GENETICS OF LEAF RUST DISEASE RESISTANCE IN WHEAT (*TRITICUM AESTIVUM* L.)

By

SURAJ SAPKOTA

(Under the Direction of Mohamed Mergoum)

ABSTRACT

Genetic resistance is the most efficient and cost-effective way to control leaf rust (LR) disease caused by *Puccinia triticina* (*Pt*) in wheat (*Triticum aestivum* L.). The objectives of this study were to map genomic loci conferring seedling and adult plant LR resistance and develop user-friendly markers for marker-assisted selection (MAS). In our first study, we developed a recombinant inbred line (RIL) population by crossing two soft red winter wheat (SRWW) lines, AGS 2038 (LR resistant) and UGA 111729 (LR susceptible) and phenotyped the parents and RIL population for adult plant LR reaction in four environments. Utilizing high-density linkage map, we detected five QTL for adult plant LR resistance. Of these, a QTL on chromosome 1AL, *QLr.ags-1AL*, was detected in all environments and explained up to 34.45% of phenotypic variation (PV). Molecular marker analysis showed that *QLr.ags-1AL* was different from *Lr59*, the only known leaf rust resistance gene on 1AL– therefore the QTL was temporarily designated as *Lr2K38*. In our second study, we utilized a different RIL population derived from SRWW cultivars, AGS 2000 and Pioneer® variety 26R61 and mapped a single common QTL, *QLr.uga.2BS*, for resistance to two *Pt* races at seedling stage. Based on the physical and genetic positions, *QLr.uga.2BS* was different from other genes/QTL reported on 2BS, and therefore, we temporarily designated it as *LrA2K*. In the third study, we evaluated a panel of 331 genetically

diverse wheat germplasm for their reaction to four *Pt* races at the seedling stage. Phenotypic analysis revealed that majority of the genotypes were susceptible and only 22 genotypes (6.6%) were resistant to all four *Pt* races. Genome-wide association study (GWAS) detected 11 QTL for LR resistance. Of these, six QTL were identified in the vicinity of known genes/QTL, therefore, more studies are warranted to determine their relationships. Five QTL, *QLr.uga-1AL, QLr.uga-4AS, QLu.uga-5AS, QLr.uga-5AL,* and *QLr.uga-7AS*, were identified on genomic regions where no LR resistance genes have been identified in wheat, representing potential novel loci for LR resistance. The germplasm and QTL identified in this research can be used in wheat breeding program to develop durable LR resistance in wheat cultivars.

INDEX WORDS: Wheat, leaf rust, race, genetic resistance, soft red winter wheat (SRWW), genetic map, genomic regions, quantitative trait loci (QTL), recombinant inbred line (RIL), marker-assisted selection (MAS)

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DEDICATION

For my parents (Khum Nath Sapkota and Sita Devi Sapkota), beloved wife (Sima Bista), and my sister Renuka Sapkota – thank you all for your love, support, and encouragement over the years.

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CHAPTER 1

LITERATURE REVIEW

Classification and evolution of wheat

Wheat (*Triticum aestivum* L.) is a member of the grass family, Poaceae, and belongs to the genus *Triticum*. The genus *Triticum* is a member of the tribe Triticeae which contains about 300 species (Clayton and Renvoize 1986). Based on the current nomenclature system, *Triticum* consists of six different species that are at three polyploidy levels, including diploids: *T. monococcum* L. (2n=14, AA genome), *T. urartu* Tumanian ex Gandilyan (2n=14, AA genome), tetraploids: *T. turgidum* L. (2n=28, AABB genome) and *T. timopheevii (Zhuk.) Zhuk.* (2n=28, AAGG genome), and hexaploids: *T. aestivum* L. (2n=42, AABBDD genome) and *T. Zhukovskyi* Menabde & Ericz (2n=42, AAAAGG genome) (Matsuoka 2011). Each species has several subspecies which exist either in a wild or cultivated form. Currently, *T. turgidum* and *T. aestivum*, known as durum and bread wheat, respectively, are the most widely cultivated wheat worldwide.

Archaeological evidence indicates that wheat was originated 10,000 years ago in the Middle East, particularly in the Fertile Crescent region (Matsuoka 2011; Dubcovsky and Dvorak 2007). The evolution of common wheat, also known as hexaploid wheat, occurred from lower to higher polyploidy levels through natural hybridization of diploid grass species, *Aegilops tauschii* (2n=2x=14, DD), and tetraploid durum wheat, *Triticum turgidum* (2n=4x=28, AABB), followed by allopolyploidization (Tsunewaki 2009). The cultivated tetraploid wheat *T. turgidum* arose from crosses between *T. monococcum* L. (AA) and a B genome donor, most likely, *A. speltoides* (Monte et al. 1993). Currently, with the advancement in tissue culture and chromosomal

doubling techniques, the common wheat can be made by artificial crossing *T. turgidum* and *A. taushii* followed by embryo rescue and chromosome doubling. This process enables scientists to transfer useful genes from *A. tauschii* to common wheat (Jiang et al. 1994).

Economic importance and production of wheat

Wheat is one of the most widely produced and consumed crops worldwide. The main reason of its popularity arises from its wide range of uses, from bread to beers. Based on the statistical data from the USDA, the total wheat production worldwide in 2018-2019 was 26,842.9 million bushels with US production of 1884.4 million bushels which is 7.02% of total world production (www.ers.usda.gov). Kansas and North Dakota are leading wheat-producing states in the US. The average yield of wheat in the US was 47.6 bushels/acres in 2018-2019. In 1981, the United States produced the greatest amount of wheat, and ranked as the number one wheat producer in the world (Economic Research Service, USDA). However, since 1984, China has become the top wheat producer in the world (Curtis, 2002).

Based on the differences in growth habit and other genetic and physiological characters, wheat is divided into five major marketing classes in the US; hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), soft white (SW), and durum. HRW is the dominant class of wheat in the US, accounting for 40% total wheat production followed by HRS (25%), SRW (15-20%), SW (10-15%), and durum (3-5%) (Vocke and Ali, 2013). Each class of wheat has a different end use, and its specific production region in the 42 states of the US. For example, HRS wheat is primarily grown in the Northern region with North Dakota as a number one wheat producer in the US, whereas SRW wheat is primarily grown in the South Eastern region (Vocke and Ali, 2013). Georgia (GA) is a major producer of wheat in the Southeast US region. However,

wheat was ranked as 39th agricultural commodities in terms of economic value produced in GA. The farm gate value of wheat in GA was $26,688,478, which is about 0.19% of the total farm gate value of agricultural commodities (Georgia Farm Gate Value Report, 2017). However, based on the acreage, wheat was ranked as 4th crop grown in GA behind cotton, peanut, and corn with 200,000 acres of land planted in 2017 (USDA-State Agriculture Overview, Georgia, 2018).

Genomics of wheat

Common wheat is a self-pollinated allohexaploid and consists of three closely related subgenomes (A, B, and D) derived from three different diploid progenitors; however, the ancestor of B-genome has always been a topic of debate (Gupta et al. 2008). Each of the three ancestral diploid parents contributed seven chromosomes making it a total of 21 chromosomes in common wheat (Gupta et al. 2008; International Wheat Genome Sequencing Consortium (IWGSC), 2014). The three diploid genomes of wheat are very large in size (about 5.5 Gb in size) and consists of more than 80% of repetitive transposable elements (TEs) (Smith and Flavell 1975; IWGSC 2014). Although the genome of common wheat is estimated to be 17 Gb in size, 14.5 Gb of the genome has been recently annotated with 107,891 genes (IWGSC 2014, 2018). Based on a study conducted to study the distribution of genes and recombination events in wheat genome it was found that genes-rich regions are located in the wheat genome indicating genes are not randomly distributed in the wheat genome (Gill et al. 1996). Furthermore, the linkage disequilibrium (LD) or non-random association between the loci is expected to be higher in wheat as compared to other cross-pollinated crops such as corn and sorghum because of high degree of inbreeding (Flint-Garcia et al. 2003).

Molecular breeding in wheat

Molecular markers

Molecular markers are small segments of a genome that are designed to detect the differences between multiple DNA sequences. The major use of DNA markers in plant breeding is for the construction of genetic linkage maps in segregation mapping populations and subsequently to use the linkage maps to detect quantitative trait loci (QTLs). Molecular markers tightly linked to the major QTL or gene can be used in breeding programs via marker-assisted selection (MAS). Several types of molecular markers, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), microsatellites or simple sequence repeat (SSR), and Single Nucleotide Polymorphism (SNP) are available in wheat (Botstein et al. 1980; Vos et al. 1995; Wang et al. 1994; Akbari et al. 2006; Somers et al. 2003); however, SSR and SNP are especially more popular and frequently used in wheat.

SSR markers are very useful in plant breeding because of their reproducibility, multiallelic nature, codominant inheritance, and relative abundance in higher organisms (Varshney et al. 2005). Several studies have demonstrated that the number of repeats within in microsatellite is highly variable even within the same population of a species. The flanking regions of the microsatellite are very conserved which enables us to design primers based on the flanking regions and use them to amplify the repetitive region (Varshney et al. 2005). Although development of SSR markers is expensive, labor intensive, and time consuming, once the primers are designed, the assays become cost-effective and user friendly (Roder et al. 1998). Many labs around the world are dedicated to the development of SSR markers from the wheat genome and related species and more than thousand SSR markers are already developed and

available. Gupta et al. (2002) developed a total of 396 markers via the Wheat Microsatellite Consortium (WMC). Song et al. (2005) developed more than 500 SSR markers and assigned identifiers prefixed with Beltsville Agricultural Research Center (BARC). Similarly, Gupta et al. (2003) developed 900 SSR primers based on the expressed sequence tags (EST) sequence of the wheat genome. These valuable wheat SSR markers are extensively used in plant breeding to develop linkage maps and QTL/gene mapping (Gupta et al. 2002; Somers et al. 2004; Hao et al. 2008).

Although SNP markers were initially discovered from the human genome, it is proven to be universal and the most abundant forms of genetic variation in all species (Rafalski 2002). Discovery and use of SNP in plant breeding has been extensively used in several crop species including rice, maize, soybean, and barley (Ravel et al. 2006). However, in wheat, SNPs development and use is limited mainly because of its large genome size and allohexaploid features (Ravel et al. 2006). Despite these obstacles, scientists around the world have made significant progress in SNP array development among hexaploid species including wheat. Cavanagh et al. (2013) initially reported a 9K iSelect SNP array from a diverse set of hexaploid wheat (2,994 accessions) including landraces and cultivars. Subsequently, Wang et al. (2014) reported 90K iSelect wheat array to detect genetic variation within hexaploid and tetraploid wheat. More recently, Winfield et al. (2016) have generated an 820K SNP array containing 64.5 and 35.5% transitions and transversions SNPs, respectively. Initially, a total of 921K putative SNPs were identified from 43 bread wheat accessions which were later validated on 475 accessions and developed a total of 820K SNP arrays for further use. Utilizing these wheat arrays, scientist have developed several linkage maps, conducted QTL mapping, and were able to detect QTL/gene associated with disease and insect resistance, physiological and agronomic

traits, yield, and quality related traits in wheat (Sapkota et al. 2019; Bassi et al. 2019; Li et al. 2019; Chen et al. 2019).

Mapping populations and linkage maps

In wheat, several types of bi-parental populations have been developed and used for QTL analysis including, but are not limited to, F2, backcross, recombinant inbred lines (RILs), and doubled haploid (DH). F2 population is easy and fast to develop, and it is most commonly used for QTL analysis. However, for quantitative traits which are influenced by environments, multiple evaluations need to be done at multiple locations/year, and F2 populations may not be applicable. RILs are developed by single seed descent method from F2 individuals until F6 or F7 generation. It takes more time to develop RILs, but results in higher resolution maps due a greater number of recombination. Also, RILs are genetically homozygous which allows for replication of the evaluation tests across locations and years, and helps to obtain more precise phenotype data. DH is another way to develop populations for QTL analysis, can be generated in short period of time, and the lines are completely homozygous. However, special tissue culture techniques are required to develop DH lines. Recently, the use of DH population has been increased in wheat genetic studies (Zhang et al. 2011; El-Hennawy et al. 2011). Genome-wide association studies (GWAS) is another approach to identify QTL associated with quantitative traits, which requires natural population rather than bi-parental populationss for QTL analysis (Zhu et al. 2008).

A linkage map or genetic map shows the position of markers relative to each other on chromosomes based on recombination frequency. For example, if there is 1% chance of recombination between two markers, the distance between them is 1 centimorgan (cM).

Construction of linkage map in wheat is relatively difficult as compared to other cereal crops including rice, maize, and barley mainly due to low level of polymorphism (Langridge et al. 2001; Chao et al. 1989; Roder et al. 1998). Furthermore, the D genome of wheat is highly conserved, and the level of polymorphism is not consistent across the wheat genomes (Langridge et al. 2001). Despite these complications, several linkage maps have been constructed in wheat for genetic studies. Liu and Tsunewaki (1991) constructed the first linkage map in common wheat using 66 F2 progenies derived from the cross of *Triticum aestivum* (chinese spring) x *T. spelta* var. *duhamelianum* (spelta). The authors used 197 RFLP markers to construct the linkage map with 82, 87, and 28 markers mapped on chromosomes A, B, and D respectively. The size of the map was 1800 cM with an average distance of 11.7 cM between the markers. In another study, 114 RILs were developed from a cross between Opata 85 x W7984, and this population was used to construct a linkage map using RFLP and microsatellite markers. Opata 85 x W7984 population, also known as International Triticeae Mapping Initiative (ITMI) population, is widely used as a reference mapping population for wheat (Langridge et al. 2001; Roder et al. 1998; Song et al. 2005). Genetic maps were also constructed in tetraploid wheat for genetic studies. Blanco et al. (1998) constructed a genetic map using 65 segregating RILs derived from the cross of Messapia and MG4343. A total of 259 markers were mapped to all 14 chromosomes with the total map length of 1352 cM. Subsquently, Peng et al. (2000), Nachit et al. (2001), and Elouafi and Nachit, (2004) constructed genetic maps in tetraploid wheat. More recently, with the advancement in sequencing technology, consensus maps with high density of markers are available for common wheat (Cavanagh et al. 2013; Wang et al. 2014; Winfield et al. 2016).

Quantitative trait loci (QTL) mapping

Broadly, two types of traits exist, qualitative and quantitative. Qualitative traits are controlled by a single gene, phenotypes of the segregating populations show discrete distribution, and are least influenced by environmental conditions. However, quantitative traits are controlled by multiple genes (also known as QTL), phenotypes of segregating populations show continuous distribution, and are influenced by environmental conditions (Young 1996; Paran and Zamir 2003). QTL mapping is the process of identifying genomic regions associated with particular quantitative traits. Although the concept of QTL mapping was first reported by Sax in 1923, not much progress was made until 1980s, probably due to the unavailability of molecular markers and suitable statistical methods (Young 1996). However, with the advancement in the field of molecular biology, and the development of highly polymorphic molecular markers throughout the genome, QTL mapping has become routine for research work on most of the crops nowadays (Young 1996).

Basically, phenotypic and genotypic data are required for QTL mapping. Phenotypic data should be precise, and if possible, the segregating population should be evaluated in multiple locations and environments to reduce the errors (Young 1996; Kearsey and Farquhar 1998). Genotypic data or markers should cover the entire genome, and only distinct polymorphic markers between the parents should be used in the mapping (Young 1996; Kearsey and Farquhar 1998). In addition to phenotypic and genotypic data, appropriate statistical tools are also needed for QTL mapping. The commonly used statistical tools for QTL mapping are single-marker analysis, simple interval mapping, and composite interval mapping (Collard et al. 2005). Single marker analysis is the simplest method of QTL detection, analysis can be performed using basic statistical software, and does not require a complete linkage map. However, the major limitation

of this method is that as the distance between the QTL and marker increases, the odds of QTL detection decreases because recombination may occur between the marker and the QTL (Collard et al. 2005). Simple interval mapping is an improvement over single marker analysis because it can map the location of QTL on the chromosome and estimate its effect on phenotypic variation more precisely. The concept of composite interval mapping was first reported by Zeng, (1993), and now it is the most widely used approach for QTL mapping. The major advantage of composite interval mapping is that it can control the effect of other markers on QTL detection by treating them as a covariate, and it is more effective mainly when QTL are linked (Kao et al. 1998; Collard et al. 2005).

Marker-assisted selection

The primary goal of plant breeding is to select superior plants with desirable traits and try to assemble more desirable genes to develop superior cultivar. This can be achieved through conventional breeding; however, it will take a considerable amount of time, typically 5-10 years in cereal crops, and is expensive (Collard and Mackill 2008). Marker-assisted selection (MAS) has a huge potential to improve the efficiency of conventional breeding using the DNA markers tightly linked to the traits. MAS uses genotypic or marker data to aid in the selection of better lines and it can be used to select for qualitative, controlled by a single major gene, and/or quantitative, controlled by multiple small effect genes, traits. There are several advantages of MAS over conventional breeding and some of them are: 1) it is simpler than phenotypic selection and saves time, resources, and effort; 2) selection can be carried out at the seedling stage; 3) single plants can be tested; and 4) can be used in the selection of certain traits which are difficult and/or costly to phenotype (Collard and Mackill 2008). There are several factors that

determine the implementation and success of MAS that includes, but are not limited to, linkage disequilibrium (LD) between the marker and the QTL/gene, heritability of the trait, cost of genotyping, type of molecular marker, and statistical analysis (Xu and Crouch 2007; Collard and Mackill 2008).

In wheat, molecular markers have been developed for several genes/QTL linked to economically important traits including disease resistance (leaf/stripe/stem rusts, Fusarium head blight, loose smut, common bunt etc.), insect resistance (Hessian fly, stem sawfly, wheat blossom midge etc.), yield, quality, and agronomic traits. Currently, 79, 81, and more than 70 genes have been formally designated for wheat leaf rust, stripe rust, and stem rust resistance, respectively, and have the potential to be used in wheat breeding program via MAS (Sapkota et al. 2019; Gessese et al. 2019; Meriem et al. 2019). Additionally, more than 100 major effect QTL have been reported for resistance to these three wheat rusts diseases and the markers linked to these QTL can be used in MAS.

Rusts disease of wheat

Rusts are the major diseases of wheat and pose a great threat to wheat production worldwide. Rust diseases are widely spread and can be found in most areas where wheat is grown (Kolmer, 2005). Leaf rust (caused by *Puccinia tritcina*), stem rust (caused by *Puccinia graminis f. sp.* Tritici), and stripe rust (caused by *Puccinia striiformis* f. sp. tritici), are the three rusts diseases known to be destructive to wheat. Of the three rust diseases, leaf rust is the most common and widely spread disease (Bolton et al. 2008). The symptoms produced by all three rust fungi are similar and have similar requirements for plant infection and colonization; however, the disease gets three different names based on their symptoms on the plant tissue

(Kolmer, 2005). Unlike other fungal pathogens, rust fungi cannot be cultured because they are obligate biotrophs and require a living host to survive and reproduce (Hovmoller et. al, 2011). The rust fungi can survive on a wide range of temperatures, with optimum temperatures from 10 to 30°C, therefore rust diseases are widely distributed throughout the world (Roelfs et al. 1992). Among the three rust species, stem rust requires the warmest temperature for an epidemic to occur. Therefore, it is more prevalent in the US Northern great plains where spring wheat is in active growth stage during the summer time. On the other hand, leaf and stripe rust epidemics occurs in cooler temperatures, hence the diseases are more common in the US Central great plains where winter wheat resumes its growth in early spring after a few months of dormancy (Eversmeyer and Kramer 2000). The life cycle of the rust fungi is complex because they require two host plants to complete their life cycle, and involves up to five spore stages. Wheat is the primary host for all three rust fungi, but *Berberis* (for stem and stripe rust fungi) and *Thalictrum* (for leaf rust fungi) are the alternate hosts. The epidemic development is very rapid on wheat because urediniospores (also known as repeating spores) are produced on wheat which can cause auto-infection (Marsalis and Goldberg, 2016).

Several management approaches can be used solely or in combination to reduce the amount of initial inoculum and/or to reduce the rate of rust disease development. Some common management practices are the removal of the alternate hosts, cultural practices, use of fungicides, and genetic resistance. The spores of the rust fungi can be wind-blown for several kilometers from the infected plants; therefore, rust epidemic can occur anytime on a continental scale (Kolmer, 2005). In addition, the airborne nature of the rust spores makes quarantine methods ineffective to control this disease. However, specialized government agencies or authorized personnel should be careful to prevent the escape of rust spores outside of their epidemiological

areas (Roelfs et al. 1992). Farmers are always recommended to implement practices that help to eradicate the volunteer plants and crop debris in the field. But, preventive cultural practice does not ensure freedom from rust disease because urediniospores are wind-blown. The use of chemical fungicides is an effective way of controlling rust diseases, but they are costly to wheat growers and have a negative impact on human health and the environment. Therefore, the use of genetic resistance is economical, environmentally friendly and the most preferred way of managing rust diseases.

Leaf rust (*Puccinia triticina*)

Distribution and importance

Leaf rust, also known as brown rust, is the most destructive foliar disease of wheat worldwide. Leaf rust is more common and widely distributed than stripe rust and stem rust diseases of wheat (Samborski 1985; Bolton et al. 2008). Leaf rust fungus can survive on wide range of climates; therefore, the disease can be found in diverse areas where wheat is grown (Roelfs et al. 1992; Bolton et al. 2008). Yield losses due to leaf rust are normally 5-15%; however, depending on the time of infection, disease severity, and level of resistance/susceptibility of wheat cultivars, losses may be even more significant (Samborski 1985; Huerta-Espino et al. 2011; Kolmer 2005; Marasas et al. 2004). Yield losses in wheat are normally due to a decreased number of kernels per spike and lower kernel weights (Bolton et al. 2008; Huerta-Espino et al. 2011). Flag leaves play a vital role in grain formation, and therefore, yield reduction is higher if the leaf rust infection is higher on flag leaves (Chester 1946). Khan et al. (1997) reported that with every 1% increase in the severity of leaf rust, the wheat yield is reduced by 1%. Leaf rust has been a problem since long time for wheat production; however, its

epidemic and effect are more damaging now since a large proportion of wheat fields worldwide are sown with genetically homogenous cultivars or closely related cultivars (Samborski 1985). Furthermore, the emergence of new virulent races every year globally (Kolmer 2005) makes leaf rust management hard and challenging.

Symptoms and management

The typical symptoms of leaf rust on wheat leaves appears as round lesions with orange-brown urediniospores on the upper leaf surface, but if the disease pressure is high, uredinia are also visible on sheaths (Roelfs et al. 1992). Susceptible wheat cultivars develop large sized uredinia without chlorosis or necrosis; however, resistant cultivars develop hypersensitive flecks with small to medium-sized uredinia (Bolton et al. 2008). Although several management strategies are available for leaf rust management i.e., genetic resistance, chemical control, and cultural method, utilization of integrated pest management (IPM) is undoubtedly the most effective means of controlling leaf rust (Roelfs et al. 1992). The use of resistant cultivars that possess both seedling and adult plant resistance (APR) genes is the most preferred method to control leaf rust. Seventy-nine leaf rust resistance genes have been identified so far, and 15 of them were APR genes (Pinto da Silva et al. 2018; Sapkota et al. 2019). *Lr34* is one of the most durable and effective genes identified for leaf rust; however, cultivars that only possess *Lr34* do not demonstrate high levels of resistance, and therefore, combining durable leaf rust resistance genes with other genes such as *Lr46* and *Lr68* would be an ideal management option (Kolmer 2013; Mehta 2014). Cultural practices such as eradication of volunteer plants, crop debris, and alternate hosts helps to reduce the amount of primary inoculum (Roelfs et al. 1992). These cultural practices do not guarantee complete freedom from rust spores in the field since

urediniospores are carried long-distances by winds. Besides genetic resistance and cultural practices, leaf rust is also controlled by the application of fungicides; however, application of chemicals is not preferred due to its adverse effects on human health and environments (Mehta 2014; Roelfs et al. 1992).

Figure 1.1 Symptom of leaf rust on flag leaves of wheat.

Host range and epidemiology of P. triticina

The primary hosts of *P. triticina* are common wheat (*T. aestivum*), durum wheat (*T. turgidum*), wild emmer wheat (*T. dicoccoides*), domesticated emmer wheat (*T. dicoccon*), triticale (X *triticosecale*), common goatgrass (*Aegilops cylindrical*), and *Ae. speltoides* (Bolton et al. 2008; Yehuda et al. 2004). Interestingly, due to high genetic diversity and distinct host ranges, isolates of *P. triticina* that occurred on durum wheat and *Ae. Speltoides* are host specific and found to be different than those that infect wheat, suggesting that these two groups may be

different formae speciales (Yahuda et al. 2004; Goyeau et al. 2006; Bolton et al. 2008). *Thalictrum speciosissimum* is the alternate host of *P. triticina* (Jackson and Mains 1921; Bolton et al. 2008). Additionally, the pathogen was also observed on another alternate host *Isopyrum fumarioides* in Siberia (Chester 1946). These two alternate hosts of *P. triticina* that are native to North America are relatively incompatible to basidiospore infection, and therefore, the contribution of a sexual cycle on leaf rust epidemics and pathogen genetic variation is not significant in North America (Jackson and Mains 1921; Saari et al. 1968; Bolton et al. 2008).

The favorable environmental conditions for the survival and infection of *P. triticina* are similar to the conditions required to grow wheat (Roelfs et al. 1992; Singh et al. 2002). Availability of moisture is crucial for leaf rust infection and the pathogen can infect wheat leaves if about 4-8 hours of dew period is available with a temperature of 10-25°C (Roelfs et al. 1992; de Vallavieille-Pope et al. 1995). However, urediniospores germination has been recorded at temperatures of 2-30°C (Roelfs et al. 1992). de Vallavieille-Pope et al (1995) reported that the germination of *P. triticina* on wheat leaves was very limited at 30°C and no germination was observed at temperature >35°C. Dry and windy days followed by the cool nights with sufficient amount of moisture (dew) helps better dispersal and germination of the *P. triticina* on wheat leaves and likely contributes to leaf rust epidemics (Roelfs et al. 1992; Eversmeyer and Kramer, 2000).

Life cycle of P. triticina

P. triticina is heteroecious and requires two taxonomically unrelated hosts to complete its life cycle (Roelfs et al. 1992; Bolton et al. 2008). It requires wheat (primary host) to complete its asexual life cycle and *Thalictrum speciosissimum* i.e., meadow rue (alternate host) to complete

the sexual life cycle. Furthermore, alike other rust pathogens, *P. triticina* is macrocyclic and has five different spore stages i.e., teliospores, basidiospore, urediniospores, pycniospores, and aeciospore (Bolton et al. 2008; Kolmer 2013). The first three stages are completed on cereal hosts and remaining two on the alternate hosts (Kolmer 2013). Urediniospores, which can re-infect wheat if optimum moisture and temperature is available, are dikaryotic (n+n) and 20 μm in width (Anikster et al. 2005; Bolton et al. 2008; Kolmer 2013). As the wheat growing season comes to an end and wheat plants mature, dikaryotic thick walled two-celled teliospores are formed which enables the pathogen to survive the hot and dry summer (Bolton et al. 2008). Early in the developmental stage, teliospores are dikaryotic, and afterwards change to diploid (2n) through a process called karyogamy. Once suitable environmental conditions are available, diploid teliospores undergo meiosis and four haploid (n) basidiospores are formed. Basidiospores are then ejected from the telial host (primary host) by wind and lands on the nearby alternate hosts where it forms pycnium on the upper side of the leaves. Each pycnium can produce pycniospores (n) and receptive hyphae which can function as male and female gametes (Bolton et al. 2008). Since pycniospores and hyphae produced from the same pycnium are sexually incompatible, pycniospores disseminated by insects or rainwater and landed on different pycnium with receptive hyphae are fertilized and dikaryotic hyphae germinates downward and forms aecia on the lower side of the leaves. Finally, the resulting dikaryotic aeciospores infect the primary hosts and the life cycle of *P. triticina* is completed (Roelfs 1985; Bolton et al. 2008; Kolmer 2013).

Genetics of leaf rust resistance

The utilization of genetic resistance is the most sustainable, economical, and environmentally safe method to control leaf rust and many genomic loci conferring resistance to leaf rust have been reported (McIntosh et al. 1995). Resistance to rust diseases in wheat is broadly classified into two categories; seedling resistance and adult plant resistance (APR) (Chen, 2005). Seedling resistance is detected in the seedling stage, and remains effective at all stage of plant growth. Therefore, seedling resistance is known as all stage resistance. Seedling resistance is race-specific, and mostly encodes a nucleotide binding leucine rich repeat (NB-LRR) class of protein (Ellis et al. 2014). Seedling resistance is race-specific, controlled by single gene and effective only for a short duration due to the continuous evolution of new races. In contrast, APR is detected at the adult stage, controlled by multiple genes, and more durable (Line and Chen, 1995; Chen, 2005). APR genes are further classified into race-specific and race non-specific resistance genes (Ellis et al. 2014).

Most of the winter wheat cultivars possess high-temperature adult plant (HTAP) resistance. Wheat cultivars with only HTAP resistance are susceptible at the seedling stage when the temperature is low, but as the temperature increases, their level of resistance also increases (Chen, 2005; Line and Chen, 1995). Apart from all stage and APR, wheat cultivars are also found to have slow rusting or partial resistance to leaf rust (Line and Chen, 1995). Like APR, studies indicated that slow rusting resistance is controlled by two or three genes with small effect, race non-specific, and more durable (Kolmer, 1996). Line and Chen (1995) mentioned that slow rusting resistance may be APR or the combination of APR and all stage resistance.

Currently, seventy-nine *Lr*-genes (*Lr1-Lr79*) have been formally cataloged for leaf rust resistance and were detected from common wheat, durum wheat, and other related species

(Qureshi et al. 2018; Cereal disease lab, http://www.ars.usda.gov/main/docs.htm? docid=10342). Of the 79 *Lr*-genes, 64 and 15 genes confer seedling and APR resistance, respectively (Pinto da Silva et al. 2018). *Lr72* (Herrera -Foessel et al. 2014), *Lr76* (Bansal et al. 2017), and *Lr79* (Qureshi et al. 2018) are some examples of seedling genes reported recently for leaf rust resistance. Among the 15 APR *Lr*-genes, seven (*Lr12*, *Lr13*, *Lr22a/b*, *Lr35*, *Lr37*, *Lr48*, and *Lr49*) and eight (*Lr34*, *Lr46*, *Lr67*, *Lr68*, *Lr74*, *Lr75*, *Lr77*, and *Lr78*) genes are reported to confer race-specific and race non-specific resistance, respectively (Pinto da Silva et al. 2018; Zhang et al. 2019). Among the *Lr*- genes, *Lr34*, a race non-specific APR gene, is probably the most commonly used in wheat breeding programs mainly because of its durability and widespread distribution (Kolmer 1996).

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CHAPTER 2

A NOVEL ADULT PLANT LEAF RUST RESISTANCE GENE *LR2K38* MAPPED ON 1AL WHEAT CHROMOSOME

Sapkota S, Mergoum M, Kumar A, Fielder JD, Johnson J, Lopez B, Sutton S, Ghimire B, Buck J, Chen Z, and Harrison S (2019). To be submitted to The Plant Genome.

Abstract

Soft red winter wheat (SRWW) cultivar AGS 2038 has a high level of adult plant leaf rust (LR) resistance. To map and characterize LR resistance in AGS 2038, a recombinant inbred line (RIL) population consisting of 225 lines was developed from a cross between AGS 2038 and susceptible line UGA 111729. The parents and RIL population were phenotyped for LR response in three field environments at Plains and Griffin, GA, in the 2017-18 and 2018-19 growing seasons, and one greenhouse environment at the adult-plant stage. The RIL population was genotyped with Illumina iSelect 90K SNP marker array, and a total of 7667 polymorphic markers representing 1513 unique genetic loci were used to construct a linkage map. Quantitative trait loci (QTL) analysis detected a total of five QTL, *QLr.ags-1AL, QLr.ags-2AS, QLr.ags-2BS1, QLr.ags-2BS2,* and *QLr.ags-2DS,* for adult plant LR resistance. Of these, a major QTL, *QLr.ags-1AL,* was detected on all tested environments and explained up to 34.45% of the phenotypic variation. *QLr.ags-1AL*, tightly flanked by *IWB20487* and *IWA4022* markers, was contributed by AGS 2038. Molecular marker analysis using a marker linked to *Lr59* showed that *QLr.ags-1AL* was different from *Lr59*, the only known LR resistance gene on 1AL – therefore the QTL was temporarily designated as *Lr2K38*. *Lr2K38* linked marker *IWB20487* was highly polymorphic among 68 SRWW lines and should be useful for deploying the *Lr2K38* gene in wheat breeding programs.

Keywords: Wheat, leaf rust, adult plant resistance, quantitative trait loci, marker-assisted selection

Introduction

Leaf rust (LR), caused by the biotrophic fungal pathogen *Puccinia triticina* (*Pt*) is one of the major foliar diseases of wheat (*Triticum aestivum* L.) worldwide causing significant yield reduction. Yield losses of up to 40% have been reported due to LR; however, losses can vary depending upon the cultivar, LR severity, infection time, rate of disease development, and disease duration (Kolmer et al. 2007; Zhao et al. 2008). Although several management practices, including the use of fungicides, are available and found effective to control wheat LR, the use of genetic resistance is undoubtedly the best and most preferred method to control LR disease (Kolmer 1996; Sapkota et al. 2019a, 2019b). To date, 79 leaf rust resistance genes (*Lr1-Lr79*) have been cataloged (Qureshi et al. 2018) and 249 QTL conferring LR resistance have been reported (Pinto da Silva et al. 2018). However, the majority of the genes/QTL reported confer race-specific resistance and can be easily overcome by changes in the pathogen population. Therefore, continuous searches for novel source of LR resistance is key to combat this devastating disease.

Resistance to rusts in wheat is broadly classified into two types: 1) race-specific resistance which is normally detected at the seedling stage, and 2) race non-specific resistance which is detected at the adult plant stage (Johnson 1988; Kolmer 2013). Seedling resistance, also known as all-stage resistance, is detected at an early growth stage (seedling) and found to be effective throughout the life of plants (Line and Chen 1995). Seedling resistance is often found effective to certain *Pt* races carrying corresponding avirulence, confers a high level of resistance, and develops hypersensitive reaction in host plants (Kolmer 2005). Of the 79 LR resistance genes (*Lr*-genes) identified so far, 64 genes are race-specific and confer LR resistance at seedling stage (Pinto da Silva et al. 2018). One of the major limitations of race-specific genes is that they are short-lived and mutation and/or recombination in *Pt* populations can easily overcome the

seedling resistance (Kolmer 2005, 2013). For instance, two seedling *Lr*-genes, *Lr10* and *Lr16*, that were deployed in Canadian wheat cultivar 'Selkirk' were ineffective after the evolution of new virulent *Pt* races (McCallum et al. 2016). Pyramiding multiple seedling *Lr*-genes could possibly prolong the life of LR resistant wheat cultivars; however, the emergence of 40-60 *Pt* races every year in the US poses a great challenge to this approach (Kolmer 2005).

Adult plant resistance (APR), also known as partial resistance or slow-rusting resistance, has been reported to be more durable as compared to seedling resistance (Kolmer 1996, 2005; Bolton et al. 2008). Currently, 15 formally cataloged *Lr*-genes are reported to confer APR. Of these, seven (*Lr12*, *Lr13*, *Lr22a/b*, *Lr35*, *Lr37*, *Lr48*, and *Lr49*) and eight (*Lr34*, *Lr46*, *Lr67*, *Lr68*, *Lr74*, *Lr75*, *Lr77*, and *Lr78*) are reported to be race-specific and race non-specific APR genes, respectively (Pinto da Silva et al. 2018; Zhang et al. 2019). Three of eight race non-specific APR genes, *Lr34*, *Lr46*, and *Lr67*, are unique and more valuable in plant breeding because they are tightly linked with other genomic loci and confer partial resistance to three rusts (leaf, stripe, and stem rusts) and powdery mildew disease caused by *Blumeria graminis* f. sp. *tritici* (William et al. 2003; Herrera-Foessel et al. 2014; Pinto da Silva et al. 2018).

Linkage mapping using bi-parental populations and association mapping using diversity panels have been used in identifying QTL for LR resistance. However, the majority of LR resistance QTL have been identified using bi-parental linkage mapping (Pinto da Silva et al. 2018). Advancement in Illumina iSelect genotyping platform, 9K, 90K, and 820K SNP arrays, and the recently released hexaploid wheat reference genome, RefSeq-v1.0 (https://www.wheatgenome.org/), enabled us to precisely locate LR resistance QTL on the wheat genome and compare identified QTL with previously reported genes/QTL using wheat consensus and physical maps (Cavanagh et al. 2013; Wang et al. 2014; Winfeld et al. 2016).

The University of Georgia Small Grain Breeding Program (UGA-SGBP) has long been dedicated to the development of wheat cultivars suitable to the Southeastern region of the US and more than 30 wheat cultivars have been released. AGS 2038 is one of the soft red winter wheat (SRWW) cultivars released in 2011 by the UGA-SGBP and licensed to AGSouth Genetics company for its high yield, good test weight, medium maturity, and resistance to current biotypes of Hessian fly in GA, USA. Additionally, we observed that AGS 2038 exhibited high levels of adult plant LR resistance in our wheat nurseries at Plains and Griffin, GA, since its release. The objectives of this study were to map genomic loci conferring adult plant LR resistance in AGS 2038 using a high-density SNP-linkage map and develop user-friendly markers that can be used in marker-assisted selection (MAS) to develop LR resistant wheat cultivars.

Materials and Methods

Mapping population development

A mapping population consisting of 225 RILs was developed from a cross between LR resistant and susceptible SRWW lines, AGS 2038 and UGA 111729, respectively. AGS 2038 developed by the UGA-SGBP and released by the Georgia Agricultural Experimental Stations in 2011 was derived from the cross between GA 961581 and PIO 26R61 which possess high level of LR resistance at adult plant stage. UGA 111729 is an elite wheat breeding line also developed by UGA-SGBP and is susceptible to LR at the adult plant stage. Additionally, a set of 68 SRWW cultivars and elite breeding lines were selected and used to test the polymorphism of the resistance-linked markers (Table 2.S1).

Phenotyping

The parents and the RIL population were evaluated for adult plant LR reaction in the field at Plains, GA during 2017-18 season (APR-PL18) and in two locations, Plains and Griffin, GA during 2018-19 growing season (APR-PL19 and APR-GRF19). The Griffin Experiment Station (Bledsoe farm) is located close to the UGA, Griffin Campus, whereas the Plains Experiment Station is located 105 miles south from Griffin, GA, and these two locations represent diverse ecological environments in GA. The plant materials were arranged in a randomized complete block design (RCBD) with two replications per location. Each entry was planted in a single row plot with 1-m long and 25 cm apart and approximately 3 gm of seeds were sown in each plot. The parents, AGS 2038 and UGA 111729, were planted after every 20 rows as resistant and susceptible checks. A highly susceptible SRWW cultivar, SS520, was planted around the research plots as a rust spreader and to uniformly increase the LR epidemic throughout the experiment. A few plants of susceptible spreader, SS520, were inoculated with *Pt* race MFGKG (current prevalent *Pt* race of Georgia) in the growth chamber and when the symptoms were visible, the infected plants were transplanted in the middle of the spreader plots to spread the LR spores in the field plots. The LR severities (the percentage of leaf tissue infected on entire plot) on the parents and RILs were recorded using a modified Cobb scale (Peterson et al. 1948) when the susceptible parent and most susceptible RILs had the highest level of LR severity.

The parents and RILs were also evaluated under greenhouse conditions for adult plant reaction in 2018 (APR-GH18) at UGA, Griffin Campus, Griffin, GA. Two seeds of each parent and RILs were planted in cone-tainers (Stuewe and Sons, Inc) filled with Sungro growing mix soil (Sun Gro Horticulture Distribution Inc.) and placed in RL98 racks. Three cone-tainers were

planted for each line with six plants in total. The parents, AGS 2038 and UGA 111729, were planted in each rack as resistant and susceptible check, respectively. After germination, the plant materials were placed in a cold room set at 5 °C for six weeks for vernalization, and subsequently transferred to the greenhouse bench set at 20 °C with a 15 h of photoperiod. The plant materials were inoculated at the anthesis stage when flag leaves were fully emerged (Feekes 10.5) using the currently predominant *Pt* race in GA, USA (Sapkota et al. 2019b). The pathogen urediniospores were collected from the susceptible wheat cultivar, SS520, from the field (Plain, GA) and maintained in the growth chamber. The inoculation was done as described by Sapkota et al. (2019b). The RILs and parents were evaluated for LR reaction two weeks after inoculation using the standard 0-4 infection types scale (Long and Kolmer 1989) and LR severity. However, only the severity data was used for subsequent analysis due to greater variation among the RILs.

Statistical analysis

Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure in SAS version 9.4 (SAS Institute Inc. 2017) to estimate the effects of variables in the experiments. The Pearson correlation coefficients (*r*) for the LR severity scores were calculated to determine the consistency of LR response across different environments using pairs.panel function in R package "psych" (Revelle 2017). The best linear unbiased predictions (BLUPs) values were calculated for LR severity across all environments using "lme4" package in R (Bates et al. 2015; R Core team 2016). Genotype, environment, genotype by environment, and replication were all treated as random effects in the model. For individual environments, only genotype and replication were used in the model and both were treated as random variables to

calculate BLUPs. BLUP values calculated for each genotype across and within environments were used in the subsequent QTL analysis as phenotypic data. Broad sense heritability (H^2) was calculated across all environments and for each single environment to determine the effect of genotype on phenotype. H^2 was calculated according to the equations $H^2 = \sigma_G^2/(\sigma_G^2 + \sigma_e^2/r)$ and $H^2 = \sigma_G^2/(\sigma_G^2 + \sigma_{GE}^2/r + \sigma_e^2/er)$ for each single environment and across all environments, respectively, where σ_G^2 is the genotypic variance, σ_{GE}^2 is the genotype by environment interaction variance, σ_e^2 is the residual variance, e is the number of environments, and r is the number of replications in each environment.

90K SNP genotyping

The high quality genomic DNA of the two parents and RILs were sent to the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Small Grain Genotyping laboratory in Fargo, ND, for 90K iSelect SNP genotyping (Wang et al. 2014). A total of 81,587 SNP markers were produced by the Illumina iSelect 90K SNP assay and alleles were called using GenomeStudio 2.0 software. Necessary corrections were made manually to minimize the errors related to the clustering of the genotypes. A total of 8800 markers were found to be polymorphic between the parents. However, of these polymorphic markers, 1133 were removed for any of these reasons: 1) inconsistent results from three replications of each parental line; 2) overlapping clustering; 3) >20% missing data, and 4) highly distorted markers i.e., that did not fit a 1:1 segregation ratio. Finally, a total of 7667 SNP markers representing 1513 unique genetic loci were used for linkage map construction and QTL analysis.

Linkage map construction and QTL analysis

Linkage maps were created by first grouping the markers with the Minimum Spanning Tree algorithm described by Wu et al. (2008), implemented in the R package "ASMap". Groups with more than 20 markers were converted into ".loc" files for import into Joinmap 5 (van Ooijen 2006). Individual population nodes were grouped again in Joinmap to generate a single grouping and a grouping consisting of sub-groups of approximately 200 markers based on LOD. Single groups were ordered based on the Maximum Likelihood (ML) algorithm using default settings. Sub-groups were ordered using the Regression algorithm utilizing a starting order from the single-group ML results and Kosambi distance calculation. Sub-group and single-group maps were combined with the published consensus map (Wen et al. 2017) via a linear programming algorithm with the R package "LPmerge". All maps were given equal weighting and constructed with max.interval parameter varying from 1 to 6. The resulting maps with the lowest mean squared error were used for QTL mapping.

QTL analysis was performed using "BIP" (QTL mapping in bi-parental populations) function in QTL IciMapping (Meng et al. 2015). The inclusive composite interval mapping of additive (ICIM-ADD) QTL method with a walk speed of 1.0 cM and 0.001 probability in stepwise regression was chosen for QTL detection. The logarithm of odds (LOD) value of 3.0 was chosen to declare significant QTL, and the LOD value was calculated from 1000 permutations with type I error of 0.01. The graphical representation of the genetic linkage map was generated using MapChart (Voorrips 2002).

To determine the physical position of the QTL detected in this study, the sequences of the SNP markers associated with the QTL were obtained from Triticeae Toolbox website

(triticeaetoolbox.org) and used to BLAST the Chinese Spring (CS) reference genome sequence (IWGSC RefSeq v1.0). Once the physical position of the QTL region was confirmed, the candidate genes within the major QTL region were downloaded from the website https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Annotations/v1.0/. If multiple transcripts existed for a single gene with the same function, only the first transcript was considered.

Conversion of SNP markers and genotyping assays

Two SNP markers, *IWB204878* and *IWA4022*, that flanked the major QTL were converted into user-friendly allele-specific primers and tested on a set of wheat materials to determine their usefulness for MAS. Primer Express® version 3.0.1 (Applied Biosystems, Foster City, CA) was used to design primers and probes. The primer pairs for each SNP were synthesized by Eurofins Genomic LLC (Louisville, KY) and are presented in Table 2.S2. Two TaqMan® probes with distinct 5′ reporter fluorophores, 3' minor grove binders (MGB), and 3' nonfluorescent quenchers (NFQ) were synthesized and obtained from Applied Biosystems (Foster City, CA). The fluorogenic probe sequences were 5'-VIC TGTTATTCTTCATCATCGC MGBNFQ-3' and 5'-6FAM TGTTATTCTTCATCATTGC MGBNFQ-3' for *IWB20487* marker and 5'-VIC ATTACTTTCCGAAGCGAGA MGBNFQ-3' and 5'-6FAM TACTTTCCGAAGCAAG MGBNFQ-3' for *IWA4022* markers. PCR assays were set up as described by Barkley et al. (2010) with minor modifications. Briefly, a 15 µl PCR reactions containing 1× TaqMan™ Genotyping Master Mix (Applied Biosystems) (7.5 µl), 0.16 µM forward primer (0.24 µl), 0.16 µM reverse primer (0.24 µl), 0.4 µM Vic probe (0.6 µl,), 0.4 µM 6Fam probe (0.6 µl), 10 ng/µl of DNA (0.6 µl), and 5.22 µl of autoclaved ddH_2O were set up.

The PCR thermal-cycling consisted of 1 cycle of 60 °C for 30 s, 1 cycle of 95 °C for 10 min, 50 cycles of 95 °C for 15 s and 60 °C for 1 min, and a final cycle of 60 °C for 30 s. The end point fluorescence data were visualized with a QuantStudio 3 real-time PCR system (Applied Biosystems).

Identification of Lr59 using linked marker

A Sequence Characterized Amplified Region (SCAR) marker, S15-T3, linked to *Lr59* (Marais et al. 2010) was used to determine its relationship with a major QTL detected in the present study. S15-T3 marker (forward primer = GTCACTTGCTTGAATTTAATG; reverse primer = TCCATAGCTGGTAGCTAGATG) amplified a 622 bp band diagnostic for *Lr59*. The genomic DNA from both parents, AGS 2038 and UGA 111729, was extracted using a modified CTAB method (Saghai-Maroof et al. 1984) and diluted to 50 ng/µl. The PCR reaction was carried out as described by Hao et al. (2008) and amplified products were separated in 1.5% agarose gel stained with ethidium bromide. A 100 bp DNA ladder (New England Biolabs, Inc.) was used to determine the size of the amplified products.

Results

Phenotypic data analysis

In both growing seasons, 2017-18 and 2018-19, AGS 2038 had LR severity of 5-10% demonstrating a highly resistant reaction while the susceptible parent, UGA 111729, had LR severity of 50-60%. In the greenhouse adult plant test, AGS 2038 developed a hypersensitive reaction with small uredia (IT=;1) and had LR severity of 10-20% whereas UGA 111729 developed medium-sized uredia with chlorosis (IT=3), with severity of 50-60%, demonstrating

resistant and susceptible reactions, respectively (Figure 2.1). The RIL population segregated well for their reaction to LR in the field and greenhouse tests at the adult plant stage. The range of LR severity of RIL population in the field and greenhouse tests was 5 to 90%, 5 to 90%, 5 to 70%, and 0 to 80% in APR-PL18, APR-PL19, APR-GRF19, and APR-GH18 environments, respectively. The H^2 for LR severity was high among four environments, with H^2 ranging from 0.82 to 0.94, indicating the non-genetic effect was minimal on LR severity (Figure 2.2). The LR severity data among the four environments were highly correlated with values from r = 0.62 (APR-GRF19/APR-GH18) to r = 0.84 (APR-PL19/APR-GRF19) and were highly significant ($p < 0.0001$) (Figure 2.2). ANOVA confirmed significant ($p < 0.0001$) variation among genotypes (RILs), environments, and genotype × environment interactions for LR response (Table 2.S3).

Construction of genetic linkage map

A genetic linkage map for the AGS 2038/UGA 111729 derived RIL population was generated using 7667 polymorphic markers covering the whole wheat genome (Table 2.1). These markers represented 1513 unique genetic loci (Table 2.1). The genetic map consisted of 27 linkage groups representing 21 wheat chromosomes. The B-genome contained the greatest number of markers followed by A and D-genomes. The number of markers and unique loci in each linkage group ranged from 11 to 1208 and 10 to 206, respectively (Table 2.1). The map covers a total of 2881.46 cM distance with an average distance of 0.38 cM between the adjacent markers. The A, B, and D-genomes covers a total of 1164.27, 965.23, and 751.96 cM length with average map density (cM/marker) of 0.36, 0.26, and 1.05 cM, respectively (Table 2.1).

QTL detection

The inclusive composite interval mapping (ICIM) in AGS 2038/UGA 11729 RIL population detected a total of five QTL for adult plant LR resistance on chromosomes 1A, 2A, 2B, and 2D (Table 2.2). Of these, a major QTL, designated as *QLr.ags-1AL*, was detected in all tested environments with a LOD value of 11.44 to 33.83 (Table 2.2, Figure 2.3). *QLr.ags-1AL* was flanked by *IWB20487* and *IWA4022* markers on the long arm of chromosome 1A and explained up to 34.45% of phenotypic variation in AGS 2038/UGA 111729 RIL population (Table 2.2). This locus was contributed by resistant parent AGS 2038 (Table 2.2). A second QTL, *QLr.ags-2DS,* was detected in all tested environments except Plains 2018 (APR-PL18), and explained up to 20.62% of phenotypic variation (Table 2.2). *QLr.ags-2DS* was contributed by resistant parent AGS 2038 and flanked by *IWA3248* and *IWB943* markers (Table 2.2, Figure 2.4). Similarly, a third QTL, designated as *QLr.ags-2AS*, was detected in Plains 2019 (APR-PL19) and combined data of all environments (APR-ALL ENV) data. *QLr.ags-2AS* was contributed by the resistant parent AGS 2038, explained up to 5.52% of phenotypic variation in the RIL population, and flanked by *IWB10896* and *IWB67304* markers (Table 2.2, Figure 2.4). Two 2B QTL, designated as *QLr.ags-2BS1* and *QLr.ags-2BS2*, were detected in Plains 2018 (APR-PL18) and Greenhouse 2018 (APR-GH18) environments, respectively, and explained up to 12.82% of phenotypic variation (Table 2.2). *QLr.ags-2BS1* and *QLr.ags-2BS2* were contributed by UGA 111729 and AGS 2038, respectively (Table 2.2, Figure 2.4).

Search for candidate genes within major QTL region

Based on the location of SNP markers, major QTL *QLr.ags-1AL* was physically mapped to 579,299,114 to 581,830,275 bp region on chromosome 1A (Figure 2.3). Within this region, a

total of 76 genes, consisting of 32 high confidence (HC) and 44 low confidence (LC) genes, were annotated (Table 2.S4). Of these, 5 genes were disease resistance protein (NBS-LLR class) family. Additionally, the *QLr.ags-1AL* region also harbors protein kinase domains i.e., protein kinase family protein, leucine-rich repeat receptor-like protein kinase family protein, and protein kinase. All of these NBS-LRR class and protein kinase domains could contribute to LR resistance in AGS 2038 and are possible candidate genes for *QLr.ags-1AL*.

Polymorphism of major QTL linked markers

Two SNP markers flanking the major QTL were converted into user-friendly allele-specific primers and tested on a set of 68 wheat lines and parental genotypes to determine their usefulness for MAS. However, only one marker, *IWB20487* linked to *QLr.ags-1AL*, showed good polymorphism among the panel of 68 SRWW, and is therefore reported here. Of the 68 wheat lines tested, only 19 lines were found to carry the AGS 2038 allele and all others produced the UGA 111729 allele (Table 2.S1). The *IWB20487* marker linked to adult plant LR resistance in AGS 2038 can be used in MAS for developing LR resistant wheat cultivars.

Discussion

The utilization of genetic resistance is undoubtedly the most preferred method to control wheat pests in general and rust diseases in particular. Although 79 *Lr* genes and more than 200 QTL have been cataloged for LR resistance, a constant search for novel gene/QTL is necessary to combat new and virulent *Pt* races emerging every year worldwide. In this study, we developed a RIL population crossing a LR resistant (AGS 2038) and susceptible (UGA 111719) SRWW cultivars. We phenotyped the parents and RIL population in four field and greenhouse

environments, genotyped the RIL population using a 90K SNP array, and mapped five QTL for adult plant LR resistance. Of these, a major QTL, *QLr.ags-1AL*, was consistently detected in all tested environments with high LOD value, ranging from 11.44 to 33.83, and explained up to 34.45% of phenotypic variation (Table 2.2). This QTL was tightly flanked by *IWB20487* and *IWA4022* markers and physically mapped at 579,299,114 to 581,830,275 bp region on the long arm of chromosome 1A (Figure 2.3).

Chromosome 1A carries two previously known *Lr* genes, *Lr10* (Feuillet et al. 1997) and *Lr59* (Marais et al. 2008) which are located on the short and long arm, respectively. We strongly believe that the major QTL, *QLr.ags-1AL*, detected in this study for adult plant LR resistance is unique and different from *Lr59*, the only gene mapped on chromosome 1AL, based on the following reasons: Firstly, *Lr59* was derived from *Aegilops peregrine* and introgressed into common wheat and thought to have replaced the complete long arm of wheat chromosome 1A (Marais et al. 2008; Pirseyedi et al. 2015). Based on the pedigree information, AGS 2038 does not share any parents that contain alien introgression. Secondly, based on molecular marker analysis using a marker (SCAR marker S15-T3) linked to *Lr59*, we confirmed that AGS 2038, donor of *QLr.ags-1AL*, was negative to *Lr59* (Figure 2.S1). Therefore, based on these facts, we believe that *QLr.ags-1AL* was different than *Lr59* and temporarily designated as *Lr2K38*.

A minor QTL, *QLr.ags-2AS*, was detected on the short arm of chromosome 2A and flanked by *IWB10896* and *IWB67304* markers (Table 2.2, Figure 2.4). Six known *Lr* genes, *Lr11*, *Lr17*, *Lr37*, *Lr45*, *Lr65*, and *LrAlt* were previously mapped in the vicinity of *QLr.ags-2AS* (McIntosh et al. 2008; Wang et al. 2010). Of these, *Lr37* and *Lr45* were derived from *A. ventricosa* and *Secale cereal*, respectively (McIntosh et al. 1995a; Helguera et al. 2003), and therefore, are unlikely to be incorporated in SRWW adopted cultivars. *Lr11* was derived from

common wheat cultivar ‘Hussar’ (Soliman et al. 1964) on chromosome 2AS; however, recent studies demonstrated that the chromosomal location of *Lr11* was erroneous (Mohler et al. 2012). *Lr17* has two resistance allele i.e., *Lr17*a and *Lr17b* (McIntosh et al. 2008). Based on the wheat consensus genetic map (Maccaferri et al. 2015), *Lr17a* and *Lr65* linked marker (*Xgwm614*) and *LrAlt* linked marker (*Xgwm636*) were mapped 39.69 and 36.51 cM distal to *QLr.ags-2AS,* respectively, and likely represent different loci for LR resistance. However, further studies such as an allelism test or molecular marker analysis are warranted to determine the relationship between *QLr.ags-2AS* and other *Lr* genes/QTL previously mapped on chromosome 2AS.

Two QTL, *QLr.ags-2BS1* and *QLr.ags-2BS2*, were detected on chromosome 2BS in the vicinity of seven known *Lr* genes i.e., *Lr13, Lr16, Lr23, Lr48, Lr73, LrZH22,* and *LrA2K* (McIntosh et al. 2008; Park et al. 2014; Wang et al. 2016; Sapkota et al. 2019a, 2019b). Of these, *Lr48* linked marker (*Xgwm429b*) was located closest to *QLr.ags-2BS1* (6.38 cM proximal), based on the wheat consensus map (Maccaferri et al. 2015), and likely represents the same locus since both confer adult plant LR resistance. However, further studies are warranted to determine the relationship between *QLr.ags-2BS1* and other previously mapped *Lr* genes. *QLr.ags-2BS2* was mapped to the same location where we detected *LrA2K* in our previous study (Sapkota et al. 2019b). Furthermore, molecular marker analysis demonstrated that AGS 2038 was positive to *LrA2K* linked marker *Xwmc770* (Sapkota et al. 2019b). Therefore, *QLr.ags-2BS2* and *LrA2K* most likely represent the same locus for LR resistance.

QTL *QLr.ags-2DS* was mapped in the vicinity of three known *Lr* genes, *Lr2, Lr22*, and *Lr39* (McIntosh et al. 2008; Raupp et al. 2001). *Lr2* has three different alleles, *Lr2a, Lr2b*, and *Lr2c*, and is linked to the centromere region of the 2D chromosome (McIntosh et al. 1995b). *Lr22* has two alleles, *Lr22a* and *Lr22b*. *Lr22a* was transferred to wheat from *Ae. tauschii* (Dyck

1979; Raupp et al. 2001). *Lr39* confers both seedling and adult plant leaf rust resistance and was transferred to wheat from *Ae. tauschii* (Raupp et al. 2001) and is unlikely to be present in adapted SRWW wheat. However, further work is needed to verify the relationship between *QLr.ags-2DS* and other known *Lr* genes/QTL previously mapped on chromosome 2DS.

Different types of disease resistance genes (R-genes) have been reported in plants; however, the majority of R-genes cloned to date encode nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins (McHale et al. 2006; Adhikari and Missaoui 2019). Among the 79 genes for LR resistance that have been cataloged so far, only 6 *Lr* genes, *Lr1, Lr10, Lr21, Lr22a*, *Lr34*, and *Lr67* have been cloned (Feuillet et al. 2003; Huang et al. 2003; Cloutier et al 2007; Krattinger et al. 2009; Moore et al. 2015; Thind et al. 2017; Sapkota et al. 2019b). Of these, *Lr1, Lr10*, and *Lr21* encode for NBS-LRR proteins (Feuillet et al. 2003; Huang et al. 2003; Cloutier et al 2007). Most of the R-genes reported in wheat-rust pathosystems confer race-specific resistance in the gene-for-gene model and have been cloned as NBS-LRR proteins (Krattinger and Keller 2016). In this study, we detected 5 NBS-LRR and several kinase-related genes in the *Lr2K38* region and most likely these genes play an important role in conferring LR resistance in AGS 2038. Similar to our study, Wang et al. (2019) and Zhou et al. (2019) also reported NBS-LRR and kinase-related genes within the QTL region detected for dwarf bunt and stripe rust resistance, respectively, in wheat. The candidate genes detected within the *Lr2K38* region will be valuable in fine mapping and cloning of *Lr2K38*.

In summary, 5 QTL for adult plant LR resistance were detected in wheat utilizing a RIL population consisting of 225 lines and 90K SNP genotyping data. A novel QTL, *QLr.ags-1AL* (*Lr2K38*), detected on the long arm of chromosome 1A was significant in all tested environments with major effects (Table 2.2, Figure 2.3). Based on the candidate gene search in the *Lr2K38*

region (IWGSC RefSeq Annotations V1.0), 5 NBS-LRR related genes were annotated which are possibly the candidate genes for the *Lr2K38* locus. *Lr2K38* linked marker *IWB20487* was highly polymorphic among the SRWW cultivars and can be used for MAS of *Lr2K38* in wheat breeding programs. Adult plant LR resistance in SRWW cultivar AGS 2038 is highly effective against current *Pt* races in the Southeastern US, and therefore, pyramiding *Lr2K38* with other *Lr* genes, such as *Lr34* and *Lr68,* could provide long lasting LR resistance.

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Tables and figures

Table 2.1 Distribution of markers and marker density across linkage groups in AGS 2038/UGA 111729 derived recombinant inbred line population.

Linkage group/ Chromosome	No. of Markers	No. of unique loci	Length (cM)	Average map density (cM/marker)	Average map density (cM/locus)
Genome A					
1A	623	106	67.27	0.11	0.63
2A	529	111	130.92	0.25	1.18
3A1	105	28	57.18	0.54	2.04
3A2	162	53	118.95	0.73	2.24
4A	357	99	167.25	0.47	1.69
5A	302	77	218.12	0.72	2.83
6A1	104	31	148.18	1.42	4.78
6A2	264	47	20.89	0.08	0.44
7A	781	127	235.51	0.30	1.85
All	**3227**	**679**	**1164.27**	**0.36**	**1.71**
Genome B					
1B	888	105	89.16	0.10	0.85
2B	1208	206	326.81	0.27	1.59
3B	206	53	107.21	0.52	2.02
4B	11	10	89.43	8.13	8.94
5B1	107	37	49.64	0.46	1.34
5B2	420	45	124.5	0.30	2.77
6B1	57	12	51.1	0.90	4.26
6B2	404	77	79.95	0.20	1.04
7B1	191	36	33.74	0.18	0.94
7B2	231	31	13.69	0.06	0.44
All	**3723**	**612**	**965.23**	**0.26**	**1.58**
Genome D					
1D	116	46	107.98	0.93	2.35
2D1	132	34	76.01	0.58	2.24
2D2	39	16	160.3	4.11	10.02
3D	150	29	145.83	0.97	5.03
4D	42	23	116.44	2.77	5.06
5D	102	27	18.17	0.18	0.67
6D	103	23	18.9	0.18	0.82
7D	33	24	108.33	3.28	4.51
All	**717**	**222**	**751.96**	**1.05**	**3.39**
Whole Genome	7667	1513	2881.46	0.38	1.90

Table 2.2 Summary of Quantitative trait loci (QTL) detected for leaf rust resistance in AGS 2038/UGA 111729 recombinant inbred line population.

QTL	Environment[a]	Flanking markers	Peak position (cM)	Peak LOD	R^2 (%)[b]	AE[c]
QLr.ags-1AL	APR-PL18	*IWB20487-IWA4022*	59.00	33.83	34.45	-8.86
	APR-PL19	*IWB20487-IWA4022*	59.00	15.57	24.33	-9.92
	APR-GRF19	*IWB20487-IWA4022*	59.00	18.19	28.94	-7.89
	APR-GH18	*IWB20487-IWA4022*	59.00	11.44	13.52	-7.99
	APR-ALL ENV	*IWB20487-IWA4022*	59.00	25.19	29.97	-8.57
QLr.ags-2DS	APR-PL19	*IWA3248-IWB943*	71.00	9.57	12.25	-7.04
	APR-GRF19	*IWA3248-IWB943*	71.00	6.44	8.11	-4.18
	APR-GH18	*IWA3248-IWB943*	71.00	19.19	20.62	-9.86
	APR-ALL ENV	*IWA3248-IWB943*	71.00	16.74	15.93	-6.25
QLr.ags-2AS	APR-PL19	*IWB10896-IWB67304*	62.00	4.74	5.52	-4.72
	APR-ALL ENV	*IWB10896-IWB67304*	62.00	3.81	3.21	-2.81
QLr.ags-2BS1	APR-PL18	*IWA2391-IWB29273*	74.00	4.33	2.73	2.58
QLr.ags-2BS2	APR-GH18	*IWB7346-IWA4673*	97.00	12.60	12.82	-7.92

[a] The four environments were field tests at Plains 2018 (APR-PL18), Plains 2019 (APR-PL19), and Griffin 2019 (APR-GRF19), and adult plant greenhouse test in 2018 (APR-GH18). APR-ALL ENV is the combined data from all tested environments.

[b] Phenotypic variation explained by the QTL.

[c] AE, additive effect. Negative and positive values indicate that the QTL was contributed by AGS 2038 and UGA 111729, respectively.

Figure 2.1 Adult plant leaf rust reaction of AGS 2038 (A and B) and GA 111729 (C and D) under greenhouse (A and C) and field (B and D) environments.

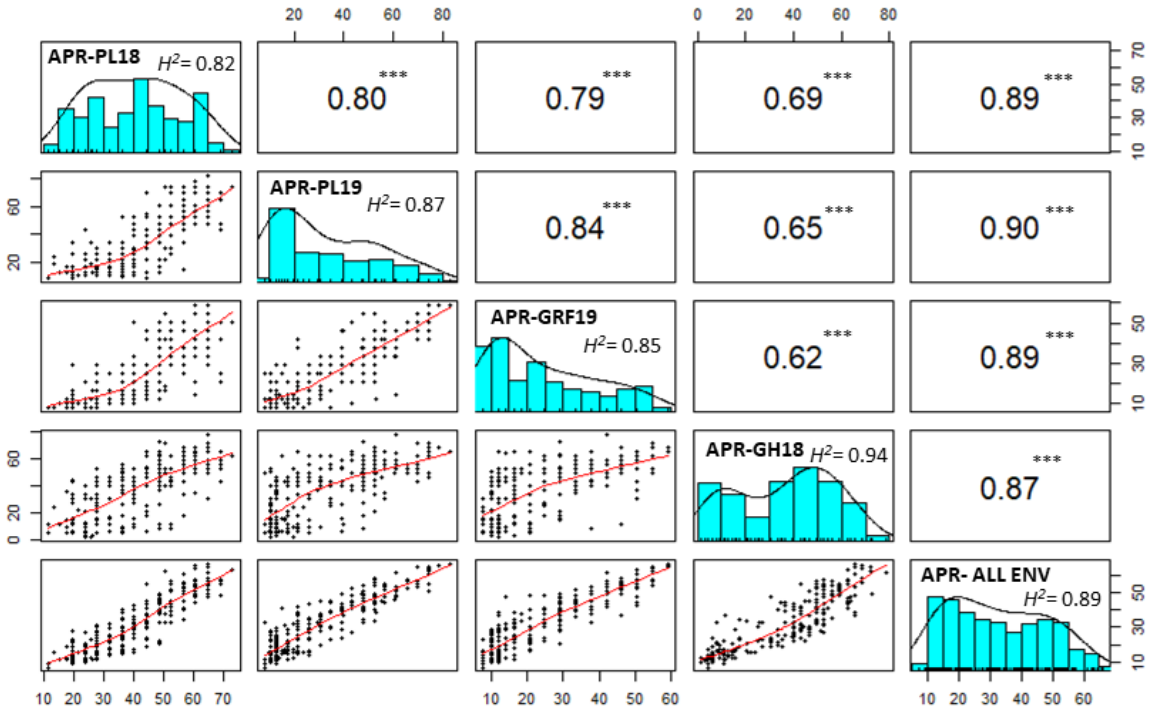

Figure 2.2 Traits distribution and correlation plots. The diagonal plots show the frequency distribution of leaf rust (LR) severity data of 225 recombinant inbred line (RIL) population in each environment (APR-PL18 = field test at Plains in 2018; APR-PL19 = field test at Plains in 2019; APR-GRF19 = field test at Griffin in 2019; APR-GH18 = greenhouse adult plant test in 2018; and APR-ALL ENV = all environments combined data). The panel above and below the diagonal represents Pearson's correlation coefficient and scatter plots, respectively. The broad-sense heritability (H^2) for each trait is shown in the diagonal histogram plots. ***$p < 0.0001$.

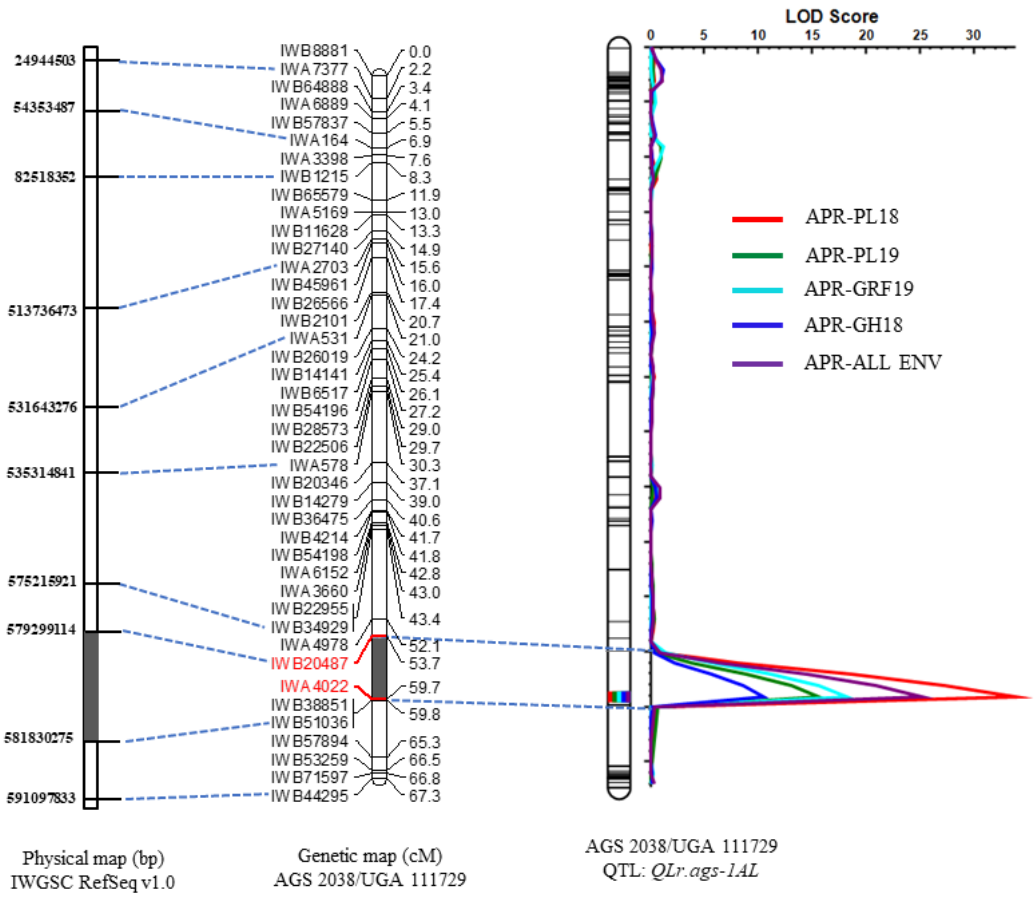

Figure 2.3 Genetic and physical map showing the location of major QTL, *QLr.ags-1AL*, detected for adult plant leaf rust resistance in AGS 2038/UGA 111729 recombinant inbred line population. The QTL region is shown by grey solid region in the genetic and physical maps and QTL flanking markers are in red font.

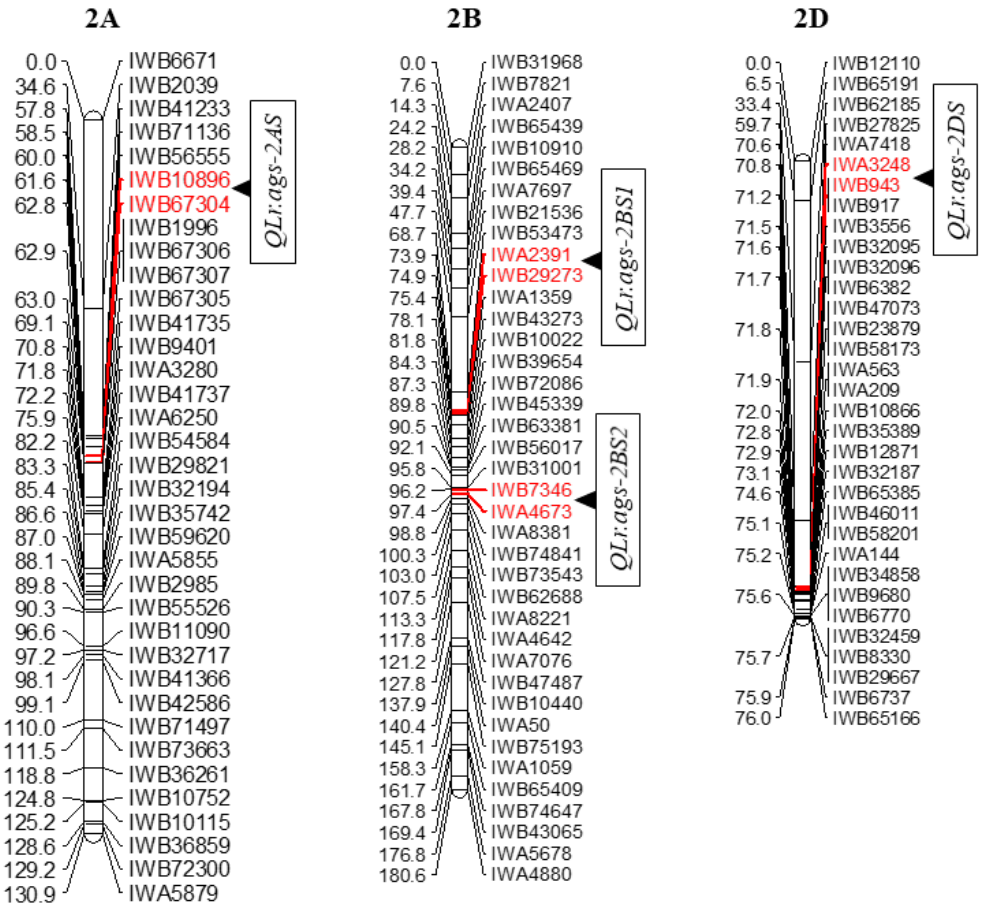

Figure 2.4 Linkage maps for adult plant resistance QTL detected on chromosomes 2A, 2B, and 2D in the AGS 2038/UGA 111729 recombinant inbred line population. The flanking markers for each QTL are in red font.

Appendix A: Supplemental figure

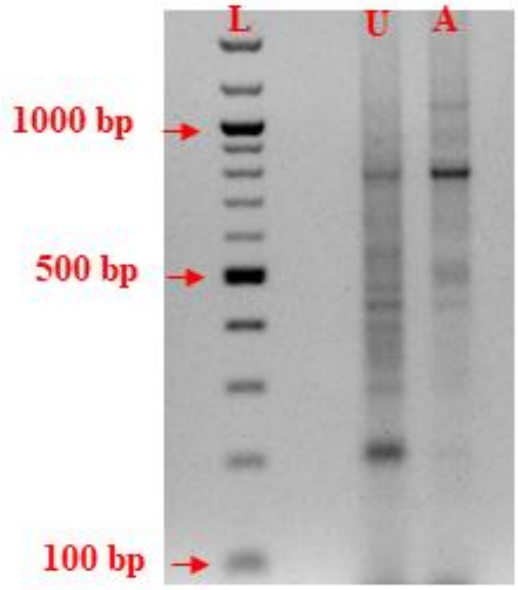

Figure 2.S1 Ethidium bromide stained agarose gel showing the amplification of SCAR marker S15-T3 on parental lines AGS 2038 (A) and UGA 111729 (U). The 100 bp DNA ladder (L) was added to determine the size of the amplified products. The S15-T3 marker amplified a 622 bp band for *Lr59* gene. Both parents, AGS 2038 and UGA 111729, did not produce a 622 bp band, and therefore, does not possess *Lr59* linked marker.

Appendix B: Supplemental tables

Table 2.S1 List of soft-red winter wheat (SRWW) cultivars used to test the polymorphism of resistance linked marker

S.N	Line Name	Pedigree	*IWB20487*
1	GA12213-16-5	04510-11LE34/081607-G1-G1-1(991227-6A33*4/SS8641)	CC
2	GA14177-3-4	AGS2024/PIO26R94//AGS2024	CC
3	GA131216-1-4	JAMESTOWN/031257-10LE34//051477-9-1-6	CC
4	GA13011ID-2-6	JAMESTOWN/031257-10E41	CC
5	GA13085-9-6	06478-23-1-3/AGS2033	TT
6	GA13264-1-6	JAMESTOWN/AGS2033	CC
7	GA131070-3-2-2	051477-9-1-6/AGS2033	CC
8	GA131187-3-9-2	061098-9-6-1/SH5550	TT
9	GA15020ID-7-7	JT152/VA11W-230//04417-12E33	CC
10	GA15040ID-8-3	AGS3000/AGS2033//AGS2033	CC
11	GA12177-8-4-4-6	SS8629/051477-206-5	CC
12	GA14317ID-11-5	JT152/PIO26R94	TT
13	GA141205ID-20-2	HILLIARD/PIO26R94	CC
14	GA131121-4-3-1	05153-16-2-4/031086-10E26//AGS2033	TT
15	GA131148-3-8-4	101700-G1-G2/031257-10LE34//031257-10E41	CC
16	GA131214-4-3-2	LA06146E-P05/JAMESTOWN/051477-9-1-6	TT
17	GA131290-13-5-4	04434-7-4-3/10041-G1-G1-G1//04434-7-4-2-1-5	CC
18	GA131331-15-4-6	04417-11E21/031257-10LE34//04570-10E46	CC
19	GA131397-6-4-7	04494-11E49/031257-10E41//041293-11E37	TT
20	GA131487-14-3-4	04434-11E49/041418-11EEL6//04417-11E21	CC
21	GA131514-5-2-6	JT/031238-7E34//051477-9-1-6	CC
22	GA131700-2-2-12	PIO26R20/04570-10E46/AGS*2/021773	TT
23	GA12485-1-3-2	031086-10E26/VAO4W-90//031086-10E26	CC
24	GA12178-2-14-3-6	03564-10E25/051477-20-6-5	CC
25	GA12212-10-4-4	041293-11LE37/031134-10E29	TT
26	GA12213-8-2-6	04510-11LE24/031134-10E29	CC
27	GA12238-9-4-2	031257-10LE34/081607-G1-G1-1/991227-6A33*4/SS8641	CC
28	GA14065I-14-3-7	06385-13-2-3/031257-10LE34	TT
29	GA14067I-12-8-7	VA09W-75/041293-11LE37//061349-13E4	CC
30	GA14119I-9-2-6	VA10W-119/041293-11LE37//041293-11E54	CC
31	GA14136I-2-4-2	051477-9-1-61031257-10E44//12421-G1-G23-G3	CC
32	GA14186I-15-3-9	041293-11LE37/06399-G2-4-1//04434-11E44	TT
33	GA14187I-4-3-4	VA10W-119/04570-10E46//041293-11E54	CC
34	GA14198I-3-7-4	TV8861/04434-11E44//061082-13E24	CC
35	GA14230I-6-4-8	04417-12E33/041052-11E51//VA10W-123	TT

36	GA14329I-17-2-3	TV8861/04434-11E44//04434-11E44	TT
37	GA14373I-12-4-2	JT/031257-10E41//04151-11E26	TT
38	GA13010-12-4-7	06033-2-7-4/031257-10E41	CC
39	GA13011-9-4-6	VA10W-119/04417-12E33	CC
40	GA13027-15-1-6	VA09W-75/041052-11E51	CC
41	GA13027-15-1-7	VA09W-75/041052-11E51	CC
42	GA13062-6-5-3	081625/2*LAO1139D-56-1//081625-3-1-3-6	CC
43	GA14119I11-2-2	VA10W-119/11LE37//SH5550	CC
44	GA131214-8-2-1	AGS3000//JT//051477-9-1-6	CC
45	GA13062-18-6-1	081625/2*LAO1139D-56-1//081625-3-1-3-6	CC
46	GA13079-8-1-7	03564-12E6/041418-11EE16/031257-10E41	CC
47	GA13080-6-2-1	051477-9-1-6/031257-10E41	CC
48	GA13087-20-6-5	06385-13-2-3/03564-12E6	CC
49	GA13153-23-6-5	101700-G1-G2-G1-G4/031257-10E41	CC
50	GA13289-6-4-3	031134-10E29/04417-11E21	CC
51	GA13375-21-1-2	06520-24-1/031086-10E26	CC
52	GA131218-1-2-1	041323-11E63/031134-10E29	CC
53	GA131289-4-5-2	041293-11E54/04434-11E44	CC
54	GA11660-1-5-3-3	SS8641/Jamestown//SS8641	TT
55	GA131482-2-2-3-5	04434-11E44*2/10041-G1-G1-G1	TT
56	GA131052-1-7-6	06110-9-2-4/051477-9-1-6	CC
57	490-17LE16	031005-20-4-5/AGS 2038	CC
58	491-17LE11	031005-20-4-5/AGS 2038	CC
59	410-18E11	041333-13-2 / AGS2038	TT
60	411-18E12	021338-9E11 / AGS2038	TT
61	423-18E25	AGS2038 / VA04W-90	TT
62	AGS 2035	89482-E7 / XW663	TT
63	USG 3024	SS 8641 / 961591-17-1-5	CC
64	GW 2032	SS 8641 / Oglethorpe // 991371-6E13	CC
65	SH 5550	PIO 26R61 / 2* SS 8641	CC
66	AGS 3030	JAMESTOWN/ AGS 2026	CC
67	USG3555	VA94-52-60/Pio2643//USG3209	CC
68	AGS 3000	Jamestown/AGS 2060	CC

"TT" and "CC" indicates AGS 2038 and UGA 111729 allele, respectively.

Table 2.S2 Primer and probe sequences of two SNP markers linked to *QLr.ags-1AL* on chromosome 1AL in wheat

SNP marker		Primer/Probe Sequences
IWB20487	Forward Primer	CCGTCGATTTGGAACAATCTC
	Reverse Primer	TGGGAAAATTAACTACCAGGAGTTTG
	Probe1	TGTTATTCTTCATCATCGC
	Probe2	TGTTATTCTTCATCATTGC
IWA4022	Forward Primer	TGAATAAGACTTTATGAGTCAGGCAAA
	Reverse Primer	GCCCATGTCGATATATCCTACTACTG
	Probe1	ATTACTTTCCGAAGCGAGA
	Probe2	TACTTTCCGAAGCAAG

Table 2.S3 Analysis of variance (ANOVA) for leaf rust severity data in AGS 2038/UGA 111729 derived recombinant inbred line population.

Source of variation	Degrees of freedom (DF)	*F*-value	P > F
Replication	1	0.16	0.68
Genotype (G)	224	25.28	<.0001
Environment (E)	3	216.25	<.0001
G × E	668	2.42	<.0001

Table 2.S4 Gene annotations within the candidate region of the major QTL identified in AGS 2038/UGA 111729 population

Gene ID	Chrom.	Position (bp)	Human-Readable-Description
TraesCS1A01G424300.1	1A	579300387	Protein ABIL1
TraesCS1A01G424400.1	1A	579533509	S-type anion channel
TraesCS1A01G424500.1	1A	579573900	S-type anion channel
TraesCS1A01G424600.1	1A	579647984	TTF-type zinc finger protein with HAT dimerization domain-containing protein
TraesCS1A01G424700.1	1A	579677386	F-box family protein
TraesCS1A01G424800.1	1A	579836462	Cellulose synthase
TraesCS1A01G424900.1	1A	579861533	Protein kinase family protein
TraesCS1A01G425000.1	1A	579864314	Mitochondrial carrier-like protein
TraesCS1A01G425100.1	1A	580183361	Indole-3-acetic acid-amido synthetase GH3.3
TraesCS1A01G425200.1	1A	580420857	Extra-large G-like protein
TraesCS1A01G425300.1	1A	580433890	Amino acid transporter family protein
TraesCS1A01G425400.1	1A	580444570	Amino acid transporter family protein
TraesCS1A01G425500.1	1A	580448575	Amino acid transporter family protein
TraesCS1A01G425600.1	1A	580453354	transmembrane protein, putative (DUF594)
TraesCS1A01G425700.1	1A	580492051	Transducin/WD40 repeat protein
TraesCS1A01G425800.1	1A	580571333	Disease resistance protein (NBS-LRR class) family
TraesCS1A01G425900.1	1A	580659531	Disease resistance protein (NBS-LRR class) family
TraesCS1A01G426000.1	1A	580739950	Disease resistance protein (NBS-LRR class) family
TraesCS1A01G426100.1	1A	580925443	Lipid transfer protein
TraesCS1A01G426200.1	1A	580959561	B-block binding subunit of TFIIIC
TraesCS1A01G426300.1	1A	580988276	Polyol transporter
TraesCS1A01G426400.1	1A	581206186	Agenet and bromo-adjacent homology (BAH) domain-containing protein
TraesCS1A01G426500.1	1A	581255339	Agenet and bromo-adjacent homology (BAH) domain-containing protein
TraesCS1A01G426600.1	1A	581256211	Ribonucleoside-diphosphate reductase large subunit
TraesCS1A01G426700.1	1A	581438178	RNA polymerase sigma factor
TraesCS1A01G426800.1	1A	581506492	Polypyrimidine tract-binding-like protein, putative
TraesCS1A01G426900.1	1A	581540710	Pentatricopeptide repeat-containing protein, putative
TraesCS1A01G427000.1	1A	581570414	Protein phosphatase 2C family protein

TraesCS1A01G427100.1	1A	581580561	CTP synthase
TraesCS1A01G427200.1	1A	581589503	Protein N-methyltransferase
TraesCS1A01G427300.1	1A	581754085	Leucine-rich repeat receptor-like protein kinase family protein
TraesCS1A01G427400.1	1A	581829492	TPR repeat-containing thioredoxin TTL1
TraesCS1A01G601100LC.1	1A	579584639	Gag polyprotein
TraesCS1A01G601200LC.1	1A	579647403	Zinc finger MYM-type-like protein
TraesCS1A01G601300LC.1	1A	579712406	Tryptophan synthase beta chain
TraesCS1A01G601400LC.1	1A	579762850	60S ribosomal protein L30
TraesCS1A01G601500LC.1	1A	579785979	LINE-1 reverse transcriptase like
TraesCS1A01G601600LC.1	1A	579826503	Transcription elongation factor Spt5
TraesCS1A01G601700LC.1	1A	579830044	Formyl-CoA:oxalate CoA-transferase
TraesCS1A01G601800LC.1	1A	579843454	RNA-binding (RRM/RBD/RNP motifs) family protein
TraesCS1A01G601900LC.1	1A	579843550	4F5 protein family protein
TraesCS1A01G602000LC.1	1A	579937793	Calcium-dependent lipid-binding (CaLB domain) family protein
TraesCS1A01G602100LC.1	1A	580167995	Protein FAR1-RELATED SEQUENCE 5
TraesCS1A01G602200LC.1	1A	580174146	Basic-leucine zipper (bZIP) transcription factor family protein
TraesCS1A01G602300LC.1	1A	580417134	Glutathione S-transferase T3
TraesCS1A01G602400LC.1	1A	580576844	Retrotransposon protein, putative, LINE subclass
TraesCS1A01G602500LC.1	1A	580579258	Endonuclease/exonuclease/phosphatase family protein
TraesCS1A01G602600LC.1	1A	580579519	Cyclin/Brf1-like TBP-binding protein
TraesCS1A01G602700LC.1	1A	580580191	Heat shock 70 kDa protein
TraesCS1A01G602800LC.1	1A	580582853	Disease resistance protein (NBS-LRR class) family
TraesCS1A01G602900LC.1	1A	580627181	Histidine--tRNA ligase
TraesCS1A01G603000LC.1	1A	580628996	Disease resistance protein (NBS-LRR class) family
TraesCS1A01G603100LC.1	1A	580637490	Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1
TraesCS1A01G603200LC.1	1A	580656814	LINE-1 reverse transcriptase
TraesCS1A01G603300LC.1	1A	580657514	RNA-directed DNA polymerase (reverse transcriptase)-related family protein
TraesCS1A01G603400LC.1	1A	580658164	JmjC domain protein JMJ24
TraesCS1A01G603500LC.1	1A	580738529	Vacuolar sorting ATPase
TraesCS1A01G603600LC.1	1A	580746488	Retrotransposon protein, putative, unclassified
TraesCS1A01G603700LC.1	1A	580893424	splicing factor PWI domain-containing protein

TraesCS1A01G603800LC.1	1A	580940247	microtubule-associated proteins 65-1
TraesCS1A01G603900LC.1	1A	580941502	B-block binding subunit of TFIIIC
TraesCS1A01G604000LC.1	1A	580947068	LINE-1 reverse transcriptase
TraesCS1A01G604100LC.1	1A	580988871	Ribonuclease Z
TraesCS1A01G604200LC.1	1A	581264937	O-fucosyltransferase family protein
TraesCS1A01G604300LC.1	1A	581449872	No-apical-meristem-associated carboxy-terminal domain protein
TraesCS1A01G604400LC.1	1A	581465447	RNA-directed DNA polymerase (Reverse transcriptase)
TraesCS1A01G604500LC.1	1A	581511230	Acetyl-CoA carboxylase 1
TraesCS1A01G604600LC.1	1A	581512723	LINE-1 reverse transcriptase-like protein
TraesCS1A01G604700LC.1	1A	581569578	Tetratricopeptide repeat (TPR)-like superfamily protein
TraesCS1A01G604800LC.1	1A	581593927	Protein kinase
TraesCS1A01G604900LC.1	1A	581637956	Ribosomal protein L11 methyltransferase
TraesCS1A01G605000LC.1	1A	581638454	Zinc transporter 5
TraesCS1A01G605100LC.1	1A	581640879	Methionine S-methyltransferase
TraesCS1A01G605200LC.1	1A	581667710	Far1-like
TraesCS1A01G605300LC.1	1A	581678836	Transposon protein, putative, Mutator sub-class
TraesCS1A01G605400LC.1	1A	581748487	Transposon protein, putative, CACTA, En/Spm sub-class

CHAPTER 3

GENETIC MAPPING OF A MAJOR GENE FOR LEAF RUST RESISTANCE IN SOFT RED WINTER WHEAT CULTIVAR AGS 2000

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Abstract

Leaf rust (LR), caused by *Puccinia triticina* (*Pt*), is a major disease of wheat worldwide. Genetic resistance is the most effective, economic, and environmentally safe method to reduce losses caused by LR. Seventy-nine LR resistance genes have been identified so far; however, only a few of them are still effective due to the constant evolution of new *Pt* races. The objectives of this study were to characterize the genetic basis of LR resistance in the soft red winter wheat (SRWW) cultivar AGS 2000 at the seedling stage, and identify markers for marker-assisted selection (MAS). A mapping population of 175 recombinant inbred lines (RILs) was developed from a cross between the susceptible cultivar Pioneer® variety 26R61 (26R61) and AGS 2000. Two out of four *Pt* races (MBTNB, MFGKG, TCRKG, and MCTNB) showed segregating reactions for the parental lines and RIL population at the seedling stage. Whole genome QTL analysis detected a single common QTL for resistance to both *Pt* races (MFGKG and MBTNB) on chromosome 2BS. The gene was flanked by *wPt-666389* and *wPt-2600* markers and explained up to 75.3 % of phenotypic variation for *Pt* race MBTNB, and is therefore a Mendelian factor. LR resistance genes, *Lr13*, *Lr16*, *Lr23*, *Lr48*, and *Lr73*, were all detected on 2BS; however, based on their physical and genetic positions the gene was distinct and therefore temporarily designated as *LrA2K*. The closely linked marker *Xwmc770* to *LrA2K* has been validated on a set of wheat cultivars and can be used in MAS for LR resistance.

Keywords: Wheat, Leaf rust, *Puccinia triticina*, Recombinant inbred line (RIL), Genetic mapping, Marker-assisted selection (MAS)

Introduction

Leaf rust (LR) disease which is also known as brown rust, caused by the fungal pathogen *Puccinia triticina* (*Pt*), is one of the major foliar diseases of wheat (*Triticum aestivum* L.) worldwide. In addition to common wheat, the primary hosts of *Pt* are durum wheat (*T. turgidum* L. var. *durum*), cultivated emmer wheat (*T. dicoccon*), wild emmer wheat (*T. dicoccoides*), goatgrass (*Aegilops speltoides*), and triticale (X *Triticosecale*) (Bolton et al. 2008). Yield losses caused by LR are usually greater than stripe and stem rusts, which may be due to the fact that LR is more common and widely distributed than stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) and stem rust (caused by *Puccinia graminis* f. sp. *tritici*) worldwide (Bolton et al. 2008). LR infection on wheat leaves reduces the photosynthetic areas as well as the pathogen utilize the nutrients from the wheat plants causing lower seed set and reduced kernel size (Bolton et al. 2008). Depending on disease severity and time of infection, yield losses of 30-40% have been recorded in wheat due to LR (McMullen et al. 2008).

Although several management practices are available for LR control, such as cultural practices and use of fungicides, genetic resistance is the most preferred and economical method to control LR with an estimated benefit-cost ratio of 27:1 (Marasas et al. 2004; Turner et al. 2017). On average, over 50 *Pt* races are detected each year in the USA and some of them are virulent to genes that were previously reported to be resistant (Kolmer 2005; Kolmer et al. 2007). This necessitates a continuous search for novel sources of LR resistance and introgressed resistant genes into elite backgrounds. So far, 79 LR resistance genes (*Lr* genes) have been identified and mapped to specific chromosomes; however, limited numbers of identified resistant genes are still effective to

a wider range of pathogen populations. Most of these genes confer race-specific resistance at the seedling stage, which is also known as all-stage resistance. All-stage resistance is controlled by a single, major gene and provides a high level of resistance; however, high selection pressure on pathogen populations annually result in an emergence of new virulent races, and single gene resistance is often easily overcome by the pathogen (Kolmer 2005). The other mode of LR resistance is adult plant resistance (APR) which is expressed at the post-seedling stage. APR is more durable, effective against many races (race-nonspecific) and controlled by multiple genes with minor effect providing partial resistance (Kolmer 1996). Some of the major seedling resistance genes, such as *Lr16* and *Lr21*, which have been widely deployed in many breeding programs have been overcome by the LR pathogen (Hiebert et al. 2014). Therefore, pyramiding multiple LR resistance genes, that includes seedling and APR genes, is an effective and sustainable strategy to provide durable control of LR disease (Bariana et al. 2007; Dakouri et al. 2013).

Two approaches are commonly used to discover loci associated with complex traits in plants: linkage and association mapping (Purcell et al. 2003). Although association mapping is a relatively recent approach and found effective to locate genomic loci associated with complex traits in plants (Zhu et al. 2008), linkage mapping has been extensively used and very successful to clone genes associated with various traits in plants. Of the 79 LR genes mapped so far, 6 genes (*Lr1*, *Lr10*, *Lr21*, *Lr22a*, *Lr34*, and *Lr67*) have been cloned (Cloutier et al 2007; Feuillet et al. 2003; Huang et al. 2003; Krattinger et al. 2009; Moore et al. 2015; Thind et al. 2017), and majority of these genes were identified using the linkage mapping approach. In addition, linkage mapping has

been also used to map major genes for other diseases of wheat, such as powdery mildew, bacterial leaf streak, and stripe rust (Hao et al. 2011, 2015; Wen et al. 2018).

In the Southeastern (SE) region of the USA, LR pathogen that survived the winter often starts infecting wheat grown in Georgia (GA) and the surrounding regions early in February at the seedling stage (Kolmer 2003), and probably remains viable through maturity. Therefore, wheat grown in GA and its neighboring states require some level of seedling and APR genes to prevent losses from LR. In the SE region of the USA where soft red winter wheat (SRWW) is widely grown, LR resistance genes *Lr9, Lr10, Lr11, Lr18,* and *Lr26* are present in most of the grown wheat cultivars (Kolmer 2003; Kolmer et al. 2007). However, these genes are expressed at the seedling stage and are not effective against current LR races at adult plant stage. In GA, the gene cluster of *Lr37*/*Yr17*/*Sr38* has been used to combat wheat rust diseases; however, with the emergence of new virulent races, the LR gene has lost its effectiveness (Mergoum et al. unpublished data). Therefore, identification of new genes effective against current races and incorporation of the genes into the wheat breeding program is essential to prevent losses from LR in the SE region of the USA.

AGS 2000 and 26R61 are SRWW cultivars widely adapted to the SE region of the US (Hao et al. 2011, 2012a; Johnson et al. 2002). A bi-parental mapping population was developed from the cross of these two cultivars, genotyped with good coverage of molecular markers, and used for genetic studies (Hao et al. 2011, 2012b). In this study we used the same mapping population because AGS 2000 and 26R61 demonstrated highly resistant and susceptible reactions, respectively to *Pt* races. Utilizing this mapping population, the objectives of this study were to characterize the genetic basis of LR

resistance in AGS 2000 at the seedling stage and identify markers for marker-assisted selection (MAS).

Materials and methods

Plant materials and Pt races

AGS 2000 (PI 612956) and 26R61 (PI 612153) were the most common SRWW cultivars in the SE region of the USA and had been used as checks for several years in the Uniform Southern SRWW Nursery. A mapping population, consisting of 175 recombinant inbred lines (RILs), was developed from the cross of 26R61 and AGS 2000 (hereafter abbreviated as PA population), and has been used to map genes for resistance to stripe rust, powdery mildew, Hessian fly, and soil-borne wheat mosaic virus (Hao et al. 2011, 2012b, 2013, and 2015). AGS 2000 (PIO2555/PF84301//Florida302) and 26R61 (Omega 78/S76/Arthur 71/3/Stadler//Redcoat/Wisconsin 1/5/Coker 747/6/Pioneer 2555sib) have different genetic backgrounds, and were developed by two different organizations. AGS 2000 was developed and released by the University of Georgia and Florida Agricultural Experimental Station in 1999 whereas 26R61 was developed and released by Pioneer Hi-Bred in 2000 (Hao et al. 2012a). A set of 20 wheat cultivars, consisting of winter and spring wheat were selected randomly and used to validate the QTL. These cultivars were obtained from the North Dakota State University (NDSU) hard red spring wheat (HRSW) breeding program and the UGA SRWW breeding program. Four of the cultivars had AGS 2000 in their pedigree (Table 3.2). Additionally, 96 elite wheat breeding lines were selected and used in the MAS experiment (Table

3.S2). These breeding lines were evaluated at seeding and adult plant stage for their reaction to the current prevalent *Pt* races of GA.

Four *Pt* races (MBTNB, MFGKG, TCRKG, and MCTNB) were used to evaluate the rust reaction phenotypes of the parents and PA population. The *Pt* races, TCRKG, MBTNB, and MCTNB are the prevalent *Pt* races in the SE region of the USA (Kolmer and Hughes 2016), and MFGKG is a current common race of *Pt* in GA (Buck and Youmans, unpublished data). The virulence profile of the four *Pt* races was characterized based on the reaction on 20 wheat lines each carrying a single LR gene (Table 3.1).

Leaf rust evaluation

Evaluation of parents (26R61 and AGS 2000) and PA population for reaction to *Pt* races was conducted on plants at the seedling stage in the University of Georgia (UGA), Griffin Campus. Plant materials were grown in cone-tainers (Stuewe and Sons, Inc.) filled with Sungro professional growing mix soil (Sun Gro Horticulture Distribution Inc.), and placed in a rack which can hold 98 cone-tainers. Osmocote Bloom 12-7-18 fertilizer (Everris NA Inc.) was applied after planting to each cone-tainer (~2 g/cone). Three seeds for each entry were planted in a cone-tainer and three cone-tainers (replications) were planted per entry. The experiment was repeated three times. The SRWW cultivar SS520, a highly susceptible check, was planted in the border to minimize the edge effects. The plant materials were arranged in a randomized complete block design (RCBD) within each rack.

Fresh urediniospores, collected from the highly susceptible wheat cultivar SS520, were suspended in sterile water and used to inoculate the 10-day-old seedlings with fully

opened secondary leaves. About 20 µl of Tween-20 (Agdia, Inc. Elkhart, IN) per 100 ml was added in the urediniospores suspension, the number of urediniospores in the inoculum was counted using a Hemocytometer (Model# BX60F5, Olympus Optical Co. Ltd, Japan), and adjusted to approximately 1×10^5 urediniospores mL^{-1}. The urediniospores inoculum was sprayed on the foliage until runoff and inoculated plants were placed in a dark chamber with high humidity for 16-18 h. Plants were then transferred to the growth chamber set at 18°C/20°C (night/day) with 15-h of light.

The reactions of parents and the PA population to *Pt* races were scored 10-12 days after inoculation using a 0-4 Stackman scale (Stackman et al. 1962) where infection type (IT) '0' = no visible uredia, ';' = hypersensitive flecks, '1' = small uredia with necrosis, '2' = small to medium sized uredia with green islands and surrounded by necrosis or chlorosis, '3' = medium sized uredia with or without chlorosis, and '4' = large sized uredia without chlorosis. Plus ('+') and minus ('-') signs were used to indicate the variation within a given IT. ITs of 0-2 and 3-4 were considered as resistant and susceptible reactions, respectively (Kertho et al. 2015). Finally, the 0-4 Stackman ITs were converted to a linearized scale (LS) of 0-9 to account for multiple infections types within a single plant, and to include non-numeric IT such as hypersensitive fleck (;) in the analysis (Zhang et al. 2014).

Data analysis and QTL mapping

The statistical analysis of the phenotypic data was done using SAS version 9.4 (SAS Institute Inc. 2017). The phenotypic data of LR evaluation for each *Pt* race was tested for normality using Shapiro-Wilk, PROC UNIVARIATE procedure (SAS Institute

Inc., Cary, NC, USA). Bartlett's or Levene's test (Levene 1960; Snedecor and Cochran 1989) was performed to check equality of variance among the experiments as described by Kariyawasam et al. (2016). A chi-square (χ^2) test was used to determine whether or not the observed segregation ratio of the phenotypic data fit the expected genetic ratios. The χ^2 analysis was performed in Microsoft Excel (version 2010) using the "chitest" function to calculate the χ^2 and *p* value.

The current study utilized the genetic map constructed by Hao et al. (2012b). Briefly, a total of 990 markers on 24 linkage groups were used for the construction of genetic maps. The maps covered a total distance of 2908.6 cM with 1125.7, 1006.2, and 776.7 cM in the A, B, and D genome, respectively. The average distance between the adjacent markers was 2.93 cM. Detection of QTL for LR resistance was performed using inclusive composite interval mapping (ICIM) in the software QTL IciMapping version 4.1 (Meng et al. 2015). The QTL was detected using 1.0 cM speed with 0.001 probability in the stepwise regression. The logarithm of odds (LOD) threshold was set at 3.00, and LOD value was calculated from 1000 permutations with type I error of 0.01. The graphical presentation of the genetic map was generated using MapChart (Voorrips 2002).

DNA extraction and marker analysis

DNA from wheat lines was extracted using a modified CTAB method (Saghai-Maroof et al. 1984) and the concentration was measured using a NanoDrop 2000C (Thermo Fisher Scientific Inc.). The DNA samples were diluted to make the final concentration of 50 ng/µl. The PCR reaction was carried out using a touchdown protocol

as described in Hao et al. (2008) and the amplified products were separated in a 1.5% agarose gel stained with ethidium bromide.

Results

Reaction of parents and RIL population to Pt races

The parents, 26R61 and AGS 2000, were evaluated for their reactions to four *Pt* races at the seedling stage. The result demonstrated that both parental lines were resistant to two *Pt* races i.e., TCRKG and MCTNB. However, they showed a differential reaction to the other two races i.e., MBTNB and MFGKG (Table 3.1, Figure 3.S1). Subsequently, the PA population was screened with all races, and no difference was observed for reaction to two races TCRKG, and MCTNB, with all lines showing resistant reaction as parents. This may indicate that 26R61 and AGS 2000 possess common alleles for resistance to each of these two races. However, PA population segregated for their reaction to two *Pt* races, MBTNB and MFGKG, and the reactions ranged from highly resistant (IT = ;1 or LS = 1) to highly susceptible (IT = 4 or LS = 9).

AGS 2000 developed hypersensitive flecks with small to medium sized uredia to both *Pt* races, MBTNB and MFGKG, indicating highly resistant reaction; however, 26R61 developed large sized uredia without chlorosis when evaluated with MBTNG and medium-sized uredia with chlorosis when evaluated with MFGKG, indicating highly susceptible reactions (Table 3.1, Figure 3.S1). The susceptible check SS520 developed large sized uredia without chlorosis (Figure 3.S1). Of the 172 RILs evaluated (3 lines were excluded due to poor germination), 76 and 84 lines were resistant and 96 and 88 lines were susceptible to MBTNB and MFGKR, respectively (Figure 3.1). The

segregation of resistant and susceptible RILs fitted a single gene segregation ratio of 1:1 to both *Pt* races ($\chi^2 = 2.32$, $p = 0.12$ for MBTNB, and $\chi^2 = 0.09$, $p = 0.76$ for MFGKG). All RILs demonstrated either resistant or susceptible reactions to both *Pt* races, and no intermediate reactions were observed (Figure 3.1). Furthermore, normality test rejected the hypothesis that the reaction of PA population to two *Pt* races fits a normal distribution. These facts suggested that LR resistance in AGS 2000 is most likely controlled by a single gene.

Discovery of novel QTL for LR resistance

The statistical analysis showed that the reactions of PA population to both *Pt* races, MFGKG and MBTNB, significantly deviated from the normal distribution. Therefore, Levene's test was performed to test homogeneity among the three experiments. This test demonstrated that the variance among the three experiments was homogeneous for both *Pt* races ($P = 0.07$ and 0.26, $df = 2$). Therefore, the average disease score across the three experiments was used for the QTL mapping. A highly significant correlation was observed between the phenotypic data of PA population evaluated against MBTNB and MFGKG races ($r = 0.82$, $P<0.0001$) suggesting that the resistance to both races was likely controlled by a common gene.

QTL analysis detected a single common QTL on short arm of chromosome 2B for resistance to *Pt* races MBTNB and MGFKG (Figure 3.2, Table 3.S1). This QTL, designated as *QLr.uga.2BS,* was contributed by the resistant parent AGS 2000, and explained 75.3 and 62. 9% phenotypic variation in the PA population to MBTNB and MFGKG races, respectively (Table 3.S1). The two DArT markers, *wPt-666389* and *wPt-*

2600 tightly flanked *QLr.uga.2BS* with 2.1 cM distance between them on 2BS map (Figure 3.2, Figure 3.S2, Table 3.S1).

Validation of QTL linked marker

The marker *Xwmc770* (Forward primer: TGTCAGACTTCCTTTGATCCCC; Reverse primer: AAGACCATGTGACGTCCAGC) located in the QTL region (2.9 cM proximal to *QLr.uga.2BS*) and tightly linked to *QLr.uga.2BS*, was chosen to be tested on a set of wheat materials (Table 3.2) to validate the new QTL and determine its diagnostic value for MAS. The results showed that the marker is present in all wheat materials which were resistant to *Pt* race MBTNB with an exception for AGS 3000 (Table 3.2). AGS 3000 was found to be highly resistant but it was negative to resistant locus. Furthermore, the wheat cultivars which have AGS 2000 in their pedigree were also found positive to *Xwmc770* (Table 3.2). This result indicated that the marker *Xwmc770* could be useful in MAS to screen germplasm and identify the sources of LR resistance.

MAS of wheat breeding lines for the development of LR resistant cultivars

To further analyze the applicability of marker in MAS, we screened a set of 96 advanced wheat breeding lines having diverse genetic backgrounds with the molecular marker *Xwmc770* tightly linked to the major QTL *QLr.uga.2BS* (Table 3.S2). Twenty-nine lines were found to possess resistant allele as resistant parent AGS 2000 (Figure 3.S3, Table 3.S2). These lines were also evaluated and phenotyped by our pathologist under both greenhouse and field conditions. Results showed that all the lines which were resistant or moderately resistant under field conditions (adult plant) and resistant under

greenhouse conditions (seedling) matched the results obtained using *Xwmc770* closely linked to the major QTL *QLr.uga.2BS*. Therefore, this marker has a great value in MAS for LR screening of large set of materials or/and segregating material. Our next step is to use this marker on all our lines in preliminary yield trials and advanced segregating generations (F4-F6). Similarly, this marker is currently being used to help us design crosses to develop populations for incorporating this LR gene, or pyramid it with other LR genes, and/or combine it with other economic traits.

Discussion

AGS 2000 and 26R61 are SRWW cultivars adapted to the SE region of the USA. These two cultivars have been commonly grown for several years in this region mainly because they have good yield and test weight, and more importantly, they possess a good level of resistance to multiple diseases, insect, and virus (Hao et al. 2011, 2012a, 2012b, 2013, and 2015; Johnson et al. 2002). Using these two SRWW cultivars, a mapping population was developed (PA population), and successfully used to map major genes for resistance to stripe rust (*YrR61*), powdery mildew (*Pm54*), Hessian fly (*HR61*), and *soil-borne wheat mosaic virus* (*Sbm*$_{P26R61}$) (Hao et al. 2011, 2012b, 2013, and 2015). However, the reaction of parents, (26R61 and AGS 2000) and PA population to the prevalent *Pt* races at seedling stage was not tested. In this present study, we evaluated the reaction of parental lines and PA population against four *Pt* races common to the Southeastern USA at the seedling stage (Table 3.1). Results showed that the parents had different reactions at the seedling stage to two *Pt* races (MBTNB and MGFKG) with AGS 2000 resistant and 26R61 susceptible. Furthermore, the PA population segregated

well to these two races allowing it to be a good population for the genetic study we conducted to determine the genetics of LR resistance in AGS 2000. The results from the QTL mapping study detected a single common QTL, *QLr.uga.2BS,* for resistance to two *Pt* races on chromosome 2BS (Figure 3.2). *QLr.uga.2BS* (*wPt-666389* - 1.1 cM - *QLr.uga.2BS*- 1 cM - *wPt-2600*) was detected with a significantly high LOD score and explained up to 75.3% of phenotypic variation in PA population suggesting that it is a major gene (Table 3.S1). Therefore, it is temporarily designated as *LrA2K*.

In addition to *LrA2K*, five LR resistance genes i.e., *Lr13* (Dyck et al. 1996), *Lr16* (McCartney et al. 2005), *Lr23* (McIntosh and Dyck 1975), *Lr48* (Bansal et al. 2008) and *Lr73* (Park et al. 2014), were detected on the short arm of chromosome 2B. *Lr16* was reported to be located on the distal end of chromosome 2B and flanked by *Xwmc764* and *Xwmc661* markers (*Xwmc764* - 9.4cM - *Lr16* - 1.4cM - *Xwmc661*) (Lan et al. 2014). The *Lr73* gene was detected close to *Lr16*, and located 8.9 cM-10.9 cM proximal to *Lr16* (Park et al. 2014). Based on the 2BS consensus map (Marone et al. 2012), the markers *Xwmc661* (linked to *Lr16*) and *wPt-5738* (linked to *Lr73*) were located 63.8 and 59.9 cM distal to *wPt-2600* (linked to *LrA2K*), respectively (Figure 3.3). Based on the 2B genetic map developed by Rosewarne et al. (2013), *Xwmc257* was reported to be located close to *Lr23* and *Lr13* (*Lr23* - 13 cM - *Xwmc257* - 39 cM - *Lr13*). The marker *Xwmc257* was located 25.7 cM distal to *LrA2K* based on the 2BS consensus map i.e., (Marone et al. 2012) (Figure 3.3). Additionally, *Lr13* was an APR gene reported from wheat cultivar Frontana (Dyck et al. 1996), but *Lr23* was a seedling gene reported from an Australian durum wheat cultivar Gaza (McIntosh and Dyck 1975). Bansal et al. (2008) reported that *Xgwm429* and *Xbarc7* markers flanked the *Lr48* gene on chromosome 2BS. *Xgwm429*

which was reported to be located 6.1 cM distal to *Lr48* is quite close to *LrA2K* (Figure 3.3); however, *Lr48* was reported from Australian spring wheat cultivar and is an APR gene (Archer and O'Brien 1987; Bansal et al. 2008). Although *LrA2K* is found to be located close to *Lr48*, it confers seedling resistance and not APR, this confirms that the two genes are different and therefore, we can argue that *LrA2K* should be a novel gene.

Based on the linked molecular markers, *Lr16* and *Lr73* were physically mapped on the terminal deletion bin of chromosome 2BS, i.e., 2BS4-0.84-1.00 (Sourdille et al. 2004). SSR markers *Xwmc257* and *Xwmc154*, which were linked to *Lr13* and *Lr23* (Rosewarne et al. 2013), were also mapped to the deletion bin 2BS4-0.84-1.00 (Hua et al. 2009). The marker *Xbarc7*, which was linked to *Lr48*, was mapped to deletion bin 2BS1-0.53-0.75 (Bansal et al. 2008; Sourdille et al. 2004). However, based on the position of closely linked marker *Xwmc770*, *LrA2K* was physically mapped to deletion bin 2BS3-0.75-0.84 (Figure 3.3). Additionally, the location of LR genes on chromosome 2BS was compared based on the location of linked markers in the physical map of Chinese Spring (RefSeq v1.0). *LrA2K* has been physically mapped to a 13.82 Mb interval on 2BS chromosome (92.16-105.99Mb). However, *Xwmc764* (*Lr16*), *wPt-4453* (*Lr73*), *Xwmc154* (*Lr13* and *Lr23*), and *Xbarc7* (*Lr48*) were physically located at 8.07, 13.48, 36.44, and 117.25 Mb positions, respectively.

MFGKG is a current virulent race of *Pt* in GA and it has been postulated that this race has been common in this region for the last several years (Buck and Youmans, unpublished data). Kolmer and Hughes (2016) reported that MBTNB was the most prevalent race of *Pt* in the USA, and most commonly present in SRWW. LR resistance genes, *Lr1*, *Lr2a*, *Lr9*, *Lr10*, *Lr11*, *Lr18*, and *Lr26*, were commonly present in the

SRWW cultivars grown in the SE region of the USA (Kolmer 2003), but *Pt* races MBTNB and MFGKG are virulent to all of them except *Lr2a* (Table 3.1). This clearly indicates the urgent need to identify additional LR resistance genes effective against current *Pt* races. LR resistance gene *LrA2K*, reported in this study, is effective against *Pt* races MBTNB and MFGKG, and therefore this gene could be used in wheat breeding programs to develop LR resistant wheat cultivars via MAS. Recently, two SRWW cultivars, Caldwell and Clark, were reported to carry APR genes for LR resistance on chromosome 3BS (Kolmer et al. 2017; Li et al. 2017). Since Caldwell and Clark were well adapted SRWW to the SE region of the USA, they can be crossed with AGS 2000 to pyramid multiple LR genes and develop new SRWW cultivars adapted to SE region with durable LR resistance.

Genotyping a set of wheat materials with *Xwmc770*, a closely linked marker to *LrA2K*, demonstrated that this marker is highly effective for MAS and pyramiding *LrA2K*. All wheat cultivars which were resistant to *Pt* race MBTNB were found to possess the resistance allele except AGS 3000 (Table 3.2) which indicates that AGS 3000 likely carries different resistance gene(s). MAS has been an effective tool in molecular breeding and has many advantages over conventional breeding which mainly accelerates the breeding cycle (Collard and Mackill 2008). *Xwmc770* has already been used in screening a set of elite wheat breeding lines and 29 lines were selected as a valuable LR resistant sources (Table 3.S2). These breeding lines are being utilized by the UGA small grain breeding program to pyramid multiple LR resistance genes and to develop durable LR resistance cultivars. Similarly, this marker is currently used to design crosses to

develop populations that segregate for LR resistance or to pyramid *LrA2K* with other effective LR genes.

In summary, a major seedling LR resistance gene (*LrA2K*) effective against two prevalent *Pt* races at seedling stage, close to *Lr48*, an APR gene, has been mapped in a SRWW cultivar AGS 2000. AGS 2000 with the *LrA2K* gene has been crossed with cultivars that possess effective APR genes such as *Lr34*, *Lr46*, and *Lr67* to develop durable LR resistant wheat cultivars. Furthermore, closely linked molecular marker (*Xwmc770*) to *LrA2K* has been validated in a set of wheat cultivars from diverse backgrounds and wheat market classes. Therefore, this marker could be used in selecting wheat cultivars and germplasm to develop LR resistant cultivars.

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Tables and figures

Table 3.1 Virulence profile of *Puccinia triticina* (*Pt*) races, and the reaction of two parents, 26R61 and AGS 2000, to each *Pt* races.

***Pt* Races**	**Virulent on genes[a]**	**Infection types [b]**		**Linearized scale [c]**	
		AGS 2000	**26R61**	**AGS 2000**	**26R61**
MBTNB	1, 3, 3ka, 11, 17, 30, B ,14a	;2	4	2	9
MFGKG	1, 3, 9, 24, 26, 11, 10, 14a, 18, 28	;2+	3	2	8
TCRKG	1, 2a, 2c, 3, 26, 3ka, 11, 30, 10, 14a, 18, 28	;1	;1+	1	1
MCTNB	1, 3, 26, 3ka, 11, 17, 30, B, 14a	;1	;3	1	3

[a] The *Pt* races were tested on 20 wheat lines carrying a single gene for LR resistance to determine the race information (Kolmer and Hughes, 2016)

[b] AGS 2000 and 26R61 were tested with each of these 4 *Pt* races at seedling stage in the growth chamber, and their infection types (ITs) were scored using a 0-4 scale with 0 being an immune reaction and 4 being a highly susceptible reaction (Stackman et al. 1962)

[c] The 0-4 ITs scale was converted to a 0-9 linearized scale to account for multiple ITs within a single plant, and to include non-numeric IT such as ';' in the analysis (Zhang et al. 2014)

Table 3.2 Validation of *LrA2K* linked marker *Xwmc770* in a set of wheat cultivars

Cultivar/line	Pedigree	Habit[a]	*Xwmc770*[b]	Phenotype[c]
AGS 2000 (control)	See "materials and method"	W	+	R
26R61 (control)	See "materials and method"	W	-	S
GA001138-8E36	GA961581/PIO26R61	W	+	R
Baldwin	AGS2485/PIO26R61	W	+	R
USG3120	GA901146/GA96004//AGS 2000	W	+	R
AGS2020	GA88151/HICKORY//AGS 2000	W	+	R
AGS3033	SS8641/4/A2000*3/931433//P2684 /3*AGS 2000	W	+	R
AGS2035	AGS 2000/PIO26R61	W	+	R
Alsen	Grandin // Grandin / Glupro // Sumai 3 / Wheaton // Grandin / ND688	S	+	R
Bolles	MN02268- 1/MN01333-A-1	S	+	R
Glenn	ND-2831/STEELE-ND	S	+	R
AGS 3000	Jamestown/AGS 2060	W	-	R
UGA111729	AGS2038/ KS89WGRC06///AGS2038	W	-	S
AGS2027	P26R24/GA961565//GA941208	W	-	S
Hillard	P25R47/Jamestown	W	-	S
USG3555	VA94-52-60/Pio2643//USG3209	W	-	S
USG3024	SS8641/961591-17-1-5	W	-	S
P26R41	26R58/WBP0287E1//8302/25R47	W	-	S
Pavon	Vicam//Cianosib/SieteCerros66/3 /Kalyansona/Bluebird	S	-	S
Prosper	ND2857/Dapps	S	-	S
Magnum	Blueboy/W-504//Arthur 71	W	-	S
Thatcher	Marquis/Iumillo durum//Marquis/ Kanred	S	-	S

[a] Growing habit of wheat cultivars: S = spring and W = winter

[b] Presence and absence of AGS 2000 allele: '+' means positive to AGS 2000 and '-' means negative to AGS 2000

[c] The wheat materials were evaluated for their reaction to *Puccinia triticina* (*Pt*) race MBTNB as described in material and methods: 'R and 'S' indicates resistant and susceptible reactions, respectively.

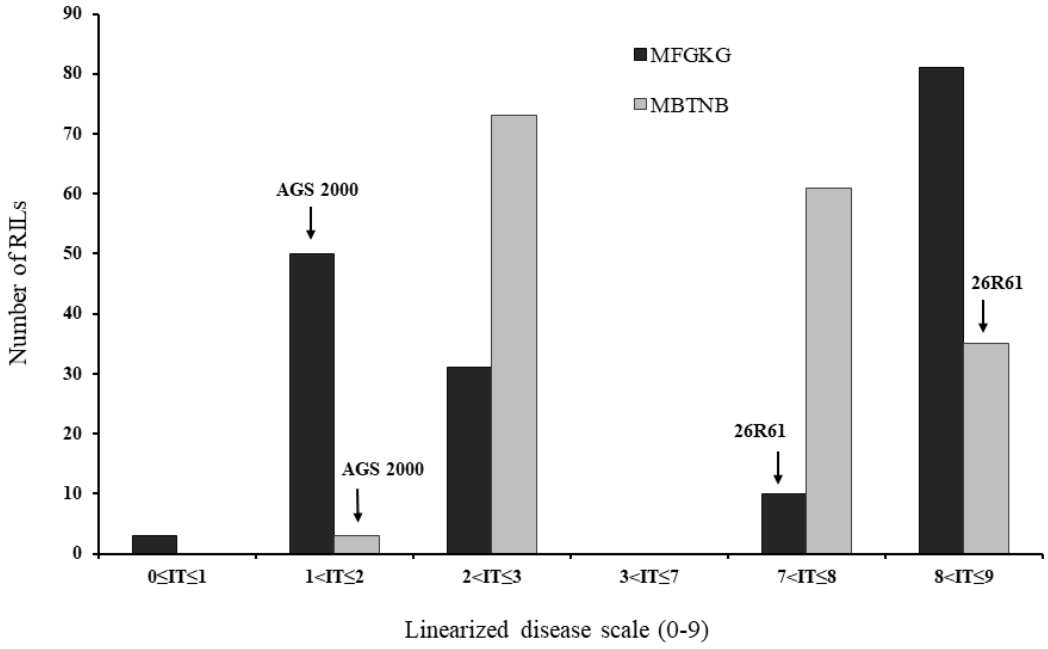

Figure 3.1 Linearized disease scale distribution of 26R61×AGS 2000 derived recombinant inbred lines (RILs) to *Puccinia triticina* races MFGKG and MBTNB. The leaf rust reactions were initially scored using a 0-4 scale (Stackman et al. 1962), and converted to a 0-9 scale (Zhang et al. 2014), with 0 being an immune reaction and 9 being a highly susceptible reaction.

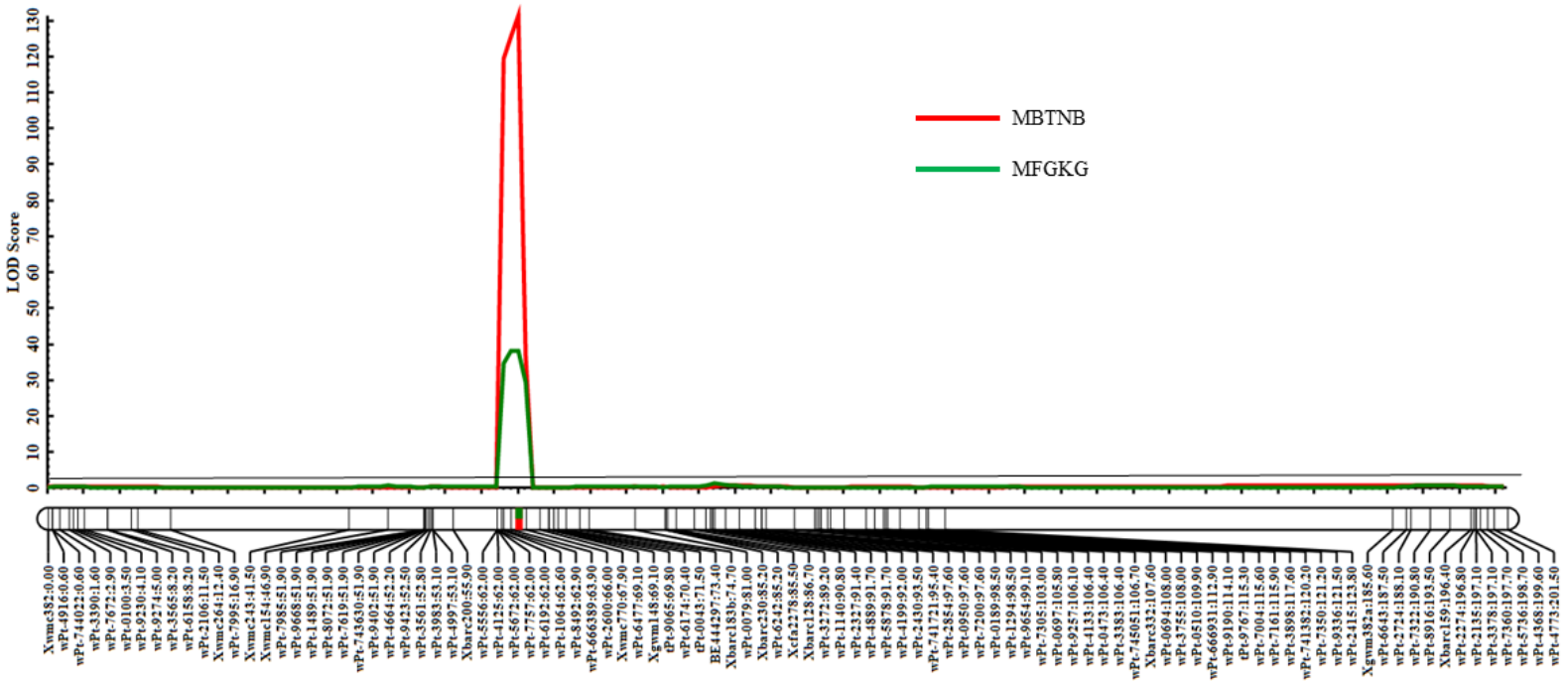

Figure. 3.2 A major QTL detected for resistance to *Puccinia triticina* (*Pt*) races MBTNB and MFGKG in the 26R61×AGS 2000 recombinant inbred line (RIL) population. The horizontal line indicates a LOD cutoff of 3.0 for QTL detection based on inclusive composite interval mapping (ICIM).

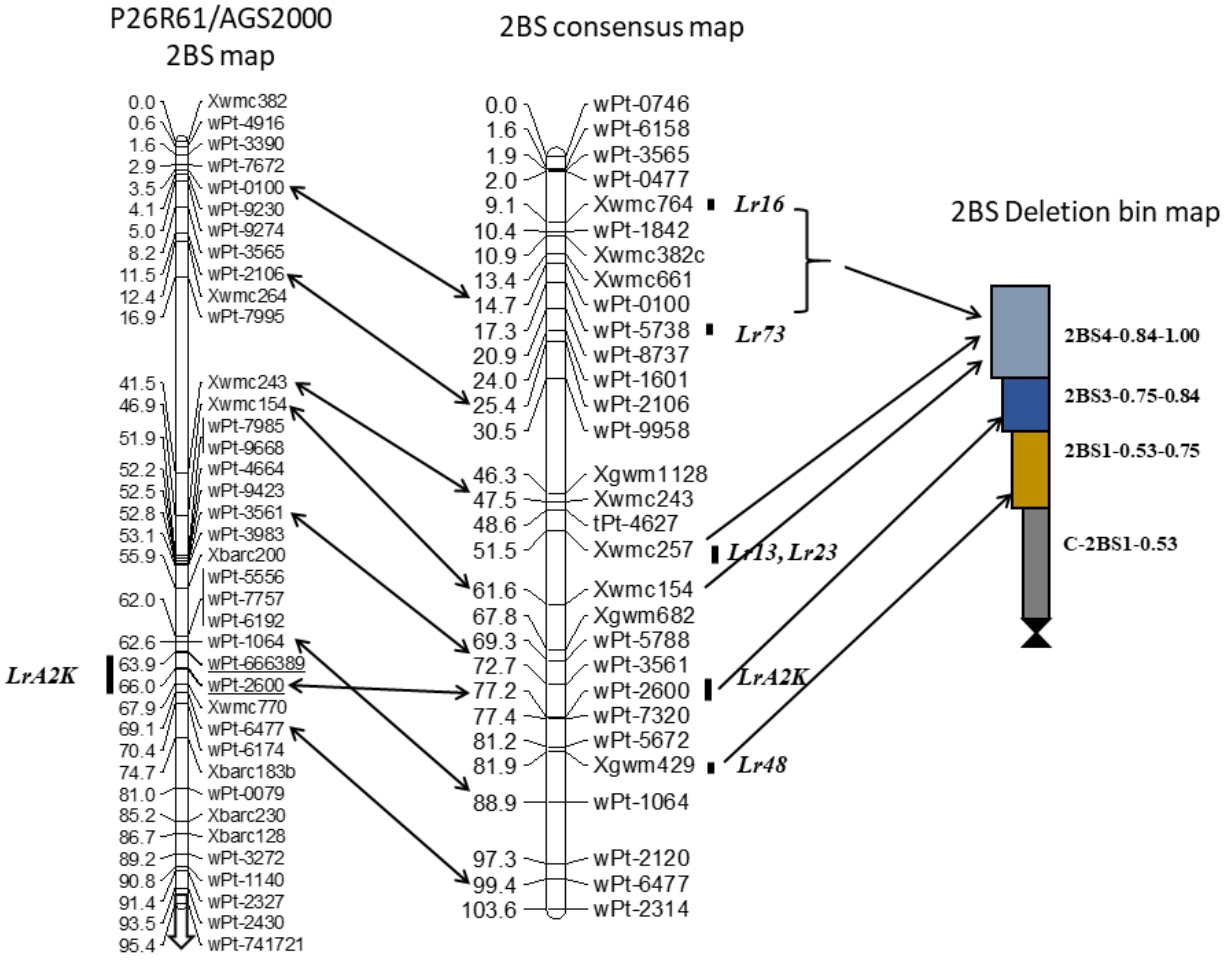

Figure 3.3 Genetic map of wheat chromosome 2BS showing the location of leaf rust resistance gene *LrA2K*. The flanking markers are underlined in the 26R61/AGS 2000 map. LR resistance genes, *Lr13*, *Lr16*, *Lr23*, *Lr48*, and *Lr73*, detected on chromosome 2BS are shown the in the 2BS consensus map. The 2BS consensus map and deletion bin maps were based the data presented in Marone et al. (2012) and Sourdillet et al. (2004), respectively.

Appendix A: Supplemental figures

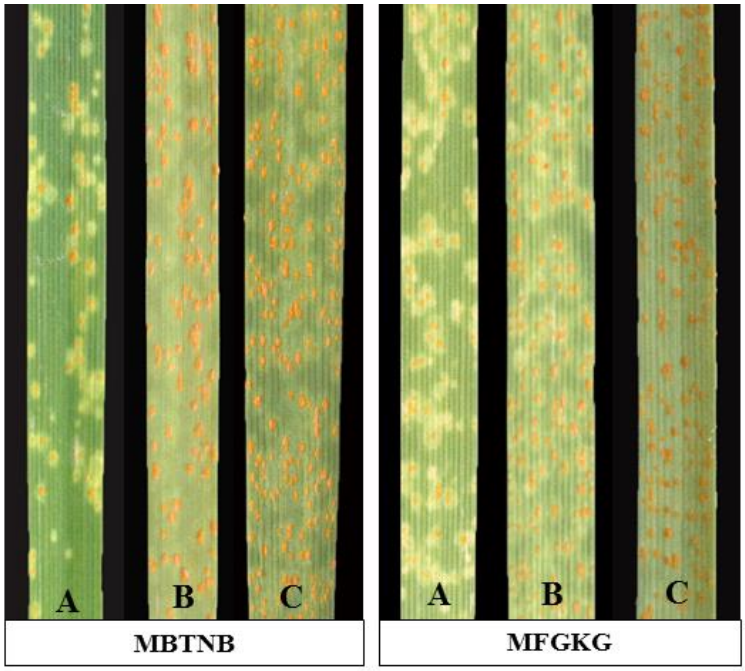

Figure 3.S1 Reaction of AGS 2000 (A), 26R61 (B), and the susceptible check SS520 (C) to *Puccinia triticina* races MBTNB and MFGKG at seedling stage.

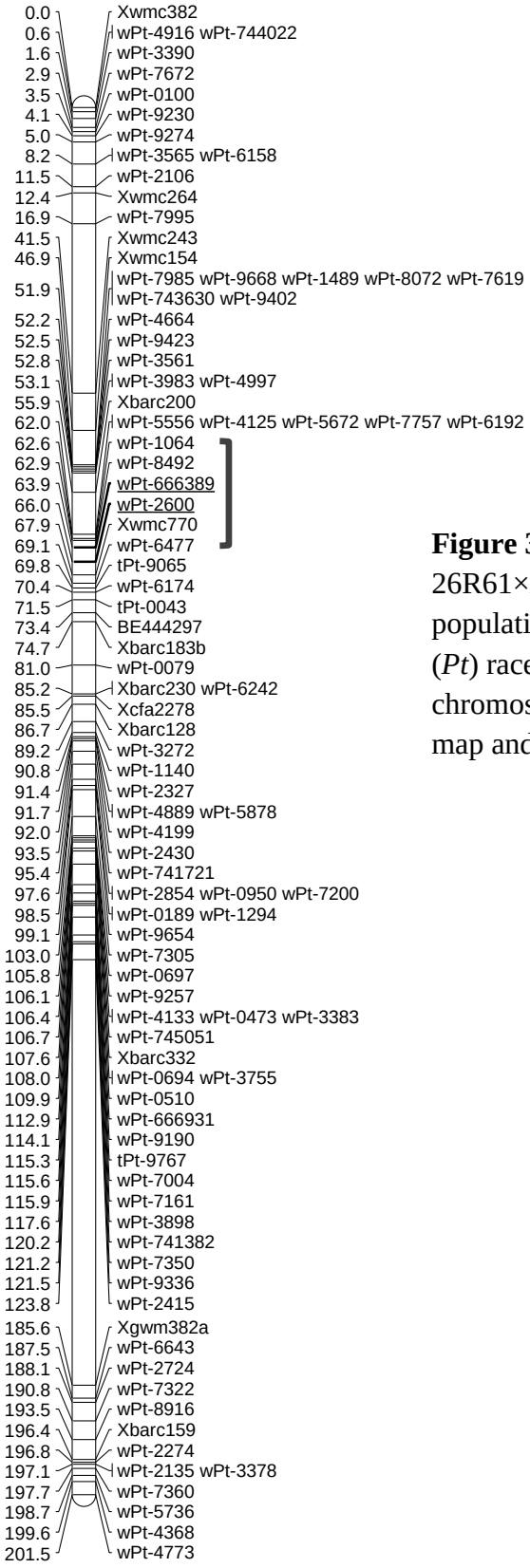

Figure 3.S2 Genetic map of wheat chromosome 2B in a 26R61×AGS 2000 derived recombinant inbred line (RIL) population. A major QTL for resistance to *Puccinia triticina* (*Pt*) races MBTNB and MFGKG was detected on 2BS chromosome. The QTL region is shown by solid line in the map and the QTL flanking markers are underlined.

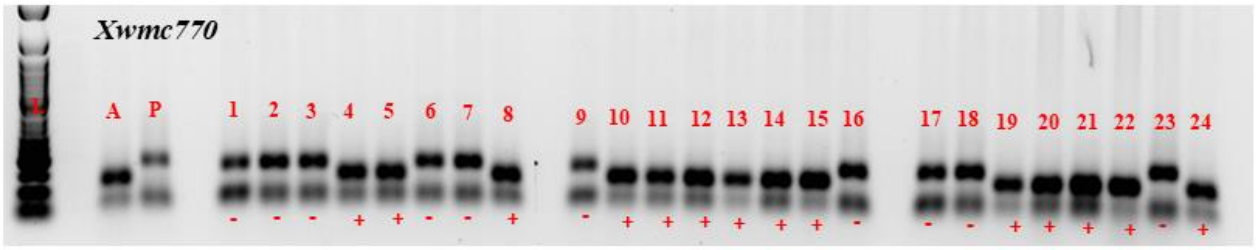

Figure 3.S3 Ethidium bromide stained agarose gel showing the amplification of SSR marker *Xwmc770* on wheat breeding lines used in marker-assisted selection (MAS) study. Only the result of 24 lines out of 96 lines are shown here; A=AGS 2000 (resistant parent), P=26R61 (susceptible parent), "+" = positive to AGS 2000 allele (resistant), "-" = negative to AGS 2000 allele (susceptible).

Appendix B: Supplemental tables

Table 3.S1 Summary of the major QTL associated with resistance to *Puccinia triticina* (*Pt*) races, MBTNB and MFGKG, in the 26R61×AGS 2000 derived recombinant inbred line population.

Pt races	QTL name	Flanking markers	Interval (cM)	LOD	PVE (%)[a]	Additive effect[b]
MBTNB	*QLr.uga-2BS*	*wPt-666389–wPt-2600*	63.9-66.0	131.2	75.3	2.6
MFGKG	*QLr.uga-2BS*	*wPt-666389–wPt-2600*	63.9-66.0	38.0	62.9	2.8

[a]Percent of phenotypic variation associated with the QTL
[b]The positive additive effect value indicated the QTL was contributed by AGS 2000

Table 3.S2 List of wheat breeding lines and their pedigree information used in marker-assisted selection (MAS) experiment.

S.N	Lines	Pedigree	*Xwcm770*[a]
1	GA12213-16-5	04510-11LE34/081607-G1-G1-1(991227-6A33*4/SS8641)	-
2	GA12213-22-2	04510-11LE34/081607-G1-G1-1(991227-6A33*4/SS8641)	-
3	GA12213-26-3	04510-11LE34/081607-G1-G1-1(991227-6A33*4/SS8641)	-
4	GA14177-3-4	AGS2024/PIO26R94//AGS2024	+
5	GA131216-1-4	JAMESTOWN/031257-10LE34//051477-9-1-6	+
6	GA13011ID-2-6	JAMESTOWN/031257-10E41	-
7	GA13011ID-5-1	JAMESTOWN/031257-10E41	-
8	GA13085-9-6	06478-23-1-3/AGS2033	+
9	GA13264-1-6	JAMESTOWN/AGS2033	-
10	GA131070-3-2-2	051477-9-1-6/AGS2033	+
11	GA131070-3-4-3	051477-9-1-6/AGS2033	+
12	GA131070-4-12-1	051477-9-1-6/AGS2033	+
13	GA131070-4-12-3	051477-9-1-6/AGS2033	+
14	GA131070-4-12-6	051477-9-1-6/AGS2033	+
15	GA131070-4-12-8	051477-9-1-6/AGS2033	+
16	GA131187-3-9-2	061098-9-6-1/SH5550	-
17	GA15020ID-7-7	JT152/VA11W-230//04417-12E33	-
18	GA15040ID-8-3	AGS3000/AGS2033//AGS2033	-
19	GA12177-8-4-4-6	SS8629/051477-206-5	+
20	GA14317ID-11-5	JT152/PIO26R94	+
21	GA14317ID-11-7	JT152/PIO26R94	+
22	GA14317ID-12-1	JT152/PIO26R94	+
23	GA141205ID-20-2	HILLIARD/PIO26R94	-
24	GA131121-4-3-1	05153-16-2-4/031086-10E26//AGS2033	+
25	GA131148-3-8-4	101700-G1-G2/031257-10LE34//031257-10E41	-
26	GA131148-3-8-9	101700-G1-G2/031257-10LE34//031257-10E41	-
27	GA131214-4-3-2	LA06146E-P05/JAMESTOWN/051477-9-1-6	+
28	GA131214-8-5-2	LA06146E-P05/JAMESTOWN/051477-9-1-6	-
29	GA131214-8-5-6	LA06146E-P05/JAMESTOWN/051477-9-1-6	-
30	GA131214-8-5-11	LA06146E-P05/JAMESTOWN/051477-9-1-6	-
31	GA131290-13-5-4	04434-7-4-3/10041-G1-G1-G1//04434-7-4-2-1-5	-
32	GA131290-13-5-10	04434-7-4-3/10041-G1-G1-G1//04434-7-4-2-1-5	-
33	GA131291-6-6-10	04434-7-4-3/0312710LE34//04434-7-4-2-1-5	-
34	GA131331-15-4-6	04417-11E21/031257-10LE34//04570-10E46	-
35	GA131397-6-4-7	04494-11E49/031257-10E41//041293-11E37	-
36	GA131487-14-3-4	04434-11E49/041418-11EEL6//04417-11E21	-
37	GA131487-14-3-7	04434-11E49/041418-11EEL6//04417-11E21	+

38	GA131487-14-5-13	04434-11E49/041418-11EEL6//04417-11E21	+
39	GA131514-5-2-6	JT/031238-7E34//051477-9-1-6	+
40	GA131514-4-1-3	JT/031238-7E34//051477-9-1-6	+
41	GA131514-4-1-10	JT/031238-7E34//051477-9-1-6	+
42	GA131514-4-1-11	JT/031238-7E34//051477-9-1-6	+
43	GA131700-2-2-12	PIO26R20/04570-10E46/AGS*2/021773	+
44	GA12485-1-3-2	031086-10E26/VAO4W-90//031086-10E26	+
45	GA12178-2-14-3-6	03564-10E25/051477-20-6-5	-
46	GA12212-10-4-4	041293-11LE37/031134-10E29	+
47	GA12212-10-4-8	041293-11LE37/031134-10E29	-
48	GA12213-8-2-6	04510-11LE24/031134-10E29	-
49	GA12213-11-5-7	04510-11LE24/031134-10E29	-
50	GA12213-11-5-7	04510-11LE24/031134-10E29	-
51	GA12238-9-4-2	031257-10LE34/081607-G1-G1-1/991227-6A33*4/SS8641	-
52	GA14065I-14-3-7	06385-13-2-3/031257-10LE34	-
53	GA14067I-12-8-7	VA09W-75/041293-11LE37//061349-13E4	-
54	GA14119I-9-2-6	VA10W-119/041293-11LE37//041293-11E54	-
55	GA14119I-11-2-9	VA10W-119/041293-11LE37//041293-11E54	-
56	GA14119I-11-2-11	VA10W-119/041293-11LE37//041293-11E54	-
57	GA14136I-2-4-2	051477-9-1-61031257-10E44//12421-G1-G23-G3	-
58	GA14186I-15-3-9	041293-11LE37/06399-G2-4-1//04434-11E44	-
59	GA14187I-4-3-4	VA10W-119/04570-10E46//041293-11E54	+
60	GA14198I-3-7-4	TV8861/04434-11E44//061082-13E24	-
61	GA14230I-6-4-8	04417-12E33/041052-11E51//VA10W-123	-
62	GA14329I-17-2-3	TV8861/04434-11E44//04434-11E44	-
63	GA14329I-2-3-3	TV8861/04434-11E44//04434-11E44	-
64	GA14373I-12-4-2	JT/031257-10E41//04151-11E26	-
65	GA13010-12-4-7	06033-2-7-4/031257-10E41	-
66	GA13011-9-4-6	VA10W-119/04417-12E33	-
67	GA13027-15-1-6	VA09W-75/041052-11E51	-
68	GA13027-15-1-7	VA09W-75/041052-11E51	-
69	GA13062-6-5-3	081625/2*LAO1139D-56-1//081625-3-1-3-6	-
70	GA13062-6-5-7	081625/2*LAO1139D-56-1//081625-3-1-3-6	-
71	GA14119I11-2-2	VA10W-119/11LE37//SH5550	+
72	GA131214-8-2-1	AGS3000//JT//051477-9-1-6	-
73	GA13062-18-6-1	081625/2*LAO1139D-56-1//081625-3-1-3-6	-
74	GA13079-8-1-7	03564-12E6/041418-11EE16/031257-10E41	-
75	GA13080-6-2-1	051477-9-1-6/031257-10E41	-
76	GA13080-6-4-2	051477-9-1-6/031257-10E41	-
77	GA13080-9-3-6	051477-9-1-6/031257-10E41	+
78	GA13087-20-6-5	06385-13-2-3/03564-12E6	-

79	GA13153-23-6-5	101700-G1-G2-G1-G4/031257-10E41	-
80	GA13289-6-4-3	031134-10E29/04417-11E21	+
81	GA13289-6-4-6	031134-10E29/04417-11E21	-
82	GA13289-11-5-1	031134-10E29/04417-11E21	-
83	GA13289-11-5-12	031134-10E29/04417-11E21	-
84	GA13289-13-6-5	031134-10E29/04417-11E21	-
85	GA13375-21-1-2	06520-24-1/031086-10E26	-
86	GA13375-21-1-5	06520-24-1/031086-10E26	-
87	GA13375-21-1-7	06520-24-1/031086-10E26	-
88	GA131218-1-2-1	041323-11E63/031134-10E29	-
89	GA131218-1-2-7	041323-11E63/031134-10E29	-
90	GA131289-4-5-2	041293-11E54/04434-11E44	-
91	GA13244-G1-10-6-2	031257-10E41/3/11243-G2//AGS2020*4/KS08-155-5H21/4/USG3024*2/081628	-
92	GA11660-1-5-3-1	SS8641/Jamestown//SS8641	-
93	GA11660-1-5-3-3	SS8641/Jamestown//SS8641	+
94	GA131482-2-2-3-5	04434-11E44*2/10041-G1-G1-G1	-
95	GA131052-1-7-6	06110-9-2-4/051477-9-1-6	-
96	GA131052-5-2-1	06110-9-2-4/051477-9-1-6	-

"+" indicates presence of AGS 2000 allele and "-" indicates absence of AGS 2000 allele

CHAPTER 4

GENOME-WIDE ASSOCIATION STUDY OF A WORLDWIDE COLLECTION OF WHEAT GENOTYPES REVEALS NOVEL QUANTITATIVE TRAIT LOCI FOR LEAF RUST RESISTANCE

Sapkota S, Hao Y, Johnson J, Buck J, Aoun M, and Mergoum M (2019). Plant Genome 12:190033. doi:10.3835/plantgenome2019.05.0033. Reprinted here with permission of the publisher.

Abstract

Wheat (*Triticum aestivum* L.) production worldwide is being challenged by several biotic and abiotic factors. Leaf rust (LR), caused by *Puccinia triticina* (*Pt*), is a major biotic constraint of wheat production worldwide. Genetic resistance is the most efficient and cost-effective way to control LR. Seventy-nine LR resistance genes have been identified to date but the frequent emergence of new virulent *Pt* races every year demands a constant search for new sources of resistance with novel quantitative trait loci (QTL) or genes. The objectives of this study were to identify putative novel sources of resistance effective against current prevalent races of *Pt* in the southeast USA and to map genomic loci associated with LR resistance using genome-wide association study (GWAS) approach. Evaluation of 331 diverse wheat genotypes against four prevalent *Pt* races (MFGKG, MBTNB, MCTNB, and TCRKG) revealed that the majority of the genotypes were susceptible and only 22 genotypes (6.6%) were resistant to all four *Pt* races. GWAS detected a total of 11 QTL on nine chromosomes for LR resistance. Of these, six QTL were identified in the vicinity of known genes or QTL; therefore, more studies are warranted to determine their relationship. Five QTL, *QLr.uga-1AL, QLr.uga-4AS, QLu.uga-5AS, QLr.uga-5AL,* and *QLr.uga-7AS*, were identified on genomic regions where no LR resistance genes have been identified in wheat, representing potential novel loci for LR resistance. The highly resistant wheat genotypes and novel QTL reported in this study could be used in breeding programs to improve LR resistance.

Keywords: Wheat, Leaf rust, *Puccinia triticina,* Genome-wide association studies (GWAS), and quantitative trait loci (QTL).

Introduction

The world's human population is projected to reach over nine billion by 2050 which demands more food production to ensure food security by that time (Tucker et al. 2017). Wheat is a major food crop contributing about 20% of calories to the human population worldwide, and an increase in wheat production is imperative to address this challenge. Although overall genetic and genomic improvement in wheat is essential to significantly increase wheat production, development of wheat cultivars that are resistant to diverse plant diseases is also important. Among the major diseases of wheat, rust diseases which includes leaf rust, stripe rust, and stem rust are the major yield-limiting factors. Of these three rust diseases, leaf rust (LR), caused by the fungal pathogen *Puccinia triticina* (*Pt*), is the most common and widely distributed worldwide (Bolton et al. 2008). *Pt* is an important wheat pathogen mainly because it can cause significant yield reduction by decreasing the number of kernels per head and lowering the kernel weight (Bolton et al. 2008; Kolmer 2005; Marasas et al. 2004).

Genetic resistance is the most efficient, cost-effective, and environmentally sound method to control losses caused by LR. Seventy-nine LR resistance genes (*Lr* genes) have been catalogued (Bolton et al. 2008; Sapkota et al. 2019). However, because of the constant evolving and emergence of new virulent *Pt* races, identification of new resistance sources to LR is vital (Kolmer 2005; Kertho et al. 2015). Resistance to rusts disease of wheat including LR has been broadly classified into two categories: seedling resistance and adult-plant resistance (APR) (Chen 2005; Kolmer 2013). Seedling resistance, also known as all stage resistance, confers resistance to only certain races (race-specific) with a high degree; however, a shift in the virulence of the pathogen population can easily overcome seedling resistance (Kolmer 2005; Kolmer 2013). *Lr72* (Herrera -Foessel et al. 2014), *Lr76* (Bansal et al. 2017), and *Lr79* (Qureshi et al. 2018) are some examples of genes for seedling resistance to LR reported recently. On the

other hand, APR is normally detected after the seedling stages, confers resistance to multiple races (race non-specific), provides a moderate level of resistance, and has been found to be more durable (Kolmer 1996; Kolmer 2013; McIntosh et al. 1995). However, there are some race-specific APR genes, such as *Lr12*, that do not fit into this category (Singh and Bowden 2011). *Lr13* (Dyck et al. 1966), *Lr34* (Dyck 1987), *Lr46* (Singh et al. 1998), and *Lr68* (Herrera-Foessel et al. 2012) are some examples of APR genes for resistance to LR. Among the APR genes, *Lr34* is probably the most effective gene mainly because of its widespread distribution, durability, and ability to produce an enhanced effect when combined with other race-specific genes (Kolmer 1996).

Recent advances in biotechnology and improvements in molecular marker development technologies have greatly facilitated the dissection of complex traits and increase the precision of plant breeding via marker-assisted selection (MAS) (Collard and Mackill 2008; Bernardo 2008). More recently, Genome-Wide Association Studies (GWAS), also known as linkage disequilibrium (LD) mapping, has become a common approach to study marker-trait associations in plants (Gupta et al. 2005; Zhu et al. 2008). Although GWAS and linkage mapping are both used in marker-trait association studies, GWAS offers several advantages over linkage mapping: 1) it utilizes natural populations, therefore, reduces research time to develop mapping population, 2) numerous recombination events that occurred during the evolutionary history of natural populations increase the mapping resolution, and 3) greater allele number (Yu and Buckler 2006; Yu et al. 2006; Zhu et al. 2008). GWAS has been successfully used in wheat germplasm to discover genomic loci associated with disease resistance (Sapkota 2015; Aoun et al. 2016; Gyawali et al. 2018; Yao et al. 2019), insect resistance (Joukhadar et al. 2013; Bassi et al. 2019),

yield (Edae et al. 2014; Sukumaran et al. 2015; Li et al. 2019), and quality (Reif et al. 2011; Zhai et al. 2018; Chen et al. 2019).

In the southeastern USA state of Georgia (GA), LR is a major disease of wheat and an epidemic occurs to some degree in almost every cropping season. With the emergence of new *Pt* races our current wheat cultivars are less effective in providing LR resistance; therefore, identification of new sources of resistance is needed. In this study, we evaluated a set of diverse wheat genotypes for their reaction to four *Pt* races including the most current common race in GA, MFGKG, with the hypothesis that these genotypes segregate in their reaction to *Pt* races and possess novel genomic loci for LR resistance. Therefore, the objectives of this study were to: (i) determine the level of resistance or susceptibility in a worldwide collection of wheat genotypes to multiple *Pt* races at the seedling stage; (ii) identify molecular markers associated with LR resistance that can be exploited in MAS breeding to develop LR-resistant wheat cultivars; and (iii) compare the resistance loci detected in this study with known genes to determine their novelty.

Materials and methods

Plant materials and Pt races

A panel of 331 worldwide diverse wheat genotypes consisting of 297 spring wheat and 34 soft red winter wheat (SRWW) accessions were used in this study to evaluate resistance to *Pt* races (Table 4.S1 and S2). Spring wheat genotypes were obtained from the United States Department of Agriculture (USDA), National Small Grain Collection (NSGC) located in Aberdeen, ID. Among the 297 spring wheat genotypes, 106, 95, 55, 38, and 3 are breeding materials, registered cultivars, cultivated germplasm, landraces, and genetic stocks, respectively,

originating from 72 countries representing diverse worldwide geographic regions. Additionally, a set of 34 SRWW genotypes, adapted to the southeastern (SE) region of the USA and collected by the University of Georgia (UGA) small grain breeding program were included in the study. Two SRWW cultivars, AGS 2000 and SS 520, which were reported to be resistant and susceptible to LR, respectively (Johnson et al. 2002; Sapkota et al. 2019) were included in the study as checks.

Four *Pt* races i.e., MFGKG, MBTNB, TCRKG, and MCTNB, representing the prevalent races of *Pt* in the SE region of the USA were used to screen the wheat genotypes. MFGKG (virulent on the genes *Lr1, Lr3, Lr9, Lr24, Lr26, Lr11, Lr10, Lr14a, Lr18,* and *Lr28*) is currently the predominant race of *Pt* in GA (Sapkota et al. 2019; Kolmer 2019). Furthermore, MBTNB (virulent on the genes *Lr1, Lr3, Lr3ka, Lr11, Lr17, Lr30, LrB,* and *Lr14a*), TCRKG (virulent on the genes *Lr1, Lr2a, Lr2c, Lr3, Lr26, Lr3ka, Lr11, Lr30, Lr10, Lr14a, Lr18,* and *Lr28*), and MCTNB (virulent on the genes *Lr1, Lr3, Lr26, Lr3ka, Lr11, Lr17, Lr30, LrB,* and *Lr14a*) are the most prevalent races of *Pt* in the SE region of the USA (Kolmer and Hughes 2016; Kolmer 2019).

Evaluation for reaction to Pt races

Phenotyping of wheat materials for reaction to *Pt* races was conducted at the seedling stage under greenhouse and growth chamber conditions at the Griffin Campus of UGA, Griffin, GA. Plant preparation, inoculum collection and preparation, inoculation, and disease scoring were done as described in Sapkota et al. (2019) with minor modifications. Briefly, three seeds per entry were each planted in a single cone-tainer (Stuewe and Sons Inc.) filled with Sungro professional growing mix soil (Sun Gro Horticulture Distribution Inc.). Approximately 2 g of Osmocote Bloom 12-7-18 fertilizer (Everris NA Inc.) was placed in each cone-tainer after

planting and the cone-tainers were kept on a greenhouse bench at 20-25°C and a 14-hr photoperiod. Resistant (AGS 2000) and susceptible checks (SS 520) were planted in each rack. Three cones (replicates) were planted for each genotype per experiment and the experiment was repeated. The plant materials were arranged in a randomized complete block design (RCBD) in all racks.

Inoculation of the plant materials was done ~ 10 days after planting when secondary leaves were fully emerged. The inoculum was prepared by mixing freshly collected urediniospores into 100 ml of sterile water with ~ 20 µl of Tween 20 (Agdia, Inc. Elkhart, IN). The number of urediniospores in the inoculum was counted by a Hemocytometer (Olympus Optical Co. Ltd. Japan) and a final concentration of 10^5 spores ml^{-1} was used for inoculation. The inoculum was sprayed onto the foliage until runoff and plants were then carefully moved to a dark chamber with high humidity. After 16-18 hr, plants were transferred to a growth chamber maintained at 18°C/20°C (night/day) with 15-hr of photoperiod.

The reaction of the plants to LR i.e., infection types (IT), was scored 10-12 days after inoculation using a 0-4 scale (McIntosh et al. 1995) where 0 = no visible uredia (immune), ; = hypersensitive fleck (very resistant), 1 = small uredia with necrosis (resistant), 2 = small- to medium-sized uredia (resistant to moderately resistant), 3 = medium-sized uredia with or without chlorosis (moderately resistant or moderately susceptible), and 4 = large sized uredia without chlorosis (susceptible reaction). The analysis of phenotypic data collected using a 0-4 scale was further transformed because it contains non-numeric IT such as ";" and does not incorporate multiple ITs developed within a single plant. Therefore, the 0-4 ITs were converted into a 0-9 linearized scale (LS) as described by Zhang et al. (2014). Wheat genotypes with LS of 0-6 and 7-9 were considered as resistant and susceptible, respectively (Kertho et al. 2015).

Statistical analysis of the phenotypic data

Statistical Analysis System (SAS) software (version 9.4, SAS Institute Inc. 2017) was used for statistical analysis of the phenotypic data. The Shapiro-Wilk test was conducted (PROC UNIVARIATE) to determine if the phenotypic data for each *Pt* races were normally distributed. Furthermore, depending on the result from the normality test, Levene's or Bartlett's test (Snedecor and Cochran 1989; Levene 1960) was performed to check the homogeneity of data among the experiments. If the data were homogenous, the overall mean was used for the GWAS analysis. Broad-sense heritability (H^2) was calculated by dividing genotypic variance by the combined sum of genotypic, block, and error variance. Pearson correlations in the phenotypic data of each *Pt* race were calculated via the PROC CORR procedure in SAS.

Marker-trait association analysis

In total, 297 of the 331 wheat genotypes used in this study had marker data available and these were included in the GWAS analysis (GWAS panel). The GWAS panel was genotyped with the 9K-SNP array by the USDA Triticeae Coordinated Agriculture Project (USDA-TCAP), and the genotypic data are publicly available on the Triticeae Toolbox website (triticeaetoolbox.org). Single-nucleotide polymorphism (SNP) markers with a minor allele frequency (MAF) of <0.05 and >10% missing data were removed from the analysis to avoid false marker-trait associations. A total of 5,025 SNP markers were used in marker-trait association analysis. Distance-based clustering and the model-based quantitative assessment of the subpopulation membership of the genotypes were performed using JMP Genomics version 9.0 and STRUCTURE version 2.3.4 (Pritchard et al. 2000). The population structure (Q matrix) and the familial relatedness within the GWAS panel were estimated through principal component

(PC) analysis and the identity-by-state matrix (K matrix), respectively. Linkage disequilibrium (LD) between SNP markers was calculated as the squared correlation coefficient (R^2) by JMP Genomics 9.0 software and comparisons were made between marker pairs within the same chromosome. Genome-wide LD decay was estimated by plotting R^2 against the corresponding pairwise genetic distance (cM) (Wang et al. 2014). A smoothing spline fit with $\lambda = 1{,}000{,}000$ was applied to LD decay.

GWAS to identify genomic regions significantly associated with LR resistance was conducted using TASSEL v.5.0 (Bradbury et al. 2007). Six different models (Naïve, PCA, Q, K, PCA+K, and Q+K) were tested in TASSEL and the mean-squared deviation (MSD) value was calculated for each model (Tamang et al. 2015; Mamidi et al. 2011). A mixed-linear model (MLM), which accounts for both population structure and relatedness, was selected on the basis of the lowest MSD value. Manhattan plots were generated for each *Pt* race by plotting –log10(*p*-value) on the *y*-axis and chromosome name and position on the *x*-axis. The *p*-value of the markers was further adjusted by calculating positive false discovery rate (pFDR) (Benjamin and Yekutieli 2001) and marker-trait association with pFDR ≤ 0.1 was considered significant.

QTL alignment and comparison with previously mapped Lr-genes

To determine the relationship of QTL detected in our study with previously mapped *Lr*-genes or QTL, we compared the position of the most significant markers representative of each QTL to previously mapped QTL or genes using wheat consensus maps (Wang et al. 2014; Maccaferri et al. 2015). The graphical presentation of the genetic map was generated via MapChart (Voorrips 2002). Furthermore, the physical positions of the representative markers were obtained from the International Wheat Genome Sequencing Consortium (IWGSC)

Reference Sequence V1.0 and used in the comparison (https://www.wheatgenome.org/News/Latest-news/RefSeq-v1.0-URGI, accessed 3 Oct. 2019).

Identification of the Lr47 and LrA2K genes via diagnostic markers

Since QTL were detected in the vicinity of known *Lr* genes (*Lr47* and *LrA2k*), molecular marker analysis was performed with diagnostic markers to determine their relationship. Twenty-eight wheat genotypes that were highly resistant to *Pt* races MFGKG and MCTNB were selected from the GWAS panel to test for the presence or absence of *Lr47* and *LrA2K* genes. *Triticum speltoides* specific marker developed for *Lr47* was used to identify *Lr47* (Helguera et al. 2000). Similarly, SSR marker linked to *LrA2K, Xwmc770,* was selected to genotype the highly resistant wheat genotypes (Sapkota et al. 2019). The cultivars CItr 17884 (pedigree: CI 15092/T. speltoides//Fletcher/3/5*Centurk) and AGS 2000 (pedigree: PIO2555/PF84301//Florida302), carrying the *Lr47* and *LrA2K* genes, respectively, were used as positive controls. The genomic DNA from wheat lines was extracted via a modified CTAB method (Saghai-Maroof et al. 1984) and diluted to 50 ng/µl to be used in a polymerase chain reaction (PCR). The PCR was carried out as described in Hao et al. (2008) and Helguera et al. (2000) and the amplified products were separated in 1.5% agarose gel stained with ethidium bromide.

Results

Variation in seedling infection types

Wheat genotypes showed a wide range of reactions to all four *Pt* races tested in this study. The LR disease scores ranged from immune reactions (IT=0, LS=0) to highly susceptible reactions (IT=4, LS=9) to all four *Pt* races (Table 4.1). The mean disease score of wheat

genotypes were 7.7, 7.6, 7.9, and 8.0 to *Pt* races MFGKG, MBTNB, MCTNB, and TCRKG, respectively (Table 4.1). The majority of the evaluated wheat genotypes were susceptible to the *Pt* races and 30 (9.1%), 47 (14.2%), 30 (9.1%) and 33 (10.0%) wheat genotypes were resistant to MFGKG, MBTNB, TCRKG, and MCTNB, respectively (Figure 4.1). Of these, only 22 genotypes (6.6%) were resistant to all four *Pt* races (Table 4.3). As expected, AGS 2000 (resistant check) and SS 520 (susceptible check) exhibited resistant and susceptible reactions to all four *Pt* races, respectively (Figure 4.1).

The Shapiro-Wilk test for normality showed that the phenotypic data of all four *Pt* races deviated significantly from a normal distribution (Table 4.1). Therefore, Levene's test was performed to test for the homogeneity within experiments. The Levene's test results indicated that the phenotypic variance of data within experiments were homogenous (P = 0.20 to 0.97) for all four *Pt* races (Table 4.1). Therefore, the overall mean value was calculated for each wheat genotype and used in the GWAS analysis. The broad-sense heritability (H^2) of the LR infection types ranged from 0.93-0.97 to four *Pt* races indicating that most of the phenotypic variation was explained by the genotypes (Table 4.1). Furthermore, Pearson's correlation coefficient (r) among all *Pt* races was highly significant ($p < 0.0001$) and the value of r ranged from 0.62 to 0.83 (Table 4.2).

Marker distribution, population structure, and LD

Of the 5,025 SNP markers used in the GWAS analysis, 2,154 (42.9%), 2,038 (40.5%), and 559 (11.1%) markers were distributed in the A, B, and D genomes, respectively; the chromosomal location of 274 (5.5%) markers was unknown. The ward clustering and cryptic relatedness analyses in the GWAS panel revealed the presence of two major clusters (Group 1

and Group 2) and Group 2 was further sub-divided into three sub-groups (Groups 2A, 2B, and 2C) with weak membership coefficients (Figure 4.2 A and B). STRUCTURE analysis also suggested two to four hypothetical subpopulations, which correlates with the grouping based on the cluster and cryptic relatedness analyses (Figure 4.2C). This structure was further supported by PC analysis (Figure 4.3). The first 4 and 10 PCs explained 18.4 and 28.7% of the genotypic variation, respectively. The first four PCs clustered the GWAS panel into four groups (Figure 4.3). Most of the wheat genotypes were clustered in Subgroup 2B (106 genotypes) originating from South America (50 genotypes), Africa (17 genotypes), Asia (17 genotypes), Europe (11 genotypes), and North America (11 genotypes). Group 1 contained the smallest number of genotypes originating from Asia (33 genotypes), Africa (8 genotypes), Europe (4 genotypes), and North and South America (1 genotype in each). Similarly, Subgroup 2A and 2C contained 58 and 86 genotypes, respectively, originating from Europe, Africa, North and South America, and Asia. This indicates that the clustering of wheat genotypes in the GWAS panel was not related strongly due to the origin of the genotypes. Therefore, other factors, such as the improvement status of the genotypes ranging from advanced released cultivars to landraces, might have contributed to this clustering. Similarly, the "spring wheat" growth habit may include different types such as "facultative" which may have played a role in the clustering as well. Based on the fitted model, LD dropped at 3 cM genetic distance, on average, which corresponds to an R^2 value of 0.2 (Figure 4.4). Therefore, ± 3 cM was used to establish confidence intervals for QTL regions. Furthermore, SNP markers with a pairwise LD (r^2) of ≥ 0.7 were considered as a single locus.

Detection of QTL for LR resistance

GWAS detected a total of 32 significant SNP markers for resistance to four *Pt* races (Table 4.S3). On the basis of their position on chromosomes and their LD value, 32 significant SNPs were assigned to 11 loci or QTL regions on chromosomes 1A, 1B, 2B, 4A, 4B, 5A, 6A, 7A, and 7B (Figure 4.5, Table 4.4). Two QTL, *QLr.uga-6AL1* and *QLr.uga-6AL2*, were detected on the long arm of chromosome 6A for resistance to *Pt* races MFGKG, MCTNB, and TCRKG; however, they are 15.6 cM and 22.9 Mb apart from each other according to genetic and physical maps, respectively (Table 4.4). Furthermore, we calculated the LD between highly significant SNP markers underlying these two loci, *IWA1497* and *IWA8431*, and found that the LD was 0.17, suggesting that these two loci are distinct QTL.

Five, three, six, and five significant QTL were detected for resistance to MFGKG, MBTNB, MCTNB, and TCRKG, respectively (Table 4.4). Among the 11 QTL detected, *QLr.uga-2BS* was significant for resistance to all four *Pt* races and explained up to 19.4% of the phenotypic variation for *Pt* race MCTNB. *QLr.uga-6AL1* was significant for resistance to three *Pt* races (MFGKG, MCTNB, and TCRKG) and explained 4.5 to 9.5% of the phenotypic variation. Three QTL (*QLr.uga-5AS, QLr.uga-7AS, and QLr.uga-*7BL) located on chromosomes 5AS, 7AS, and 7BL, were significant for resistance to two of the four *Pt* races and explained in total of 14.0, 10.2, and 12.6% of the phenotypic variation, respectively (Table 4.4).

Six QTL were significant for resistance to one of the four *Pt* races tested (Table 4.4). Two QTL, *QLr.uga.1AL* and *QLr.uga.6AL2*, were significant for resistance to *Pt* race TCRKG and collectively explained 11.7% of the phenotypic variation. *QLr.uga-1BL* and *QLr.uga-4BL* were found to be significant for resistance to *Pt* race MCTNB and collectively explained 12.7% of the phenotypic variation. *QLr.uga-4AS* and *QLr.uga-5AL* were found to be significant for

resistance to *Pt* race MBTNB and collectively explained 11.2% of the phenotypic variation (Table 4.4).

Discussion

The discovery of novel sources of resistance with novel genes or QTL is a constant challenge and critical in plant breeding to combat threats to crop production caused by pests. In wheat, gene pyramiding to develop durable LR resistant cultivars is of paramount importance. GWAS is a powerful approach in plants to detect QTL associated with complex traits (Zhu et a. 2008; Hall et al. 2010). GWAS has been successfully used in wheat germplasm to detect several genomic loci or regions conferring resistance to LR at seedling and adult-plant growth stages (Kertho et al. 2015; Aoun et al. 2016; Turner et al. 2017; Riaz et al. 2018). In this study, we identified 17 spring and 5 SRWW lines carrying race-specific resistance to four *Pt* races that are prevalent in the SE region of the US. The study also detected 11 QTL significant for resistance to LR; five of them were uncharacterized previously in *T. aestivum*, and therefore, these loci are likely to represent novel QTL for LR resistance (Table 4.4).

Among the 2192 spring wheat genotypes collected globally and deposited in the USDA-TCAP, Turner et al. (2017) selected 1032 genotypes and conducted GWAS for resistance to LR at the seedling and adult plant stages. Of these, a subset containing 113 genotypes was evaluated at the seedling stage (the seedling resistance panel) with a mixture of 10 *Pt* races. Of the 297 wheat genotypes used in our study, 27 genotypes overlapped with seedling resistance panel (Turner et al. 2017); however, different *Pt* races were used in these two studies. Eight of 17 spring wheat genotypes found to be resistant to all four *Pt* races in this study were also reported to be resistant by Turner et al. (2017) study (Table 4.3). Interestingly, a marker on chromosome

2BS, *IWA8221*, was found to be significant for resistance to LR in both studies. The resistant genotypes and the significant marker reported from both studies could be used in a wheat breeding program as resistance sources to a wider range of *Pt* races.

Correlation analysis revealed the presence of significant correlations between the LR infection types of four *Pt* races used in this study (Table 4.2). Several factors may contribute to this high correlation in the phenotypic data of the four *Pt* race. Firstly, based on the virulence profile test conducted on 20 wheat lines carrying a single *Lr* gene, all four *Pt* races were virulent to *Lr1*, *Lr3*, *Lr11*, and *Lr14a* (Sapkota et al. 2019), therefore, correlation among the phenotypic data can be expected. Secondly, the GWAS panel utilized in this study probably had common genomic loci conferring resistance to *Pt* races and this was further confirmed by the GWAS analysis result that allowed the identification of common QTL, e.g., *QLr.uga-2BS*, that conferred resistance to all four *Pt* races (Table 4.4). Similar results were reported earlier by Desiderio et al. (2014b) and Sapkota et al. (2019) where high correlations were observed in the phenotypic data evaluated with multiple *Pt* races followed by the identification of common genomic loci for resistance to those races.

Of the 11 QTL detected through GWAS, *QLr.uga-2BS* was found to be significant for resistance to all four *Pt* races (Table 4.4, Figure 4.6), thus providing a wide spectrum of resistance. Seven known *Lr* genes and two QTL, *Lr13* (Dyck et al. 1966), *Lr16* (McCartney et al. 2005), *Lr23* (McIntosh and Dyck 1975), *Lr48* (Bansal et al. 2008), *Lr73* (Park et al. 2014), *LrZH22* (Wang et al. 2016), *LrA2K* (Sapkota et al. 2019), *QLr.cimmyt-2BS* (Rosewarne et al. 2012), and *QLr.hebau-2BS* (Zhang et al. 2017) were also detected on chromosome 2BS in the vicinity of *QLr.uga-2BS* identified here (Figure 4.6). Of these seven known genes, *Lr13* and *Lr48* are APR genes reported in the wheat cultivars Frontana and CSP44, respectively (Dyck et

al. 1966; Bansal et al. 2008). Given that *QLr.uga-2BS* is a seedling QTL, it is unlikely to be any of these APR genes. *Lr16*, *Lr23*, *Lr73*, and *QLr.cimmyt-2BS* confers resistance at the seedling stage; however, according to the consensus genetic map (Maccaferri et al. 2015), they are 89.6, 47.1, 76.8, and 29.7 cM distal to *QLr.uga-2BS*, respectively (Figure 4.6). Two loci, *QLr.hebau-2BS* and *LrZH22*, were found to be located in the same position. According to the consensus map, these two loci were mapped 33.1 cM proximal to *QLr.uga-2BS* (Figure 4.6). In our recent study (Sapkota et al. 2019), we used a bi-parental mapping population consisting of 175 RILs and mapped a single major gene, *LrA2K*, on chromosome 2BS that conferred resistance to two *Pt* races (MFGKG and MBTNB). Since *LrA2K* and *QLr.uga-2BS* are very close on 2BS map (Figure 4.6) and confer resistance to the same races, they are likely to be the same locus. Furthermore, molecular marker analysis with the a diagnostic marker, *Xwmc770*, closely linked to *LrA2K* demonstrated that 10 of the 28 resistant wheat genotypes in the GWAS panel carry *LrA2K* (Figure 4.S1). This further provides more strong evidence that *LrA2K* and *QLr.uga-2BS* represent the same locus for LR resistance.

QLr.uga-1BL was detected in the vicinity of six known *Lr* genes, *Lr26*, *Lr33*, *Lr44*, *Lr46*, *Lr51*, and *LrZH84* and two QTL, *QLr.pser-1BL* and *QLr.caas-1BL* (Figure 4.6) (Zhao et al. 2008; Li and Bai 2009; Ren et al. 2012). Of these, *Lr26*, *Lr44*, and *Lr51* were derived from *Secale cereal*, spelt wheat, and *Aegilos speltoides*, respectively (Dyck and Skyes 1994; Zhao et al. 2008). Given that no *S. cereal*, spelt wheat, and *A. speltoides* accessions were included in our GWAS panel, these three genes are unlikely to be *QLr.uga-1BL*. *Lr33* conferred seedling resistance, was derived from a common wheat, and was mapped close to the centromere region 2.6 cM away from *Lr26* (Dyck et al. 1987). Similarly, *LrZH84* conferred seedling resistance, was derived from the Chinese wheat cultivar Zhao 8425B, and

was mostly effective against Chinese *Pt* races (Zhao et al. 2008). According to the consensus genetic map (Maccaferri et al. 2015), *LrZH84* was mapped 57.6 cM distal to *QLr.uga-1BL* (Figure 4.6). *Lr46* is an APR gene that conferred slow rusting resistance and was originally derived from the CIMMYT spring wheat cultivar Pavon 76 (Singh et al. 1998). Both QTL detected on chromosome 1BL, *QLr.pser-1BL* and *QLr.caas-1BL*, confer APR and were derived from the Chinese wheat cultivars Ning7840 and Bainong 64, respectively (Li and Bai 2009; Ren et al. 2012). According to the consensus genetic map, *QLr.pser-1BL* was mapped 80.3 cM distal to *QLr.uga-1BL* indicating that they may represent two separate loci for LR resistance. However, *QLr.caas-1BL* and *QLr.uga-1BL* were mapped very close (<1cM) on the 1BL map (Figure 4.6), therefore, further studies are warranted to determine their relationship.

One known gene, *Lr49*, and two QTL, *QLr.pbi-4BL* and *QLr.cimmyt-4BL*, were previously mapped on chromosome 4BL which harbors *QLr.uga-4BL* (William et al. 2006; Bansal et al. 2008; Singh et al. 2009). All these previously reported genes and QTL, *Lr49*, *QLr.pbi-4BL*, and *QLr.cimmyt-4BL*, confer APR and were reported from wheat cultivars VL404, Beaver, and Avocet S, respectively. The markers *Xbarc163* and *Xwmc349*, which flanked *Lr49*, were mapped 14.2 and 16.7 cM away from *QLr.uga-4BL*, respectively, according to the consensus genetic map developed by Maccaferri et al. (2015). Similarly, the markers *wPt-1708* and *gwm495* linked to *QLr.pbi-4BL* and *QLr.cimmyt-4BL*, respectively, were mapped 20.4 and 2.5 cM away from *QLr.uga-4BL* (Figure 4.6). Given that all three genomic loci previously mapped on 4BL confers APR, they are unlikely to be *QLr.uga-4BL*. However, since *QLr.uga-4BL* was located close to *QLr.cimmyt-4BL* (2.5 cM), it is hard to tell if they are the same or different QTL and- therefore further studies are warranted to determine the relationship between these QTL.

Two QTL, *QLr.uga-6AL1* and *QLr.uga-6AL2*, were found to be significant for resistance to LR on chromosome 6AL (Figure 4.5, 3.6; Table 4.4). Two named *Lr* genes, *Lr56* and *Lr64*, and two QTL, *QLr.hbau-6AL* and *QLr.cimmyt-6AL*, were previously reported on chromosome 6AL for LR resistance (Bansal et al. 2013; Aoun et al. 2016). *Lr56* is a seedling resistance gene derived from *Aegilops sharonensis* (Marais et al. 2006). Since genetic materials carrying *Lr56* were not included in our GWAS panel, it is unlikely that *QLr.uga-6AL1* and *QLr.uga-6AL2* are *Lr56*. Similarly, *Lr64* is also a seedling resistance gene detected in *Triticum dicoccoides* (Desiderio et al. 2014a). According to the consensus genetic map (Maccaferri et al. 2015), *QLr.uga-6AL1* and *QLr.uga-6AL2* were mapped 26.7 and 57.4 cM distal to *Lr64*, respectively (Figure 4.6). Both QTL previously reported on chromosome 6AL, *QLr.hbau-6AL* and *QLr.cimmyt-6AL*, confer APR and were reported in the wheat line Avocet (William et al. 2006; Zhang et al. 2009). Based on the position of the genes and QTL on the consensus genetic map and their source, it is likely that the two QTL detected in this study represent distinct loci for LR resistance; however, further studies are required to elucidate their relationship.

Four known *Lr* genes (*Lr14a*, *Lr14b*, *Lr68*, and *LrBi16*) and five QTL (*QLr.cimmyt-7BL*, *QLr.csiro-7BL.2*, *QLr.osu-7BL*, *QLrlp.osu-7BL*, and *QLr.ubo-7B.2*) were previously mapped on chromosome 7BL in the vicinity of where *QLr.uga-7BL* was detected (Figure 4.6) (McIntosh et al. 1995; Xu et al. 2005a; 2005b; Maccaferri et al. 2008; Rosewarne et al. 2008, 2012; Herrera-Foessel et al. 2008, 2012; Zhang et al. 2011; Li et al. 2014). Interestingly, all previously mapped genes or QTL were detected proximal to *QLr.uga-7BL*, according to the consensus genetic map (Figure 4.6; Maccaferri et al. 2015). Of the four *Lr* genes previously mapped on 7BL, *Lr14a* was derived from durum wheat (*T. turgidum*), and three genes, *Lr68, Lr14b*, and *LrBi16*, were derived from common wheat (McIntosh et al. 1995; Herrera-Foessel et al. 2008, 2012; Zhang et

al. 2011). Since *Lr68* is an APR gene and confers a high level of slow rusting resistance (Herrera-Foessel et al. 2012), it is unlikely that *QLr.uga-7BL* is *Lr68*. All four *Pt* races used in this study are virulent to *Lr14a* (Sapkota et al. 2019) indicating that *QLr.uga-7BL* is unlikely to be *Lr14a*. *Lr14b* and *LrBi16* both confer race-specific resistance and were mapped >100 cM away from *QLr.uga-7BL*, according to the consensus genetic map. All five previously reported QTL on chromosome 7BL, *QLr.cimmyt-7BL, QLr.csiro-7BL.2, QLr.osu-7BL, QLrlp.osu-7BL,* and *QLr.ubo-7B.2,* confer APR to LR (Li et al. 2014), and were mapped 48.8 to 101.9 cM proximal to *QLr.uga-7BL* (Figure 4.6). Further studies, such as allelism tests or diagnostic marker analysis, may help to determine the relationship between *QLr.uga-7BL* and previously reported genes or QTL on chromosome 7BL.

Among the 11 QTL detected, five QTL (*QLr.uga-1AL, QLr.uga-4AS, QLr.uga-5AS, QLr.uga-5AL,* and *QLr.uga-7AS*) were detected on genomic regions where no catalogued *Lr* genes have been reported in *T. aestivum*. Only one *Lr* gene, *Lr59,* has been cataloged and designated on chromosome 1AL which was transferred from *A. peregrine* (Marais et al. 2008). Given that no genetic material carrying *A. peregrine* was included in our GWAS panel, *QLr.uga-1AL* probably represents a novel locus for LR resistance. Likewise, only one known *Lr* gene, *Lr47,* was reported on chromosome 7AS close to *QLr.uga-7AS* (Figure 4.6). *Lr47* was originally derived from *A. speltoides and transferred to chromosome 7AS of common wheat (Dubcovsky et al. 1998)*. To facilitate marker-assisted selection (MAS) and introgress the *Lr47* into commercial wheat cultivars, *Lr47* linked restriction fragment length polymorphism (RFLP) marker, *Xabc465,* was converted to PCR-based diagnostic marker (Helguera et al. 2000). To determine the relationship between *Lr47* and *QLr.uga-7AS,* we used a diagnostic marker developed for *Lr47* and genotyped on 28 resistant wheat genotypes. The result demonstrated that

none of the resistant genotypes in our GWAS panel carried the *Lr47* gene (Figure 4.S1). Therefore, *QLr.uga-7AS* is likely to be a novel locus for LR resistance.

Chromosome 5A has not been previously reported to carry *Lr* genes (McIntosh et al. 1995; Aoun et al. 2016). The two QTL detected on chromosome 5A in this study, *QLr.uga-5AS* and *QLr.uga-5AL*, are certainly novel genomic regions for LR resistance (Table 4.4; Figure 4.6). Similarly, *QLr.uga-4AS* has been mapped on chromosome 4AS where no other named *Lr* gene has been detected according to the LR gene catalogue (McIntosh et al. 1995; 2014). Therefore, *QLr.uga-4AS* represents a novel locus associated with MBTNB resistance.

Conclusions

In this study, we evaluated a diverse set of wheat germplasm for their reaction to four *Pt* races, MFGKG, MBTNB, MCTNB, and TCRKG, and identified 22 wheat genotypes that were highly resistant to all four *Pt* races (Table 4.3). The *Pt* races used in this study are the prevalent races in the Southern region of the US including MFGKG, the current common race in Georgia. A GWAS detected 11 QTL (32 SNP markers) that were significant for resistance to LR on chromosomes 1A, 1B, 2B, 4A, 4B, 5A, 6A, 7A, and 7B (Figure 4.5, Table 4.4). Among these, five QTL, *QLr.uga-1AL*, *QLr.uga-4AS*, *QLr.uga-5AS*, *QLr.uga-5AL*, and *QLr.uga-7AS*, were detected on genomic regions where no previously catalogued *Lr* genes had been reported in *T. aestivum* representing potential novel loci for LR resistance. Six other QTL, *QLr.uga-1BL*, *QLr.uga-2BS*, *QLr.uga-4BL*, *QLr.uga-6AL1*, *QLr.uga-6AL2*, and *QLr.uga-7BL*, were detected in the vicinity of known *Lr* genes or QTL, and therefore, further studies such as allelism test are warranted to determine their relationship. *QLr.uga-2BS* was found to be significant for resistance to all four *Pt* races with a major effect and explained up to 19.4% of the phenotypic variation.

Based on molecular marker analysis, we found that *QLr.uga-2BS* and *LrA2K* may represent the same loci for LR resistance. By using the highly resistant wheat genotypes discovered in this study, we have developed bi-parental mapping populations to validate the genomic loci found to be significant for LR resistance and develop tightly linked molecular markers to be used in wheat breeding programs.

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Tables and figures

Table 4.1 Summary of the phenotypic data of 331 wheat genotypes evaluated for their reaction to four *Puccinia triticina* (*Pt*) races.

***Pt* race**[a]	**Mean**	**Min**	**Max**	**SD**	**Shapiro-Wilk test**[b]	**Leven's test**[c]	H^2
MFGKG	7.7	0.0	9.0	2.1	$p<0.0001$	$P=0.20$	0.93
MBTNB	7.6	0.0	9.0	2.3	$p<0.0001$	$P=0.26$	0.96
MCTNB	7.9	0.0	9.0	1.8	$p<0.0001$	$P=0.86$	0.97
TCRKG	8.0	0.0	9.0	2.2	$p<0.0001$	$P=0.97$	0.97

[a]A total of 331 wheat genotypes were evaluated for their reaction to four *Pt* races and the infection types (ITs) were recorded 10-12 days after inoculation using a 0-4 scale. The 0-4 ITs scale was converted to a 0-9 linearized scale and used in the analysis.

[b]Shapiro-Wilk test was performed to determine if the phenotypic data were normal or not. $p<0.05$ indicates a non-normal distribution.

[c]Leven's test was performed to determine if the data among the experiments are homogenous or not. $P>0.05$ indicate equal variance.

SD = standard deviation
H^2 = broad-sense heritability

Table 4.2 Correlation analysis among the phenotypic data of 331 wheat genotypes evaluated for reaction to *Puccinia triticina* (*Pt*) races MFGKG, MBTNB, MCTNB, and TCRKG.

Pearson Correlation Coefficients				
Pt race	MFGKG	MBTNB	MCTNB	TCRKG
MFGKG	1.00	0.62	0.76	0.81
MBTNB	*	1.00	0.73	0.71
MCTNB	*	*	1.00	0.83
TCRKG	*	*	*	1.00

Table 4.3 List of wheat genotypes that are resistant to all four *Puccinia triticina* (*Pt*) races.

					Disease score[d]			
Genotypes[a]	**Pedigree[b]**	**Origin[b]**	**Type[c]**	**Habit[b]**	**MFGKG**	**MBTNB**	**MCTNB**	**TCRKG**
CItr 12835	RL 2265/2*Redman	Canada	Breeding line	S	2.00	2.2	2.2	2.2
CItr 15634	II-55-10/4/Pembina/II-52-329/3/II-53-388/III-58-4//II-53-546	United States	Breeding line	S	1.3	1.8	2.0	2.0
CItr 15850	Sonora 64A//Tezanos Pintos Precoz/Nainari 60	Mexico	Breeding line	S	1.3	2.0	2.2	1.7
PI 134348	N/A	Australia	Breeding line	S	1.8	2.0	2.8	2.0
PI 184845	N/A	Guatemala	Cultivated	S	1.5	1.7	3.0	1.3
PI 235221	Tinstein/Chugoku No. 79	Japan	Breeding line	S	2.0	2.5	3.0	2.8
PI 266148	San Pastore/Funo	Italy	Cultivar	S	1.8	2.8	3.0	2.5
PI 414625	N/A	China	Cultivated	S	1.2	2.0	2.5	1.3
PI 422282	N/A	India	Cultivar	S	1.3	2.0	2.3	2.3
PI 508385	Bluebird Tordo//Lachish/3/Nursi/T. dicoccoides (strain G-25)	Israel	Breeding line	S	2.0	2.0	2.8	2.7
PI 518648	BW15(Manitou/Tobari66)/BW517(Carazinho/CT 763//Atlas 66/CT 262	Canada	Cultivar	S	1.7	2.0	2.5	1.7
PI 519612	Platifen/Toquifen sib	Chile	Breeding line	S	2.7	2.2	3.0	2.7
PI 519805	ND 457*3/T.durum//Estanzuela Dakuro	Uruguay	Breeding line	S	2.5	2.8	1.8	3.0
PI 520108	Hork/Kalyansona	Mexico	Breeding line	S	1.2	1.0	1.7	1.0
PI 520350	Butte*2/ND 507	United States	Breeding line	S	1.0	1.5	1.8	1.2
PI 572730	fraternal selection	Mexico	Cultivar	S	3.0	3.0	3.0	2.7
CItr 14362	Frocor//Yaqui/Kentana	Chile	Breeding line	S	1.3	2.4	2.7	1.7
AGS 2035	AGS 2000/PIO26R61	United States	Cultivar	SRWW	3.0	2.0	2.0	1.0
AGS 3000	Jamestown/AGS 2060	United States	Cultivar	SRWW	0.0	0.0	0.0	0.0
Pioneer 26R94	N/A	United States	Cultivar	SRWW	2.0	1.0	1.3	1.0
GA081446-15E47	02655-7-7 / 02328-G1-G1	United States	Breeding line	SRWW	1.0	1.7	2.0	1.0
Dyna-Gro Baldwin	AGS2485/PIO26R61	United States	Cultivar	SRWW	2.0	1.8	2.0	1.0

[a] The wheat genotypes in bold were also reported resistant when tested with a mixture of 10 *Pt* races in Turner et al. (2017) study.

[b]The information about the pedigree origin and growing habit of spring (S) and soft red winter wheat (SRWW) genotypes were obtained from the U.S National Plant Germplasm System website (https://npgsweb.ars-grin.gov/gringlobal/search.aspx) and The University of Georgia small grains breeding program respectively. N/A indicated the information is not available

[c]Improvement status of the wheat genotypes.

[d]The wheat genotypes were evaluated for their reaction to four *P. triticina* races (detail explained in Materials and Methods section) and the infection types (ITs) were recorded 10-12 days after inoculation using a 0-4 scale. The 0-4 IT scale was further converted into a 0-9 linearized scale (LS) to include non-numeric IT in the analysis. Genotypes with an average LS score of 0-6 and 7-9 were considered as resistant and susceptible, respectively

Table 4.4 Summary of quantitative trait loci (QTL) detected significant for resistance to *Puccinia triticina* (*Pt*) races MFGKG, MBTNB, MCTNB, and TCRKG.

Pt race	QTL	Marker[a]	Chr	SNP allele[b]	*p*-value	R^2(%)[c]	pFDR	Position (cM)[d]	IWGSC RefSeq v1.0[e]	
									Start	End
MFGKG	*QLr.uga-2BS*	IWA8221	2BS	A/**C**	2.1E-09	13.6	1.05E-05	90.2	135692007	135692070
	QLr.uga-5AS	IWA2143	5AS	**T**/C	8.27E-05	7.0	5.19E-02	22.6	4849258	4849458
	QLr.uga-6AL1	IWA1497	6AL	T/**C**	3.69E-06	7.9	4.64E-03	106.1	586324094	586324294
	QLr.uga-7AS	IWA7201	7AS	T/**C**	1.39E-04	5.3	6.99E-02	101.4	76499417	76499534
	QLr.uga-7BL	IWA6401	7BL	**T**/C	1.00E-05	7.2	8.38E-03	77.7	544138937	544139137
MBTNB	*QLr.uga-2BS*	IWA8221	2BS	A/**C**	1.47E-07	10.4	7.39E-04	90.2	135692007	135692070
	QLr.uga-4AS	IWA1766	4AS	**A**/G	9.21E-05	5.6	7.71E-02	-	1892987	1893187
	QLr.uga-5AL	IWA5929	5AL	**A**/G	9.05E-05	5.6	7.71E-02	-	670407096	670407213
MCTNB	*QLr.uga-1BL*	IWA415	1BL	A/**G**	8.09E-05	6.9	5.19E-02	103.5	627946628	627946743
	QLr.uga-2BS	IWA8221	2BS	A/**C**	1.73E-12	19.4	7.69E-09	90.2	135692007	135692070
	QLr.uga-4BL	IWA4055	4BL	**A**/C	6.74E-05	5.8	5.19E-02	66.5	469016622	469016822
	QLr.uga-6AL1	IWA1497	6AL	T/**C**	4.42E-04	4.5	9.58E-02	106.1	586324094	586324294
	QLr.uga-7AS	IWA7201	7AS	T/**C**	2.67E-04	4.9	7.63E-02	101.4	76499417	76499534
	QLr.uga-7BL	IWA6401	7BL	**T**/C	1.24E-04	5.4	5.19E-02	77.7	544138937	544139137
TCRKG	*QLr.uga-1AL*	IWA1952	1AL	A/**G**	3.41E-05	6.3	2.85E-02	71.1	343409113	343409313
	QLr.uga-2BS	IWA8221	2BS	A/**C**	1.15E-10	16.0	5.77E-07	90.2	135692007	135692070
	QLr.uga-5AS	IWA2143	5AS	**T**/C	9.00E-05	7.0	5.65E-02	22.6	4849258	4849458
	QLr.uga-6AL1	IWA1497	6AL	T/**C**	5.09E-07	9.5	1.06E-03	106.1	586324094	586324294
	QLr.uga-6AL2	IWA8431	6AL	**T**/C	1.33E-04	5.4	7.23E-02	90.5	563377848	563377948

Abbreviations: QTL = quantitative trait loci; Chr = chromosome; pFDR = positive false discovery rate

[a]The most significant single-nucleotide polymorphic (SNP) marker in the QTL region

[b]The favorable alleles are bold and underlined

[c]The proportion of the phenotypic variation associated with the markers.

[d]Marker position on wheat consensus map

[e]http://www.wheatgenome.org/News/Latest-news/RefSeq-v1.0-URGI

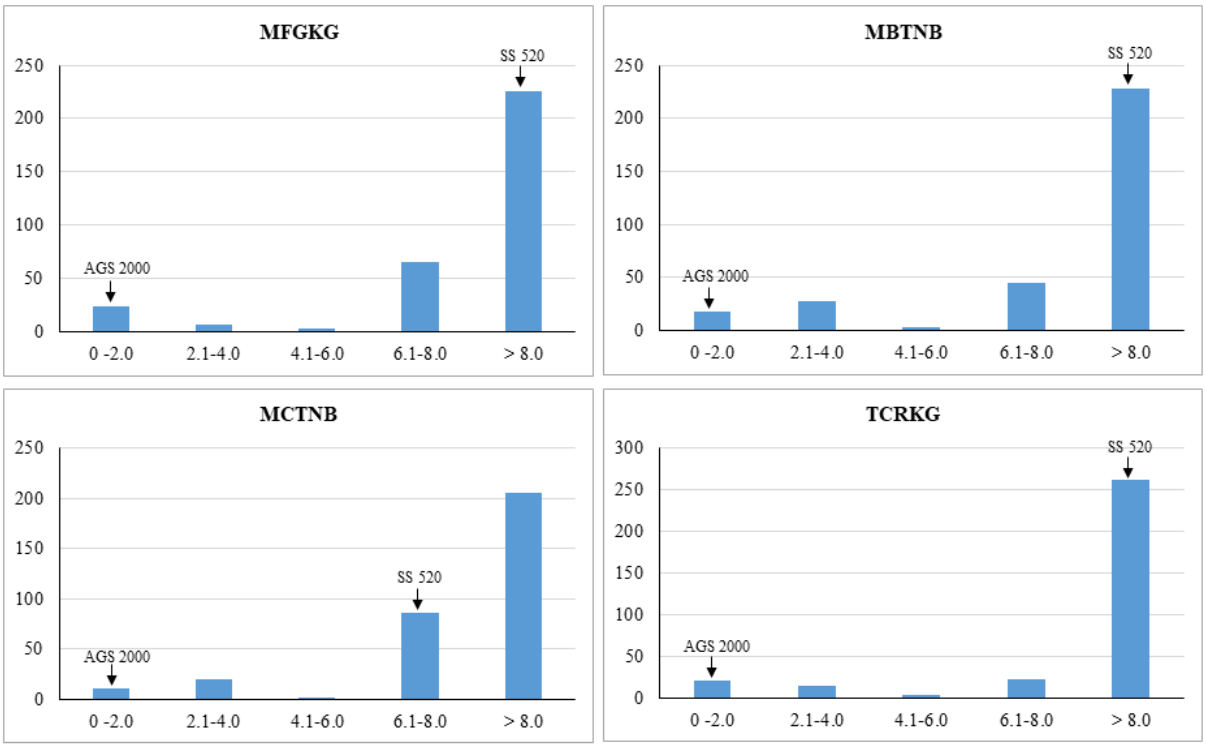


Figure 4.1 Histogram plot showing the distribution of phenotypic data of 331 wheat genotypes to each *P. triticina* (*Pt*) race. A total of 4 *Pt* races i.e., MFGKG, MBTNB, MCTNB, and TCRKG, were used in the evaluation. Soft red winter wheat cultivars AGS 2000 and SS 520 were included as resistant and susceptible checks, respectively. The *x*-axis is the linearized disease scale and the *y*-axis is the number of wheat genotypes.

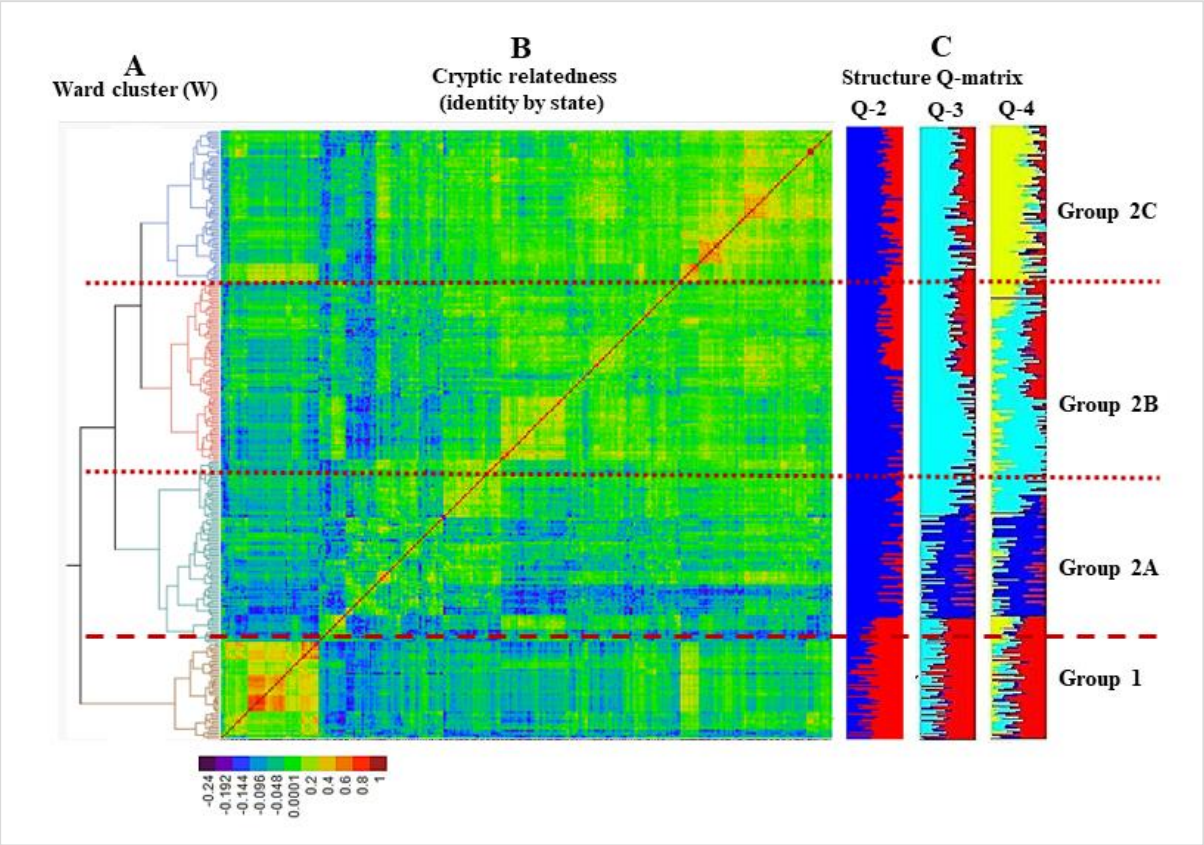


Figure 4.2 Population structure analysis of 297 spring wheat genotypes; (A) Ward clustering of the 297 wheat genotypes; (B) 297 x 297 cryptic relatedness matrix based on genetic distance. A horizontal dashed line separates the two major groups (Group 1 and 2) and the dotted lines separate the subgroups within group 2 (2A, 2B and 2C); (C) Matrices of membership coefficients corresponding to 2 to 4 hypothetical subpopulations derived from the STRUCTURE analysis.

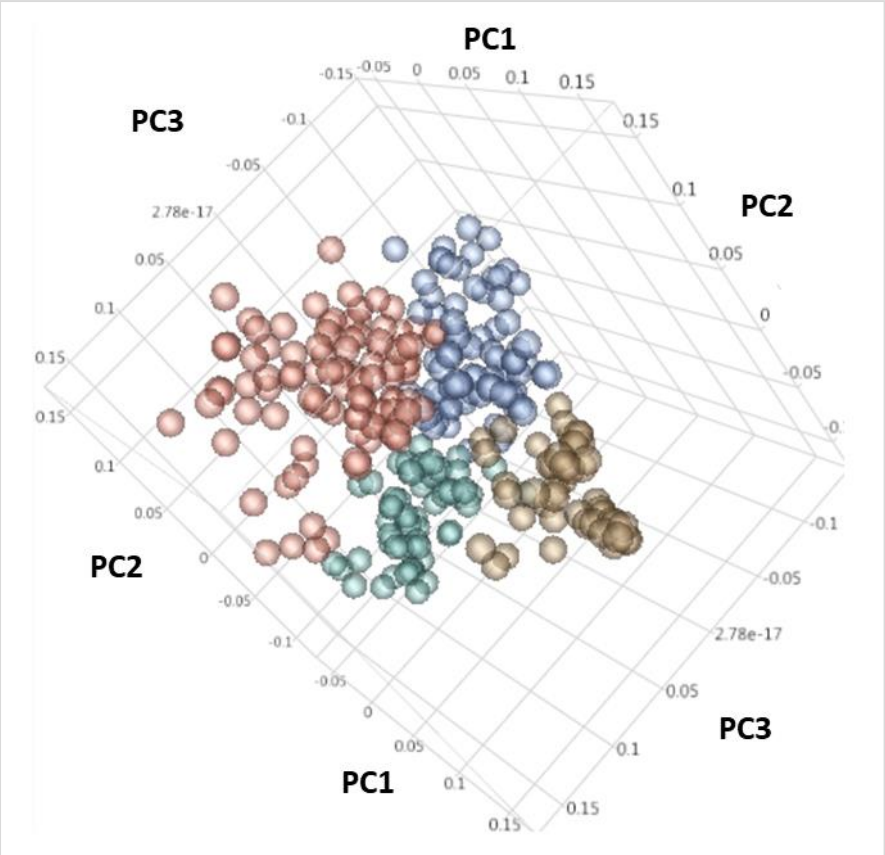


Figure 4.3 Population structure based on principal component (PC) analysis in 297 spring wheat genotypes. Four different colors represent clusters: brown = cluster 1 (group 1), green = cluster 2 (group 2A), red = cluster 3 (group 2B), and blue = cluster 4 (group 2C).

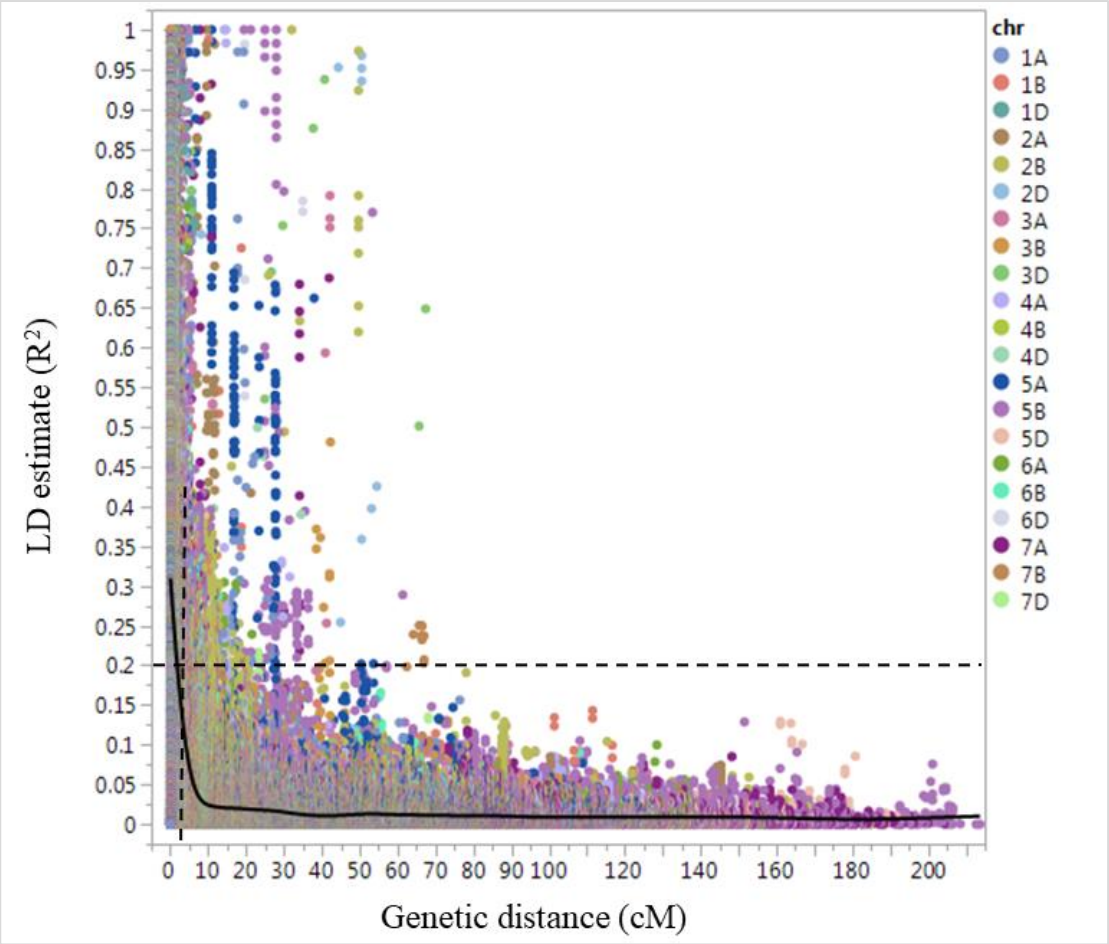


Figure 4.4 Scatter plot demonstrating linkage disequilibrium (LD) decay across the chromosomes for 297 spring wheat genotypes. The LD estimate (R^2) for pairs of single-nucleotide polymorphisms was plotted against corresponding map distances based on the consensus map (Wang et al. 2014).

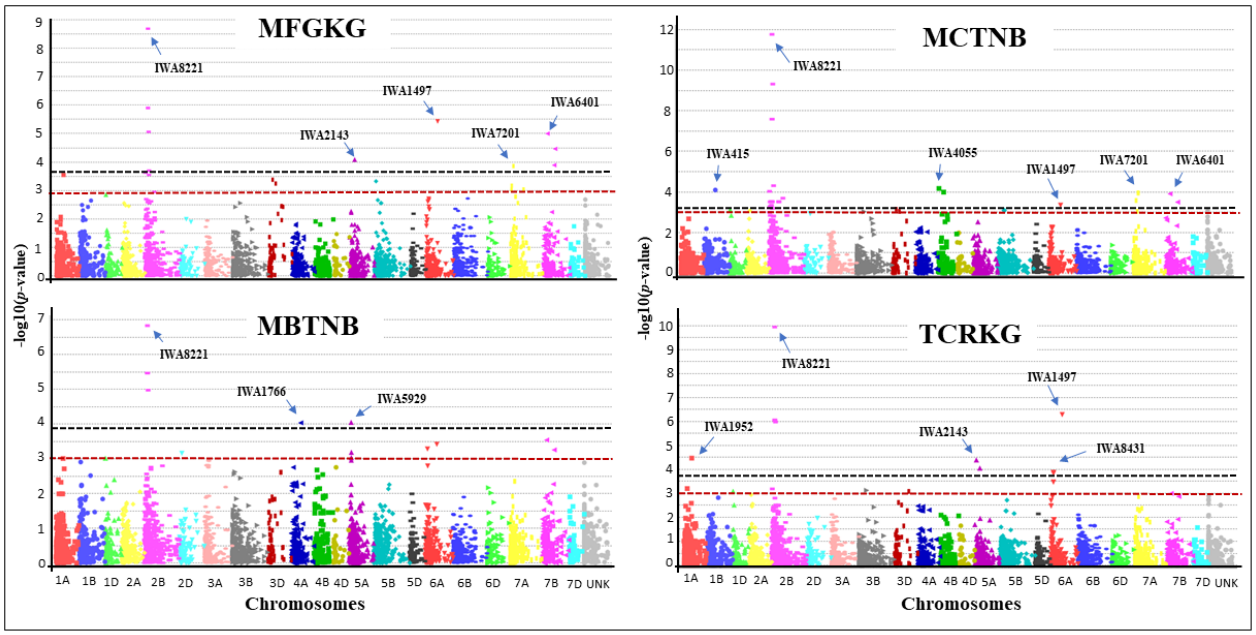
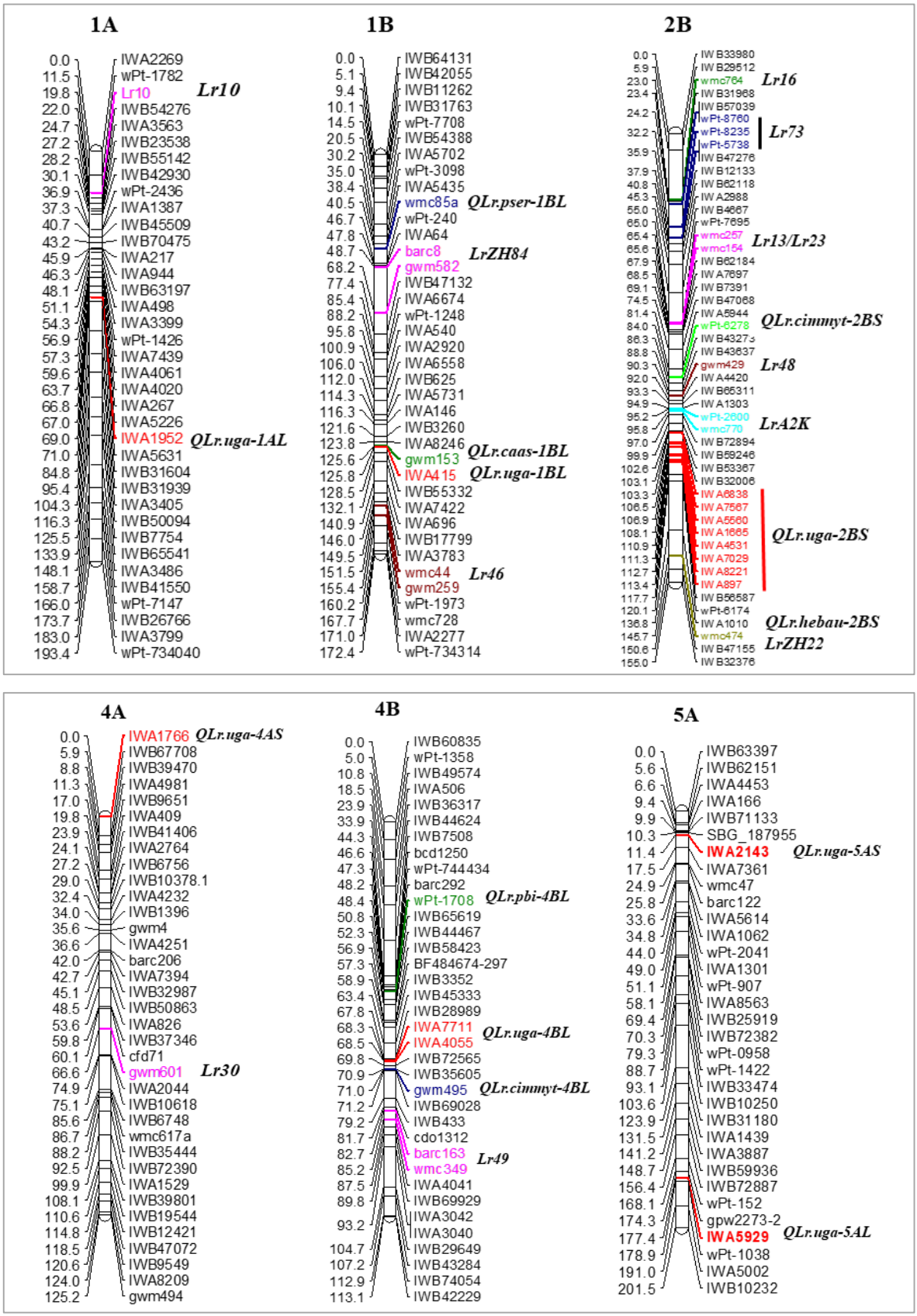


Figure 4.5 Manhattan plot showing the genome-wide scan of markers associated with resistance to *P. triticina* races MFGKG, MBTNB, MCTNB, and TCRKG. The red horizontal dashed line indicates significant threshold at *p*-value = 0.001 and the black dashed line indicates significant threshold at false positive discovery rate (pFDR) = 0.1. The most significant single-nucleotide polymorphism (SNP) markers in each quantitative trait loci (QTL) region are shown by arrows.


1A
0.0 IWA2269
11.5 wPt-1782
19.8 Lr10 Lr10
22.0 IWB54276
24.7 IWA3563
27.2 IWB23538
28.2 IWB55142
30.1 IWB42930
36.9 wPt-2436
37.3 IWA1387
40.7 IWB45509
43.2 IWB70475
45.9 IWA217
46.3 IWA944
48.1 IWB63197
51.1 IWA498
54.3 IWA3399
56.9 wPt-1426
57.3 IWA7439
59.6 IWA4061
63.7 IWA4020
66.8 IWA267
67.0 IWA5226
69.0 IWA1952 QLr.uga-1AL
71.0 IWA5631
84.8 IWB31604
95.4 IWB31939
104.3 IWA3405
116.3 IWB50094
125.5 IWB7754
133.9 IWB65541
148.1 IWA3486
158.7 IWB41550
166.0 wPt-7147
173.7 IWB26766
183.0 IWA3799
193.4 wPt-734040
1B
0.0 IWB64131
5.1 IWB42055
9.4 IWB11262
10.1 IWB31763
14.5 wPt-7708
20.5 IWB54388
30.2 IWA5702
35.0 wPt-3098
38.4 IWA5435
40.5 wmc85a QLr.pser-1BL
46.7 wPt-240
47.8 IWA64
48.7 barc8 LrZH84
68.2 gwm582
77.4 IWB47132
85.4 IWA6674
88.2 wPt-1248
95.8 IWA540
100.9 IWA2920
106.0 IWA6558
112.0 IWB625
114.3 IWA5731
116.3 IWA146
121.6 IWB3260
123.8 IWA8246 QLr.caas-1BL
125.6 gwm153
125.8 IWA415 QLr.uga-1BL
128.5 IWB55332
132.1 IWA7422
140.9 IWA696
146.0 IWB17799
149.5 IWA3783
151.5 wmc44 Lr46
155.4 gwm259
160.2 wPt-1973
167.7 wmc728
171.0 IWA2277
172.4 wPt-734314
2B
0.0 IWB33980
5.9 IWB29512
23.0 wmc764 Lr16
23.4 IWB31968
24.2 IWB57039
32.2 wPt-8760 Lr73
wPt-8235
wPt-5738
35.9 IWB47276
37.9 IWB12133
40.8 IWB62118
45.3 IWA2988
55.0 IWB4667
65.0 wPt-7695
65.4 wmc257 Lr13/Lr23
65.6 wmc154
67.9 IWB62184
68.5 IWA7697
69.1 IWB7391
74.5 IWB47068
81.4 IWA5944
84.0 wPt-6278 QLr.cimmyt-2BS
86.3 IWB43273
88.8 IWB43637
90.3 gwm429 Lr48
92.0 IWA4420
93.3 IWB65311
94.9 IWA1303
95.2 wPt-2600 LrA2K
95.8 wmc770
97.0 IWB72894
99.9 IWB59246
102.6 IWB53367
103.1 IWB32006
103.3 IWA6838
106.5 IWA7567
106.9 IWA5560
108.1 IWA1665 QLr.uga-2BS
110.9 IWA4531
111.3 IWA7029
112.7 IWA8221
113.4 IWA897
117.7 IWB56587
120.1 wPt-6174
136.8 IWA1010 QLr.hebau-2BS
145.7 wmc474 LrZH22
150.6 IWB47155
155.0 IWB32376
4A
0.0 IWA1766 QLr.uga-4AS
5.9 IWB67708
8.8 IWB39470
11.3 IWA4981
17.0 IWB9651
19.8 IWA409
23.9 IWB41406
24.1 IWA2764
27.2 IWB6756
29.0 IWB10378.1
32.4 IWA4232
34.0 IWB1396
35.6 gwm4
36.6 IWA4251
42.0 barc206
42.7 IWA7394
45.1 IWB32987
48.5 IWB50863
53.6 IWA826
59.8 IWB37346
60.1 cfd71
66.6 gwm601 Lr30
74.9 IWA2044
75.1 IWB10618
85.6 IWB6748
86.7 wmc617a
88.2 IWB35444
92.5 IWB72390
99.9 IWA1529
108.1 IWB39801
110.6 IWB19544
114.8 IWB12421
118.5 IWB47072
120.6 IWB9549
124.0 IWA8209
125.2 gwm494
4B
0.0 IWB60835
5.0 wPt-1358
10.8 IWB49574
18.5 IWA506
23.9 IWB36317
33.9 IWB44624
44.3 IWB7508
46.6 bcd1250
47.3 wPt-744434
48.2 barc292
48.4 wPt-1708 QLr.pbi-4BL
50.8 IWB65619
52.3 IWB44467
56.9 IWB58423
57.3 BF484674-297
58.9 IWB3352
63.4 IWB45333
67.8 IWB28989
68.3 IWA7711 QLr.uga-4BL
68.5 IWA4055
69.8 IWB72565
70.9 IWB35605
71.0 gwm495 QLr.cimmyt-4BL
71.2 IWB69028
79.2 IWB433
81.7 cdo1312
82.7 barc163 Lr49
85.2 wmc349
87.5 IWA4041
89.8 IWB69929
93.2 IWA3042
IWA3040
104.7 IWB29649
107.2 IWB43284
112.9 IWB74054
113.1 IWB42229
5A
0.0 IWB63397
5.6 IWB62151
6.6 IWA4453
9.4 IWA166
9.9 IWB71133
10.3 SBG_187955
11.4 IWA2143 QLr.uga-5AS
17.5 IWA7361
24.9 wmc47
25.8 barc122
33.6 IWA5614
34.8 IWA1062
44.0 wPt-2041
49.0 IWA1301
51.1 wPt-907
58.1 IWA8563
69.4 IWB25919
70.3 IWB72382
79.3 wPt-0958
88.7 wPt-1422
93.1 IWB33474
103.6 IWB10250
123.9 IWB31180
131.5 IWA1439
141.2 IWA3887
148.7 IWB59936
156.4 IWB72887
168.1 wPt-152
174.3 gpw2273-2
177.4 IWA5929 QLr.uga-5AL
178.9 wPt-1038
191.0 IWA5002
201.5 IWB10232

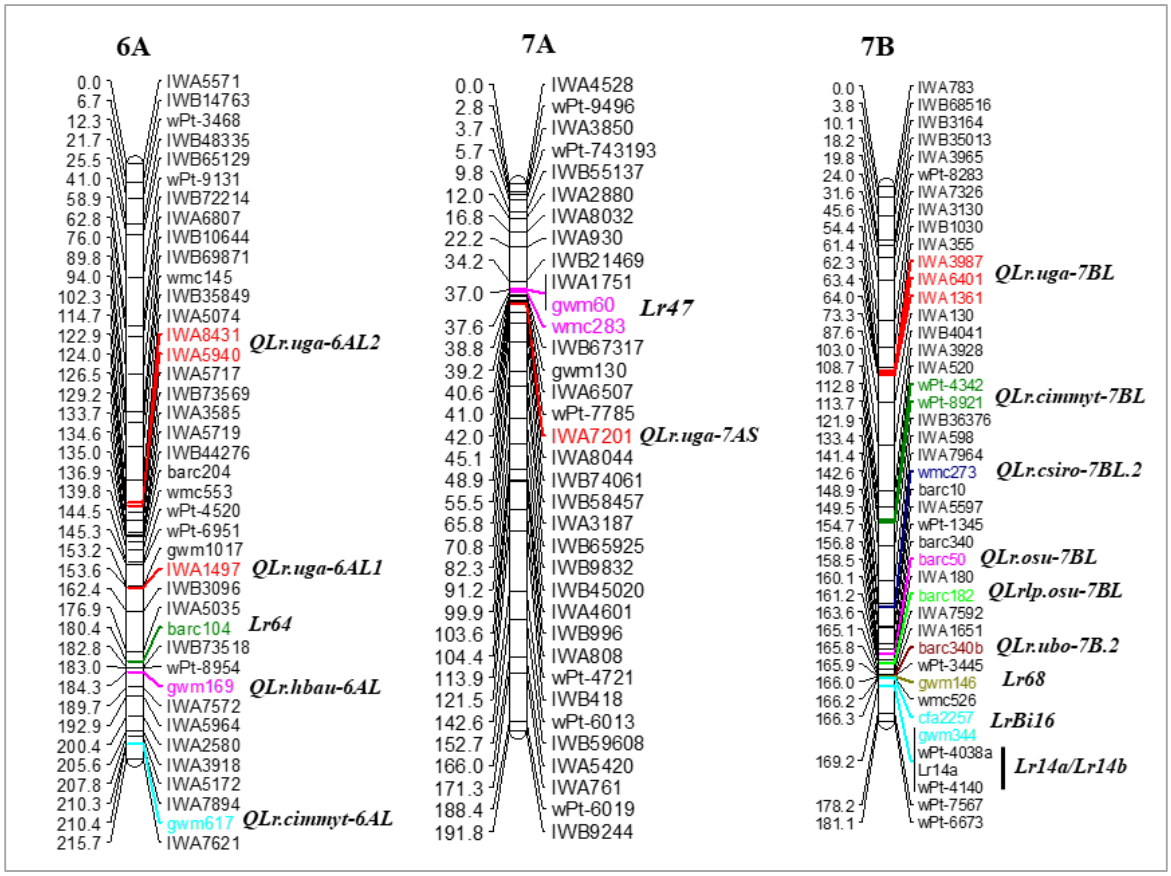


Figure 4.6 Chromosomal locations of quantitative trait loci (QTL) detected significant for resistance to leaf rust (LR) in this study relative to known *Lr* genes or QTL on those chromosomes. Markers detected significant for LR resistance in this study are all in red font. Marker order and location (left side of chromosome bars) are as reported by Maccaferri et al. (2015). For better readability, not all markers are presented in this figure.

Appendix A: Supplemental figure

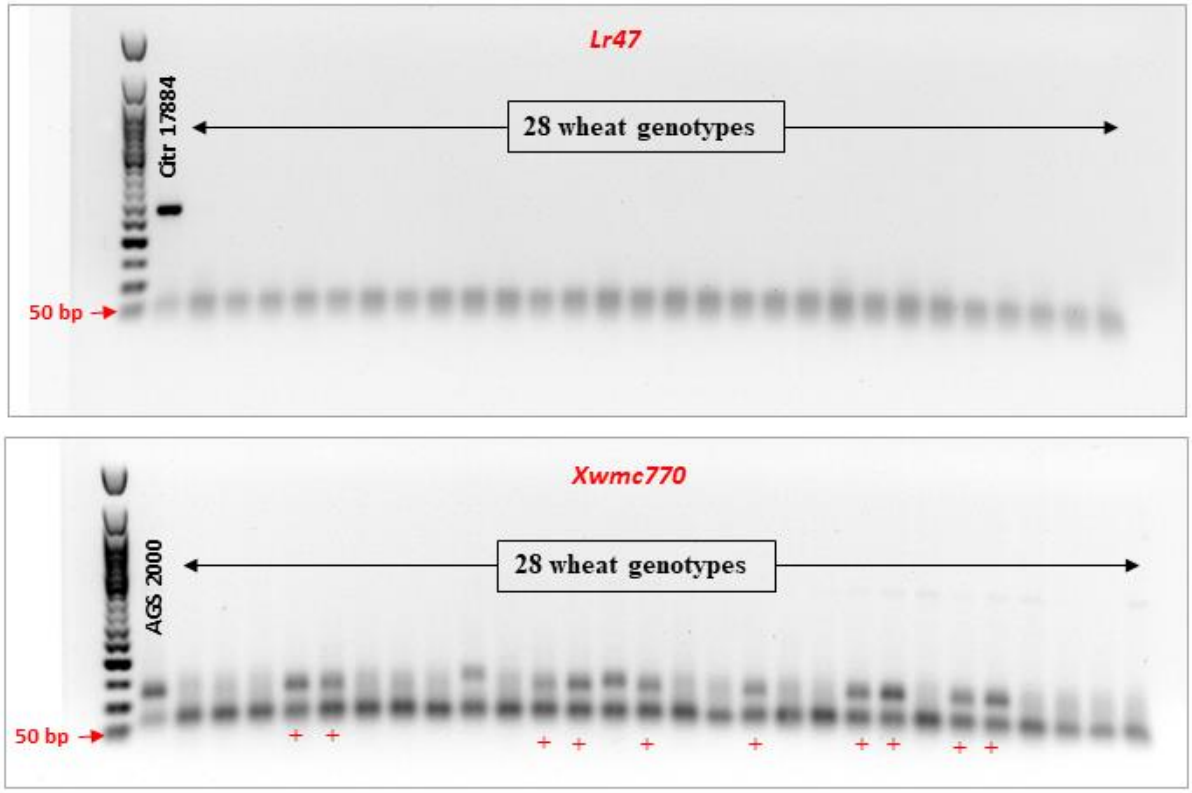


Figure 4.S1 Ethidium bromide stained agarose gel showing the amplification of diagnostic markers of *Lr47* and *LrA2K* (*Xwmc770*) on 28 wheat genotypes resistant to *P. triticina* races MFGKG and MCTNB. Wheat lines Citr 17884 and AGS 2000 were used as positive control for *Lr47* and *LrA2K*, respectively. "+" indicate positive to AGS 2000 allele which carry *LrA2K* gene.

Appendix A: Supplemental tables

Table 4.S1 List of 297 spring wheat genotypes and their average disease score to four *Puccinia triticina* (*Pt*) races

Accession no.	Name	Origin	*Puccinia triticina* race[a]			
			MFGKG	MBTNB	MCTNB	TCRKG
CItr 3008	WOL_KOREN|CITR3008	S. Africa	9.00	9.00	9.00	9.00
CItr 8442	FAVORITO	Argentina	8.67	8.33	9.00	9.00
CItr 12170	S691B	Canada	8.83	8.83	8.83	9.00
CItr 12814	III-46-15	United States	9.00	9.00	9.00	9.00
CItr 12835	RL2661	Canada	2.00	2.25	2.17	2.20
CItr 14346	BETMARK	S. Africa	8.83	8.33	8.67	8.67
CItr 14371	8475-59	Brazil	8.00	9.00	9.00	8.17
CItr 15088	SR13	Canada	9.00	8.83	8.67	8.83
CItr 15136	AMERICAN378|CITR15136	Sudan	8.33	9.00	9.00	8.83
CItr 15212	DEIR_ALLA	Jordan	3.17	2.75	8.33	8.33
CItr 15382	SYRIMEX	Syria	8.00	8.50	9.00	1.33
CItr 15634	II-62-71	United States	1.33	1.80	2.00	2.00
CItr 15850	II-18889-4M-1Y-4M-2Y-3C	Mexico	1.33	2.00	2.17	1.67
PI 24486	PI24486	Turkmenistan	9.00	8.17	8.83	9.00
PI 41032	ALLORCA	Tunisia	8.67	8.17	9.00	8.50
PI 68281	PI68281	Azerbaijan	8.33	8.50	9.00	8.33
PI 83729	MAGYAROVAR81	Hungary	8.83	8.67	9.00	8.17
PI 86198	KRASNAYA_OSTISTAYA_NO.65	Ukraine	8.50	8.50	9.00	8.67
PI 94341	PI94341	Armenia	8.17	8.00	NA	NA
PI 94367	61BP	Armenia	8.83	8.50	8.50	9.00
PI 94757	PI94757	Armenia	8.67	9.00	8.33	9.00
PI 106202	G124-15-0	Australia	9.00	9.00	8.00	8.50
PI 107608	FIRWHILL	Australia	8.67	8.00	8.83	8.67
PI 117757	3085	Australia	9.00	3.00	8.00	8.33
PI 124847	G-29-14-0-3-1-0	Australia	8.83	8.00	9.00	8.80
PI 126822	KENYA_CROSSBRED	Kenya	9.00	9.00	8.50	9.00
PI 129518	O.S.JAKOWSKI	Poland	8.67	9.00	9.00	8.67
PI 130647	81005	Australia	8.50	9.00	9.00	9.00
PI 131273	C10444	Australia	8.50	2.50	8.00	8.00
PI 131401	0-27-5-0	Australia	9.00	8.00	8.00	8.40
PI 134348	AD21/1-3-5	Australia	1.83	2.00	2.83	2.00
PI 163571	1017	Guatemala	8.67	9.00	8.00	8.80
PI 170907	153-M-M-3	South Africa	7.33	9.00	8.17	9.00
PI 175517	SAMPO|PI175517	Finland	7.50	8.83	8.00	8.83

PI 176325	10056	India	8.33	8.83	9.00	8.33
PI 177167	184P.2.A.1.F	Kenya	8.83	9.00	8.00	8.80
PI 180617	CARSTENS_SOMMERWEIZEN	Germany	9.00	9.00	8.00	8.50
PI 181470	PROGRESS|PI181470	Finland	8.67	9.00	8.50	8.83
PI 182673	SALAMOUNI	Lebanon	8.00	9.00	8.00	8.00
PI 184575	AUSSIE	Australia	7.33	8.00	8.00	9.00
PI 184598	URUGUAY|PI184598	Uruguay	8.00	9.00	9.00	9.00
PI 184631	WEBSTER	Russian Fed	2.00	8.17	8.67	9.00
PI 184634	HEINES_KOLBEN	Germany	8.67	9.00	8.17	8.60
PI 184845	CRIOLLO|PI184845	Guatemala	1.50	1.67	3.00	1.33
PI 184993	M-36	Norway	8.67	9.00	8.00	8.83
PI 185272	H836SEL47_259	Argentina	6.33	8.00	8.00	8.00
PI 185356	FLORENCE193	Portugal	7.50	8.33	8.50	8.67
PI 185713	IDEAL|PI185713	Portugal	7.50	8.67	8.50	8.83
PI 185836	BH3145	Brazil	8.17	8.60	8.67	8.83
PI 185909	II-1989	Mexico	8.67	8.83	8.50	8.50
PI 185932	II-888	Mexico	9.00	8.50	8.50	8.33
PI 186033	II-1442-4C-1C-11C	Mexico	8.33	9.00	8.00	8.67
PI 189384	PI189384	Finland	NA	8.50	3.00	8.50
PI 189794	SEL.49-2825H557	Argentina	9.00	8.17	8.00	8.50
PI 189799	SEL.49-4807H603	Argentina	6.17	9.00	9.00	8.83
PI 189826	SEL.49-2810H1070	Argentina	9.00	8.50	8.83	8.33
PI 190450	JO3|PI190450	Norway	8.67	8.67	8.00	8.17
PI 190914	PONDUS	Sweden	8.67	8.83	8.33	8.83
PI 191323	FYLGIA	Sweden	7.50	9.00	9.00	8.00
PI 191453	HUNDI	Spain	8.17	8.67	8.33	8.83
PI 191576	H3B12699	Portugal	7.50	8.67	8.33	8.50
PI 191638	EQUATOR_KTI	Kenya	8.67	8.83	8.67	8.83
PI 191961	MAGVAROVAX	Hungary	7.67	8.67	8.33	9.00
PI 192014	SERRANO	Portugal	8.83	8.50	8.50	9.00
PI 192027	3430	Mozambique	9.00	8.50	9.00	8.67
PI 192123	CJLUGAS	Mozambique	8.67	8.67	9.00	9.00
PI 192131	RENEW	Mozambique	8.67	8.33	8.33	9.00
PI 192155	3579	Mozambique	7.83	8.67	8.83	9.00
PI 192169	PRECOCE|PI192169	Portugal	8.33	8.50	8.50	9.00
PI 192208	RUMANIEN|PI192208	Romania	7.17	9.00	8.17	9.00
PI 192282	HATVANI5612	Hungary	8.83	9.00	8.83	8.83
PI 192299	INDISKT	India	8.83	9.00	8.83	8.50
PI 192312	3781	Sweden	8.67	2.17	8.00	8.80
PI 192348	KADOLZER|PI192348	Czechoslovakia	8.67	8.83	8.83	9.00
PI 192539	H19D12716	Portugal	7.50	9.00	8.00	8.83

PI 192557	H_N_B_C13739	Portugal	8.17	8.60	8.50	8.17
PI 192623	HINDI12	Egypt	6.67	9.00	8.83	9.00
PI 193937	INTER-GENERIC	Colombia	8.67	8.83	9.00	9.00
PI 202672	PI202672	Finland	9.00	8.17	NA	NA
PI 205738	MARIACHE50|PI205738	Argentina	8.67	8.33	9.00	8.80
PI 207104	65O	Iran	9.00	8.33	8.33	8.60
PI 210866	3777-50	Brazil	9.00	9.00	8.33	9.00
PI 210972	T.A.622-3SASL2176	Egypt	8.33	8.00	8.17	9.00
PI 213601	D.I.V.6722	Argentina	1.83	8.50	NA	1.33
PI 214396	COLOTANA2107/50	Brazil	8.67	9.00	8.83	9.00
PI 221361	LEDA|PI221361	Belgium	8.67	9.00	8.67	9.00
PI 223185	ATACATZO_NO.1	Ecuador	8.50	9.00	8.17	8.83
PI 225412	51-491	Uruguay	8.83	8.33	8.33	8.83
PI 230652	JHIAVEVA	Paraguay	8.67	8.50	8.83	8.83
PI 231120	II-2809-1C1XMX45X1X	Guatemala	8.67	8.50	9.00	9.00
PI 234163	INDUSTRIAL_ARGENTINO	Peru	8.67	8.00	8.50	9.00
PI 234236	IDAHO1877NR_HD	Zambia	8.00	8.67	9.00	9.00
PI 234239	IDAHO1880NR_BB	Zambia	8.67	8.17	8.33	9.00
PI 234968	PI234968	Italy	9.00	8.50	9.00	9.00
PI 235221	HIMEKEI_NO.428-1	Japan	2.00	2.50	3.00	2.80
PI 237655	KENYA_GOVERNOR|PI237655	Kenya	8.00	8.50	8.83	9.00
PI 237658	RHODESIAN_SABANERO|PI237658	Kenya	8.83	8.67	9.00	8.50
PI 238403	358-P.6.A	Kenya	8.50	8.67	9.00	8.50
PI 241596	TAICHUNG_NO.23|PI241596	Taiwan	8.83	8.83	8.00	9.00
PI 243679	5401	Iran	9.00	8.50	8.83	8.80
PI 245394	3064	Afghanistan	7.33	8.50	8.00	8.40
PI 247907	ANDES55	Colombia	7.83	8.00	8.50	9.00
PI 247914	MAIPOFEN	Chile	8.33	9.00	8.67	8.50
PI 249817	NTF5-1	Isrel	8.83	9.00	8.33	8.50
PI 253803	K1761	Afghanistan	9.00	9.00	8.50	9.00
PI 254124	505.M.I.D.7	Kenya	9.00	9.00	8.83	8.67
PI 254126	559.L.2.C.1	Kenya	7.50	8.83	8.17	9.00
PI 254128	559.O.1.L.2	Kenya	9.00	3.00	8.50	8.20
PI 254132	604.L.1.B.4	Kenya	9.00	9.00	8.83	9.00
PI 254824	JANETZKIS_JABO	Germany	8.67	8.17	9.00	8.83
PI 255140	LOOSDORFER_MANFRED	Austria	8.67	9.00	8.67	9.00
PI 260805	1406-3683	Egypt	9.00	8.50	8.33	9.00
PI 266148	LEONE	Italy	1.83	2.83	3.00	2.50
PI 268009	LIR	Portugal	8.17	8.83	8.00	9.00
PI 268305	PI268305	Iran	8.50	9.00	8.67	8.83
PI 270044	C591	Pakistan	8.67	8.50	8.00	8.83

PI 271129	MULT760	Peru	7.33	9.00	8.67	8.83
PI 271130	MULT764	Peru	6.67	8.17	8.00	9.00
PI 272331	IAS-43	Brazil	8.33	8.17	9.00	8.67
PI 276705	KRASNOZERNAJA	Russian Fed	8.67	8.50	9.00	9.00
PI 278213	SINAI1	Egypt	9.00	8.67	9.00	9.00
PI 278545	ALEPPO28	Syria	8.17	8.50	8.67	9.00
PI 278655	SARRUBRA	Russian Fed	8.67	9.00	8.83	8.83
PI 279454	RITCHIE	United Kingdom	8.67	9.00	9.00	9.00
PI 282922	I-1039	Argentina	8.83	8.17	8.50	8.50
PI 283147	DORZIYEH_KARAK	Jordan	7.83	8.67	8.00	9.00
PI 283874	HILGENDORF1961	New Zealand	8.50	8.50	8.00	8.17
PI 284547	FYLBY	Belgium	9.00	9.00	8.67	8.33
PI 285944	GORZOWSKA_SZTYWNA	Poland	8.67	9.00	9.00	8.17
PI 286544	COLORADO	Ecuador	8.83	8.83	8.67	8.50
PI 294911	KARNOBATSKA_RANASREIKA	Bulgaria	8.50	8.33	8.83	8.60
PI 294970	KRASNAJA_ZVEZDA|PI294970	Kazakhstan	8.67	8.00	8.50	9.00
PI 297021	184P	Kenya	8.83	2.50	8.17	9.00
PI 298603	BETANA|PI298603	South Africa	9.00	8.50	8.67	9.00
PI 299414	1013B.1.KJ	Kenya	8.83	8.20	8.17	8.67
PI 306529	HARISON_BARBU	Romania	9.00	8.83	8.00	8.67
PI 308674	WHITE_SPITZKOP	South Africa	9.00	2.67	8.33	8.67
PI 312115	KWARTA	South Africa	8.67	2.50	8.00	9.00
PI 312116	RHEEBOK	South Africa	6.50	2.50	3.00	2.60
PI 315837	OPAL	Germany	8.83	9.00	8.00	8.67
PI 321700	KOLBEN_II	Germany	9.00	8.67	8.17	9.00
PI 321889	B-858	Turkey	9.00	8.50	8.00	8.83
PI 323607	PI323607	Australia	8.83	8.17	8.00	8.17
PI 324151	CATCHER	Kenya	5.67	8.00	8.33	8.00
PI 326331	KHAMI	Zimbabwe	8.33	8.50	8.83	8.67
PI 326336	SUGAMUXI68	Colombia	8.83	9.00	8.33	9.00
PI 337147	MAGNIF96	Argentina	7.50	2.50	2.60	2.33
PI 338417	NP757	India	9.00	8.50	8.00	8.83
PI 343730	KOLIBRI|PI343730	Germany	8.00	8.50	8.50	8.67
PI 343737	ATACAZO	Ecuador	9.00	8.67	8.00	8.67
PI 343738	RUMINAHUI	Ecuador	8.83	8.67	8.00	8.60
PI 344170	IAO4	Brazil	8.67	2.67	8.67	9.00
PI 344190	MISSIONEIRO	Brazil	8.50	9.00	8.00	9.00
PI 344203	ANHANGUERA	Brazil	9.00	8.50	9.00	9.00
PI 345693	MINSKAJA	Belarus	9.00	8.00	9.00	8.83
PI 347171	FAO26.430	Afghanistan	NA	8.00	8.00	8.75
PI 351504	MASSAUX_NO.3	Argentina	8.33	8.83	8.00	8.50

PI 351536	OTTAWA2780E	Canada	8.83	8.83	8.00	8.67
PI 351704	BELORUSSKAJA15	Belarus	8.67	8.50	8.00	8.40
PI 351758	FASAN	Germany	8.33	8.50	8.67	9.00
PI 351874	145-7	Burundi	8.67	9.00	8.00	8.67
PI 351878	KISKA9	Burundi	9.00	8.50	8.00	8.67
PI 351903	B205	Switzerland	9.00	8.50	8.50	8.50
PI 351994	Z.88.116	Switzerland	8.33	8.50	8.17	8.50
PI 352183	MEX16	Mexico	7.33	5.67	8.50	8.67
PI 352204	B580	Switzerland	8.50	8.17	9.00	8.80
PI 352206	B669	Switzerland	9.00	9.00	8.00	9.00
PI 352250	NAPO	Ecuador	8.00	8.83	9.00	9.00
PI 358339	2020/70	Croatia	8.83	8.00	8.50	8.60
PI 366063	GIZA156	Egypt	9.00	2.50	8.00	8.83
PI 366923	1196	Afghanistan	NA	NA	NA	NA
PI 372137	LUTESCENS491	Ukraine	9.00	8.83	8.50	9.00
PI 378910	13340	Colombia	7.67	9.00	9.00	9.00
PI 378915	18127	Philippines	8.67	8.67	8.67	9.00
PI 382162	16-52-3	Brazil	9.00	9.00	8.83	9.00
PI 384352	DIKWA7	Nigeria	9.00	9.00	8.83	9.00
PI 384378	DIKWA33	Nigeria	9.00	8.67	8.17	9.00
PI 384379	DIKWA34	Nigeria	8.83	8.67	8.50	9.00
PI 387594	IAR/W/128-5	Ethiopia	9.00	8.67	8.67	9.00
PI 388036	LINE99	Isrel	9.00	8.50	8.17	8.83
PI 388037	539/21	Isrel	8.67	8.67	8.17	9.00
PI 388038	PI388038	Isrel	5.67	9.00	8.50	9.00
PI 388082	FAO33.218	Pakistan	8.67	8.83	9.00	9.00
PI 410899	GLORIA	Morocco	9.00	8.67	8.67	9.00
PI 410914	3297	Morocco	9.00	8.67	8.83	9.00
PI 410954	PI410954	South Africa	8.50	1.60	8.00	4.50
PI 411132	GOGATSU_KOMUGI	Japan	8.50	8.17	8.50	8.50
PI 412985	RED_BOBS	Canada	8.67	8.83	8.33	9.00
PI 414538	SIBIRIACKA4	Russian Fed	9.00	8.33	8.00	9.00
PI 414625	3311	China	1.17	2.00	2.50	1.33
PI 418575	BURIATSKAJA34	Russian Fed	8.50	9.00	9.00	9.00
PI 422282	HI588	India	1.33	2.00	2.33	2.33
PI 422440	MYSEGEJA	Albania	8.67	9.00	9.00	9.00
PI 427285	SAKIGAKE_KOMUGI	Japan	7.83	8.50	9.00	9.00
PI 428666	DETENICKA_VOUSKA	Czech Republic	8.67	9.00	8.67	8.83
PI 428668	DVORSKEHO_ZORO	Czechoslovakia	8.67	8.67	8.33	8.83
PI 428690	LEUCURUM3|PI428690	Uzbekistan	9.00	9.00	9.00	9.00
PI 429318	MESRI	Yemen	9.00	9.00	9.00	9.00

PI 434987	ESTANZUELA_YOUNG	Uruguay	1.50	8.33	8.50	1.50
PI 435132	H-78270	Spain	8.50	8.17	8.00	8.50
PI 438961	AL_BORUBRUM50	Kazakhstan	8.50	9.00	9.00	9.00
PI 438966	PAVLODARSKAJA_I	Kazakhstan	9.00	8.50	9.00	8.80
PI 438967	PIROTRIKS28|PI438967	Kazakhstan	7.83	8.67	8.67	8.80
PI 438968	SNEGURKA	Kazakhstan	9.00	9.00	8.83	9.00
PI 438969	SHORTANDINSKAJA25	Kazakhstan	9.00	9.00	8.33	9.00
PI 447353	LUNG_CHUN_NO.6	China	8.67	8.00	8.00	9.00
PI 447384	XIN_CHUN_NO.1	China	8.50	8.67	8.17	8.83
PI 449296	MT-7	Spain	7.83	8.50	8.50	9.00
PI 449298	7020	Spain	9.00	8.67	8.83	9.00
PI 462111	PI462111	Yemen	8.83	9.00	8.67	9.00
PI 468988	MG27041	Greece	8.83	9.00	8.33	9.00
PI 468990	MG27043	Greece	8.50	8.50	8.50	9.00
PI 469072	MG27959	Greece	8.17	1.50	8.83	9.00
PI 480480	R-124	Bolivia	8.67	8.50	8.00	8.83
PI 502627	RED_STAR	Uzbekistan	2.67	2.83	8.00	1.25
PI 508385	V764-14-J2-B2-J2	Isrel	2.00	2.00	2.83	2.67
PI 508387	V882-F22-F2-F3-F2-J2	Isrel	8.67	2.50	8.50	9.00
PI 508388	1108/83	Isrel	9.00	9.00	8.83	8.83
PI 518648	LAURA	Canada	1.67	2.00	2.50	1.67
PI 519011	N-1254-5C-2C-2C	Chile	7.67	NA	8.00	9.00
PI 519357	BLUEBIRD_S|PI519357	Mexico	8.83	8.00	8.00	8.17
PI 519421	VI.106-5B-2T-1B-1T-1B	Ecuador	7.17	8.17	9.00	8.67
PI 519484	12584-8B-2T-3B1T	Colombia	8.33	8.67	8.67	8.67
PI 519503	L1360-3838	Egypt	7.33	8.17	9.00	9.00
PI 519512	ND71-12-111	United States	1.17	2.17	8.00	2.83
PI 519580	CH-7790-12P-9P-1P-1P	Chile	8.50	8.00	8.00	8.83
PI 519612	A5292-23P-1P-1P	Chile	2.67	2.17	3.00	2.67
PI 519683	A4920-52P-2P-2P	Chile	8.50	8.17	8.00	8.50
PI 519792	QP330-1C-1C-1C-1C	Chile	8.67	8.33	8.83	9.00
PI 519805	LE2096	Uruguay	2.50	2.80	1.83	3.00
PI 519842	F8-4	Mexico	7.00	8.33	8.67	7.83
PI 519904	W5865-2-M-3-LM	South Africa	8.67	9.00	9.00	8.60
PI 519908	W5672-3-M-6-TM	South Africa	8.50	8.17	8.00	9.00
PI 519912	W5885-2-M-1-LM	South Africa	9.00	5.17	8.00	1.67
PI 520033	KENYA4135-H3D5	Kenya	7.33	8.50	8.50	8.83
PI 520108	CM39714-5S-2AP-OAP	Mexico	1.17	1.00	1.67	1.00
PI 520265	ND598	United States	8.67	8.00	8.00	9.00
PI 520282	CEP75336	Brazil	7.33	8.00	8.00	8.25
PI 520350	ND587	United States	1.00	1.50	1.83	1.17

PI 520374	CM2281-13M-1Y-3M-1Y-1M-OY	Mexico	6.67	9.00	8.00	8.33
PI 520375	CM23091-1M-2Y-OY	Mexico	8.33	8.00	8.00	9.00
PI 520377	SE381-4S-1S-6S-OS	Syria	7.50	NA	8.00	NA
PI 520386	CM32285-3S-4AP-OAP	Syria	6.33	2.33	2.17	2.50
PI 520498	JACUI	Brazil	8.67	9.00	8.00	9.00
PI 520557	BOBWHITE_S|PI520557	Mexico	9.00	2.60	8.33	2.80
PI 525282	1130	Morocco	9.00	9.00	9.00	9.00
PI 525326	1359	Morocco	9.00	8.50	8.50	9.00
PI 532056	PI532056	Egypt	9.00	8.50	9.00	8.40
PI 532070	1014	Egypt	9.00	9.00	9.00	9.00
PI 532255	MUFSEGHA	Oman	9.00	8.83	8.67	9.00
PI 532284	7442	Oman	8.67	8.00	8.50	9.00
PI 534448	MG18237	Algeria	8.33	8.17	9.00	8.83
PI 542661	BOURBA	Algeria	8.67	8.83	8.50	9.00
PI 559704	ULJBINKA25	Kazakhstan	9.00	9.00	8.50	9.00
PI 565222	GABO|PI565222	Bolivia	8.83	2.80	8.50	9.00
PI 572630	KAZAHSTANSKAJA4	Kazakhstan	8.83	8.83	8.50	9.00
PI 572632	URAL_SKAJA_JUBILEJNAJA	Kazakhstan	8.83	9.00	8.50	9.00
PI 572636	RANJAJA73	Ukraine	8.50	9.00	8.50	9.00
PI 572730	CHAPINGO_VF74	Mexico	3.00	3.00	3.00	2.67
PI 572822	86PK1311-001.02	Pakistan	8.67	8.17	8.33	8.83
PI 574348	15040	Saudi Arabia	8.67	8.00	9.00	NA
PI 576639	2262-12	Tunisia	8.67	8.00	8.83	9.00
PI 577777	MG18017|PI577777	Algeria	8.83	8.83	9.00	9.00
PI 583670	SPINKCOTA	United States	8.67	8.50	8.00	8.83
PI 583705	COXILHA	Brazil	8.67	9.00	9.00	9.00
PI 583715	OBREGON	Mexico	8.67	9.00	9.00	8.83
PI 591942	SST124	South Africa	8.67	2.50	9.00	9.00
PI 592983	POMERELLE	United States	9.00	8.00	9.00	9.00
PI 593658	AC_BARRIE	Canada	2.00	NA	3.00	2.60
PI 595661	8644-057-I	Canada	8.33	8.50	8.83	9.00
PI 596367	P8921-Q4C5	Canada	8.67	9.00	9.00	9.00
PI 610755	CIGM90.483	Mexico	8.17	8.50	8.67	9.00
PI 613317	CIGM90.412	Mexico	7.33	8.83	9.00	8.00
PI 614012	CIGM98.748-1	Mexico	3.33	8.83	3.00	1.50
PI 614040	CIGM98.752-1	Mexico	8.83	8.83	8.17	8.50
PI 620714	OR9630064	United States	8.83	8.17	9.00	8.83
PI 623352	IWA8603039	Iran	8.83	9.00	8.50	9.00
PI 623820	IWA8607143	Iran	8.67	NA	NA	NA
PI 624156	IWA8607793	Iran	7.00	8.50	8.00	9.00
PI 624226	IWA8607919	Iran	9.00	9.00	9.00	8.83

PI 624292	IWA8608045	Iran	8.33	NA	8.00	9.00
PI 624883	IWA8609067	Iran	9.00	8.00	8.00	7.50
PI 624979	IWA8609318	Iran	8.83	8.50	8.00	8.40
PI 625571	IWA8611725	Iran	8.83	8.00	9.00	9.00
PI 625642	IWA8611964	Iran	8.67	8.33	8.00	8.50
PI 625778	IWA8612362	Iran	7.50	8.50	8.83	9.00
PI 638576	99CF635	United States	9.00	9.00	9.00	9.00
CItr 12302	RL1527	Canada	8.67	9.00	9.00	9.00
PI 191882	BXRA8_10142	Argentina	9.00	9.00	9.00	8.50
CItr 12691	POSO48	United States	9.00	9.00	8.83	8.67
CItr 14362	2809-2B-4B-1B-3T	Chile	1.33	2.40	2.67	1.67
CItr 14400	3.18.1957	Peru	8.67	8.67	9.00	8.83
PI 191261	BLANDO588	Spain	8.67	8.83	9.00	9.00
PI 278375	KENYA131	Kenya	NA	9.00	9.00	8.83
PI 519418	VI-36-2-30B-3T-2B-2T	Ecuador	9.00	9.00	9.00	9.00
PI 344154	IJUI	Brazil	8.67	9.00	9.00	9.00
PI 351870	10180-54-29	Burundi	9.00	9.00	9.00	9.00

Table 4.S2 List of 34 soft red winter wheat (SRWW) genotypes and their average disease score to four *Puccinia triticina* (*Pt*) races

No.	Name	*Puccinia triticina* race[a]			
		MFGKG	**MBTNB**	**MCTNB**	**TCRKG**
1	SS 8641	8.00	8.00	8.00	8.00
2	AGS 2035	3.00	2.00	2.00	1.00
3	AGS 3000	0.00	0.00	0.00	0.00
4	Pio26R41	8.00	8.67	8.00	8.00
5	Pio26R94	2.00	1.00	1.00	1.00
6	Hilliard	8.33	8.00	8.00	8.00
7	USG 3555	8.00	8.00	8.00	8.00
8	AGS 2024	8.00	8.00	8.00	8.33
9	GA04510-11LE24	8.33	8.00	8.00	9.00
10	GA 041293-11E54	8.33	8.00	8.00	9.00
11	GA 03564-12E6	8.00	8.00	8.00	8.00
12	GA 051102-13LE43	8.33	9.00	8.00	9.00
13	GA 061349-13LE31	8.00	9.00	8.00	8.00
14	GA 07353-14E19	8.00	1.83	2.00	8.00
15	GA JT141-14E45	8.00	8.00	8.00	8.00
16	GA 051207-14E53	8.00	8.00	8.00	8.67
17	GA081446-15E47	1.00	1.67	1.33	1.00
18	GA071171-15E64	8.00	8.00	8.00	8.00
19	GA05450-15E52	8.00	8.00	8.33	8.33
20	GA08261-15E7	8.00	8.00	8.00	8.00
21	GA06283-15LE25	1.00	8.00	8.00	8.00
22	GA081113-15E8	8.00	2.67	8.00	8.00
23	AGS 2027	8.00	8.00	8.33	8.33
24	AGS 2033	8.00	2.83	2.67	8.00
25	Dyna-Gro 9171	8.00	8.00	8.00	8.00
26	Dyna-Gro 9522	8.67	8.67	8.00	8.00
27	Dyna-Gro Baldwin	2.00	1.83	2.00	1.00
28	Dyna-Gro Savoy	8.67	8.67	8.33	8.33
29	Progeny 870	8.00	8.00	8.00	9.00
30	Southern Harvest 550	8.33	8.00	8.00	8.33
31	SS 8415	8.00	8.00	8.33	8.00
32	Syngenta Cypress	8.00	8.00	8.00	8.00
33	Syngenta Viper	8.33	8.33	8.00	8.33
34	USG 3024	8.83	8.00	8.00	9.00

Table 4.S3 Summary of SNP markers associated with resistance to four Puccinia triticina (*Pt*) races.

QTL	Marker	Chr.	*p*-value	R^2	pFDR	Position (cM)	IWGSC RefSeq v1.0		*Pt* race
							Start	End	
Q.Lr.uga-1AL	IWA1952	1AL	0.0000341	0.06338	2.85E-02	71.1	343409113	343409313	TCRKG
Q.Lr.uga-1BL	IWA415	1BL	0.0000809	0.06941	5.19E-02	103.6	627946628	627946743	MCTNB
QLr.uga-2BS	IWA8221	2BS	2.1E-09	0.13595	1.05E-05	90.2	135692007	135692070	MFGKG
	IWA6838	2BS	0.00000127	0.08698	2.12E-03	90.9	139814755	139814904	MFGKG
	IWA1665	2BS	0.00000876	0.07275	8.37E-03	89.3	122754955	122754755	MFGKG
	IWA2624	2BS	0.00000127	0.08698	2.13E-03	90.9	139811917	139812117	MFGKG
	IWA8221	2BS	0.000000147	0.10394	7.39E-04	90.2	135692007	135692070	MBTNB
	IWA6838	2BS	0.00000341	0.08023	5.71E-03	90.9	139814755	139814904	MBTNB
	IWA2624	2BS	0.00000341	0.08023	5.71E-03	90.9	139811917	139812117	MBTNB
	IWA1665	2BS	0.0000105	0.07193	1.32E-02	89.3	122754955	122754755	MBTNB
	IWA8221	2BS	1.73E-12	0.19421	7.69E-09	90.2	135692007	135692070	MCTNB
	IWA1665	2BS	4.79E-10	0.14822	1.20E-06	89.3	122754955	122754755	MCTNB
	IWA6838	2BS	2.58E-08	0.11687	3.24E-05	90.9	139814755	139814904	MCTNB
	IWA2624	2BS	2.58E-08	0.11687	3.24E-05	90.9	139811917	139812117	MCTNB
	IWA4531	2BS	0.0000495	0.06049	4.97E-02	89.6	131571865	131572065	MCTNB
	IWA897	2BS	0.000206	0.05035	7.63E-02	92.2	146955997	146956097	MCTNB
	IWA7030	2BS	0.000293	0.0597	7.63E-02	88.9	118285264	118285404	MCTNB
	IWA5560	2BS	0.000306	0.04757	7.63E-02	88.9	121368647	121368472	MCTNB
	IWA763	2BS	0.000306	0.04757	7.63E-02	88.9	121371147	121370960	MCTNB
	IWA607	2BS	0.000308	0.04771	7.63E-02	88.9	115381667	115381787	MCTNB
	IWA608	2BS	0.000534	0.04368	9.58E-02	88.9	115381251	115381131	MCTNB
	IWA7029	2BS	0.000452	0.0449	9.58E-02	88.9	118286701	118286501	MCTNB
	IWA7567	2BS	0.000534	0.04368	9.58E-02	88.9	115968466	115968614	MCTNB
	IWA2557	2BS	0.000306	0.04757	7.63E-02	NA	121676936	121677065	MCTNB
	IWA4655	2BS	0.000526	0.04379	9.58E-02	NA	118060896	118060696	MCTNB
	IWA2312	2BS	0.000534	0.04368	9.58E-02	NA	115381290	115381161	MCTNB

	IWA4456	2BS	0.000306	0.04757	7.63E-02	NA	121680512	121680641	MCTNB
	IWA8221	2BS	1.15E-10	0.16032	5.77E-07	90.2	135692007	135692070	TCRKG
	IWA6838	2BS	0.000000873	0.09041	1.07E-03	90.9	139814755	139814904	TCRKG
	IWA2624	2BS	0.000000873	0.09041	1.07E-03	90.9	139811917	139812117	TCRKG
	IWA1665	2BS	0.00000106	0.08895	1.07E-03	89.3	122754955	122754755	TCRKG
QLr.uga-4AS	IWA1766	4AS	0.0000921	0.05624	7.71E-02	NA	1892987	1893187	MBTNB
QLr.uga-4BL	IWA4055	4BL	0.0000674	0.05826	5.19E-02	66.6	469016622	469016822	MCTNB
	IWA7711	4BL	0.000102	0.0553	5.19E-02	69.8	459922034	459922168	MCTNB
QLr.uga.5AS	IWA2143	5AS	0.0000827	0.06992	5.19E-02	22.6	4849258	4849458	MFGKG
	IWA2143	5AS	0.00009	0.06981	5.65E-02	22.6	4849258	4849458	TCRKG
	IWA7361	5AS	0.000041	0.07485	2.94E-02	25.6	8243722	8243590	TCRKG
QLr.uga-5AL	IWA5929	5AL	0.0000905	0.05637	7.71E-02	NA	670407096	670407213	MBTNB
QLr.uga.6AL1	IWA1497	6AL	0.00000369	0.07909	4.64E-03	106.1	586324094	586324294	MFGKG
	IWA1497	6AL	0.000442	0.04501	9.58E-02	106.1	586324094	586324294	MCTNB
	IWA1497	6AL	0.000000509	0.0945	1.06E-03	106.1	586324094	586324294	TCRKG
QLr.uga.6AL2	IWA8431	6AL	0.000133	0.05365	7.23E-02	90.5	563377848	563377948	TCRKG
	IWA5940	6AL	0.000144	0.05307	7.23E-02	90.5	563378317	563378468	TCRKG
QLr.uga-7AS	IWA7201	7AS	0.000267	0.04858	7.63E-02	101.4	76499417	76499534	MCTNB
	IWA7201	7AS	0.000139	0.05303	6.99E-02	101.4	76499417	76499534	MFGKG
QLr.uga-7BL	IWA6401	7BL	0.00001	0.07174	8.38E-03	77.7	544138937	544139137	MFGKG
	IWA3987	7BL	0.000129	0.05349	6.99E-02	77.7	538877041	538876898	MFGKG
	IWA1361	7BL	0.0000344	0.06284	2.47E-02	76.0	538869193	538868993	MFGKG
	IWA6401	7BL	0.000124	0.05393	5.19E-02	77.7	544138937	544139137	MCTNB
	IWA1361	7BL	0.000319	0.04727	7.63E-02	76.0	538869193	538868993	MCTNB

CHAPTER 5

SUMMARY

Wheat (*Triticum aestivum* L.) is a major food crop grown worldwide contributing about 20% calories to the human population food. An increase in wheat production is imperative to ensure food safety to the human population, which is projected to reach nine billion by 2050. Rusts diseases of wheat, which consist of leaf, stripe, and stem rusts are major biotic constraints of wheat production worldwide. Among the three rust diseases, leaf rust (LR) caused by *Puccinia triticina* (*Pt*) is the most common and widely distributed worldwide. Chemical fungicides and cultural practices can help control LR disease; yet genetic resistance is the most economical and preferred method to manage this disease However, the continuous emergence of new and virulent *Pt* races every year poses a great challenge and demands for the continuous search of novel sources of resistance to combat this devastating disease. The research presented in this dissertation dissects the genetics of LR resistance in wheat germplasm, provides valuable genetic information, and new tools for wheat breeding in the Southeastern (SE) region of the USA.

Soft-red winter wheat (SRWW) cultivars AGS 2000 and AGS 2038 have been leading cultivars in the SE region of the USA possessing good level of LR resistance. Utilizing two recombinant inbred line (RIL) population derived from AGS 2000/26R61 and AGS 2038/UGA 111729, we mapped and characterized LR resistance genes, *LrA2K* and *Lr2K38*, on chromosomes 2BS and 1AL for seedling and adult plant LR resistance, respectively. *LrA2K* was detected in the SRWW cultivar AGS 2000 and is highly effective against two prevalent *Pt* races,

MFGKG and MBTNB, in the SE region of the US. The molecular marker *Xwmc770*, which is closely linked to *LrA2K*, was validated on a set of wheat cultivars, and therefore can be used in marker-assisted selection (MAS). *Lr2K38* was mapped in the SRWW cultivar AGS 2038 and confers adult plant LR resistance. Of the two SNP markers that flanked *the Lr2K38* gene, *IWA20487* is highly polymorphic among SRWW cultivars and should be useful for deploying the *Lr2K38* gene in wheat breeding programs. Pyramiding the *LrA2K* and *Lr2K38* genes identified from our study with other LR resistance genes could help develop durable LR resistance wheat cultivars. Screening 331 wheat lines with four *Pt* races, MFGKG, MBTNB, MCTNB, and TCRKG, at the seedling stage identified 22 lines highly resistant to all four *Pt* races. A total of 297 among 331 wheat genotypes had marker data available, and therefore, were used in the genome-wide association study (GWAS) study. The GWAS detected 11 QTL significant for LR resistance. Of these, five QTL were uncharacterized previously in common wheat, and therefore, these loci are likely to represent novel QTL for LR resistance.

In summary, this research identified wheat germplasm and QTL/genomic regions of the wheat genome significantly associated with LR resistance allowing wheat scientists to efficiently incorporate these resistances into elite wheat germplasm. Additionally, the findings of this research have provided additional understanding of the genetics of LR resistance in wheat. The highly resistant wheat genotypes discovered in this study can serve as new parental lines to use in crosses to develop LR resistant wheat cultivars.