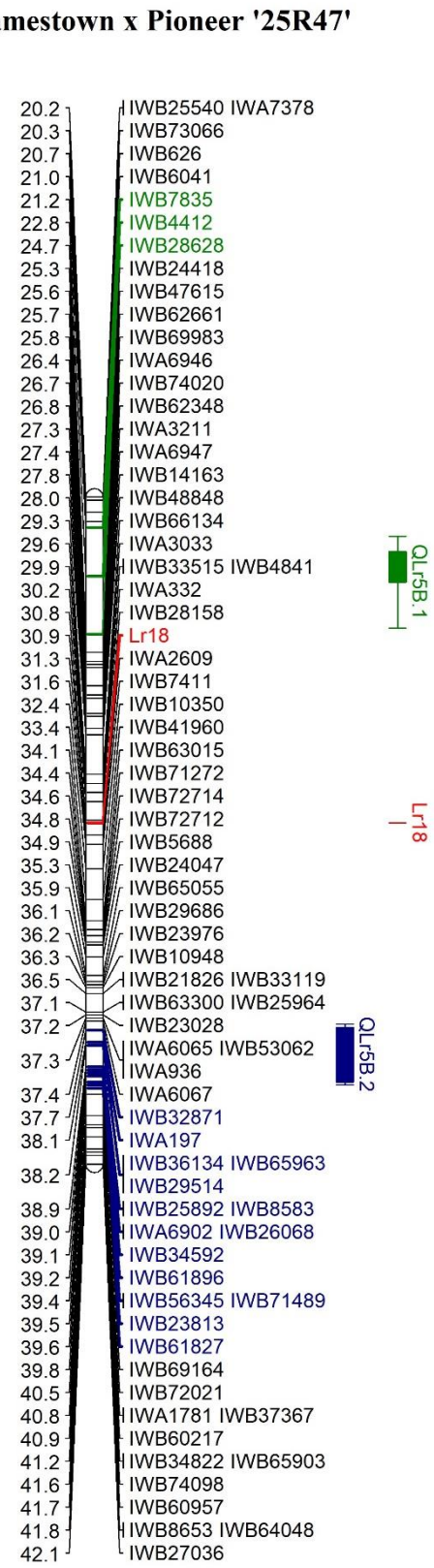

Figure 3.2. Partial linkage map of chromosome 5B from Carpenter et al. (2017) with *Lr18*, *QLr5B.1*, and *QLr5B.2*.

Table 3.1. Soft red winter wheat cultivars postulated to carry gene *Lr18* and prospective parental donor ancestors.

Release	Cultivar	Pedigree
1956	Bledsoe (CItr 13238)	Frondoso//Redhart 3/Noll/3/3*Purplestraw/4/ Steinweidel/ **T.timopheevii** //W38/Hope/3/Purplestraw
1961	GA1123 (CItr 13292)	Trumbull/Red Wonder//Steinweidel/**T. timopheevii** /3/W38/ Illinois No. 1/ Hope//Purplestraw/Thatcher// Leap/4/ Chancellor
1960	Redcoat (CItr 13170)	Surpreza/Fultz sel. CI 11845/7/Kawvale/5/Fultz/Hungarian //W38 /3/ Wabash/4/Fairfield/6/Trumbull*3//Hope/Hussar
1970	Holley (CItr14579)	Georgia 1123*2//Knox 62/Suwon 92/3/Redcoat/Bledsoe
1970	McNair 2203 (CItr15228)	Redcoat sib//Norin 10/Brevor/6/Seneca/5/Asosan /3/ Supreza/Redhart // Chancellor/4/Transfer
1975	McNair 1813 (CItr15289)	Seneca/6/Redcoat sib/5/Redcoat sib/Kenya 338/3/(Purple Straw/Trumbull /Steintin/Thorn)//Kenya 338/4/Norin 10/ Brevor
1977	McNair 1003 (PI552975)	McNair 2203/Blueboy
1980	Stacy (CItr17861)	Purdue 4946/McNair 1813
1980	Coker 797 (CItr17722)	Coker 68-15/5/Coker 57-6//Hadden/4/Nadadores 63/3/Hadden/Purdue 4946A4-18-2-10-1//Coker 57-6*2/Purdue 4946A4-18-2-10-1
1980	Coker 916 (PI600974)	Purdue 6028A2-5-9/3/Coker 61-19*3/Purdue 4946A4-18-2-10-1//Blueboy
1982	Hunter (PI468977)	Coker 68-15/4/Potomac/3/Coker 61-19*3/Purdue 4946A4-18-2-10-1 // Blueboy
1983	Coker 983 (PI601076)	Coker 68-15/4/Potomac/3/Coker 61-19*3/Purdue 4946A4-18-2-10-1 // Blueboy
1990	GA-Gore (PI561842)	Coker 797 / Stacy
1990	Coker 9803 (PI548845)	McNair 1003/Coker 916
1993	2684 (PI566923)	Pioneer Line W9057B/Caldwell//Hunter
1994	2643 (PI583739)	Pioneer line W9032B/Pion. line W1074B//Pion. line W1039B/Coker 983
1995	2691 (PI590941)	Pioneer line W9016A/'2551'//Hunter
1999	26R24 (PI614110)	WBA084D5(Aurora/Tyler//2550sib/Coker87-13)/Coker 983//Coker 87-13
2000	38158 (PI619052)	FFR555W/GA-Gore
2000	Renwood3260 (PI635148)	SC861562/Coker 9803
2004	Choptank (PI639724)	Coker 9803/Freedom
2007	Jamestown (PI653731)	Roane/Pioneer 2691
2015	Hilliard (PI66271)	Pioneer 25R47/Jamestown

Table 3.2. Soft Red Winter Wheat RIL and F_2 populations for goodness of fit chi-square analysis.

P1†	P2†	Pedigree	Generation	Number of Individuals	*df*	SEG PATTERN	χ^2	*p*
R	R	RL6009 / Jamestown	F_2	200	1	-	-	-
R	S	Jamestown / MCIA Venus	F_2	200	1	3:1	0.04	0.84
R	S	Jamestown / VA10W-21	F_2	200	1	3:1	2.47	0.12
R	S	RL6009 / VA10W-21	F_2	200	1	3:1	2.19	0.14
R	S	Jamestown / P0537A1-12	F_2	200	1	3:1	0.79	0.37
S	R	VA08MAS-369 / Jamestown	F_2	200	1	3:1	2.32	0.13
R	R	RL6009 / VA10W-119	F_2	200	1	3:1	1.18	0.28
R	R	RL6009 / VA09W-110	F_2	200	1	3:1	2.47	0.12
S	R	Pioneer 25R47/ Jamestown	F_5:F_9	186	1	3:1	2.59	0.11
R	R	RL6009 / Shirley	F_2	200	1	15:1	0.69	0.40

† P1, P2 are classified as R as they are postulated to be resistant and contain *Lr18* ,or as S as they are postulated to be susceptible and not contain *Lr18*

Table 3.3. KASP primers developed for SNP loci polymorphic among all population linked with Lr18 through genotyping using the iSelect 90K wheat assay

SNP ID	iSelect 90K SNP Name	Primer Sequence		
		Allele 1[a]	Allele 2[b]	Reverse
IWB 5688	BobWhite_s66049_223	gccgaccccaagaaaaca	gccgaccccaagaaaacg	gctttgaacaagtgcagccta
IWB 41960	Kukri_c18410_193	tgaacatctcaacaccagtaact	tgaacatctcaacaccagtaacc	tggtcacagagtggtccg
IWB 4875	BobWhite_rep_c50349_139	gaccgaggtggagctcga	gaccgaggtggagctcgg	atccgactgaagcccacg

[a]A1 primer labeled with FAM: 5' GAAGGTGACCAAGTTCATGCT 3'

[b]A2 primer labeled with VIC: 5' GAAGGTCGGAGTCAACGGATT 3'

Table 3.4. Soft Red Winter wheat cultivars used as parental lines in the Virginia Tech small grains breeding program examined for seedling resistance to *P. triticina*

Line	IWB41960[†]	2017 TNRJJ[‡§]	2016 TNRJJ[‡§]	2015 TNRJJ[‡§]	2014 TNRJJ[‡§]
Hilliard	Lr18	12;	23;	23;	1;
VA11W-108PA	Lr18	12;	2;	-	-
VA11W-106	Lr18	12;	23;	23;	;1
VA11W-279	Lr18	0;	0;	0;	0;
VA11W-313	Lr18	1;	3;	23;	;1
VA12W-72	Lr18	;1	23;	2;	1;
VA12W-68	Lr18	;1	23;	23;	1;
VA13W-174	Lr18	23/0;	0;/3	23/0;	0;1;
VA14W-29	Lr18	12;	3	12;	12;
VA14W-28	Lr18	;1	23;	1;	;1
VA14W-32	Lr18	12;	23;	12;	1;
VA09MAS3-34-2-1	Lr18	1;	23	23	;12
VA07MAS1-7047-1-1-4-2	Lr18	;1	12;	1;	;1
VA09MAS1-12-5-1	Lr18	2;	23	3	23;
VA09MAS1-12-5-1-1	Lr18	1;	3;	3-	-
VA09MAS1-12-5-1-3	Lr18	1;	23;	23;	-
VA09MAS1-12-8-4	Lr18	;1	23;	12;	;1
VA09MAS6-122-7-1	Lr18	12;	23	2;	12;
VA09MAS6-122-7-1-1	Lr18	12;	12;	12;	-
VA09MAS6-122-7-1-4	Lr18	12;	12;	12;	-
VA14FHB-22	Lr18	3	3	3	23;
VA14FHB-29	Lr18	3	3	3	3
VA15W-94	Lr18	3/0;	3	3	-
VA15W-101	Lr18	23;	3	3	-
DH11SRW061-16	-	3	3	3	-
DH11SRW065-23	Lr18	3	3	3	-
DH11SRW065-26	Lr18	0;	0;	0;	-

DH12SRW057-006	Lr18	23;	3	3	-
Pioneer Brand 26R59	-	2;	3;	-	-
SY Viper	Lr18	0;	0;	-	-
NC13-20332	Lr18	12-;	2;	-	-
NC13-21213	Lr18	12;	3;	-	-
GA07169-14LE24	Lr18	-	3	-	-
GA07353-14E19	Lr18	0;	12;	-	-
GAJT 141-14E45	Lr18	1;	12;	-	-
TX EL2	-	1;	2;	-	-
TN1604	Lr18	1;/3	23;	-	-
15 MW 133	Lr18	0;	-	1;	;1
MDC07026-F2-19-13-1	Lr18	-	-	12;	;1
L11541	Lr18	-	23;	1;	12;
MD272-8-4-14-8	Lr18	0;	0;	0;	-
MD07W478-14-6	Lr18	-	3;	23;	-
CROPLAN 8550	-	23;	3	-	-
OH09-207-68	Lr18	-	3	-	-
X08C-1077-11-18-3	-	-	;1	-	-
VA09MAS8-34-5-2	-	;1/3	0;23	0;/3	;1
VA09MAS2-131-6-2	-	;1	0;	0;	0;
VA09MAS2-131-6-2-4	Lr18	0;	0;	;1	-
VA15W-63	-	0;	0;	0;	-
DH11SRW069-70	Lr18	;1	;1	0;	-
DH12SRW056-058	-	23;	3	3	-
VA13W-38	Lr18	;1/3	;12	;12	;1
VA12W-31	Lr18	;1	1;	1;	;1
VA12FHB-8	-	3	3	3	3
DH11SRW070-14	-	12;	2;	23;	;1
VA08MAS1-188-6-4-1	Lr18	0;	0;	;1	0;
Shirley	-	0;	;1	0;	0;
KY07C-1145-94-12-5	-	-	12;	-	-

KWS 074	Lr18	-	3	-	-
P0762A1-2-8	-	3/;1	3/;1	12;	-
P04620A1-1-7-4-17	-	-	3	-	-

†Indicates the presence or absence of Lr18

‡Seedlings of parental lines screened with TNRJJ from 2013-2017. Lines were rated using Roelf's scale.

§ Roelf's scale was scored as: 0 was scored as no visible spores, 1 as small spores with hypersensitive responses, 2 as small to medium sized spores that are bordered by chlorosis of leaf tissue, 3 as medium sized spores without chlorosis, and 4 as large spores with no chlorosis. Those scores with a ; included indicates a hypersensitive response often referred to as a "fleck" response.

CHAPTER IV

Identification of Quantitative Trait Loci for Adult Plant Resistance to *Puccinia triticina* in the Soft Red Winter Wheat Cultivar 2013412

Neal Carpenter, Carl Griffey, Gina Brown-Guedira, Priyanka Tyagi, J. Paul Murphy, Marla Barnett, Mark Christopher

Neal Carpenter and Carl Griffey, Dept. of Crop and Soil Environmental Sciences, Virginia Tech, Blacksburg, VA 24061; Gina Brown-Guedira and Priyanka Tyagi, Eastern Regional Small Grains Genotyping Laboratory USDA-ARS, North Carolina State University, Raleigh, NC 27695; J. Paul Murphy, Department of Crop Science, North Carolina State University, Raleigh, NC 27695; Marla Barnett, Limagrain Genetics, Wichita, KS 67204; Mark Christopher, KWS Cereals USA LLC, Champaign, IL 61822.

ABSTRACT

Disease resistance is critical in soft red winter wheat (*Triticum aestivum* L.) cultivars. Leaf rust caused by *Puccinia triticina* Eriks is a destructive pathogen of wheat. Phenotypic data was collected at diverse locations for resistance to leaf rust (Illinois, North Carolina, and Virginia) in a '2013412' (PI 667644) / VA10W-21 (PI 676295) population (412/21) composed of 157 doubled haploid (DH) lines. The 412/21 DH lines were genotyped via genotyping by sequence (GBS). Analysis of the 412/21 population identified one quantitative trait loci (QTL) region associated with adult plant resistance to leaf rust on chromosome 1B. This QTL is associated with both infection type and disease severity. Phenotypic variation (%) explained by the putative leaf rust resistance QTL of 2013412 on 1B was as high as 40.1%. Introgression and pyramiding of this QTL with other genes conferring resistance to leaf rust via marker-assisted selection will facilitate development of soft red winter wheat cultivars having more durable resistance. Kompetitive allele-specific (KASP) markers KASP_S1B_8414614 and KASP_S1B_8566239 were developed as markers for use in marker assisted selection.

INTRODUCTION

Leaf rust (*Puccinia triticina* Eriks) is the most common type of rust of wheat (*Triticum aestivum* L.) worldwide (Bolton et al., 2008) and can be found on every continent with the exception of Antarctica (Huerta-Espino et al., 2011). In the southeastern United States soft red winter (SRW) wheat region, disease severity will typically peak during April in Georgia and at the end of May in Virginia (Kolmer and Hughes, 2013). Leaf rust is a destructive pathogen of wheat that reduces photosynthetic area and limits photosynthesis, and ultimately affects seed development and yield (Roelfs, 1992). Control of leaf rust through deployment of diverse and durable genetic resistance in cultivars as opposed to reliance upon fungicides, has been reported to be the most cost effective method with an estimated 27:1 benefit to cost ratio (Marasas et al. 2003).

Host resistance is the most economical solution for providing full season control and reducing damage due to diseases as opposed to use of multiple fungicide applications. Most leaf rust (*Lr*) resistance genes operate on a gene for gene basis (Bolton et al., 2008) and their action and effectiveness is race-specific. Pyramiding of multiple genes into single cultivars and use of quantitative trait loci (QTL) conferring adult plant resistance (APR) is the best strategy to achieve durable resistance. Adult plant resistance or quantitative resistance is more durable and effective against multiple races of a pathogen than race specific resistance.

Genotyping-by-sequence (GBS) allows for the complexity of genomes to be reduced by use of restriction enzymes and multiplex sequencing (Elshire et al., 2011; Poland et al., 2012). Methylation-sensitive restriction enzymes reduces the repetitive sequences while allowing for the genotyping of large genomes without a sequenced genome (Poecke et al., 2013). This allows

access to important genomic regions that are inaccessible by sequence capture approaches (Mir et al. 2013).

Soft red winter (SRW) wheat cultivars 2013412 (VA06W-412) and VA10W-21 were crossed and used to develop doubled haploid lines in order to map QTL associated with resistance to leaf rust. Cultivar 2013412 (*SS8412*) is a broadly adapted, high yielding, full-season, short height semi-dwarf producing grain has exceptional adult plant resistance to *Puccinia triticina*. It has performed well in diverse regions of the SRW wheat production area from Louisiana to Wisconsin. Cultivar 2013412 is resistant to leaf rust, stem rust (*Puccinia graminis* f. sp. *tritici* Pers.) powdery mildew (*Blumeria graminis* (DC) Speer), and *Barley Yellow Dwarf Virus*. On the basis of preliminary research, 2013412 was postulated to have gene *Lr46*. Wheat genotypes with combinations of adult plant resistance genes such as *Lr34*, *Lr46*, and *Lr68* have exhibited slow rusting durable leaf rust resistance, although these genes are most effective when pyramided with other seedling resistance genes (Lillemo et al., 2008). Slow rusting describes resistance mechanisms typically that do not completely stop fungal infection, but slow the infection rate and reduce the number and size of pustules and spores (Singh et al., 2000). A number of these slow-rusting genes need to be pyramided together to achieve effective genetic control since a single gene usually does not have enough effect to significantly limit disease progress.

In this study a doubled haploid population was derived from a 2013412 / VA10W-21 cross, and was genotyped using GBS technology. The objectives of this study were to (1) map QTLs for leaf rust resistance traits in 2013412 using a high-density GBS-SNP map, (2) develop KASPar markers closely linked to the QTL for deploying marker-assisted selection in wheat breeding programs.

MATERIALS AND METHODS

Plant Materials

One hundred and fifty seven doubled haploid (DH) lines were derived from the cross of 2013412 (PI 667644) by VA10W-21 (PI 676295). The cultivar 2013412 was derived from the cross 'Tribute' (PI 632689) / 'AGS 2000' (PI 612956) // VAN99W-20 (VA90-54-631 / VA90-52-49). Parentage of VA90-54-631 is 'Tyler' / 'Coker 78-23' // 'McNair 1003' /3/ 4* 'Massey' /8/ 'Saluda' /7/ FL737G3- 12-2-12 / Tyler /5/ CItr13836 / 9* 'Chancellor' // 'Wheeler' /3/ 'Severn' /4/ 'Feland' /6/ Tyler. Parentage of VA90-52-49 is 'Hunter' / Wheeler. The pedigree of FL737G3-12-2-12 is Vogel 5 / 'Anderson' // Purdue Dwarf / 'Hadden' /3/ Purdue 6562A1-4-2 /4/ 'Blueboy II' / 'Coker 68-8'. Parentage of P6562A1-4-2 is 'Siete Cerros' / 'Arthur'.

Field Assessment

The DH population was evaluated in replicated field tests comprised of 1.2 m rows as the experimental unit arranged in a randomized complete block design. Disease resistant and susceptible checks along with both parents of the population were spaced throughout the design. Disease spreader strips comprised of a mixture of the leaf rust susceptible cultivars Massey (PI 17953) (Starling et al., 1984) , Sisson (PI 617053) (Griffey et al., 2003), and FFR 555W (PI 560318) bordered the RIL population at each location.

Leaf rust assessments of the DH population were conducted at Warsaw, VA (1 replicate in 2015/16 and 2 replicates in 2016/2017) where susceptible borders were inoculated with leaf rust races TNRJJ and TCRKG using Soltrol light mineral oil (Chevron Phillips Chemical Company, Woodlands TX) in addition to natural infection. The RIL population was also evaluated at Kinston and Plymouth, NC (2 replications), and Champaign, IL (1 replication) under

natural infection in 2017. Disease ratings were taken when the susceptible checks displayed no less than 30% severity. The population was assessed for infection type using a 0 to 9 scale (Singh et al., 1992) and disease severity from 0 to 100 percent based on the modified Cobb scale (Peterson et al., 1948) for *P. triticina*. Leaf rust was rated one to three times at each location per growing season depending on crop growth stage when optimal infection levels of *P. triticina* occurred.

GBS library construction and SNP identification

Genotyping by sequencing (Elshire et al., 2011) using the protocol described by Poland et al. (2012) was conducted for all 157 lines by the USDA-ARS Eastern Small Grains Regional Genotyping Center. DNA was extracted from tissue collected from 10 day old plants using DNEasy 96 Plant Kits (Qiagen Group, Crawley, Sussex, UK). Genome complexity was reduced using a combination of two enzymes, MspI (CCGG) a common cutter and PstI (CTGCAG) a rare cutter, and barcoded adaptors were ligated to each sample. Ninety-six individual samples were pooled into a single library and polymerase chain reaction amplified, each library was sequenced on an Illumina HiSeq 2500 (Illumina, San Diego, CA, USA). Single Nucleotide Polymorphism (SNP) calling on raw sequence data was done with Tassel5GBSv2 pipeline (Bradbury et al., 2007) using the alignment method of BWA version 0.7.12 (Li and Durbin, 2009) for aligning SNPs to a reference sequence. The International Wheat Genome Sequencing Consortium (IWGSC) genome assembly v0.4 was used as a reference genome to align the SNP with a physical position. A total of 20,590 polymorphic SNP were identified and a configured in HapMap format. The sequenced data was first filtered in TASSEL 5.2.30 for a minor allele frequency less than 5%. Each SNP was then calculated for percentage of missing data, any SNP above 20% missing data was dropped due to the poor quality of the SNP.

Genetic map construction and QTL analysis

The BIN function in IciMapping 4.1 (Wang et al., 2012) was used to delete redundant markers. In R/QTL the ASMap v0.4 (Taylor et al., 2017) package was used to create the linkage groups with the MSTmap algorithm (Wu et al. 2008). IciMapping 4.1.0 (Wang et al., 2012) was used to identify QTL via interval mapping (IM) and composite interval mapping (CIM). The critical LOD value of 3.0 to declare a QTL significant ($p = 0.05$) was based on 1000 permutations (Doerge and Churchill, 1996) for all traits and linkage groups. MapChart 2.2 (Voorrips, 2002) was used to draw linkage maps.

KASPar SNP and QTL Validation

Tissue of each DH was collected when seedlings reached the three-leaf stage and placed into 2 ml test tubes, each containing two stainless steel beads for tissue grinding. Tissue samples were frozen in an ultra-low temperature (−80°C) freezer and then subsequently ground using a Spex CertiPrep 2000 Geno-Grinder (Metuchen, NJ, USA) for 15 s or until finely ground. The DNA extraction was then implemented using a modified CTAB method (Saghai-Maroof et al., 1984). KASPar oligos were ordered from Integrated DNA Technologies (Coralville, IA), with primers carrying standard FAM or VIC compatible tails and the target SNP in the 3' end (Table 4.1). Primer mix was set up as recommended by LGC Genomics (Middlesex, UK) (46 µl dH_2O, 30 µl common primer (100 µM), and 12 µl of each tailed primer (100 µM)). KASPar primers were used for a validation of results found in the QTL analysis. Linkage maps were constructed using IciMapping 4.1.0 (Wang et al., 2012), the Kosambi mapping function (Kosambi, 1943) was used to estimate map distance, and linkage groups were constructed based upon a minimum logarithm of odds (LOD) threshold value of 6.0.

RESULTS

Linkage Maps

Markers were placed on all 21 chromosomes of genomes A, B, and D. A total of 20,590 SNPs were polymorphic between the parents 2013412 and VA10W-21, and 4,569 SNPs were used to create a high density linkage map after filtering and deletion of redundant markers.

QTL Analysis

One QTL was identified in 2013412 associated with leaf rust resistance on chromosome 1B. Markers linked to the QTL with the highest LOD scores in each year for each trait are presented in Tables 4.1 and 4.2. The QTL on chromosome 1B conferring leaf rust resitance has a large effect and was identified in each year for both infection type and disease severity.

QTL Associated with Leaf Rust Resistance on Chromosome 1B

The LOD peak of the QTL located on chromosome 1B, designated *QLr.vt-1B* (Fig. 4.1) was located at SNP marker interval S1B_8414614 – S1B_9571857 between positions 161 and 165, respectively. Phenotypic variation in infection type explained by *QLr.vt-1B* was highest (49.6%) in the 2016 Blacksburg test, 28.7% in the 2017 Plymouth test, 26.5% in the 2016 Warsaw test, and 25.2% in the 2017 Kinston test (Table 4.2). The LOD scores were 35.3, 12.0, 11.5, and 10.3, respectively. Phenotypic variation in leaf rust severity explained by *QLr.vt-1B* also was highest (40.1%) in the 2016 Blacksburg test, 36.5% in the 2017 Warsaw test, 29.2% in the 2016 Warsaw test, 29.1% in the 2017 Plymouth test, and 19.7% in the 2017 Kinston test. The LOD scores were 32.8, 15.3, 15.8, 12.3, and 6.5, respectively.

To confirm the KASP markers would produce similair results CIM was performed with the KASP markers that were polymorphic. The LOD peak of the QTL located on chromosome 1B, designated *QLr.vt-1B* (Fig. 4.1) was located at SNP marker interval KASP_S1B_8414614 – KASP_S1B_8566239. Phenotypic variation in infection type explained by *QLr.vt-1B* was highest (25.5%) in the 2016 Warsaw test, 22.9% in the 2016 Blacksburg test, 20.4% in the 2017 Kinston test, 20.0% in the 2017 Warsaw test, and 19.3% in the 2017 Plymouth test (Table 4.3). The LOD scores were 10.5, 9.3, 7.9, 7.7, and 7.2, respectively. Phenotypic variation in leaf rust severity explained by *QLr.vt-1B* also was highest (29.7%) in the 2016 Warsaw test, 19.9% in the 2017 Kinston test, 15.8% in the 2017 Plymouth test, and 14.2% in the 2016 Blacksburg test. The LOD scores were 12.6, 7.8, 5.7, and 5.5, respectively.

Minor variability in the amount of phenotypic variation explained by QTL *QLr.vt-1B* is mostly likely due to differences in infection type and severity in the different environments and subsequently via environment by genotype interactions (Fig. 4.2).

Phenotypic Effects of QTL Marker Loci on Leaf Resistance

Prior marker information from the 2009 – 2010 Uniform Southern Soft Red Winter Wheat Nursery report (Brown-Guiedira, 2010) and the current marker data confirm that cultivar 2013412 possess genes *Lr24* and *Lr46* in addition to *QLr.vt-1B*. Gene *Lr46* was likely inheritied from the cultivar 'McCormick' as it is known to posess *Lr46* (Griffey et al., 2005). To predict the effect of individual versus pyramided genes / QTL on leaf rust resistance, individual DH lines of the mapping population containing different allelic combinations (e.g. + or -) of *QLr.vt-1B* , *Lr24*, and *Lr46* were delineated into separate haplotype. The means of each DH group associated with disease infection type and severity were compared using a Students t test (Table

4.4). Cultivar 2013412 and DH lines containing *QLr.vt-1B*, *Lr24* and *Lr46* or only *QLr.vt-1B* and *Lr24* were most resistant to leaf rust and did not differ significantly ($P < 0.05$) for mean infection type or severity. This indicates that there may be some positive interaction between *QLr.vt-1B* and *Lr24*. Lines containing *QLr.vt-1B* and *Lr46* were similar to lines having only *QLr.vt-1B* and had a significantly ($p > 0.05$) lower mean infection type than lines containing only *Lr46*. This indicates that there is little to no interaction between *QLr.vt-1B* and *Lr46*. Lines having only *QLr.vt-1B* also had a significantly ($p > 0.05$) lower mean infection type than lines having *Lr24* and *Lr46* or either gene alone. Lines having only *Lr24* had the highest infection type and severity means as expected since virulence for this gene is widespread.

DISCUSSION

Leaf Rust Resistance QLr.vt-1B on Chromosome 1B

Six known leaf rust genes, *LrZH84*, *Lr26*, *Lr33*, *Lr44*, *Lr46* , *Lr71*, and *Lr75*, have previously been reported to reside on chromosome 1B (Dyck et al., 1987; Singh et al., 2013; Singh et al., 1990; Singla et al., 2017; William et al., 2003; Yang et al., 2004; Zhao et al., 2008). It is unlikely that the source of resistance in cultivar 2013412 is *Lr26* as this gene resides on the 1BL/1RS translocation (Singh et al., 1990), which 2013412 lacks. Genes *Lr33*, *Lr44*, and *Lr46* are known to reside on the termial end of chromosome 1B (Dyck et al., 1987; William et al., 2003; Yang et al., 2004); whereas, *QLr.vt-1B* resides on the proximal end of chromosome 1BS. Genes *LRZH84* and *Lr75* are located close to the centromere of chromosome 1B (Singla et al., 2017; Zhao et al., 2008). Gene *Lr71* maps a significant distance away from *QLr.vt-1B* (Supplemental Table 4.1). There are several other regions on 1B that have been reported to be a

cluster of QTL associated with leaf rust resistance (Soriano and Royo, 2015). Additional information is available in the T3 database (Blake et al., 2016; Peng and Yang, 2017) and Supplementary Table 4.1. However, most of these QTL confer resistance to leaf rust in both the seedling and adult plant stages, while *QLr.vt-1B* confers only adult plant resistance. The QTL detected in 'Forno' and 'Oberkulmer' (Messmer et al., 2000), 'MG5323' (Desiderio et al., 2014), 'Pastor' (Rosewarne et al., 2012), and 'Francolin#1' (Lan et al., 2014), were mapped to the short arm of chromosme 1B but are closer to the centromere than the proximal end where *QLr.vt-1B* resides. The QTL mapped in 'Sujata' (Lan et al., 2015), 'Bainong 64' (Ren et al., 2012), and 'Pavon 76' (William et al., 2006) were mapped to the long arm of chromosome 1B, while *QLr.vt-1B* resides on chromosome 1BS. Based upon this information it is likely that *QLr.vt-1B* is a novel resistance QTL.

Breeding Applications

One QTL region identified on chromosome 1B was associated with adult plant resistance to leaf rust (Tables 4.2 and 4.3). Once markers closely linked to this QTL are validated, they can be used in marker-assisted selection (MAS) to incorporate and pyramid *QLr.vt-1B* with other effective resistance genes. Lines having *QLr.vt-1B* and *Lr24* either with or without *Lr46* had similar leaf rust infection type and severity means as 2013412 (Table 4.4). Lines having only *QLr.vt-1B* had similar means for leaf rust infection type and severity as lines having *QLr.vt-1B* and *Lr46*. Lines having *Lr46* either with or without *Lr24* did not differ significantly from lines lacking any resistance genes / QTL for leaf rust resistance. While pyramiding *QLr.vt-1B* and *Lr24* likely will be most advantageous, the addition of *Lr46* may also be beneficial in reducing leaf rust severity and further enhancing resistance durability. Future work will include the validation of kompetitive allele-specific markers KASP_S1B_8414614 and

KASP_S1B_8566239 to validate *QLr.vt-1B* in other genotypes having cultivar 2013412 in their genetic backgrounds, and for routine use by the USDA Small Grains Genotyping Labs in obtaining haplotypes of cultivars, breeding lines and parents, and deployment in MAS breeding.

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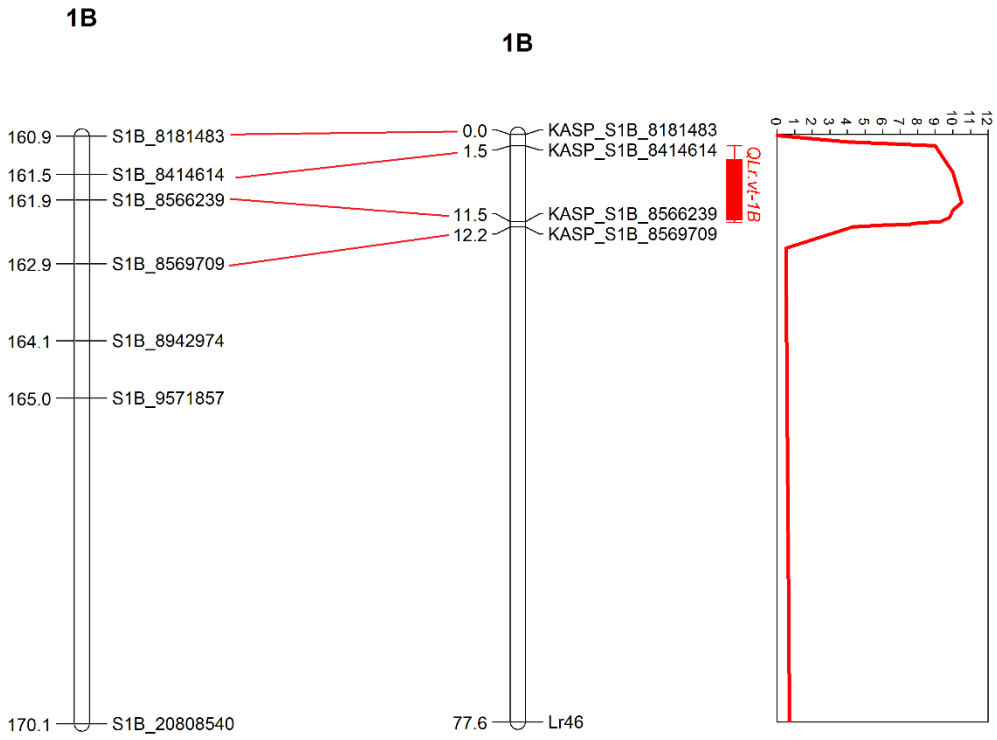

Figure 4.1. Partial linkage map of chromosome 1B in the 2013412 / VA10W-21 DH wheat population showing the putative location of *QLr.vt.1B* conferring adult-plant resistance to leaf rust. GBS map (left). KASPar map (right)

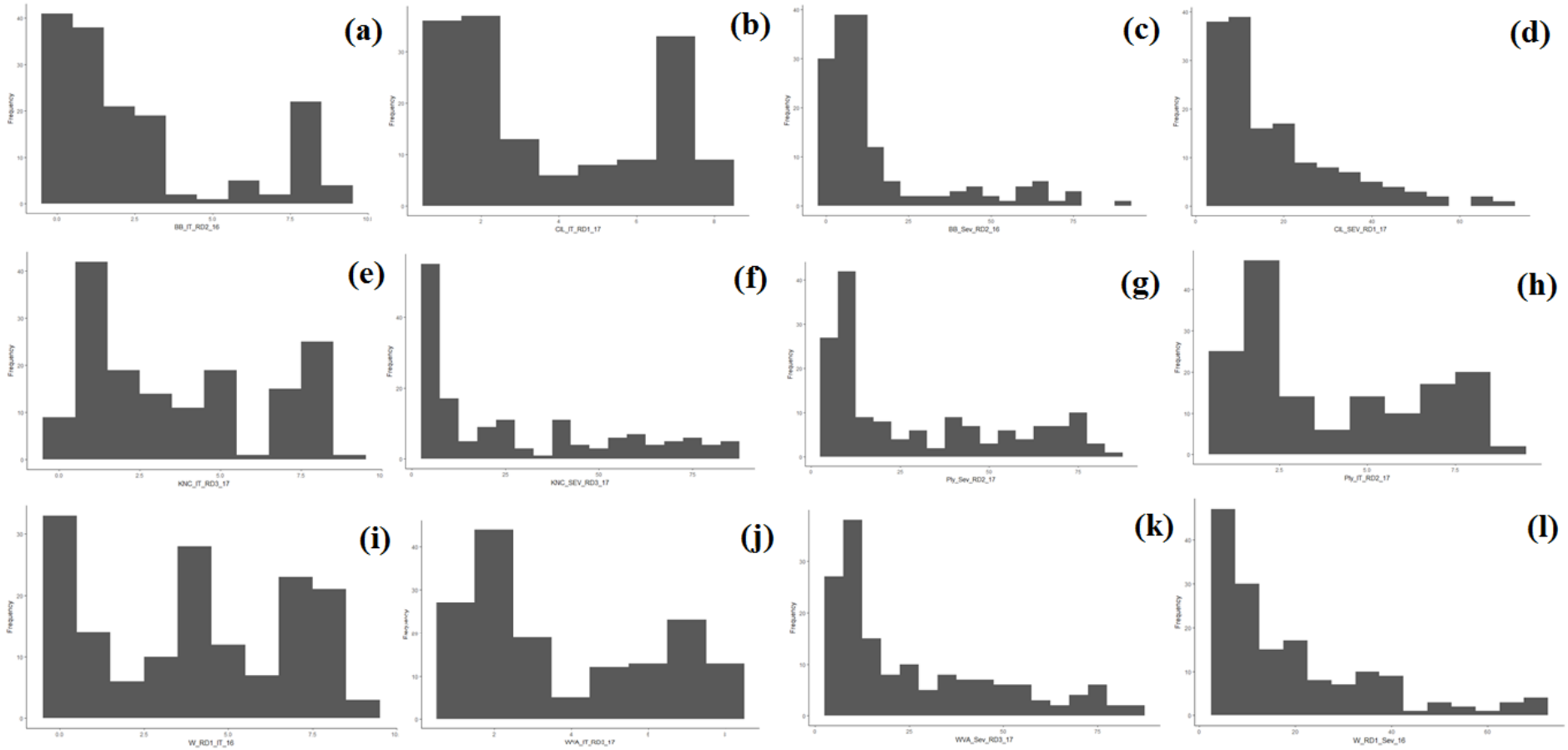

Fig 4.2. Histograms of leaf rust infection type and severity for the 2013412 / VA10W-21 DH wheat population at locations used in mapping leaf rust resistance conferred by QLr.vt-1B. First two letters indicate states (WVA = Warsaw, VA; KNC = Kinston, NC; PNC = Plymouth, NC); letters following underscore represent traits (IT = infection type; SEV = severity); the last two digits indicate rating date (RD, each rating date was analyzed separately for each location).

Table 4.1. Significant GBS SNPs associated with resistance to Puccinia *triticina* in the 2013412/VA10W-21 doubled haploid wheat population based on marker-trait association analysis converted to KASPar sequences.

Primer†	Sequence
S1B_8181483_ALC_FAM	GAAGGTGACCAAGTTCATGCTGGATTCGTAACCACATGTAACATTTC
S1B_8181483_ALT_VIC	GAAGGTCGGAGTCAACGGATTCTTGGATTCGTAACCACATGTAACATTTT
S1B_8181483_C1	GACGACGACGAGGAGGAGGTT
S1B_8414614_ALA_FAM	GAAGGTGACCAAGTTCATGCTCATCCTCGGTTCACTCTTGTTACA
S1B_8414614_ALT_VIC	GAAGGTCGGAGTCAACGGATTCATCCTCGGTTCACTCTTGTTACT
S1B_8414614_C1	CAGTAGCCTGCAGGCAAGCCAA
S1B_8566239_ALT_FAM	GAAGGTGACCAAGTTCATGCTTACTGACGCCTCCATCTGCGA
S1B_8566239_ALC_VIC	GAAGGTCGGAGTCAACGGATTCTGACGCCTCCATCTGCGG
S1B_8566239_C1	TTGGTATATTCGCAATGAAGTGGTGCATA
S1B_8569709_ALA_FAM	GAAGGTGACCAAGTTCATGCTCCGTGTGATGCGGCAAGTGCA
S1B_8569709_ALG_VIC	GAAGGTCGGAGTCAACGGATTCGTGTGATGCGGCAAGTGCG
S1B_8569709_C1	CTCAACACCAAACTGAACATGCACGTA
S1B_8942974_ALC_FAM	GAAGGTGACCAAGTTCATGCTGAGCTGCAGCACATGTTCGAG
S1B_8942974_ALT_VIC	GAAGGTCGGAGTCAACGGATTGGAGCTGCAGCACATGTTCGAA
S1B_8942974_C1	CGCCACCATCCATGGGAGTGAT
S1B_9571857_ALC_FAM	GAAGGTGACCAAGTTCATGCTGTACCAGCCGTACGGACACG
S1B_9571857_ALT_VIC	GAAGGTCGGAGTCAACGGATTGGTACCAGCCGTACGGACACA
S1B_9571857_C1	TTTCAATCTGGCCTCTTCCTCCCTT
S1B_20808540_ALG_FAM	GAAGGTGACCAAGTTCATGCTGATCTTCCGTGGCGCCAGC
S1B_20808540_ALA_VIC	GAAGGTCGGAGTCAACGGATTCGATCTTCCGTGGCGCCAGT
S1B_20808540_C1	GCAACTCCGACTCTGACGGAGAA

†GBS SNP in KASPar marker format with two allele specific primers (AL) and one reverse primer (C1).

Table 4.2. Quantitative trait loci (QTL) and GBS SNPs associated with leaf rust infection type and severity in 2013412/VA10W-21 doubled haploid wheat lines evaluated in 2016 and 2017 seasons.

Trait name†	Chr‡	Confidence Interval	Left marker	Right marker	LOD§	PVE¶	Add#
BVA_IT_RD1_16	1B	160.9-165.0	S1B_8414614	S1B_9571857	35.3	49.6	-2.8
BVA_IT_RD2_16	1B	160.9-165.0	S1B_8414614	S1B_9571857	34.2	46.3	-2.7
KNC_IT_RD2_17	1B	160.9-165.0	S1B_8414614	S1B_9571857	10.3	25.2	-1.5
PNC_IT_RD1_17	1B	160.9-165.0	S1B_8414614	S1B_9571857	12.0	28.7	-1.6
WVA_IT_16_RD1	1B	160.9-165.0	S1B_8414614	S1B_9571857	9.3	26.4	-1.5
WVA_IT_16_RD2	1B	160.9-165.0	S1B_8414614	S1B_9571857	11.5	26.5	-1.9
BVA_Sev_RD1_16	1B	160.9-165.0	S1B_8414614	S1B_9571857	28.7	25.1	-15.7
BVA_Sev_RD2_16	1B	160.9-165.0	S1B_8414614	S1B_9571857	32.8	40.1	-17.9
KNC_Sev_RD1_17	1B	160.9-165.0	S1B_8414614	S1B_9571857	6.5	19.7	-4.8
KNC_Sev_RD2_17	1B	160.9-165.0	S1B_8414614	S1B_9571857	8.2	4.4	-8.2
PNC_Sev_RD1_17	1B	160.9-165.0	S1B_8414614	S1B_9571857	10.0	13.6	-12.6
PNC_Sev_RD2_17	1B	160.9-165.0	S1B_8414614	S1B_9571857	12.3	29.1	-15.4
WVA_Sev_RD1_16	1B	160.9-165.0	S1B_8414614	S1B_9571857	15.8	29.2	-10.3
WVA_Sev_RD2_16	1B	160.9-165.0	S1B_8414614	S1B_9571857	16.1	29.1	-17.5
WVA_Sev_RD1_17	1B	160.9-165.0	S1B_8414614	S1B_9571857	12.1	24.7	-8.8
WVA_Sev_RD2_17	1B	160.9-165.0	S1B_8414614	S1B_9571857	21.1	27.8	-14.9
WVA_Sev_RD3_17	1B	160.9-165.0	S1B_8414614	S1B_9571857	15.3	36.5	-15.6

† First two letters indicate states (WVA = Warsaw, VA; KNC = Kinston, NC; PNC = Plymouth, NC); letters following underscore represent traits (IT = infection type; SEV = severity); the last two digits indicate rating date (RD, each rating date was analyzed separately for each location).

‡ Chromosome.

§ Logarithm of odds value.

¶ Phenotypic variation explained.

Level of additivity.

Table 4.3. Quantitative trait loci (QTL) and KASPar markers associated with leaf rust infection type and severity in 2013412/VA10W-21 doubled haploid wheat lines evaluated in 2016 and 2017 seasons.

Trait name†	Chr‡	Left marker	Right marker	LOD§	PVE¶	Add#
						%
BB_IT_RD1_16	1B	KASP_S1B_8414614	KASP_S1B_8566239	9.3	22.9	-1.8
BB_IT_RD2_16	1B	KASP_S1B_8414614	KASP_S1B_8566239	9.3	22.8	-1.8
CIL_IT_RD1_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	4.1	11.7	-1.0
KNC_IT_RD1_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	7.8	20.3	-1.3
KNC_IT_RD2_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	4.8	13.3	-1.1
KNC_IT_RD3_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	7.9	20.4	-1.6
Ply_IT_RD1_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	6.9	16.9	-1.3
Ply_IT_RD2_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	7.2	19.3	-1.4
W_IT_RD1_16	1B	KASP_S1B_8414614	KASP_S1B_8566239	10.5	25.5	-1.8
W_IT_RD2_16	1B	KASP_S1B_8414614	KASP_S1B_8566239	8.6	21.4	-1.9
WVA_IT_RD1_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	6.4	16.9	-1.2
WVA_IT_RD2_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	7.7	20.0	-1.3
WVA_IT_RD3_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	7.2	19.0	-1.3
BB_Sev_RD1_16	1B	KASP_S1B_8414614	KASP_S1B_8566239	5.1	13.3	-9.0
BB_Sev_RD2_16	1B	KASP_S1B_8414614	KASP_S1B_8566239	5.5	14.3	-9.9
CIL_Sev_RD1_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	5.6	15.6	-6.9
KNC_Sev_RD1_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	6.6	15.9	-5.7
KNC_Sev_RD2_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	4.1	11.7	-6.4
KNC_Sev_RD3_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	7.8	19.9	-14.6
Ply_Sev_RD1_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	4.2	11.9	-9.4
Ply_Sev_RD2_17	1B	KASP_S1B_8414614	KASP_S1B_8566239	5.7	15.8	-12.1
W_Sev_RD1_16	1B	KASP_S1B_8414614	KASP_S1B_8566239	12.6	29.7	-11.7
W_Sev_RD2_16	1B	KASP_S1B_8566239	KASP_S1B_8569709	8.6	8.1	-12.4

† First two letters indicate states (WVA = Warsaw, VA; CIL=Champaign, IL; KNC = Kinston, NC; PNC = Plymouth, NC); letters following underscore represent traits (IT = infection type; SEV = severity); the last two digits indicate rating date (RD, each rating date was analyzed separately for each location).

‡ Chromosome.

§ Logarithm of odds value.

¶ Phenotypic variation explained.

Level of additivity.

Table 4.4. Mean leaf rust infection type and severity of parent 2013412 and double haploid wheat lines having different haplotype combinations of *QLr.vt-1B, Lr24*, and *Lr46*.

QTL combination†	Infection type	Severity	Individuals‡
2013412	2.10a	10.5a	–
QLr.vt-1B, Lr24, Lr46	2.26ab	11.6ab	16
QLr.vt-1B, Lr24	2.79ab	14.9ab	20
QLr.vt-1B, Lr46	3.32b	18.3abc	27
QLr.vt-1B	3.21b	18.6bc	48
Lr46	5.65c	31.9cd	16
Lr24, Lr46	6.08c	32.4cd	8
None	5.30c	34.7d	16
Lr24	7.15c	56.3e	6

† Mean phenotype of QTL combination of DHs. Those with the same letter are not significantly different ($p < 0.05$). All markers within the respective QTL were used.

‡ Denotes number of DHs in each QTL combination.

Supplement Table 4.1. Physical positions of QLr.vt-1B and various leaf rust resistance QTL on chromosome 1B from the T3 database.

SNP marker	Chromosome	IWGSC RefSeq v1.0[†]		%[‡]
S1B_8181483	1B	8181383	8181583	100
S1B_8414614	1B	8414514	8414714	100
S1B_8566239	1B	8566139	8566339	100
S1B_8569709	1B	8569609	8569809	100
S1B_8942974	1B	8942874	8943074	100
S1B_9571857	1B	9571757	9571957	100
IACX7107	1B	11859343	11859224	99
GENE-0815_140	1B	15142189	15142280	98
BS00074962_51	1B	15658734	15658634	100
S1B_20808540	1B	20831230	20831430	100
BS00110463_51	1B	28485911	28486011	100
IACX7219	1B	28561192	28561311	100
BobWhite_rep_c55186_299	1B	28561281	28561381	100
BS00004789_51	1B	28763603	28763503	100
Excalibur_c58433_839	1B	41087205	41087105	100
wmc230(Lr75)	1B	42183378	42183140	95
Barc8 (LrZH84)	1B	42329355	42329355	100
BS00079450_51	1B	43043569	43043469	100
IAAV7845	1B	45737900	45737700	100
BS00084021_51	1B	50184218	50184118	100
BS00031417_51	1B	50186284	50186184	100
IWB46280	1B	58558123	58558023	99
RAC875_c42113_93	1B	60108250	60108150	100
Barc80	1B	68755062	686755235	99
Tdurum_contig25384_255	1B	69825104	69825004	100
IACX13974	1B	69826757	69826626	100
BS00083533_51	1B	69906151	69906250	100
RAC875_c5544_725	1B	70318074	70318150	100
BS00022745_51	1B	70711205	70711105	100
IAAV6799	1B	74229258	74229064	98
Excalibur_c45655_318	1B	86705825	86705925	100
Tdurum_contig100344_184	1B	87188426	87188326	99
Tdurum_contig100344_160	1B	87188450	87188350	98
BS00031056_51	1B	90710804	90710904	100
BS00094467_51	1B	92871683	92871783	100
BS00094469_51	1B	92871702	92871802	100
IAAV9176	1B	93914935	93915096	100
Kukri_c7647_1122	1B	94831684	94831784	100
tplb0038o14_241	1B	94956280	94956380	100
tplb0038o14_634	1B	94956673	94956773	100

SNP marker	Chromosome	IWGSC RefSeq v1.0[†]		%[‡]
TA001211-0664	1B	94977326	94977379	100
GENE-1623_147	1B	98168644	98168736	98
RAC875_rep_c108757_136	1B	98722083	98722183	100
BS00022736_51	1B	98722356	98722283	100
BS00069316_51	1B	98904315	98904215	100
Kukri_c5299_462	1B	99598026	99597926	100
BS00098730_51	1B	101222351	101222451	100
JD_c6297_810	1B	104465487	104465387	99
IACX6397	1B	104721235	104721354	100
Excalibur_c2541_262	1B	104772866	104772766	100
RAC875_c46581_420	1B	107133499	107133599	100
RAC875_rep_c69176_194	1B	107134173	107134273	100
BobWhite_rep_c49610_521	1B	107135690	107135590	100
BobWhite_rep_c50112_99	1B	107136494	107136587	97
GENE-0403_266	1B	109728540	109728632	98
GENE-0403_110	1B	109728753	109728652	98
Kukri_rep_c108883_577	1B	109729494	109729594	100
IACX20130	1B	111368361	111368161	100
IAAV2848	1B	111368418	111368218	100
Tdurum_contig19251_352	1B	112864418	112864518	100
Tdurum_contig19251_515	1B	112864581	112864681	100
BS00069054_51	1B	115285489	115285389	100
BS00088767_51	1B	115958113	115958189	100
IACX20344	1B	117183520	117183643	100
IAAV4559	1B	119099212	119099371	100
BS00004981_51	1B	119766038	119766126	100
BS00093945_51	1B	119767691	119767791	100
BS00093946_51	1B	119767719	119767819	100
BS00084722_51	1B	119885937	119886036	99
GENE-0041_182	1B	120881220	120881322	97
BS00093740_51	1B	120884101	120884001	100
BS00093736_51	1B	120884238	120884138	100
BS00107597_51	1B	123343401	123343301	100
BS00038368_51	1B	130636903	130636803	100
BS00087988_51	1B	132937636	132937736	100
BS00073034_51	1B	136645710	136645810	100
GENE-4436_150	1B	136650010	136649923	98
JD_c1532_462	1B	137645771	137645871	98
BS00004316_51	1B	137646953	137646868	100

SNP marker	Chromosome	IWGSC RefSeq v1.0[†]		%[‡]
BS00040342_51	1B	139671222	139671122	100
GENE-0121_95	1B	140297030	140296939	97
RFL_Contig2670_718	1B	142523014	142522914	100
GENE-4608_406	1B	144274345	144274441	99
BobWhite_c5757_260	1B	145453071	145453171	98
JD_c5757_605	1B	145453416	145453516	99
Excalibur_rep_c103847_565	1B	145453641	145453741	100
BS00021870_51	1B	145455115	145455015	99
BS00022507_51	1B	145456209	145456109	100
BS00083237_51	1B	145456236	145456164	100
Excalibur_c42764_741	1B	145720338	145720238	100
Kukri_c9105_1184	1B	146616138	146616038	100
IAAV587	1B	148894211	148894310	100
IAAV268	1B	148898412	148898220	100
GENE-0189_45	1B	150367783	150367877	100
BobWhite_c14526_271	1B	151468295	151468195	99
BS00033738_51	1B	151468674	151468587	100
GENE-0489_91	1B	151470283	151470377	99
TA004235-0297	1B	151470906	151470857	100
BobWhite_c45771_129	1B	152798229	152798129	97
Jagger_c7740_104	1B	154598386	154598286	100
BobWhite_c29659_339	1B	154598741	154598841	100
BS00093102_51	1B	155407625	155407525	100
IAAV5364	1B	155750197	155750048	100
IACX6356	1B	157902130	157902018	91
GENE-0366_124	1B	159917083	159916995	97
RAC875_c37025_2027	1B	161210809	161210709	100
BobWhite_c12695_394	1B	161212035	161212135	100
BS00087939_51	1B	162781414	162781514	100
GENE-0456_190	1B	162811401	162811309	98
GENE-0456_163	1B	162811428	162811336	98
BobWhite_c45790_683	1B	163097068	163096968	100
BS00106579_51	1B	166078425	166078325	100
BS00092426_51	1B	166590938	166591038	100
BS00087444_51	1B	166593090	166593008	100
BS00087441_51	1B	166593812	166593712	100
Jagger_c6107_116	1B	166657551	166657651	100
Kukri_c8943_1381	1B	166657822	166657722	100
BS00109001_51	1B	167108704	167108604	100
BS00023185_51	1B	167108717	167108617	100
BobWhite_rep_c53595_274	1B	167713641	167713741	100

SNP marker	Chromosome	IWGSC RefSeq v1.0[†]		%[‡]
Kukri_c24684_134	1B	167714104	167714179	100
BS00081963_51	1B	169662066	169661966	100
Kukri_rep_c115647_349	1B	171044974	171045069	100
GENE-2762_144	1B	173975550	173975454	99
tplb0035p13_560	1B	179629833	179629933	100
RAC875_s117310_106	1B	183878165	183878265	100
IWA5636	1B	186462898	186463007	99
RAC875_c30367_736	1B	186799598	186799505	100
GENE-0040_121	1B	187209953	187210045	98
GENE-0040_194	1B	187210022	187210110	97
TA005710-0827	1B	187210693	187210742	100
BS00095751_51	1B	188262333	188262433	100
Tdurum_contig29891_62	1B	188263106	188263006	99
Kukri_c9721_1525	1B	188793760	188793860	100
BobWhite_rep_c49149_530	1B	189704680	189704584	99
BS00015169_51	1B	189715586	189715486	99
TA003235-0794	1B	189715956	189716009	100
GENE-0561_101	1B	193287241	193287341	100
RAC875_c68119_594	1B	193628379	193628479	100
BS00027514_51	1B	193630123	193630223	100
GENE-0181_607	1B	195593694	195593782	98
TA005699-1050	1B	196464167	196464114	100
BS00035794_51	1B	198644455	198644355	100
Kukri_c17055_189	1B	199010827	199010927	100
GENE-0433_66	1B	199574985	199574896	99
RAC875_c25100_89	1B	199575788	199575688	100
IAAV3801	1B	200300052	200300252	100
IWA3740	1B	201755648	201755551	98
BS00021904_51	1B	207271011	207271111	100
BS00091993_51	1B	207272442	207272542	100
Tdurum_contig27840_865	1B	209130705	209130605	100
Tdurum_contig27840_304	1B	209133171	209133071	100
Excalibur_c24828_1288	1B	212433595	212433495	100
Barc187 (Lr71)	1B	212509755	212510251	83
RAC875_s110045_62	1B	212723504	212723404	100
BobWhite_c10399_401	1B	213326293	213326193	100
Kukri_rep_c111568_656	1B	213911937	213912037	100
BobWhite_c14258_434	1B	217564277	217564180	100
BobWhite_c14258_383	1B	217564302	217564228	100
Excalibur_c18009_66	1B	218421514	218421414	100
GENE-2766_500	1B	223800526	223800622	99

SNP marker	Chromosome	IWGSC RefSeq v1.0†		% ‡
Kukri_rep_c105316_262	1B	230943835	230943735	100
BS00019225_51	1B	234252136	234252036	100
BobWhite_c11036_842	1B	236958751	236958829	100
TA002525-0992	1B	236959829	236959776	100
IWA8230	1B	242735769	242735669	100
RFL_Contig4059_310	1B	249236188	249236288	100
BS00062965_51	1B	251056199	251056299	100
RAC875_c29054_192	1B	260838950	260838850	99
IWA4508	1B	263271041	263271157	99
RAC875_c40579_501	1B	266089723	266089802	100
IAAV1851	1B	269219703	269219903	100
IWA3339	1B	288809971	288810171	99
IACX7921	1B	299974561	299974361	100
Kukri_c31093_387	1B	304510315	304510215	100
Ku_c11813_215	1B	307428472	307428372	99
TA004365-0417	1B	324355801	324355748	100
TA003382-0407	1B	324790666	324790719	100
IWA4389	1B	326779824	326780024	100
IWA7737	1B	331423967	331424131	100
IWA8065	1B	442179828	442179711	99
Ku_c70461_480	1B	463532019	463532119	100
IWA140	1B	465699951	465700071	100
IWA7734	1B	465699980	465700111	100
IWA2753	1B	480368008	480367808	100
IWA540	1B	543012537	543012417	99
BS00003934_51	1B	564908991	564909091	100
RAC875_c55891_659	1B	564909183	564909283	100
IWA5382	1B	586292367	586292499	100
Barc81	1B	627913626	627913935	99
Excalibur_c63243_361	1B	634150615	634150715	99
IWA3095	1B	640849627	640849731	100
wmc44-1B	1B	662194991	662195326	99
IWA802	1B	667918373	667918573	99
wPt-1770-1B	1B	671741402	671741057	99

† Physical Position from IWGSC RefSeq v1.0
‡ Percentage aligned from IWGSC RefSeq v1.0

CHAPTER V

Conclusions and Future Directions

A total of four QTL associated with either leaf or stripe rust resistance in wheat were identified in field studies conducted during 2014 and 2015 growing season. Phenotypic data was collected at diverse locations for resistance to leaf rust (North Carolina, Texas, and Virginia) and stripe rust (Arkansas, North Carolina, Georgia, Texas, and Virginia). Analyses identified two quantitative trait loci (QTL) for leaf rust resistance on chromosome 5B and two QTL for stripe rust resistance on chromosomes 3B and 6A. These QTL were associated with both infection type and disease severity. Phenotypic variation (%) explained by the putative leaf rust resistance QTL of Jamestown on 5B was as high as 22.1%. Variation explained by the putative stripe rust resistance QTL of Jamestown on 3B and 6A was as high as 11.1 and 14.3%, respectively. For QTL *QLr.vt-5B.1* and *QLr.vt-5B.2*, WRKY like transcription factors were identified within both QTL. WRKYs are one of the largest families of transcriptional regulators in plants and are involved in biotic and abiotic stress. Future efforts should include development of near isogenic lines (NIL) using markers from *QLr.vt-5B.1* and *QLr.vt-5B.2* to study expression levels of WRKYs during disease response to leaf rust in the adult plant stage. This should give a better indication whether these WRKY genes are acting a positive regulator against leaf rust and/or working in conjunction with other genes that may be highly expressed by plants in response to the pathogen. A few other genes that the WRKY genes may be working with are a multi-drug resistance protein from the ABC transporter family proteins and a vesicle associated membrane protein (VAMP) that were conserved in *QLr.vt-5B.1* and *QLr.vt-5B.2*, respectively. VAMPs operate in an exocytic plant defense pathway that often form a tertiary complex. The ABC transporter family protein is involved in regulating gene response and control of the stomata,

likely during the infection process. This conserved domain also contributes to plant signaling defenses much like WRKY genes. In QTL *QYr.vt-6A* the conserved domain of a CBL interacting serine/threonine protein kinase was observed. NILs using markers from *QYr.vt-6A* should be developed to also study expression of this gene as described above.

Seedlings of recombinant inbred lines (RILs) from a cross of Pioneer '25R47' / Jamestown and 200 F_2 seedlings from eight other crosses including Jamestown and/or the *Lr18* host differential line RL6009 (Thatcher*6/Africa 43) were screened with *P. triticina* race TNRJJ. Genetic analysis of the populations was conducted to validate the presence of gene *Lr18* in Jamestown. Linkage analysis conducted with SNP markers in the Pioneer 25R47 / Jamestown population identified markers that were tightly linked with *Lr18*, and these were validated in Jamestown / VA10W-21 and RL6009 / VA10W21 F_2 populations. Results of linkage analysis identified SNP maker IWB41960 linked within 5 cM of gene *Lr18* in all three populations. A conserved domain of the GTP binding elongation factor Tu family protein was identified in the marker (IWB41960) region that is tightly linked with *Lr18*. The Tu family protein is often involved in pathogen associated molecular pattern resistance which is involved in defense signaling to prevent disease infection. This is interesting as it is not the typical NB-LRR motif which is part of effector triggered immunity that is common among resistance genes. This could explain why *Lr18* has been maintained through indirect selection over numerous breeding cycles. Isolation and cloning of this gene could provide breeders with a moderately and more durable source of seedling resistance. Future efforts should include development of near isogenic lines in order to develop closer linked markers and/or clone *Lr18*.

One QTL on chromosome 1B of wheat cultivar 2013412, detected in field studies conducted during 2016 and 2017, was associated with adult plant resistance to leaf rust in diverse

locations (Illinois, North Carolina, and Virginia). This QTL is associated with both infection type and disease severity. Phenotypic variation explained by the putative leaf rust resistance QTL of 2013412 on 1B was as high as 40.1%. A TIR-NBS-LRR domain was conserved within *QLr.vt-1B.* This is a resistance motif that is often found among race specific genes. This is interesting as 2013412 lacks seedling resistance to the predominant races of *P. triticina*. Measuring expression of this gene during the period of plant infection may give insights as to whether there are other genes in the defense pathway contributing to the uniqueness of 2013412's resistance. Introgression and pyramiding of this QTL with other genes conferring resistance to leaf rust via marker-assisted selection will facilitate development of soft red winter wheat cultivars having more durable resistance. KASP markers KASP_S1B_8414614 and KASP_S1B_8566239 were developed as markers for use in marker assisted selection. KASP markers linked to the QTL associated with adult plant resistance to leaf rust will be used routinely in the breeding program to haplotype parents, design crosses, and in marker assisted selection.

Identification and cloning of the genes associated with resistance to leaf and stripe rust should provide insight on gene action and the mechanisms governing resistance. This information could be used in the development of high yielding inbred and hybrid wheat cultivars having more effective and durable resistance. Private and public breeding programs have begun development and testing of inbred lines for hybrid wheat production, and the characterization and cloning of these genes/QTL will allow for introgression into heterotic groups for durable resistance.