GENETIC CHARACTERIZATION OF WHEAT GENES RESISTANCE TO TAN SPOT AND LEAF RUST

by

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Abstract

Tan spot, caused by *Pyrenophora tritici-repentis* (Ptr), is an economically important foliar disease worldwide. Race 1 of the fungus, which produces the necrosis toxin Ptr ToxA and the chlorosis toxin Ptr ToxC, is the most prevalent race in the Great Plains of the United States. The purposes of this study are to 1) identify and map novel quantitative trait loci (QTL) involved in resistance to tan spot race 1 in common wheat (*Triticum aestivum* L.) and 2) explore the inverse gene-for-gene interaction in the wheat-*P. tritici-repentis* pathosystem. A population of 288 $F_{2:6}$ recombinant inbred lines (RILs) developed from the cross between Chinese landrace WSB (resistant) and Ning7840 (highly susceptible) was firstly used to identify genomic regions harboring novel sources of resistance. Two QTLs associated with resistance to chlorosis were mapped to the short arm of chromosome 1A and 2B in the WSB/Ning7840 population. No interaction was found between the two QTL. To further explore the specific wheat-ToxC model, three other populations were developed based on two susceptible parents, Ning7840 and Wheaton. QTL analysis revealed that common QTL were detected in populations shared with the same susceptible parents. The observations suggested that susceptibility rather than resistance for tan spot chlorosis is specific and presented evidence for the inverse gene-for-gene theory in the WSB-ToxC pathosystem.

Leaf rust, caused by *Puccinia triticina* Eriks., is another important foliar disease of common wheat worldwide. The rust-resistance genes *Lr41* and *Lr42* from *T. tauschii* accessions TA2460 (*Lr41*) and TA2450 (*Lr42*) have been used as sources of rust resistance in breeding programs. Molecular markers linked to these genes are essential tools for gene pyramiding. Two $BC_3F_{2:6}$ mapping populations were evaluated for leaf rust resistance at both seedling and adult plant stages and analyzed with simple sequence repeat (SSR) markers. Both genetic and physical mapping confirmed that markers linked to *Lr41* and *Lr42* were on chromosome arm 2DS and 1DS, respectively. Marker analysis in a diverse set of wheat germplasm indicated that tightly linked markers for *Lr41* and *Lr42* can be used for marker-assisted selection (MAS) in breeding programs.

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CHAPTER 1 - LITERATURE REVIEW

Tan spot

Disease Symptoms, Cycle and Epidemiology and its impact on wheat production

Tan spot, caused by the ascomycete fungus *Pyrenophora tritici-repentis* (Died.) Drechsler (anamorph: *Drechslera tritici-repentis* (Died.) Shoemaker), is an important disease of common wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum L. var. durum*) throughout the world. It also occurs in many other species of native and cultivated grasses (Ali and Francl, 2003; Cox et al., 1992). The symptoms of the disease initially display as small dark-brown spots, and then become tan elliptical or diamond-shaped lesions surrounded by a chlorotic halo with a small dark-brown spot in the center characterized by the outgrowth of conidia and conidiophores (Balance and Lamari, 1998; De Wolf et al., 1998).

The life cycle of *P. tritici-repentis* involves two forms, ascospores produced during the sexual stage and conidia produced during the asexual stage (Schilder and Bergstrom, 1992). The ascospores (primary inoculum) are released from pseudothecia which occur on crop residue and are dispersed by the wind to initiate the primary infection cycle (Hosford, 1971), while conidia are produced on the infected plants and dispersed by wind and rain to function as secondary inoculum (Hosford, 1972). Tan spot epidemics during the primary infection cycle in early spring can cause up to 17% of the total yield losses (Shabeer and Bockus, 1988). Conidia, as the secondary inoculum, produce the remainder of yield loss. Therefore, ascospores initiate early season disease

infection and conidia are mainly responsible for late season disease development and most of the yield loss.

Tan spot epidemics are largely dependent on temperature, humidity, and available initial inocula from crop residues left on the soil surface. The optimum temperatures for disease to develop are from 20ºC to 25ºC (da Luz and Bergstrom, 1986; Lamari and Bernier, 1994). Resistant reactions can be induced in susceptible cultivars due to loss of toxin activity when the temperature reaches 27ºC or higher (Lamari and Bernier, 1994). The optimum conditions for initial infection require 12 to 48 hours of leaf wetness for growth of *P. tritici-repentis* under favorable conditions, but the wet duration can be as short as 6 hours for establishment of an infection (Hosford et al., 1987). Prolonged leaf wet periods more than 48 hours can sometimes induce tan spot in resistant cultivars (Hosford, 1982). In fields, reduced tillage considerably increases the severity of the disease (Bockus and Claassen, 1992), and it can cause approximately 50% yield loss in highly susceptible cultivars (Riede et al., 1996).

Race Differentiation and Disease Quantification

Isolates of *P. tritici-repentis* differ in virulence (da Luz and Hosford, 1980). Lamari and Bernier (1989) developed a qualitative virulence analysis technique and initially grouped isolates of *P.tritici-repentis* into five races based on their virulence patterns on five differential hexaploid wheat genotypes (Table 1). Race 1 (nec+, chl+) caused both necrosis and chlorosis on susceptible host genotypes, race 2 caused necrosis only, race 3 and race 5 induced chlorosis on different wheat genotypes, and race 4 was avirulent on wheat. The five races have all been found in North America (Ali et al., 1999; Lamari et al., 2003), with races 1 and 2 being the most prevalent (Ali and Francl, 2003;

Lamari and Bernier, 1989; Lamari et al., 1998). Three additional races were discovered by Lamari et al. (2003), with race 6 combining the virulence of race 3 and race 5; race 7 combining the virulence of race 2 and race 5, and race 8 combining the virulence of races 2, 3 and 5.

For disease evaluation, several rating systems have been used to characterize reactions to *P. tritici-repentis.* For example, lesion size and percent infection (da Luz and Hosford, 1980; Nagle et al., 1982), lesion type (Gilchrist et al., 1984), and an index to combine lesion size, percent leaf area diseased (%LAD) and leaf location (Raymond et al., 1985). Lamari and Bernier (1989) later introduced a 1 to 5 rating scale on the bases of lesion types. Type 1 (resistant type) has small dark brown to black spots without any surrounding chlorosis or tan necrosis, type 2 (moderately resistant) has small dark brown to black spots with very little chlorosis or tan necrosis, type 3 (moderately resistant to moderately susceptible) has small dark brown to black spots completely surrounded by a distinct chlorotic or tan necrotic ring and the lesions are generally not coalescing, type 4 (moderately susceptible) has small dark brown to black sports completely surrounded by chlorotic or tan necrotic zones and some of the lesions are coalescing, and type 5 (highly susceptible) has dark brown to black centers that may or may not be distinguishable and most infected zones consist of coalescing chlorotic or tan necrotic lesions.

Table 1.1 A set of differential host used to differentiate the five races of *Pyrenophora tritici-repentis*

(Lamari et al., 1995; Lamari et al., 2003; Strelkov and Lamari, 2003; Strelkov et al., 2002)

Genotype	Race 1	Race 2	Race 3	Race 4	Race 5
Glenlea	necrosis[b]	necrosis	R[a]	R	R
Katepwa	necrosis	necrosis	R	R	chlorosis
Salamouni	R	R	R	R	R
6B365	chlorosis[c]	R	chlorosis	R	R
6B662	R	R	R	R	chlorosis

[a]R represents resistant reaction
[b]necrosis represents susceptible necrotic reaction
[c]chlorosis represents susceptible chlorotic reaction

Host-Selective Toxins

P. tritici-repentis produces toxins during infection. The toxins produced by certain races of the pathogen are host genotype specific, thus called host-selective toxins (HST), and responsible for inducing disease symptoms on susceptible wheat cultivars. To date, four HST, Ptr ToxA (Tomas and Bockus 1987; Tomas et al., 1990), Ptr ToxB (Orolaza et al., 1995; Strelkov et al., 1999), Ptr ToxC (Effertz et al., 2002) and Ptr ToxD (Ali et al., 2002; Manning et al., 2002), have been characterized in different races of *P.tritici-repentis*. All toxins are proteinaceous in nature except for ToxC which is a polar, nonionic, and low molecular weight compound.

Ptr ToxA

Lamari and Bernier (1989) reported the occurrence of a toxic compound within culture filtrates from isolates of race 1 and race 2, and suggested that the toxin of *P. tritici-repentis* was cultivar specific and a potential pathogenicity factor. The toxin was responsible for the development of the necrosis symptoms in susceptible wheat genotypes. Later, four research groups independently isolated and purified the necrosis toxin from culture filtrates of necrosis-inducing *P. tritici-repentis* isolates (Balance et al., 1989; Tomas et al., 1990; Tuori et al., 1995; Zhang et al., 1997) and described the toxin (designated as the Ptr necrosis toxin) as a large monomeric and heat-labile protein with a molecular weight of 13.9 kDa (Balance et al., 1989). The toxin induces necrosis on sensitive host tissue at a minimum active concentration of 0.2 nM. Tomas et al. (1990) further purified a 14.7 kDa heat-stable protein and designated it as Ptr toxin. This toxin

produced necrosis at a minimum active concentration of 90 nM. Later, Tuori et al. (1995) identified a 13.2 kDa heat labile protein, called Ptr ToxA, responsible for major necrosis and several other chromatographically and immunologically distinct toxins in less abundant amount. The Ptr ToxA induced necrosis in sensitive host tissue at a minimum active concentration of 60 nM. They further concluded that Ptr ToxA was most likely the same as Ptr toxin purified by Tomas et al. (1990), and the minor cationic toxins were most likely the same toxin as that purified by Balance et al (1989). It was hypothesized that multiple toxins might be produced by *P. tritici-repentis* in different amounts depending on the isolates used (Tuori et al., 1995). Balance et al. (1996) and Ciuffetti et al. (1997) independently cloned and sequenced the same toxin encoding gene, *ToxA*, and found that the gene could produce toxins with different biochemical properties but the same virulence through different post-translation processes (Ciuffetti and Tuori, 1999).

Infiltration of Ptr ToxA into sensitive host leaves alone resulted in tan spot symptoms (Balance et al., 1989), suggesting Ptr ToxA is more likely a major pathogenicity factor. A dominant gene, *Tsn1*, on the chromosome 5BL was identified to condition the sensitivity to Ptr ToxA (Anderson et al., 1999; Faris et al., 1996; Gamba et al., 1998). Further investigation revealed the interaction between Ptr ToxA and the product of *Tsn1* gene (Anderson et al., 1999). The insensitivity of a host to the toxin is due to lack of the toxin sensitivity gene in the host. In a susceptible host, the toxin sensitivity gene probably produces a host specific receptor or binding site for the toxins to cause tan spot symptoms, whereas in a resistant host, a host specific receptor can not be generated due to lack of the toxin sensitivity gene and this leads to a disruption of the signaling cascade required for toxin activity in the host. By assaying the role of wheat

metabolism in the host-pathogen interaction, Kwon et al. (1998) hypothesized that Ptr ToxA requires active transcription, translation and functional host H^+-ATPase enzymes for toxin activity.

By tagging Ptr ToxA with green fluorescent protein (GFP), Manning and Ciuffetti (2005) examined the different actions of Ptr ToxA in insensitive and sensitive wheat lines and found that Ptr ToxA was internalized into cells of toxin-sensitive lines but not the insensitive lines. The internalization may protect Ptr ToxA from being degraded by proteinase K in sensitive wheat cells. Therefore, *Tsn1*, the sensitivity gene, most likely behaves as a receptor and is responsible for the uptake of toxin into the cell.

Studies on the Stagonospora nodorum blotch pathogen suggested that Ptr ToxA is not the only toxin to interact with *Tsn1*. *S. nodorum* ToxA produced by *S. nodorum* has also shown interaction with *Tsn1*. An interspecific transfer of the *ToxA* gene from *S. nodorum* to *P. tritici-repentis* resulted in the emergence of tan spot symptoms (Friesen et al., 2006). Sequence analysis of *Ptr ToxA* and *S. nodorum ToxA* identified 99.7% homology between the two genes.

Ptr ToxB

Another type of HST is a chlorosis-inducing toxin(s). Orolaza et al. (1995) designated this toxin as Ptr ToxB which induces chlorosis in sensitive wheat cultivars. That culture filtrates from isolates of race 5 caused chlorosis symptoms on susceptible hosts, but an insensitive reaction on resistant hosts suggested Ptr ToxB as a pathogenicity factor (Orolaza et al., 1995). The same chlorosis toxin, Ptr ToxB, was isolated by Strelkov et al. (1999) and was characterized as a 6.61 kDa heat stable, hydrophilic and

monomeric protein. This toxin can induce chlorosis at a minimum active concentration of 14 nM.

Martinez et al. (2001) firstly cloned and characterized the *ToxB* gene from a North Dakota isolate of race 5, and found that *ToxB* is a gene with multiple-copies. Ten open reading frames (ORF) were identified in Algerian and North Dakota isolates of race 5, nine were cloned, and all had identical ORFs (Martinez et al., 2004; Strelkov et al., 2005), indicating recent duplication of the gene.

Similar to *Tsn1*, a single dominant gene, *Tsc2*, on chromosome 2BS was reported as the sensitivity gene to Ptr ToxB (Friesen and Faris, 2004). This gene accounts for 70% of the phenotypic variation for resistance to *P. tritici-repentis* race 5. The cloning of *Tsc2* may provide insight into understanding of interaction between *ToxB* and *Tsc2*.

Ptr ToxC

Another toxin produced by *P. tritici-repentis* race 1 has been reported to induce extensive chlorosis in the wheat genotype 6B-365 (Effertz et al., 1998). This toxin, designated as Ptr ToxC, is a polar, nonionic, low molecular weight molecule (Effertz et al., 2002). It showed similar pathogenicity as race3 and has been partially purified from culture filtrate of *P. tritici-repentis* race 1, but its chemical structure still has not been fully characterized.

Effertz et al. (2002) located the gene conferring insensitivity to partially purified Ptr ToxC, *tsc1*, to the short arm of chromosome 1A, the same chromosome location where a major quantitative trait loci (QTL) associated with resistance to extensive chlorosis caused by *P. tritici-repentis* race 3 was mapped (Faris et al., 1997). The results

suggest that Ptr ToxC produced by *P. tritici-repentis* race 1 may be the same as that by *P. tritici-repentis* race 3.

Ptr ToxD

Toxin Ptr ToxD was named independently by two research groups, but reported with different properties (Ali et al., 2002; Manning et al., 2002). Ali et al. (2002) reported that Ptr ToxD induced extensive chlorosis on a wheat genotype ND495 that showed insensitivity to Ptr ToxC, while Manning et al. (2002) described Ptr ToxD as a necrosis-inducing toxin. Further work is needed to resolve the discrepancy.

Inheritance of resistance to *P. tritici-repentis* races

Tan spot resistance has been reported as either monogeneic or polygenic inheritance in wheat. Nagle et al. (1982) evaluated percentage leaf area infected in six hexaploid and five tetraploid sources and concluded that the F_2 segregation did not fit simple Mendelian ratios. Rees et al. (1988) reported that more than four recessive genes might be involved in eight sources of resistance. Other studies also provide evidence to support polygenic control of tan spot resistance in bread wheat (Faris and Friesen, 2005; Friesen et al., 2003) and durum wheat (Elias et al., 1989).

In contrast, several studies suggested monogenic inheritance of resistance to tan spot. Lamari and Bernier (1991) reported that wheat resistance to two distinct tan spot symptoms, necrosis and chlorosis, were controlled by two independent genes. One single recessive gene was reported to be responsible for resistance to tan spot in diploid and tetraploid wheat (Sykes and Bernier, 1991), durum wheat (Lamari and Bernier, 1989), and hexaploid common wheat (Lee and Gough, 1984; Ma et al., 1998). Faris et al. (1997) further mapped a major QTL associated with resistance to extensive chlorosis on

chromosome 1AS which explained a large proportion of phenotypic variation. In addition, two recessive genes conferring resistance have also been reported in tetraploid (*T. timpheevi*) wheats (Ma et al., 1998) and hexaploid wheats (Sykes and Bernier, 1991).

Researches on characterization of HSTs produced by isolates of *P. tritici-repentis* and the relationship between tan spot resistance and insensitivity to HST have advanced the understanding of wheat-*P.tritici-repentis* host-pathogen system (Effertz et al., 2002; Friesen and Faris, 2004; Friesen et al., 2003; Lamari and Bernier, 1989; Orolaza et al., 1995; Tomas and Bockus 1987; Tuori et al., 1995). The gene(s) for host susceptibility to disease infection and sensitivity to HST are more likely the same and have been shown as dominant over that for resistance and insensitivity, respectively (Gamba and Lamari, 1998; Gamba et al., 1998; Lamari and Bernier, 1989; Lamari and Bernier, 1991; Orolaza et al., 1995; Stock et al., 1996). However, the gene for the susceptibility/sensitivity in pathogen /Ptr ToxA system is different from that in pathogen/Ptr ToxB system (Balance and Lamari, 1998). *Tsn1,* the dominant gene on chromosome 5BL, conditions sensitivity to Ptr ToxA (Anderson et al., 1999; Faris et al., 1996; Lamari and Bernier, 1989) and susceptibility to necrosis caused by isolates of races 1 and 2 (Cheong et al., 2004; Friesen et al., 2003; Lamari and Bernier, 1989), while *Tsc2,* the dominant gene on chromosome 2BS, controlled sensitivity to Ptr-ToxB, and susceptibility to extensive chlorosis was induced by isolates of race 5 (Friesen and Faris, 2004; Orolaza et al., 1995; Strelkov and Lamari, 2003). In addition to these two wheat-*P.tritici-repentis* pathosystems, *Tsc1*, the sensitivity gene to Ptr ToxC, has been mapped to the short arm of chromosome 1A (Effertz et al., 2002). Both *Tsc1* and *Tsc2* control resistance to extensive chlorosis (Gamba et al., 1998) but with independent functions. The resistance allele of *Tsc1* gene is

highly correlated with the effects of a major QTL for resistance to tan spot caused by *P. tritici-repentis* race 3, suggesting that insensitivity to Ptr ToxC is also associated with tan spot resistance (Effertz et al., 2002).

In addition to the race-specific genes/QTL, two race-nonspecific resistance QTL were also identified on chromosome 1BS and 3BL. These QTL showed resistance to most races identified to date including races 1, 2, 3 and 5 (Faris and Friesen, 2005). In this case, *Tsn1*-Ptr ToxA interaction might not be involved in the disease resistance (Friesen et al., 2002; Friesen et al., 2003; Riede et al., 1996; Singh et al., 2006), therefore QTL with a broad spectrum of resistance to different races might contribute to the resistance. The results also suggest that the wheat-*P.tritici-repentis* pathosystem may not simply follow a gene-for-gene model and be more complicated than expected.

Molecular mapping and QTL analysis in wheat

Molecular Markers

As rapid development of molecular technologies, molecular markers are becoming more and more important in wheat genetic research and breeding. Different types of DNA markers have been used for QTL mapping, trait tagging and marker-assisted breeding. Because conventional disease screening is laborious, time consuming, and ineffective for these traits with polygenic control and low genetic heritability (Wechter et al., 1995), DNA markers make it possible to dissect complicated traits into simple Mendelian traits and have been successfully used as biomarkers for marker-assisted selection (MAS), linkage map construction, and association mapping in many crop species. It can provide quicker, more reliable, earlier disease or other trait screening

than conventional methods and lay a solid foundation for map-based cloning of the genes/QTL of interest.

Commonly used DNA markers for linkage maps include restriction fragment length polymorphism (RFLP) (Botstein et al., 1980), random amplified polymorphic DNA (RAPD) (Williams et al., 1990), amplified fragment length polymorphism (AFLP) (Vos et al., 1995) and simple sequence repeat (SSR) (Beckman and Weber, 1992; Wang et al., 1994). Among them, SSR are the widely used markers for genetic mapping and MAS in wheat due to their high reproducibility, ease of use, high level of polymorphism, co-dominant nature, and high chromosome-specificity (Roder et al., 1998). An SSR consists of nucleotide repeats of 1-6 base pairs sequence in length. To date, more than 2000 SSR primers have been developed and most of them have been assigned to wheat chromosomes (Gupta et al., 2002; Guyomarc'h et al., 2002; Somers et al., 2004; Sourdille et al., 2004), and are rich sources of markers for wheat research.

Mapping populations

A population segregating for a desired trait can be developed from a bi-parental cross for construction of genetic linkage maps. Several types of populations can be used in genetic mapping, including F_2, backcross (BC_1), doubled haploid (DH) and recombinant inbred line (RIL) populations. Since many economically important traits in wheat are quantitative traits that are easily influenced by environment, repeated experiments in different years and locations may be required for accurately estimating phenotypic values. Genetically stable populations, such as DH and RIL populations are preferred to F_2 and BC_1 because the former populations are permanent and the same genotypes can be evaluated in multiple environments although it usually takes several

years to develop. DH population has the advantage of RIL populations because it can be generated more rapidly in 1-2 years. Both DH and RIL population have been extensively used in wheat genetic mapping (Chalmers et al., 2001; Groos et al., 2002; Liu et al., 2005; Paillard et al., 2003; Quarrie et al., 2005; Somers et al., 2004; Sourdille et al., 2003; Torada et al., 2006). Considering several rounds of recombination underwent during the process of RIL development and only one round of recombination in DH line population, RIL populations are better populations for mapping if available.

Genetic and physical mapping

The traits with categorical phenotype values and discrete distributions of phenotypes are qualitative traits and usually show single gene segregation ratios in a mapping population. Phenotypic data can be integrated with molecular markers to assemble into linkage groups. The genetic distances calculated from recombination frequency between markers or marker and traits are used to measure the distance among marker/traits (Lander et al., 1987). In a mapping study, increasing population size and the marker density both can enhance map resolution and increase the possibility of finding tightly linked markers to a trait. The tightly linked or co-segregated markers to a trait can be further used as useful tag in marker-assisted selection and map-based cloning.

In the past half century, researches on wheat cytogenetics have been advanced by exploiting the compensating ability of homoeologous chromosomes to develop aneuploid wheat genetic stocks such as monosomics, nulli-tetrasomics (NT), ditelosomics (DT), intervarietal disomic substitution (IDS) and deletion lines (Endo and Gill, 1996; Sears, 1954; Sears, 1966; Sears and Sears, 1978). Monosomic lines have been extensively employed to identify chromosomes carrying traits/genes of interest and to localize them

to a chromosome. NT and DT lines have been widely used to assign molecular markers such as RFLPs and SSRs to specific chromosomes and chromosome arms, respectively. Deletion lines have been extensively used to map genes or molecular markers to sub-chromosomal regions or bins (Faris and Gill, 2002; Gill et al., 1996; Sandhu et al., 2001; Sourdille et al., 2004; Sutka et al., 1999).

QTL analysis

Many economically important traits are quantitative traits that show continuous distributions of phenotype values in a segregating population and are usually controlled by multiple loci or quantitative trait loci (QTL). Several methods have been developed for QTL analysis. The earliest and simplest method is single-marker analysis that assesses association between markers and the quantitative trait of interest. Although single marker analysis has been successfully used to identify a potential QTL and roughly estimate its effect, it is unable to determine the relative location of a QTL in a chromosome and potential recombination frequency between the marker and QTL.

Interval mapping (IM) is another method to analyze QTL which utilizes all markers information of a linkage group to calculate the logarithm of the odds (LOD) score, permitting the detection of QTL in each marker interval (Lander and Botstein, 1989). The LOD score predicts the most plausible position of the QTL within the interval by detecting if the maximized LOD score exceeds the threshold value. This method can predict the number, effect and location of QTL, but may not be able to separate a ghost QTL from a real QTL due to QTL effects from the neighboring region**.**

To compensate the disadvantage of IM, composite interval mapping (CIM) introduced by Zeng (1994), uses additional markers as cofactors to remove the impact

from other QTL in the genome. This method also can detect QTL with minor effects. Despite of higher accuracy in CIM than IM, results from both mapping methods are usually reported in many QTL mapping studies. In either method, usage of a correct threshold to declare significant QTL is very important. Permutation test has been widely accepted for determination of the threshold value (Churchill and Doerge, 1994). In this method, trait data are repeatedly shuffled, or assigned at random to the individuals in the population. At each iteration, the chosen QTL-mapping method is recomputed and the maximum value for the test statistic is recorded. Finally these maximum values are sorted and a threshold declared as the percentile above which only a chosen proportion of scores fall: say the 95th, for a 0.05 significance threshold. The method works for any distribution of the phenotype, including non-normal distribution.

The power of QTL detection could be affected not only by changing statistical methods used for QTL analysis, but also by other experimental factors, such as population type and size and the number of observations per genotype. Accurate estimation of phenotype data from repeated experiments could greatly enhance the reliability of the QTL detected by the statistical methods.

CHAPTER 2 - Identification of QTLs associated with resistance to *Pyrenophora tritici-repentis* race 1

Introduction

Tan spot is one of the most important foliar diseases of wheat in the Great Plains of the United States. Several strategies have been proposed for reducing yield losses caused by tan spot, including burying or destroying crop residues (Bockus and Shroyer, 1998), nonhost species rotations (Bockus and Claassen, 1992), and foliar fungicide application. However, usage of host resistance is a preferred approach when farmers want to grow wheat under reduced tillage to conserve soil and soil moisture with minimum investment in fungicide application. Currently used cultivars cannot completely eliminate the damage caused by the disease (Bockus et al., 2001). Therefore, new sources of resistance to *P. tritici-repentis* need to be identified for genetic improvement of commercial cultivars.

Resistance to tan spot has been reported to cosegregate with insensitivity to host selective toxins (HST) produced by *P. tritici-repentis* races (Gamba et al., 1998). Currently, eight races have been defined based on their virulence patterns on five differential hexaploid wheats and the specific HST they produced (Andrie et al., 2007; Lamari et al., 2003). Among four known HST, Ptr ToxA is the best characterized toxin and produces necrosis symptoms on the leaves of susceptible wheat (Balance et al., 1989; Tomas et al., 1990). A single recessive gene, *tsn1*, was discovered to confer insensitivity to Ptr ToxA and located on chromosome 5BL in hexaploid wheat (Faris et al., 1996; Stock et al., 1996). Although both Ptr ToxB and Ptr ToxC produce extensive chlorosis on

susceptible wheat, they have different chemical characteristics. Ptr ToxB is a 6.6 kDa protein and a recessive gene, *tsc2*, on the short arm of chromosome 2B controls insensitivity to the toxin (Friesen and Faris, 2004; Strelkov et al., 1999). While Ptr ToxC is a nonionic, polar, low molecular weight molecule, and specifically interacts with *tsc1*, a recessive insensitivity gene on chromosome 1AS (Effertz et al. 2002).

Currently, sources of resistance to tan spot have been mainly found in American and European cultivars. The ITMI population derived from the cross between W-7984 (synthetic hexaploid wheat) and 'Opata 85' a CIMMYT-bred hard red spring wheat (P1591776) has been extensively used for mapping tan spot resistance genes (Effertz et al., 2002; Faris et al., 1997; Faris et al., 1999; Friesen and Faris, 2004). Furthermore, Singh et al. (2008) detected novel resistance QTL in Indian lines. However, there are no reports about detection of novel QTL in Chinese landraces. In this study, we develop a recombinant inbred lines (RIL) population from the cross between the Chinese landrace Wangshuibai (WSB) and Ning7840 to explore the genetic sources of resistance to *P. tritici-repentis* race 1, which produces Ptr ToxA and Ptr ToxC and is the most prevalent race in the North American (Ali and Francl, 2003).

Materials and Method

Plant materials and disease evaluation

A population of 288 $F_{2:6}$ recombinant inbred lines (RILs) was developed from the cross between Chinese landrace WSB and Ning7840 by single seed descend. WSB is resistant to *P. tritici-repentis* race 1 and Ning7840 is highly susceptible. WSB had a similar leaf area diseased (54% LAD) to the resistant check Karl 92 (55% LAD) and Ning7840 had a similar LAD (84%) to the susceptible check TAM 105 (88% LAD)

(Table 1). A subset of 96 RI lines was randomly selected for preliminary QTL scan. To evaluate tan spot resistance, all RILs, along with the two parents and two checks, were planted in a rack containing 100 RLC4 66-ml plastic cone tubes (Stuewe and Sons, Corvallis, OR) filled with a mixture of steamed soil:vermiculite (50/50) with a cotton ball plugged in the bottom of each tube. One seed per RIL or cultivar was planted in each tube and the experiment was arranged in a randomized-complete-block design with 20 blocks (racks) (Bockus et al. (2007). Due to limitation in mist chamber space, four racks were planted each day for five consecutive days. To further validate the initial QTL mapping result, the remainder of the 192 RILs were phenotyped in the same way as described for the initial population but with 10 replicates per RIL.

Plants were grown under light for 12 h at 25ºC and darkness for 12 h at 21ºC. At the four-leaf stage (about four weeks after planting), plants were inoculated with a spore suspension (~5,000 spores/ml) from the isolate AZ-00 of *P. tritici-repentis*. The isolate AZ-00 belongs to *P. tritici-repentis* race 1 (Andrie et al., 2007). Spores were produced by transferring a small disc of mycelium plugs of the fungus from a 1/4 potato-dextrose agar (PDA) plate to the center of V-8 agar plates (150 ml V-8 juice, 3 g $CaCO_3$, 15 g agar, 850 ml water), flattening aerial hyphae with a sterile, bent-glass rod around the perimeter when the colony reached about 4-5 cm in diameter (about 5 days in the dark at 21- 24ºC). the plates were placed at 21-24ºC for 12 h under light (about 40 cm below four fluorescent tubes) followed by 12 h dark at 16ºC. Spores were harvested by flooding plates with distilled water, scraping the surface of the colony with a fungal transfer spatula, pouring and rinsing the suspension through one layer of cheesecloth into a container, and diluting to the desired concentration with distilled water. A DeVilbis

atomizer (Micromedics Inc., St. Paul, MN) connected to an air compressor was used to uniformly apply 35 ml of the suspension to the seedlings in each rack. After inoculation, the plants were immediately placed in a mist chamber with 100% relative humidity created by a cool humidifier for 48 h at 20 to 28ºC with a 12-h photoperiod. After the mist period, plants were returned to the original greenhouse benches. Seven days after inoculation, the bottom three leaves of each plant were scored for the percentage leaf area with necrosis and/or chlorosis, and an average %LAD across three leaves of the same plant was used as the overall disease score for the plant. The scores from all plants in each genotype were combined for QTL analysis in each experiment.

Analysis of variance was conducted by using the PROC GLM procedure of SAS (SAS Institute, Inc. Version 9.1) with an average %LAD across 20 plants in the first or 10 plants per line in the second experiment. Fisher's least significant difference (LSD) at $\alpha = 0.05$ was performed to separate the genotype means.

Toxin infiltration

Toxin Ptr ToxA was extracted from *P. tritici-repentis* race 1 (isolate Pt-1c) as described by Tomas et al. (1990). After wheat plants were scored for tan spot symptoms, approximately 100 µl Ptr ToxA was infiltrated into the middle of the youngest fully expanded leaves of the two parents and two checks using a Hagborg device (Hagborg, 1970). The water-soaked area of the infiltrated region was immediately delimited with a permanent felt marker. Three to five days after infiltration, presence or absence of tan necrosis on the infiltrated leaves was recorded to reflect sensitive (+) or insensitive (-) to Ptr ToxA, respectively. Infiltrations were performed in at least three plants per genotype.

Marker analysis

One-week-old leaf tissue was collected in 1.1-ml 8-strip tubes, dried for two days in a freeze drier (Thermo Fisher, Waltham, MA), and ground to fine powder in a Mixer Mill (Retsch GmbH, Rheinische Strasse 36, Germany) by shaking strip tubes with a 3.2-mm stainless-steel bead at 25 times/sec for 5 min. Genomic DNA was extracted from parents and RILs by using cetyltrimethyl ammonium bromide (CTAB) method (Saghai-Maroof et al., 1984). PCR amplifications were performed in a Tetrad Peltier DNA Engine (Bio-Rad Lab, Hercules, CA). A 12-µl PCR mixture contained 1.2 µl of 10X NH_4 buffer (Bioline Inc. Taunton, MA), 2.5 mM of $MgCl_2$, 200 µM of each dNTP, 100 nM of forward tailed primer, 200 nM of reverse primer, 100 nM of M13 fluorescent-dye labeled primer, 1 U of *Taq* DNA polymerase, and 50 ng template DNA. A modified touchdown program (Ma et al., 2005) was used for amplification of PCR. In brief, the reaction was incubated at 95ºC for 5 min then continued for five cycles of 1 min of denaturing at 96ºC, 5 min of annealing at 68ºC with a decrease of 2ºC in each of subsequent cycles, and 1 min of extension at 72ºC. For another five cycles, the annealing temperature started at 58ºC for 2 min with a decrease of 2ºC for each subsequent cycle. PCR went through an additional 25 cycles of 1 min at 96ºC, 1 min at 50ºC, and 1 min at 72ºC with a final extension at 72ºC for 5 min. PCR products were separated in an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA) and data were scored using GeneMarker version 1.6 (SoftGenetics LLC. State College, PA)

Bulked segregant analysis was used for screening polymorphic SSRs associated with tan spot resistance. Equal amounts of DNA were pooled separately from five resistant and five susceptible RILs. A total of 1500 microsatellite markers (SSR) covering

all 21 wheat chromosomes (Roder et al., 1998; Somers et al., 2004) were screened between the parents and between two bulks. Polymorphic markers between the bulks were further analyzed for linkage mapping and QTL analysis in the populations.

Linkage and QTL analysis

Linkage map was constructed by using JoinMap program version 3.0 (Van Ooijen and Voorrips, 2001). LOD threshold value was set at 3.0 for grouping linked markers in linkage groups. Ripple was performed each time after adding a new marker. Kosambi (1944) centimorgans (cM) was used to convert recombination frequencies into genetic distance.

Combined means were used for interval mapping (IM) and composite interval mapping (CIM) by using QTL Cartographer V2.5 (Wang et al., 2007). Composite interval mapping was implemented by using the standard model 6, starting with default values of five cofactors obtained by a forward regression to control genetic background. A window size of 10 cM was used to block a chromosome region between the markers flanking the test site. In both IM and CIM, the walking speed for genome wide QTL scan was set at 1.0 cM and the LOD thresholds to declare a significant QTL were determined based on the result of 1000 permutations. For each QTL, coefficient of determination (R^2), which was the proportion of total phenotypic variance explained by a QTL, was determined based on the R^2 for the single marker that was the closest to the target QTL. The total R^2 that represents the phenotypic variation explained by the model was calculated through multiple linear regressions using SAS REG procedure. All loci that had significant main effects were tested against all other markers to detect significant interactions ($P < 0.01$).

Results

Reactions of the RILs to isolate AZ-00 and toxin bioassay

All parents, their RILs in WSB/Ning7840 population, and two checks were inoculated with conidia of AZ-00 (nec+ chl+). The reactions of both parents to isolate AZ-00 were not significantly different from their corresponding resistant or susceptible checks (Table 2.1). The RILs segregated for reactions to isolate AZ-00. Resistant genotypes developed small dark-brown spots with no or little encompassing chlorosis, while susceptible ones had extensive chlorosis over the entire leaf. Some RILs showed moderate resistance or susceptibility with about 60%-70% LAD. Frequencies of %LAD in the population showed continuous, but bimodal distributions with two major peaks, indicating that a QTL with a major effect may contribute to tan spot resistance in the population and other QTL with minor effects and environmental factors may also be involved in the population (Figure 2.1).

In the toxin infiltration assay, phenotypes of susceptibility to ToxA were observed on leaves of the susceptible check TAM 105, but not on the resistant check Karl 92. However, no lesions typical of toxin susceptibility appeared on the parents WSB and Ning7840 (Table1), suggesting that ToxA was not the virulence factor responsible for the tan spot symptoms in Ning7840.

Initial QTL localization in WSB

After 1500 SSR markers were screened, 169 were polymorphic between parents and 58 were polymorphic between bulks, therefore, they were used for screening the initial population for linkage mapping. Linkage analysis identified nine linkage groups (Data not shown). Further QTL scan using the linkage maps and %LAD means across 20

plants identified three and two QTL on two chromosomes by IM and CIM methods, respectively. IM detected two QTL with a major effect (R^2=0.56 and 0.74) on the short arm of chromosome 1A. These QTL were flanked by markers *Xcfa2153* and *Xgwm33,* and by *Xwmc818* and *Xbarc148*, respectively (Table 2.2), but only the QTL linked to *Xcfa2153* was detected by CIM, and designated as *QTs.ksu-1AS*. This QTL explained up to 40% of phenotypic variation for tan spot resistance. A third QTL with a minor effect was detected on 2BS, and designated as *QTs.ksu-2BS*. This QTL was flanked by *Xbarc7* and *Xbarc55*, and explained 13% and 10% phenotypic variation by IM and CIM, respectively (Table 2.2). A slight difference in QTL locations was observed between IM and CIM. *Xbarc7* was the closest marker to the QTL in IM and *Xbarc55* was the closest marker in CIM. In either case, alleles decreasing the %LAD were from the resistant parent WSB. Since CIM reduces the genetic noise and enhances the QTL detection power by incorporating cofactors in the model, the confidence intervals determined by the drop of the LOD value around the maximum LOD value tend to be smaller in CIM than in IM (Table 2.2). For this reason, the results from CIM were used for further data interpretation.

Validation of QTL in a large population

An additional population of 192 F_6 RILs from the same cross was used to validate the results derived from the initial mapping population. All markers linked to QTL on chromosome 1AS and 2BS were screened on the population. Both *QTs.ksu-1AS* and *QTs.ksu-2BS* were significant in IM and CIM. The *QTs.ksu-1AS* explained about 39% and 40% phenotypic variation in both IM and CIM (Table 2.2, Figure 2.2a). The *QTs.ksu-2BS* explained 5% and 4% phenotypic variation in IM and CIM, respectively

(Table 2, Figure 2.2b). All alleles decreasing the %LAD were from resistant parent WSB. Both markers *Xcfa2153* and *Xbarc7* were assembled into multiple regression models and together explained 39% of the phenotypic variation for resistance to isolate AZ-00 and interaction between the two loci was not significant (Table 2.4). The markers *Xcfa2153* and *Xbarc7* associated two loci had significant effects for the %LAD means (Table 2.2), indicating the significant differences in disease development among RI lines that have only WSB alleles of marker *Xcfa2153* or *Xbarc7* compared to those that have Ning7840 alleles of both markers as described below and shown in Table 2.3.

Two marker loci closely linked to the two QTL were chosen to estimate their effectiveness for marker-assisted selection (Table 2.3). The replacement of the Ning7840 allele of *Xcfa2153* with the corresponding WSB allele led to an 18% decrease in mean %LAD and the replacement of the Ning7840 allele of *Xbarc7* with the corresponding WSB allele led to a 5.4% decrease in mean %LAD. When both markers alleles of Ning7840 were replaced by corresponding WSB alleles, the mean %LAD decreased 21.4%. The results indicated that marker-assisted selection for the two QTL using a single marker per QTL can significantly decrease %LAD and *QTs.ksu-1AS* is likely the major QTL responsible for tan spot resistance in WSB.

Discussion

Tan spot is one of the important foliar diseases of wheat in the Great Plains of the United States, and yield losses of up to 50% have been reported (Riede et al., 1996). Breeding for tan spot resistance has been regarded as the most economic approach for disease control. In the last two decades, resistance to tan spot has been reported to be under either polygenic (Cheong et al., 2004; Elias et al., 1989; Faris and Friesen, 2005;

Faris et al., 1997; Friesen and Faris, 2004) or oligogenic control (Duguid and Brûlé-Babel, 2001a; Duguid and Brûlé-Babel, 2001b; Gamba and Lamari, 1998; Gamba et al., 1998; Lamari and Bernier, 1989; Lamari and Bernier, 1991; Singh and Hughes, 2005; Tadesse et al., 2007). Currently, QTL associated with resistance to tan spot race 1, 2, 3 and 5 have been reported (Effertz et al., 2001; Faris et al., 1996; Faris et al., 1997; Friesen and Faris, 2004). In this study, we identified two quantitative trait loci (QTL), *QTs.ksu-1AS* and *QTs.ksu-2BS,* associated with tan spot resistance to *P. tritici-repentis* race 1 (isolate AZ-00) in a recombinant inbred population derived from two Chinese landraces WSB and Ning7840.

Although many environmental factors may influence tan spot symptom development, testing of seedlings in a greenhouse is highly repeatable if proper experimental design is employed. The randomized complete block design deployed in this study controlled experimental variation by explaining spatial and temporal effects. Analysis of variance has shown that the %LAD means of resistant and susceptible checks generated from two experiments are not significantly different (data not shown). Thus, with no significant experiment by treatment interactions, the disease data from two experiments can be combined for further QTL analysis.

The effects of *QTs.ksu-1AS* and *QTs.ksu-2BS* varied with population size and mapping methods used (Table 2.2). Markers *Xcfa2153* and *Xbarc7* are the two markers closest to *QTs.ksu-1AS* and *QTs.ksu-2BS,* respectively. An additional QTL was detected on 1AS beside *QTs.ksu-1AS* by IM in the initial population of 96 RILs (Table 2.2). However, this QTL disappeared for IM in the large population and was not detected by CIM in either population. Thus this QTL could be a ghost QTL. In the initial mapping,

inconsistent results were obtained using CIM and IM, e.g. the phenotypic variations and confidence intervals of two QTL (Table 2.2). However, the differences between IM and CIM were trivial when population size increased from 96 to 288 RILs, suggesting that the QTL detection power may increase with the population size and a large population significantly improves precision for QTL detection. CIM appears to be more reliable in predicting QTL because it considers the background effect by taking cofactors into account, with which the total variance that is determined by other linked QTL is reduced, therefore increasing the relative variance explained by the target QTL.

To date, four HST, Ptr ToxA (Tomas and Bockus 1987; Tomas et al., 1990), Ptr ToxB (Orolaza et al., 1995; Strelkov et al., 1999), Ptr ToxC (Effertz et al., 2002) and Ptr ToxD (Ali et al., 2002; Manning et al., 2002), have been characterized in different races of *P. tritici-repentis*. *P. tritici-repentis* race 1 produces both ToxA and ToxC during infection. In the present study, the toxin infiltration results excluded Ptr ToxA as the toxin responsible for tan spot resistance segregation in the current mapping population. Meanwhile, the population segregated for extensive chlorosis which is the typical symptom caused by Ptr ToxC (Lamari and Gilbert, 1998). Therefore, Ptr ToxC was most likely the toxin responsible for tan spot resistance segregation in WN population.

Genetic and mapping studies have revealed a major QTL (*QTsc.ndsu-1A*) and an insensitivity gene (*tsc1*) on chromosome 1AS associated with resistance to chlorosis caused by *P. tritici-repentis* race 1 and insensitivity to partially purified chlorosis toxin, Ptr ToxC, respectively, in the ITMI population (Effertz et al., 2002; Faris et al., 1997). A RFLP marker *XGli1* was identified to be closely linked to the resistance QTL and had a distance of 5.7 cM to the insensitivity gene. The QTL *QTs.ksu-1AS* was mapped to a

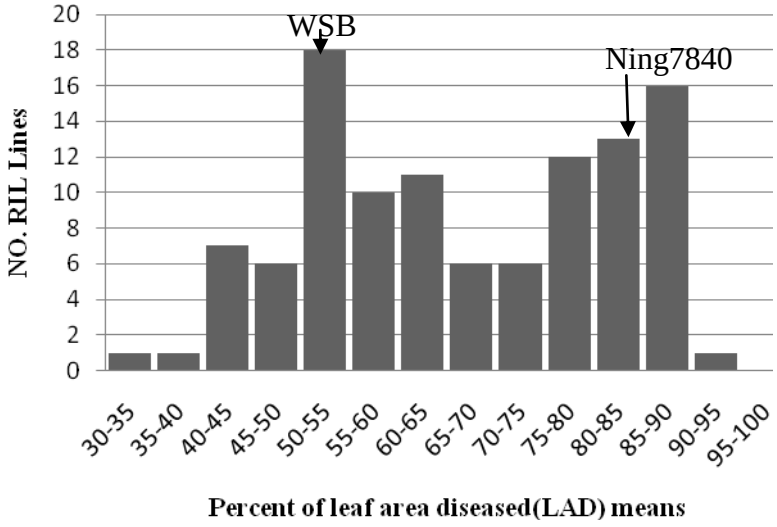
similar position as that for *QTsc.ndsu-1A*. After comparing our map (Figure 2.2a) with the one generated by Faris et al. (1997) and others (Roder et al., 1998; Somers et al., 2004; Sourdille et al., 2004), SSR markers *Xgwm136*, *Xgwm33* and *Xcfa2153* reside in the region flanked by RFLP markers *XksuD14.1* and *XksuD14.2* at the distal end of chromosome 1A (data not shown). Therefore, the two QTL are most likely the same QTL mapped in different populations. Furthermore, Chu et al. (2006) mapped the *Ne2* gene on chromosome 2BS with a distance of 3.2 cM away from marker *Xbarc55*, which was in the vicinity of *QTs.ksu-2BS* in this study. However, *Ne2* gene is believed to interact with *Ne1* which is a complementary gene on chromosome 5BL for controlling hybrid necrosis (Chu et al., 2006). In this study, hybrid necrosis was not observed in the WN population, and *Ne2* has not yet been found to be associated with resistance to chlorosis caused by *P. tritici-repentis* race 1, therefore, *QTs.ksu-2BS* may not be the same gene as *Ne2*.

The results of this study could facilitate marker-assisted selection to use the tan spot resistance QTL from chromosome 1AS and 2BS in breeding programs. SSR markers *Xcfa2153* and *Xbarc7*, which are closely linked to *QTs.ksu-1AS* and *QTs.ksu-2BS*, respectively, are highly polymorphic in a collection of germplasm (Sun unpublished data) and could be readily used for marker-assisted breeding. Since *Xcfa2153* is mapped in a similar location as *XGli1*, it can also be used to screen for the *tsc1* gene. Primer CFA2153 amplified a 212-bp fragment in WSB and a 218-bp fragment in Ning7840. And Primer BARC7 amplified a 293-bp and a 288-bp fragment in WSB and Ning7840, respectively. Breeders could choose to select the allele from a resistance parent or select against the allele from a susceptible parent to increase the level of tan spot resistance. Furthermore, we have mapped the *QTs.ksu-1AS* within a relatively small interval of approximately 3

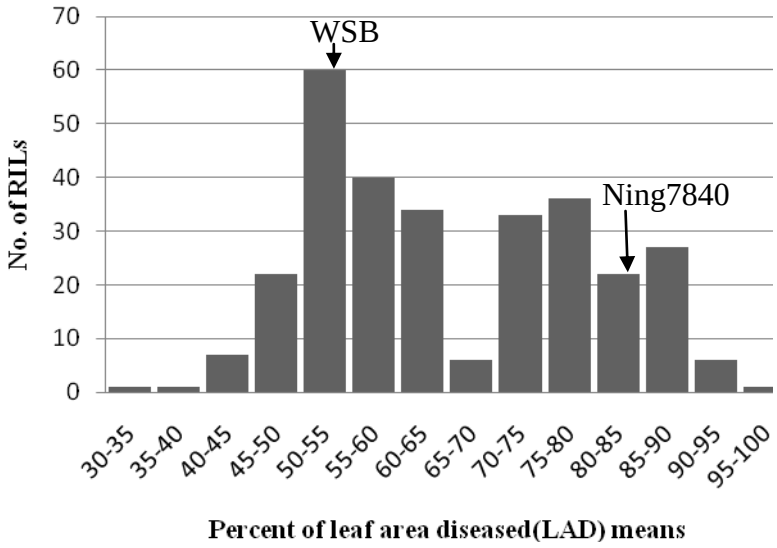
cM, which should help for further high-resolution mapping, assuming that there are enough polymorphic markers at the distal end of chromosome 1AS.

Figure 2.1 Distribution of recombinant inbred lines (RIL) in a WSB/Ning7840 population using percentage diseased leaf area (%LAD) means caused by *Pyrenophora tritici-repentis* race 1.

(a) Percentage diseased leaf area means across the subset of WN population; (b) Percentage diseased leaf area means across the entire WN population.



(a)



(b)

Figure 2.2 Composite interval mapping (CIM) of QTLs associated with tan spot resistance contributed by WSB.

The positions of markers and centimorgan (cM) distances between loci are shown to the right of the linkage maps. The vertical solid line represents the logarithm of the odds (LOD) significance threshold of 2.5. The LOD, additive effect and R^2 values for each QTL are listed in Table 2.2.

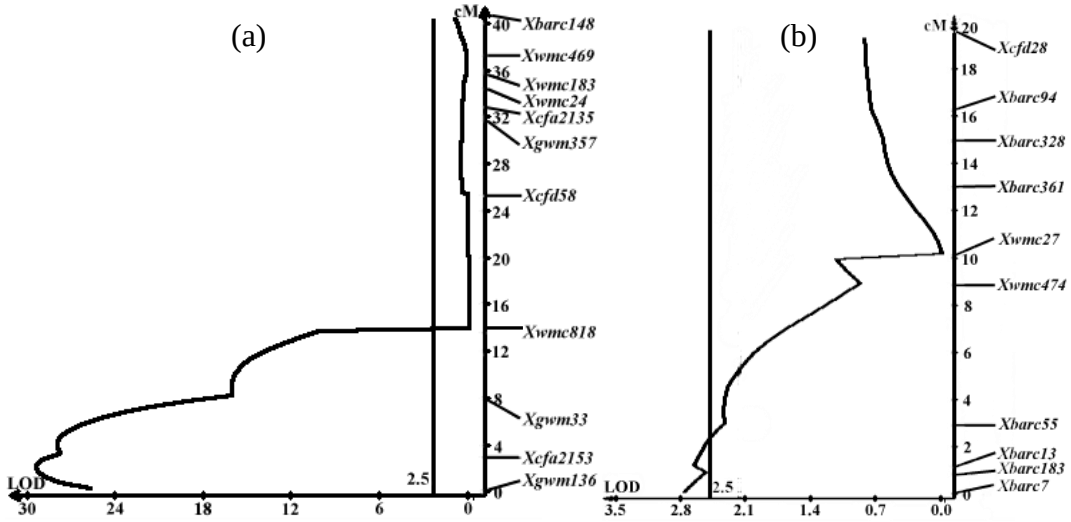


Table 2.1 Range and means of percent leaf area diseased after inoculation with *Pyrenophora tritici-repentis* race 1 and reaction to toxin infiltration for two parents and two check cultivars.

	Leaf area diseased range (%)	Leaf area diseased means (%)	ToxA reaction
WSB	47.9-64	53.9±4.1	Negative
Ning7840	70.3-86.3	83.8±4.1	Negative
Karl92	46.6-62.7	54.7±4.1	Negative
TAM105	79.6-95.7	87.7±4.1	Positive
LSD (α=0.05)		11.1	

Table 2.2 Interval mapping (IM) and Composite interval mapping (CIM) analysis of QTLs associated with resistance to race 1 of *Pyrenophora tritici-repentis*.

QTL	Method	Chr[a]	Position (cM)	Marker[b]	LOD	Additive Effect	Threshold (5%, 1%)	R^2	LOD1 Interval (cM)[c]	LOD2 Interval (cM)
Initial population (96 RILs)										
QTs.ksu-1AS	IM	1AS	3.4	*Xcfa2153*	15.5	-11.6	2.2, 3.6	0.56	1.8-4.4	1.1-4.4
		1AS	16.7	*Xwmc818*	7.7	-13.3	2.2, 3.6	0.74	14.7-19.2	14.1-20.3
	CIM	1AS	3.4	*Xcfa2153*	11.9	-11.5	2.2, 3.2	0.40	0-10	0-16.6
QTs.ksu-2BS	IM	2BS	0.0	*Xbarc7*	3.2	-5.7	2.2, 3.6	0.13	0-2.5	0-8
	CIM	2BS	2.9	*Xbarc55*	4.1	-8.2	2.2, 3.2	0.10	1.2-6.2	0.8-6.9
Full size population (288 RILs)										
QTs.ksu-1AS	IM	1AS	2.0	*Xcfa2153*	28.9	-8.9	1.6, 2.4	0.40	0.8-2.8	0.4-3.1
	CIM	1AS	2.0	*Xcfa2153*	29.5	-8.9	1.6, 2.5	0.39	0.8-2.6	0.5-3.1
QTs.ksu-2BS	IM	2BS	0.0	*Xbarc7*	2.5	-2.9	1.6, 2.4	0.05	0-2.7	0-2.7
	CIM	2BS	0.0	*Xbarc7*	2.8	-3.0	1.6, 2.5	0.04	0-2.7	0-2.7

[a] Chr and Position refer to the chromosome and map position of the QTL corresponding to the maximum LOD score for IM and CIM.

[b] The closest markers to the LOD peaks are given.

[c] LOD1 and LOD2 are confidence intervals for the position of each QTL estimated by a drop of 1 and 2, respectively, from the maximum LOD score.

Table 2.3 Number of observations and percent lead area diseased (%LAD) means of RILs of the WSB/Ning7840 population for the four allelic state combinations for markers *Xcfa2153* and *Xbarc7* after inoculation with conidia of *Pyrenophora tritici-repentis* race 1.

Xcfa2153	*Xbarc7*	N[a]	%LAD means[b]
WSB	WSB	69	55.6a*
NING	WSB	53	71.6b
WSB	NING	69	59.0a*
NING	NING	61	77.0c

LSD (P=0.05) = 3.92

[a]N represents Number of observations.

[b]Means with the same letter are not significantly different.

* Significantly different at P=0.075 level.

Table 2.4 Analysis of variance for percent leaf area diseased estimated from greenhouse experiments conducted in Manhattan, KS.

R^2=0.39

Source	DF[a]	SS	MS	F value	Pr > F
Model	3	19655.26279	6551.75426	53.11	<.0001
Xcfa2153	1	17980.47711	17980.47711	145.75	<.0001
Xbarc7	1	1197.17395	1197.17395	9.7	0.0021
*Xcfa2153 * Xbac7*	1	66.66608	66.66608	0.54	0.463
Error	248	30594.71068	123.36577		
Corrected total	251	50249.97347			

[a] Abbreviations: DF = degree of freedom, SS = Sum of Squares, MS = Mean square.

CHAPTER 3 - Genetic analysis of susceptibility to extensive chlorosis caused by *Pyrenophora tritici-repentis* race 1 in Chinese accessions of common wheat

Introduction

As discussed in Chapter 1, different from ToxA, ToxC is not a protein or a stable molecule, therefore purification of Ptr ToxC is difficult. A previous study suggested that sensitivity gene *Tsn1* was most likely involved in protein import to govern the sensitivity to ToxA (Manning and Ciuffetti, 2005). The wheat sensitivity gene (*Tsc1*) to ToxC might be different, and the interaction between *Tsc1* and ToxC may be more complicated than that between *Tsn1* and ToxA. However, to date, only a few reports mapped insensitivity genes to Ptr-ToxC (Effertz et al., 2002). The interaction between sensitivity genes and Ptr ToxC has not been reported. The objectives of this study were to investigate the interaction between the host sensitivity gene(s) and race 1 of the pathogen through QTL mapping and to test the inverse gene-for-gene interaction hypothesis in the wheat-*Pyrenophora tritici-repentis* pathosystem.

Materials and Method

Plant materials and disease evaluation

Five wheat accessions including two Chinese resistant landraces ‘WSB’ and ‘HYZ’, one Korean moderately resistant cultivar ‘Chokwang’, one Chinese susceptible line ‘Ning7840’, and one US susceptible cultivar ‘Wheaton’ were selected as parents to develop four mapping populations for this study based on their reactions to race 1 of *P.*

tritici-repentis (Table 3.1). Four populations of recombinant inbred lines (RILs) were created by crossing a resistant parent to a susceptible parent and advancing generations by using single seed decent. The population WSB/Ning7840 (WN) consisted of 288 $F_{2:6}$ RILs; the population of Chokwang/Ning7840 (CN) consisted of 96 $F_{2:6}$ RILs; the population WSB/Wheaton (WW) had 138 $F_{2:6}$ RILs (Yu et al., 2008); and the population HYZ/Wheaton (HW) had 96 F_6 RILs. All populations were evaluated for tan spot severity in an independent experiment according to Bockus et al. (2007) using two US hard winter wheat cultivars Karl 92 and TAM 105 as resistant and susceptible checks, respectively. In each experiment, one population, including their parents and two checks, was arranged in a randomized-complete-block design in a greenhouse at Kansas State University, Manhattan, KS, with 20 blocks for populations CN and WW and 10 blocks for WN and HW. An additional 380 accessions from different wheat grown regions of the USA and several other countries were also evaluated for tan spot resistance and toxin reaction using the same design with 10 blocks. Due to space limitation in the mist chamber, only four racks of 100 entries were planted each day. In each block, a single high quality seed was planted in each RLC4 66 ml plastic tube (Stuewe and Sons, Corvallis, OR) filled with a 50:50 mixture of steamed soil:vermiculite. A cotton ball was placed in the bottom of each tube to prevent soil leaking from the holes at the bottom of tube.

Plants were grown at 25ºC for 12 h under light and 21ºC for 12 h in dark prior to inoculation. Four weeks after planting, a spore suspension (~5,000 spores/ml) of the isolate AZ-00 of *P. tritici-repentis* was used to inoculate plants at the four leaf stage. The isolate AZ-00 was an isolate of race 1 based on race differential assay (Andrie et al.,

2007). Spores were produced by inoculating a small disc of one-fourth strength potato-dextrose agar containing fungal mycelium to the center of V-8 agar plates (150 ml V-8 juice, 3 g $CaCO_3$, 15 g agar, 850 ml water) and incubating in the dark at 21-24°C. Aerial hyphae in the plate was flattened with a sterile, bent-glass rod around the perimeter when the colony reached about 4-5 cm in diameter (about 5 days), and the plates were placed at 21-24ºC for 12 h under light (about 40 cm below four fluorescent tubes) and at 16ºC for 12 h in dark. Spores were harvested by flooding plates with distilled water, scraping the surface of the colony with a spatula, pouring and rinsing the suspension through one layer of cheesecloth into a container and diluting to the desired concentration with distilled water. A DeVilbis atomizer (Micromedics Inc., St. Paul, MN) connected to an air compressor was used to uniformly apply 35 ml of the suspension to each rack. The inoculated plants in the racks were immediately placed into a mist chamber with a cool humidifier to maintain 100% relative humidity for 48 h at 20-28ºC with a 12-h photoperiod. After the mist period, plants were returned to the greenhouse bench. Seven days after inoculation, the bottom three leaves of each plant were scored for the percentage leaf area diseased (%LAD) with necrosis and/or chlorosis symptoms and a mean %LAD across three leaves of the same plant was used as the overall disease score for the plant. The scores from 10 (WN, HW and 380 natural populations) or 20 (WW and CN populations) individual plants per genotype were used individually for statistical analysis and the overall mean for a genotype was used for QTL analysis.

Toxin infiltration

The toxin Ptr ToxA was extracted from *P. tritici-repentis* race 1 (isolate Pt-1c) as described by Tomas et al. (1990). After wheat plants were scored for tan spot symptoms,

approximately 100 µl Ptr ToxA was infiltrated into the middle of the youngest fully expanded leaves of the two parents and two checks using a Hagborg device (Hagborg, 1970). The edges of the infiltration spot were immediately delimited with a permanent felt marker. Three to five days after infiltration, absence or presence of tan necrosis on the infiltrated areas was scored to reflect insensitivity (-) or sensitivity (+) to Ptr ToxA, respectively. Infiltrations were repeated at least three times per genotype in different plants.

Statistical analysis

Analysis of variance on disease severities from each experiment was conducted by using the mean %LAD across three leaves of each plant. Fisher's least significant difference (LSD) at $\alpha = 0.05$ was used to separate the genotype means.

Marker analysis

One-week-old leaf tissue was collected in 1.1-ml 8-strip tubes, dried in a freeze drier (Thermo Fisher, Waltham, MA) for two days, and ground to fine powder in a Mixer Mill (Retsch GmbH, Rheinische Strasse 36, Germany) by shaking strip tubes with a 3.2-mm stainless steel bead at 25 times/sec for 5 min. Genomic DNA was extracted from parents and RILs by using the cetyltrimethyl ammonium bromide (CTAB) (Saghai-Maroof et al., 1984). PCR amplifications were performed in a Tetrad Peltier DNA Engine (Bio-Rad Lab, Hercules, CA). A 12-µl PCR mixture contained 1.2 µl of 10X NH_4 buffer (Bioline Inc. Taunton, MA), 2.5 mM of $MgCl_2$, 200 µM of each dNTP, 100 nM of forward tailed primer, 200 nM of reverse primer, 100 nM of M13 fluorescent-dye labeled primer, 1 U of *Taq* DNA polymerase, and 50 ng template DNA. A modified touchdown program (Ma et al., 2005) was used for PCR reactions. In brief, the reaction was

incubated at 95ºC for 5 min then continued for five cycles of 1 min of denaturing at 96ºC, 5 min of annealing at 68ºC with a decrease of 2ºC in each of subsequent cycles, and 1 min of extension at 72ºC. For another five cycles, the annealing temperature started at 58ºC for 2 min with a decrease of 2ºC for each subsequent cycle. PCR went through an additional 25 cycles of 1 min at 96ºC, 1 min at 50ºC, and 1 min at 72ºC with a final extension at 72ºC for 5 min. PCR products were separated and sized using the ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). SSR data was further processed using GeneMarker version 1.6 (SoftGenetics LLC. State College, PA)

Bulked segregant analysis was used for screening polymorphic SSR markers associated with tan spot resistance. Equal amounts of DNA were pooled separately from five resistant and five susceptible RILs. A total of 1500 microsatellite markers (SSRs) covering all 21 wheat chromosomes (Roder et al., 1998; Somers et al., 2004) were screened between the parents and between two bulks in the WN population. In the WW population, approximately 1300 SSRs and additional 110 primer pairs of amplified fragment length polymorphism (AFLPs) were used for a whole genome screen between parental lines and two bulks (Yu et al., 2008). Polymorphic markers between bulks were further analyzed in the two mapping populations for linkage analysis. Markers from the QTL regions identified in the WN and WW populations were further analyzed in the CN and HW populations to validate QTL in different backgrounds.

Linkage and QTL analysis

Linkage analysis was performed by using the JoinMap program version 3.0 (Van Ooijen and Voorrips, 2001). The LOD threshold for grouping started at 3.0 and increased to separate linked groups to match with chromosome information from the published

wheat reference maps (Roder et al., 1998; Somers et al., 2004; Sourdille et al., 2004). Ripple was performed each time after adding one locus to test local rearrangements and refine the map. Recombination frequencies were converted to centimorgans (cM) with the Kosambi mapping function (Kosambi, 1944).

QTL Cartographer V2.5 (Wang et al., 2007) was used to perform interval mapping (IM) and composite interval mapping (CIM). CIM was implemented by using the standard model 6, starting with default values of five cofactors which were obtained by a forward regression to control genetic background. The walking speed scanning the genome for both methods was set at 1.0 cM. The LOD threshold used to declare a significant QTL was estimated from 1000 permutations of the data. For each QTL, coefficient of determination (R^2), which was the proportion of total phenotypic variance explained by a QTL, was determined by the marker closest to the identified QTL. The total R^2 representing the phenotypic variation explained by each model was conducted through multiple linear regression using the SAS REG procedure. All of the loci with significant main effects were tested against all of the other markers to detect significant interactions ($P < 0.01$).

Results

Reaction of the RILs to isolate AZ-00 and toxin bioassay

Four populations, WN, WW, CN and HW, were inoculated with isolate AZ-00 (nec+ chl+). Disease severity was evaluated by %LAD. The reaction of parents to inoculation was not significantly different from that of their corresponding checks, except that WSB showed significantly higher resistance than Karl92 in the experiment with the WW population and Wheaton had significantly lower susceptibility than TAM 105 in the

experiment with the HW population (Table 3.1). The difference in %LAD was not significant among different experiments for both controls (Table 3.1), indicating the environments for tan spot evaluation were well controlled among the experiments and results from different experiments were comparable.

All four populations segregated for tan spot resistance with broad phenotypic variations among the RILs. Resistant genotypes developed small dark-brown spots with no or little encompassing chlorosis, whereas susceptible ones had extensive chlorosis on an entire leaf. The mean %LAD ranged from 30% to 100% in the WN population (Figure 3.1a), 40% to 95% in the CN population (Figure 3.1b), 35% to 90% in the WW population (Figure 3.1c), and 40% to 90% in the HW population (Figure 3.1d). The frequencies of %LAD showed continuous but bimodal distributions in all four populations (Figure 3.1), among which the population WN demonstrated the deepest valley between the two peaks, indicating a major QTL for tan spot resistance in the population (Figure 3.1a).

To determine whether Ptr ToxA was responsible for pathogenicity in the populations, all parents and two checks were infiltrated with the toxin. The typical toxin-sensitivity phenotype showed up only on the susceptible check TAM 105 and not on any parent or the resistant check Karl92, indicating ToxA was not the virulence factor toward the parents tested (Table 3.1). Because all four populations segregated for extensive chlorosis, but not necrosis, also indicated that these populations may segregate for sensitivity to ToxC.

Expression of QTL from WSB in different populations

In the WSB/Ning7840 (WN) population, two QTL were detected to be associated with tan spot resistance as reflected by mean %LAD. One QTL, *QTs.ksu-1AS,* with a larger effect was detected on the short arm of chromosome 1A and explained 39% of the phenotypic variation (Table 3.2, Figure 3.2a). This QTL was flanked by *Xcfa2153* and *Xgwm33.* Another minor QTL, *QTs.ksu-2BS,* was detected on 2BS and explained 5% phenotypic variation (Table 3.2). This QTL was flanked by *Xbarc7* and *Xbarc55*. All marker alleles that showed decreased disease severity were from the resistant parent WSB. Multiple linear regression showed that two markers, *Xcfa2153* and *Xbarc7,* together explained 39% of the phenotypic variation for resistance to isolate AZ-00. Interaction between the two marker loci was not significant (Table 3.3).

None of the two QTL detected in the WSB/Ning7840 population were detected in the WSB/Wheaton (WW) population. Instead, composite interval mapping detected two QTL on chromosome 3BS (Table 3.2, Figure 3.2c). One QTL on the distal end of the 3BS chromosome, designated as *QTs.ksu-3BS.1*, explained 12% of the phenotypic variation and was flanked by markers *Xbarc147* and *Xgwm493*. Another QTL close to the centromere on 3BS, designated as *QTs.ksu-3BS.2,* explained 10% of the phenotypic variation and was flanked by markers *Xbarc218* and *Xwmc612*. Multiple linear regression using the markers *UMN10* and *XpAGT-mCTA172* from 3BS indicated that the two markers together explained about 26% of the phenotypic variation for resistance to isolate AZ-00. Epistatic interaction was not significant between the two markers closely linked to the QTL (Table 3.3). In all cases, the marker alleles of a genotype with a higher %LAD were from the susceptible parents, i.e. Ning7840 and Wheaton. That

different QTL were mapped in the two populations suggests that QTL for resistance may not be a specifically genetic factor to determine segregation of tan spot resistance. Rather, the QTL for susceptibility from Ning7840 and Wheaton may specifically interact with the pathogen to determine segregation of tan spot resistance in the two mapping populations.

QTL for tan spot susceptibility

To test the hypothesis that susceptible QTL may regulate segregation of tan spot resistance in a mapping population, two additional populations were developed by crossing two different resistant parents, a Korean cultivar Chokwang and a Chinese landrace HYZ, to Ning7840 (Chokwang/Nin7840 or CN) and Wheaton (HYZ/Wheaton or HW), respectively. As in population WN, one QTL with a major effect on tan spot resistance was identified on 1AS in population CN. This QTL was flanked by markers *Xgwm136* and *Xgwm33* and explained 63% of the phenotypic variation (Table 3.2). Ning7840 carried the marker alleles for susceptibility. Many markers mapped in the WN population including the closest marker to 1A QTL, *Xcfa2153*, were not polymorphic in the CN population (Figure 3.2b). The 2BS QTL detected in the WN population and the 3BS QTL detected in the WW population were not detected in this population, albeit that all mapped markers from the previous two populations were segregating in this population.

Two QTL on chromosome 3BS mapped in the WW population were also detected in the HW population. *Xbarc87* was the closest marker to the QTL in the distal end of chromosome 3BS and explained 7% of the phenotypic variation (Table 3.2, Figure 3.2d), while *Xbarc164* was the tightly linked marker to the QTL close to centromere and explained 9% of the phenotypic variation. A significant interaction was detected between

Xbarc87 and *Xbarc164*. The multiple linear regression analysis revealed that two markers together explained 28% of the phenotypic variation (Table 3.3), suggesting QTL interaction contributed to lower %LAD in the population. In both QTL, Wheaton contributed alleles for susceptibility. QTL on 1AS and 2BS were not detected in this population. The results support that QTL for susceptibility in Wheaton and Ning7840 might be responsible for segregation of tan spot resistance in these populations.

Tan spot resistance and toxin reactions in a worldwide wheat collection

A total of 378 wheat lines from different classes and origins were evaluated for resistance to *P. tritici-repentis* race 1 and Ptr ToxA reaction (Table 3.4). A total of 229 accessions were only impacted by ToxC due to their insensitivity to Ptr ToxA. Among these lines, 106 were from China and other Asian countries; 110 lines were from the US; and 13 lines were from Europe and South American. The resistant and susceptible checks, Karl 92 and TAM 105, had %LAD means of 43 and 79, respectively. Based on mean %LAD of checks, the 229 accessions were classified into four categories: resistant (≤45 %LAD means), moderately resistant (45 to 60), moderately susceptible (60 to 75) and susceptible (>75). Among 229 accessions, 76% (174) were resistant or moderately resistant. These included 78 (71%) US accessions and 87 (82%) Asian accessions. The result indicated that most of the accessions that showed resistance to ToxA were resistant to ToxC because race 1 of *P. tritici-repentis* presumably only produces ToxA and ToxC.

Discussion

Host selective toxins (HST) produced by *P. tritici-repentis* are the major virulence factors for tan spot in wheat (Lamari and Bernier, 1989). To date, four types of HST have been reported, among them proteinaceous ToxA and ToxB have been isolated and well

characterized (Martinez et al., 2001; Strelkov et al., 1999; Tomas et al., 1990; Tuori et al., 1995). However, ToxC is a polar, nonionic, low-molecular-weight molecule and has not been fully characterized due to difficulty in purification and its instability (Effertz et al., 2002). Also, ToxC is not readily produced in culture filtrate (Lamari et al, 2003), which makes it unavailable for researchers to directly use it for infiltration assays. Although *P. tritici-repentis* race 3 can produces ToxC only, the isolate was not available for this study. Thus an isolate of *P. tritici-repentis* race 1 (Andrie et al., 2007) that produces both ToxA and ToxC was used for QTL mapping of tan spot resistance in the four mapping populations. Because all parents of the four populations in this study were insensitive to ToxA (Table 3.1), we excluded the possible effect of ToxA as a virulence factor in these populations. Also, extensive chlorosis was the typical symptom observed in the parents, which is an indication of ToxC as a major causal agent for disease in these parents (Lamari and Gilbert, 1998). Thus, we assume that the extensive chlorosis segregating in the four populations was caused by ToxC, not ToxA. Moreover, in previous studies, the major QTL resistance to extensive chlorosis (Faris et al., 1997) and the gene, *tsc1*, conferring insensitivity to partially purified ToxC (Effertz et al., 2002) were mapped at the same locus by using *P. tritici-repentis* race 1 and race 3, respectively. Singh and Hughes (2006) also reported that resistance to chlorosis caused by races 1 and 3 in hexaploid wheat was controlled by the same gene. These results indicated that race 1 can be used to identify resistance to chlorosis or sensitivity to Ptr-ToxC when resistance to necrosis or sensitivity to Ptr-ToxA is not responsible for segregation in the population. Therefore, race 1 used in this study should provide accurate prediction of the Ptr-ToxC effect since Ptr-ToxA was not the causal agent of tan spot in these populations.

An inverse gene-for-gene model has been proposed for gene interaction in the wheat-*P. tritici-repentis* host-pathogen system based on the fact that the pathogen produces multiple toxins that can differentially attack different genotypes of a single host species (Lamari et al., 2003). In this model, compatibility is the basis for specificity, which results from the interaction between a HST produced by the fungus and its respective host receptor coded by a toxin sensitivity gene (susceptibility gene) in the host plants (Lamari et al., 2003). The compatibility determines whether the pathogen isolate can cause tan spot infection in a host plant. This is different from the classic gene-for-gene model in which incompatibility is the basis for specificity. However, this hypothesis has not been validated in the wheat-ToxC pathosystem with emphasis on host sensitivity genes. Only one report showed a line of evidence to support the hypothesis. By testing Chinese Spring 5B deletion and substitution lines, Anderson et al. (1999) found that Chinese Spring missing the host sensitivity gene *Tsn1* showed insensitivity to HST Ptr-ToxA. In the current study, there is the first to use a QTL mapping approach to demonstrate that host susceptibility or toxin sensitivity QTL determine the specificity between a HST / fungal race and its respective genes for toxin sensitivity / host susceptibility.

In the initial mapping experiment, the QTL *QTs.ksu-1AS* on chromosome 1AS showed a major effect on tan spot resistance in the population from the cross WSB/Ning7840. This QTL was mapped at the same region as the major QTL, *QTsc.ndsu-1A*, mapped for resistance to chlorosis (Faris et al. 1997) and the gene *tsc1* for ToxC insensitivity (Effertz et al. 2002). Both *QTsc.ndsu-1A* and *tsc1* were mapped in the ITMI population of W-7984 / Opata 86, with the allele for tan spot resistance and toxin

insensitivity from W-7984. The comparison of common markers between maps of this study and theirs suggests that the two QTL are the same, and both responsible for insensitivity to ToxC. Another QTL on 2BS was detected in the same population in this study. This QTL showed a minor effect on resistance to extensive chlorosis and has not been reported previously. Multiple regression analysis with a combination of markers linked to the two QTL indicated insignificant increase in coefficient of correlation (R^2). Thus, *QTs.ksu-1AS* is likely the most important QTL for tan spot reaction in the WN population.

According to the inverse gene-for-gene model, specific interaction between host and pathogen in this population should be determined by the HST in race 1 and toxin sensitivity QTL in the susceptible parent Ning7840, not insensitivity QTL from WSB. If this is the case, the same QTL would be identified in a mapping population with the same susceptible parent but a different resistant parent. As expected, *QTs.ksu-1AS* identified in the WN population was detected from the RIL population derived from the cross between Ning7840 and Chokwang, a Korean cultivar which has no relation to WSB, in light of genetic and geographic differences between the two resistant parents. This result suggests that the segregation of wheat resistance/susceptibility to extensive chlorosis in the two populations is determined by the same major QTL for susceptibility in Ning7840.

Another QTL on 2BS with a minor effect on resistance to chlorosis was not detected in the CN population despite the fact that all the markers in the linkage group mapped in the WN population were polymorphic. This may be due to the effects of different genetic backgrounds on expression of the minor QTL. The result indicates that wheat sensitivity to ToxC is most likely conferred by a major QTL/gene with possibly

one or a few modifier QTL with a minor effect, which agrees with several other studies (Effertz et al., 2002; Faris et al., 1997; Gamba et al., 1998). Our results also propose that QTL for host susceptibility to a pathogenic race that produces a toxin controls the segregation of plant resistance/susceptibility in the populations.

To further test the hypothesis with a population having a different susceptible parent, another RIL population (WW) was developed by crossing the Chinese landrace WSB to Wheaton, a susceptible parent from the U.S.A. A total of 187 markers including SSRs and AFLPs were used for linkage map construction based on their high correlation with tan spot data. Four linkage groups with 51 markers were further used for QTL analysis. As expected, the QTL on chromosome 1A was not detected despite of the fact that most of markers around *QTs.ksu-1AS* were mapped in the WW population. Instead, two new QTL, *QTs.ksu-3BS.1* and *QTs.ksu-3BS.2*, were identified on chromosome 3BS after a genome-wide scan of QTL in this population (Table 3.2, Figure 3.2c, d). They are new QTL for susceptibility and have not been previously reported. This result provides strong evidence to support the hypothesis that susceptible QTL in susceptible parents determine the interaction specificity between this host and its pathogen.

To validate two 3BS QTL for susceptibility in Wheaton, a RIL population derived from the cross between another resistant Chinese landrace HYZ and Wheaton was used. Although WSB and HYZ are two unrelated Chinese landraces, two 3BS QTL detected in the WW population were also identified in similar chromosomal regions of the HW population. The results further confirmed that Wheaton harbors the two susceptible QTL on chromosome 3BS. The mapping results from all four populations demonstrate that the alleles for resistance in a resistant parent might not be specific in different populations,

and the compatible interactions between susceptible QTL and specific HST/races were specific and determined the occurrence of tan spot in a host plant genotype.

Most pathogen-host systems follow the classical gene-for-gene model of host-pathogen interaction (Flor, 1947), in which the interaction of a resistance gene allele in a resistant host to a corresponding avirulence gene allele in a pathogen race is specific and dominant over other susceptible genes to determine the resistance phenotype of the host genotype. Whereas in the tan spot system, the situation appears to be opposite: specificity exists between a allele for susceptibility in a host plant and a corresponding allele from a virulenct race, a mirror image of the gene-for-gene model (Lamari et al. 2003). In this model, it is hypothesized that the elicitor protein produced from the pathogen recognizes the specific host receptor produced by the host sensitivity gene to develop susceptibility. Based on this model, we can speculate that susceptibility will be gained only if a mutation occurs in a resistance gene allele or a neutral gene allele to gain a new function of producing a host receptor for an existing HST; or the pathogen race develops a new elicitor (HST) to be compatible with the product of the resistance gene or neutral gene allele. In the both cases, it is a gain of a function, thus should be rare in nature. In fact, only four different HST associated with *P. tritici-repentis* have been found to date. This scenario suggests that resistance to tan spot may be durable and not be easily defeated by new races.

To further test the above scenario, 378 lines from all over the world were screened for ToxA sensitivity and resistance to race 1. As expected, the majority of the accessions that were insensitive to ToxA (76%) were resistant and moderately resistant. In 110 U.S. accessions, 78 (71%) showed resistance and moderate resistance. Most of the

U.S. accessions are elite breeding lines from major hard and soft winter wheat breeding programs and some cultivars that have recently been released. They have not been selected for tan spot resistance. For 106 Asian accessions, 87 (82%) were resistant and moderately resistant to tan spot. Most of these Asian accessions are landraces from China and Japan. The results indicate that most wheat accessions are resistant or moderately resistant to ToxC and susceptible genotypes are much fewer than resistant genotypes. Landrace populations even have fewer susceptible genotypes than modern breeding populations. These results support the hypothesis that interaction between host resistance and pathogen avirulence genes is not specific, and establishment of specificity between a host susceptibility/HST sensitivity gene and pathogen virulence gene might result from long time evolution.

After comparing the QTL locations for tan spot susceptibility with that for resistance to Fusarium head blight (FHB) in the WSB/Wheaton population, we found that the two QTL for tan spot resistance on 3BS were also significant for FHB resistance (Yu et al., 2008). In the HW population, the QTL *QTs.ksu-3BS.2* was also significant for FHB resistance with R^2 of 0.06 (Tao Li et al., unpublished data). The results suggest a pleiotropic effect of the 3BS QTL on resistance to tan spot race 1 and FHB Type II resistance. One possible explanation is that the same genes may control the resistance to tan spot race 1 and FHB type II resistance since they are both affected by toxins (ToxC and DON, respectively). Additional evidence for this is that the 3AS genomic region carrying the QTL for resistance to tan spot caused by *P. tritici repentis* race 1 also contains a QTL for resistance to FHB according to the common SSR marker *Xbarc45* (Chen et al., 2007; Singh et al., 2008); It is also possible that the two 3BS QTL are

general disease defense genes and may be involved in both tan spot and FHB resistance. This is supported by the observations that there are two race-nonspecific QTL for resistance to tan spot caused by races 1, 2, 3, and 5 on 3BS and 3BL, respectively (Faris and Friesen (2005). Either *QTs.ksu-3BS.1* or *QTs.ksu-3BS.2* may be the same locus reported on 3BS. However, an accurate comparison is not possible due to unavailability of common markers between the two maps. Furthermore, Czembor, et al. (2003) found a QTL close to marker *Xgwm533* located on 3BS controlling partial resistance to *Stagonospora nodorum blotch* (SNB) which also produces an HST similar to those of tan spot (Friesen et al., 2006). And Singh et al (2006) mapped the gene for resistance to necrosis, *tsn2*, caused by race 3 in durum wheat populations on chromosome 3B close to the marker *Xgwm285* which is in the vicinity of *QTs.ksu-3BS.2* in the WW population (Yu et al., 2008).

In this study, we are the first to demonstrate that QTL in susceptible parents determine the specific compatible interaction between a pathogen (HST) and host by comparing QTL expression in the same and different genetic backgrounds. This finding provided solid evidence to support the inverse gene-for-gene model in the wheat-ToxC system and may help increase the efficiency in breeding selection strategies. The usual selection method for breeding disease resistance is to select for resistant genotypes. Based on the results from this study, susceptible rather than resistant genotypes carried QTL that interacted specifically to HST produced by the pathogen race inoculated. Therefore, to improve tan spot resistance, breeders need to avoid the use of highly susceptible parents with a HST sensitivity gene in their crossing programs. Additionally, markers for susceptibility QTL should be identified and used for selection against

susceptible alleles to remove highly susceptible genotypes. In addition, a clone of the susceptible gene/QTL such as *QTs.ksu-1AS* would greatly help address the questions regarding the specific host recognition of ToxC, the role of sensitivity genes in the plant defense system, and their interactions with HST.

Figure 3.1. Histograms of percentage diseased leaf area (%LAD) means caused by *Pyrenophora tritici-repentis* race 1 in recombinant inbred lines (RILs) for four populations.

(a) Percentage diseased leaf area means across the entire WSB/Ning7840 (WN) population; (b) Percentage diseased leaf area means across the entire Chokwang/Ning7840 (CN) population; (c) Percentage diseased leaf area means across the entire WSB/Wheaton (WW) population; and (d) Percentage diseased leaf area means across the entire HYZ/Wheaton (HW) population.

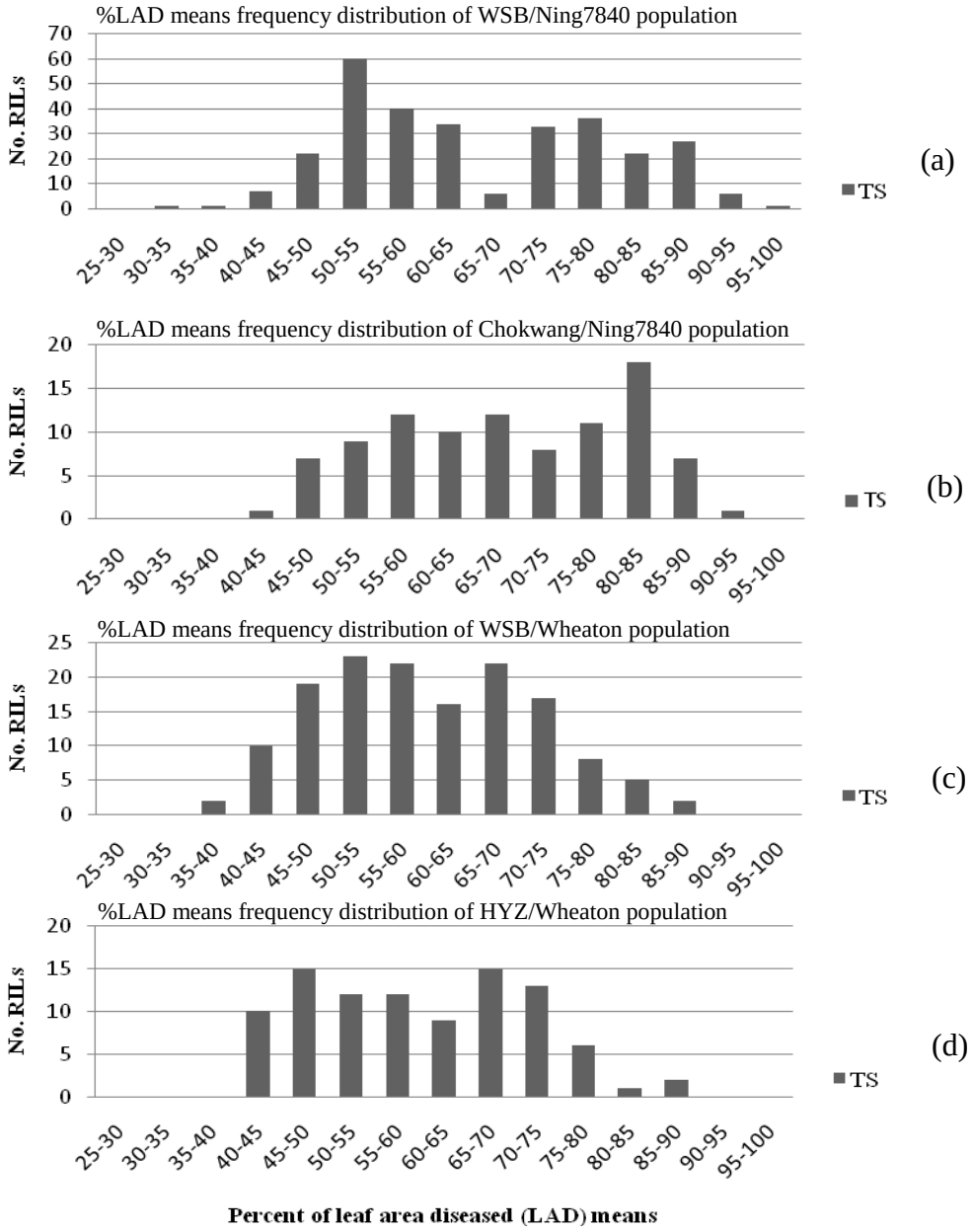


Figure 3.2. Composite interval mapping (CIM) of QTLs associated with tan spot resistance in recombinant inbred populations WSB /Ning7840 (a) Chokwamg / Ning7840 (b), WSB/Wheaton (c), HYZ / Wheaton (d).

The marker positions are displayed between two linkage maps and centimorgan (cM) distances between loci are shown at the left and right margins. The vertical solid line represents the logarithm of the odds (LOD) significance threshold. The LOD, additive effect, LOD threshold and R^2 values for each QTL are listed in Table 3.2. The common markers between two populations are underlined.

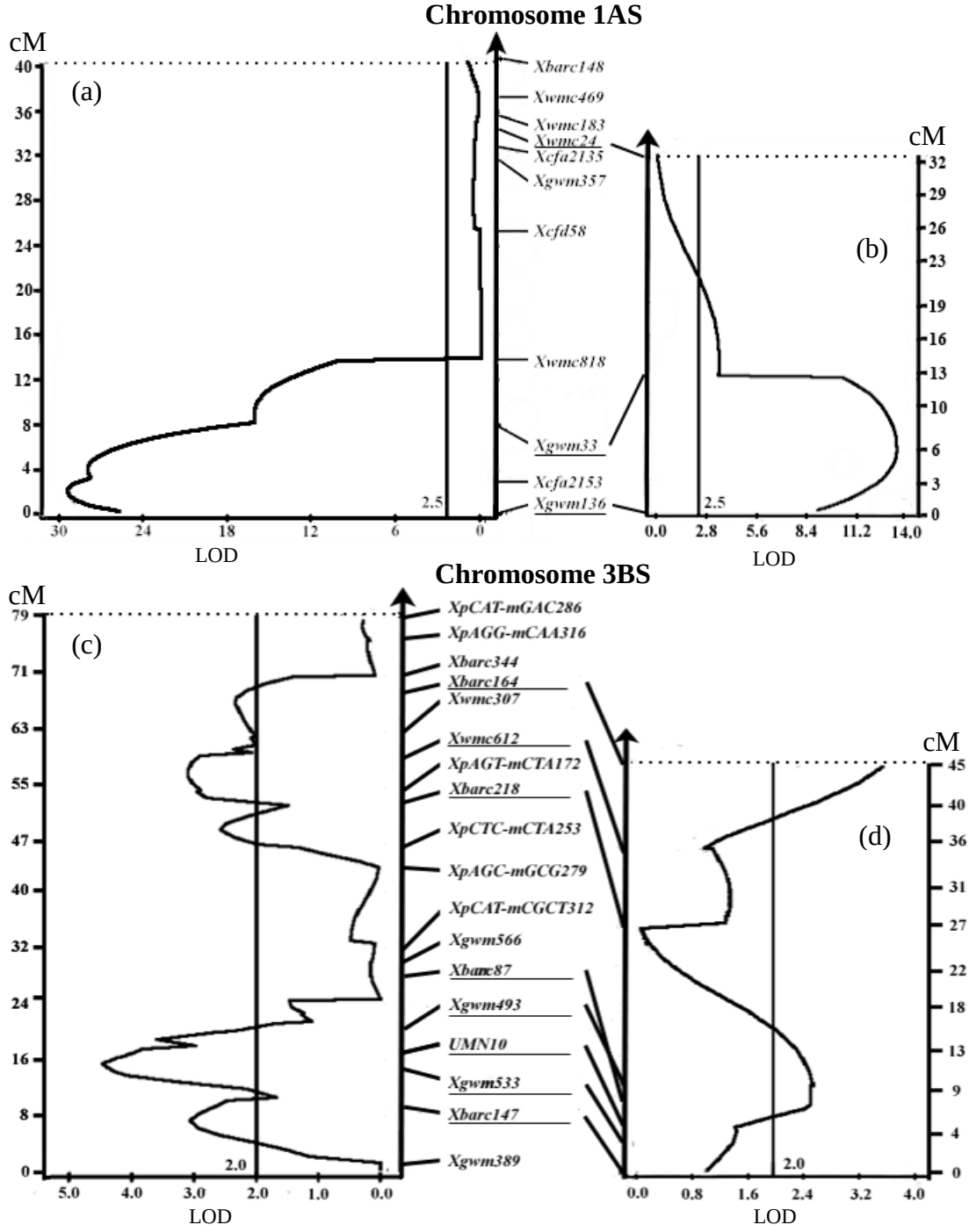


Table 3.1. Reactions of the parents of wheat populations (POP) and two check cultivars to *Pyrenophora tritici-repentis* and the host specific toxin Ptr ToxA.

POP	Cultivars	%LAD[x]	ToxA reaction
WN	WSB	52.1[y] a	-
(288)	Ning7840	84.4 b	-
	Karl 92[z]	54.1 a	-
	TAM 105	82.9 b	+
	LSD(α=0.05)	14.1	
CN	Chokwang	60.6 a	-
(96)	Ning7840	83.4 b	-
	Karl 92	52.9 a	-
	TAM 105	84.1 b	+
	LSD(α=0.05)	12.1	
WW	WSB	41.5 a	-
(138)	Wheaton	83.2 c	-
	Karl 92	53.3 b	-
	TAM 105	90.0 c	+
	LSD(α=0.05)	10.3	
HW	HYZ	41.6 a	-
(96)	Wheaton	75.5 b	-
	Karl 92	49.2 a	-
	TAM 105	90.3 c	+
	LSD(α=0.05)	11.3	

[x] Mean percentage leaf area diseased (%LAD).
[y] Means followed by the same letter are not significantly different according to ANOVA and LSD (P=0.05).
[z] Karl 92 is the resistant check cultivar and TAM 105 is the susceptible check.

Table 3.2. Composite interval mapping (CIM) of QTL associated with resistance to *Pyrenophora tritici-repentis* race 1 in four RIL populations.

Pop	QTL	Chr.	Position (cM)[a]	Marker[b]	LOD	Additive Effect	Threshold (5%, 1%)	R^2	LOD1 Interval[c]	LOD2 Interval
WN										
	QTs.ksu-1AS	1AS	3.4	*Xcfa2153*	29.5	-8.88	1.7, 2.6	0.39	0.4-3.1	0.8-2.8
	QTs.ksu-2BS	2BS	0.0	*Xbarc7*	2.8	-3.07	1.7, 2.6	0.05	0-3.1	0-3.1
CN										
	QTs.ksu-1AS	1AS	5.5	*Xgwm136*	13.5	-10.01	1.5, 2.2	0.63	1.6-11.1	2.6-9.7
WW										
	QTs.ksu-3BS.1	3BS	15.1	*UMN10*	4.5	-3.95	2.0, 2.6	0.13	12.7-17.3	11.8-17.7
	QTs.ksu-3BS.2	3BS	53.7	*XpAGT-mCTA172*	3.1	-3.14	2.0, 2.6	0.10	54.5-59.3	52.2-59.7
HW										
	QTs.ksu-3BS.1	3BS	14.3	*Xbarc87*	2.5	-3.2	1.9, 2.3	0.07	12.8-23.3	4.8-26.8
	QTs.ksu-3BS.2	3BS	44	*Xbarc164*	3.5	-4.03	1.9, 2.3	0.09	39-44	35-44

[a] Chr and Position refer to the chromosome and map position of the QTL corresponding to the maximum LOD score for IM and CIM.

[b] The closest markers to the LOD peaks are given.

[c] LOD1 and LOD2 are confidence intervals for the position of each QTL estimated by a drop of 1 and 2, respectively, from the maximum LOD score.

Table 3.3. Phenotypic effects of markers closely linked to the QTL for resistance to *Pyrenophora tritici-repentis* race 1 and possible interactions between QTL in the four recombinant inbred populations.

Population	Chr	Marker or interaction	LOD	R^2
WN	1AS	*Xcfa2153*	29.5	0.39
	2BS	*Xbarc7*	2.8	0.05
	1AS/2BS	*Xcfa2153*Xbarc7*	…	NS[b]
Total R^{2a}	…	...	…	0.39
CN	1AS	*Xgwm136*	13.5	0.63
WW	3BS	*UMN10*	4.5	0.13
	3BS	*XpAGT-mCTA172*	3.1	0.10
	3BS	*UMN10* XpAGT-mCTA172*	…	NS
Total R^2	…	...	…	0.26
HW	3BS	*Xbarc87*	2.5	0.07
	3BS	*Xbarc164*	3.5	0.09
	3BS	*Xbarc87*Xbarc164*	…	0.09
Total R^2	…	...	…	0.28

[a] Total R^2 was obtained from multiple regression model which included only significant markers and interactions.

[b] NS represents non-significant.

Table 3.4. Frequency of percentage leaf area diseased (%LAD) means segregated for extensive chlorosis caused by *Pyrenophora tritici-repentis* race 1 in three natural populations. Karl 92 and TAM 105 serve as resistant and susceptible checks.

%LAD range	US[a]	Asian	Other	Total
20-25	1	0	0	1
25-30	3	1	0	4
30-35	11	1	1	13
35-40	13	7	1	21
40-45	12	15	2	29
45-50	15	26	0	41
50-55	12	21	3	36
55-60	11	16	2	29
60-65	5	9	0	14
65-70	6	2	1	9
70-75	9	3	1	13
75-80	8	3	0	11
80-85	4	0	2	6
85-90	0	2	0	2
Total accessions	110	106	13	229
Karl92=43% TAM105=79%				

[a] US represents accessions from US; Asian represents accessions from Asia, Other represents accessions from South American and Europe.

CHAPTER 4 - Molecular Markers for Wheat Leaf Rust Resistance Gene *Lr41*

Introduction

Leaf rust, caused by *Puccinia triticina* Eriks. can cause yield losses up to 40% in susceptible wheat cultivars (Knott, 1989) and is one of the most important diseases of common wheat (*Triticum aestivum* L.) worldwide (Kolmer, 1996). Breeding for leaf rust resistance in wheat is challenging because resistance can be completely overcome by a shift in predominant pathogen races in a rust population. Successful control of rust epidemics using genetic resistance has two dimensions: monitoring dynamic changes of rust pathogen populations to identify new virulent races, and deploying resistance genes to defeat the new pathogen races. However, another approach to deter rapid changes in pathogenicity within the leaf rust population is using cultivars with multiple genes resistant to different pathogen races. Successful examples are spring wheat cultivars with combinations of three resistance genes that withstood virulence changes in *P. triticina* over extended periods of time (Kolmer et al., 2008a).

Currently, more than 60 leaf rust-resistance genes have been identified from wheat and its relatives. Many leaf rust-resistance genes have been identified in the wild wheat relative *T. tauschii* including *Lr21* (1DS), *Lr22a* (2DS), *Lr32* (3D), *Lr39*/*Lr41* (2DS), *Lr42* (1D), and *Lr43* (7DS) (Cox et al., 1994; Gill et al., 1991; Hiebert et al., 2007; Huang and Gill, 2001; Huang et al., 2003; Hussien et al., 1997; Kerber, 1987; Raupp et al., 2001; Rowland and Kerber, 1974). Recombination between the corresponding chromosomes of *T. tauschii* and the D genome of *T. aestivum* occurs at a level similar to

that within the cultivated hexaploid species (Fritz et al., 1995). This lessens the challenge of gene introgression from *T. tauschii* with minimal linkage drag, and as such many disease-resistance genes from *T. tauschii* have been transferred into common wheat.

Pyramiding resistance genes in new cultivars may greatly increase durability of wheat resistance to leaf rust. However, pyramiding several resistance genes into a single genetic background using traditional phenotypic analysis requires time-intensive evaluation of a large breeding population exposed to several different races. Thus, molecular markers linked to these resistance genes, either race-specific or non-race-specific, would be essential tools for successfully and rapidly pyramiding new combinations of resistance genes through marker assisted selection (MAS) in breeding programs.

Lr41 was originally mapped on chromosome 1DS in an early cytogenetic study (Cox et al., 1994) and relocated on 2DS through molecular mapping (Singh et al., 2004). Marker *Xgdm35* was reported to be closely linked with *Lr41* (Singh et al., 2004). This marker has been used in MAS for *Lr41* in hard winter wheat breeding programs in the southern Great Plains of the USA in the past 2 years. Unfortunately, a low frequency of the resistance marker allele in hard winter wheat germplasm generated many false negative results. Therefore, better markers are needed for this gene to be deployed in hard winter wheat through MAS. Objectives of this study were to 1) confirm the chromosome location of the resistance gene *Lr41* using molecular markers and a near-isogenic line (NIL) population, 2) identify molecular markers closely linked to *Lr41* to facilitate effective deployment of the gene in breeding programs, and 3) genotype diverse wheat cultivars and breeding lines for polymorphism at the marker loci.

Materials and Methods

Plant materials and rust evaluation

Wheat breeding line KS93U62 was developed by backcrossing an *Lr*41-containing *T. tauschii* accession TA2460 to cv Century (Cox et al., 1994; Martin et al., 2003) and then crossed to OK92G205 and OK92G206, two Century-backcross-derived NILs for the presence or absence of awns, both without the *Lr41* resistance gene (Carver et al., 1993). The corresponding F_2 population was artificially inoculated with *P. triticina* in a greenhouse at Kansas State University, Manhattan, KS, USA to identify plants with *Lr41* resistance (Martin et al., 2003). More than 200 resistant F_2 seedlings were selected from each cross, and their $F_{2:3}$ progeny were further evaluated to identify non-segregating families derived from F_2 plants homozygous for the *Lr41* allele for leaf rust resistance. Selected $F_{2:4}$ and $F_{2:5}$ families were further evaluated for adult-plant resistance in the field in Oklahoma in 1998 and 1999. According to their leaf rust reactions in the presence of natural field infection, 51 $F_{2:6}$ NILs were selected and used in this experiment.

To check seed purity from field-grown plants and to verify resistance of selected NILs, all 51 $F_{2:6}$ NILs were evaluated twice for adult-plant resistance in March (spring) and November (fall) 2007 and for seedling resistance in spring 2008 in a greenhouse at Kansas State University. In the 2007 greenhouse experiments, all NILs were inoculated at early anthesis with isolate PRTUS25 (race MDB, avirulence/virulence formula: 2a, 2c, 3ka, 9, 11, 16, 17, 18, 19, 26, 30, 39, 41/1, 3, 10, 24), which is avirulent to *Lr41* and virulent to *Lr24* in Century, and a few lines with heterogeneous rust reactions were observed. Plants of those lines from the spring 2007 experiment were harvested separately as a seed source for the fall 2007 and spring 2008 greenhouse experiments.

Leaf rust symptoms on leaves of adult plants were scored as resistant and susceptible compared with both parents two weeks after inoculation. The experiments used a randomized complete block design with two replicates and five plants per replicate. In the spring 2008 seedling resistance test, six plants per NIL were planted in a tray with soil mix (Hummert International, Erath City, MO, USA) and grown in a growth chamber maintained at 20ºC at Kansas State University. Seedlings were inoculated at the two-leaf stage with rust isolates PRTUS25 and PRTUS35 (race TNR, avirulence/virulence formula: 16, 17, 19, 26/1, 2a, 2c, 3, 3ka, 9, 10, 11, 24, 30, 39, 41), which is virulent to both *Lr24* and *Lr41*. Inoculated seedlings were kept in a moist chamber at 20ºC with 100% humidity for 12 h. Before scoring for disease, plants were kept in a growth chamber for 10 d at 20ºC with 12 h light. Seedling infection types were determined according to McIntosh et al. (1995).

Chinese Spring nullitetrasomic and ditelosomic genetic stocks, Nulli-1D/Tetra-1B (abbreviated as N1D-T1B), N2D-T2A, N2D-T2B, Ditelo1DS (abbreviated as DT1DS), DT1DL, and DT2DL, were used to determine the physical location of *Lr41*. The new markers developed from this study were further surveyed for polymorphism using 73 accessions from different classes and origins. Among them, OK Bullet, Thunderbolt, AP03T6115, Postrock, TX01V5719, Overley, Fuller, Bullet06ERU, and three sister selections of OK Bullet—OK02522W, OK05737W, and OK05741W—were expected to carry *Lr41*.

Marker analysis

Seedlings from the fall 2007 experiment were used as the plant source for DNA isolation. Leaf tissue was collected in 1.1 ml strip tubes and dried in a freezer-drier

(Thermo Fisher, Waltham, MA, USA) for 2 d. Tubes containing a 3.2-mm stainless bead and dried tissue were shaken in a Mixer Mill (Retsch GmbH, Rheinische Strasse 36, Germany) at 25 times per second for 5 min. Genomic DNA was extracted from parents and NILs using the cetyltrimethyl ammonium bromide (CTAB) protocol (Saghai-Maroof et al., 1984). PCR amplifications were performed in a Tetrad Peltier DNA Engine (Bio-Rad Lab, Hercules, CA, USA) with a total volume of 12 µl containing 1.2 µl of 10x NH_4 buffer (Bioline, Taunton, MA, USA), 2.5 mM of $MgCl_2$, 200 µM of each dNTP, 50 nM of forward tailed primer, 250 nM of reverse primer and 200 nM of M13 fluorescent-dye labeled primer, 0.6 U of *Taq* DNA polymerase, and 100 ng template DNA. A touchdown program modified from Ma et al. (2005) was used for PCR reactions. The reaction was incubated at 95ºC for 5 min then continued for five cycles of 1 min of denaturing at 96ºC, 5 min of annealing at 68ºC with a decrease of 2ºC in each subsequent cycle, and 1 min of extension at 72ºC. For another five cycles, the annealing temperature started at 58ºC for 2 min with a decrease of 2ºC for each subsequent cycle. Reactions went through an additional 25 cycles of 1 min at 96ºC, 1 min at 50ºC, and 1 min at 72ºC with a final extension at 72ºC for 5 min. PCR products were analyzed on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Bulked segregant analysis was used for screening polymorphic SSR markers associated with *Lr41*. Equal amounts of DNA were pooled from five *Lr41*-resistant and five *Lr41*-susceptible NILs. Sixty SSR markers from chromosome 1D and 55 markers from 2D (Roder et al., 1998; Somers et al., 2004) were selected for screening the parents and two bulks. Polymorphic markers between the parents and bulks were further analyzed on all NILs for linkage relationship.

Data analysis

Data collected from the ABI DNA Analyzer were further processed using GeneMarker version 1.5 (SoftGenetics LLC., State College, PA, USA) and rechecked twice manually for accuracy. Genetic linkage among SSR makers and the leaf rust locus was determined by Joinmap 3.0 (Van Ooijen and Voorrips, 2001) using the Kosambi mapping function (Kosambi, 1944) with an LOD threshold of 3.0.

Results

Reactions of NILs and parents to leaf rust infection

Adult and seedling plants of NILs and parents showed expected reactions to leaf rust infection when inoculated with the isolate PRTUS25, which is avirulent to *Lr41*, at two different growth stages. *Lr41*-resistant NILs produced small, hypersensitive, necrotic or chlorotic flecks, whereas leaves of susceptible NILs were covered with medium-sized uredinia (Table 4.1). Seedlings of NILs were also inoculated with PRTUS35, an isolate virulent to *Lr41*, to verify that resistance in the NILs was due to *Lr41*, and not other genes. KS93U62 and the check cultivar TAM 110 were susceptible to PRTUS35 (Table 4.1), and all NILs showed highly susceptible symptoms with infection types (IT) of 3 to 3+ (data not shown). Nine heterogeneous NILs were identified based on their infection types from the first experiment. Both genotypes (*Lr41* and non-*Lr41* types) from these nine lines were harvested separately and evaluated for rust resistance in the second and third experiments. Consistent results were obtained for all nine lines among three experiments. These results indicated that rust ratings for NILs at different stages under different conditions were consistent and leaf rust data were appropriate for gene mapping.

Linked markers to Lr41

When 115 SSR markers from chromosome 1D and 2D were screened on parents and bulks, four SSR primers (BARC124, GWM210, GDM35, and CFD36) showed polymorphism between parents and between bulks. For example, primer BARC124 amplified 261-bp fragments in KS93U62 and the resistant bulk (Figure 4.1A & C) and 271-bp fragments in the susceptible parents (OK92G205 and OK92G206) and susceptible bulk (Figure 4.1B & D). These markers were further used to analyze the 51 NILs. Linkage analysis using the four markers and rust data identified *Xbarc124* as the marker linked most closely, at 1 cM apart, to *Lr41* (Figure 4.2B). Three other markers were also close to *Lr41*: *Xgwm210*, *Xgdm35* and *Xcfd36* were 1.6, 2.8, and 4.1 cM proximal to *Lr41*, respectively. The flanking marker for *Lr41* was not identified. Comparative analysis with published maps (Singh et al., 2004; Somers et al., 2004; Sourdille et al., 2004), suggested that *Lr41* was distal to the centromere near the telomere of chromosome 2DS (Figure 4.2).

Marker *Xgdm35* was selected to verify the physical location of *Lr41*. Primer GDM35 amplified a clear band in N1D-T1B, DT1DS, and DT1DL, indicating that the marker was not on 1D (Figure 4.3). However, primer GDM35 did not amplify any band in N2D-T2A, N2D-T2B, and DT2DL, indicating that the marker was on the short arm of chromosome 2D. This result provided further evidence to support that *Lr41* was on chromosome 2DS, not 1DS as originally reported.

Because all four markers were closely linked to *Lr41*, they all have potential to be used in MAS. To evaluate the polymorphism of those markers in diverse wheat germplasm, 73 additional wheat cultivars or breeding lines from different wheat classes and several countries were analyzed with these markers (Table 4.2). Each primer amplified at least one fragment in each of 73 wheat accessions (Table 4.2). For most

markers, many resistant accessions amplified the banding patterns of KS93U62 that harbors *Lr41* while most susceptible accessions amplified different banding patterns from the ones of KS93U62.

Primer BARC124 amplified five fragments among 73 accessions, but only the 261-bp fragment was associated the *Lr41* allele from *T. tauschii,* and all others were different from that amplified in KS93U62. Most accessions amplified a single fragment, but eight accessions amplified two fragments. Among five accessions amplifying the 261-bp fragment of KS93U62, only two hard winter wheat accessions, Thunderbolt and Tx01V5719, were expected to have *Lr41*. The other three accessions, IL94-1909 from Illinois, USA, and 117.92 and Sumai 3 from China, did not carry the *Lr41* gene based on available information and amplified two fragments: one 261-bp fragment plus another fragment from wheat.

Primer GWM210 amplified three different banding patterns in 73 accessions, 182/206 bp, 182/184 bp, and 182/184/206 bp. Because the 182-bp fragment was monomorphic across all accessions and KS93U62 amplified 182-bp and 206-bp fragments, not the 184-bp fragment, the 184-bp fragment was considered non-KS93U62 marker allele. Excluding the monomorphic 182-bp fragment, marker allele *Xgwm210* (206-bp fragment) was shown in all 11 hard winter wheat accessions that carry *Lr41* and five other accessions without *Lr41*. Among accessions carrying the 206-bp allele without *Lr41,* three were hard winter wheat lines from Oklahoma (OK05903C, OK04525, and OK05830) and two were spring wheat accessions from China (Sumai 3) and Japan (Shinchunaga) (Table 4.2). An additional 11 accessions that amplified the 206-bp fragment also amplified the 184-bp fragment; they all were susceptible accessions.

Primer GDM35 amplified 18 alleles in 73 accessions, and KS93U62 amplified a 182-bp fragment. Thunderbolt was the only cultivar with *Lr41* that amplified the 182-bp fragment. However, the 182-bp fragment appeared in 21 accessions that do not carry *Lr41*. All 21 accessions with the 182-bp fragment had an additional fragment of varied sizes. Among them, 18 were soft winter wheat lines from the USA and three were soft spring wheat accessions from China. Primer CFD36 amplified five fragments in 73 accessions. The 213-bp fragment amplified in KS93U62 appeared in most soft wheat accessions.

Discussion

Because of rapid changes in predominant rust pathogen races in nature, single-gene resistance in a cultivar may become ineffective soon after it is released. Stacking two or more genes in one cultivar can enhance durability and the level of rust resistance. For example, a gene combination *Lr9* and *Lr24* provided relatively long-lasting resistance (McVey and Long, 1993). Also, the combination of seedling resistance gene(s) with an adult-plant resistance gene such as *Lr34* expressed a high level (Kolmer, 2003) and durable rust resistance that has proven to be effective over time (Kolmer et al., 2008a). Therefore, pyramiding *Lr41* with other durable adult-plant genes such as *Lr34* or *Lr46* or some broadly effective resistance gene such as *Lr21* could be an effective strategy for minimizing losses caused by rust epidemics.

Knowledge of chromosome locations of genes is essential for using gene pyramiding to develop multiple-gene resistant cultivars in breeding programs. In an early cytogenetic study, *Lr41* was located on the short arm of chromosome 1D (Cox et al., 1994). More recently, molecular mapping relocated the gene on 2DS, and established the

close linkage, or possibly allelism with *Lr39* (Singh et al., 2004). In the present study, using ditelosomic and nullitetrasomic genetic stocks and markers linked to *Lr41*, we physically confirmed *Lr41* on chromosome 2DS. Marker *Xgdm35*, rather than the more closely linked marker *Xbarc124*, was selected to verify the physical location of *Lr41* in this study because *Xbarc124* is not chromosome specific and thus amplified loci on other chromosomes besides 2DS (http://wheat.pw.usda.gov/). Confirming the physical location of *Lr41* provides useful information, allowing breeders to make decisions about what other genes can be combined to enhance durable resistance in a cultivar.

In this study, four markers were mapped within 4.1 cM from *Lr41*. A distal flanking marker was not found, even when all published SSR markers from 2DS (Somers et al., 2004) were screened between parents. Slight differences in marker order were observed between linkage groups constructed in this study and those reported in previous studies (Singh et al. 2004, Somers et al. 2004). In the map by Singh et al. (2004), *Xgdm35* was the closest marker to *Lr41*; in our study *Xbarc124* was the closest marker to *Lr41*, which agrees with Somers et al. (2004). However, *Xcfd56* was located between *Xbarc124* and *Xgwm210* in the Somers et al. (2004) map but was the furthest marker from *Lr41* in our study. The slight differences in marker order and marker interval among three maps could be due to differences in mapping population and population type used for map construction. In this study, we used a backcross population; other studies used a recombinant inbred line population (Somers et al. 2004) or F_2 population (Singh et al. 2004). Nevertheless, the four markers are very closely linked to *Lr41* (<8 cM) based on maps from this study and Singh et al. (2004). Therefore, markers identified in this study

would be good candidates for use in MAS, assuming that sufficient polymorphism exits between parents used in breeding programs.

The actual utility of markers developed from linkage mapping in MAS will depend on the frequency of the resistance marker allele in resistant parents and the level of polymorphism between resistant and adapted parents. To indirectly assess the usefulness of these markers for *Lr41* in future MAS, markers linked to *Lr41* were analyzed in a collection of 73 accessions from Argentina, Brazil, USA, Austria, France, China, and Japan. Among the four markers, *Xbarc124* resided the closest to *Lr41*. Of 11 cultivars thought to carry *Lr41*, only two (TX01V5719 and Thunderbolt) amplified a single 261-bp allele of *Xbarc124* as present in KS93U62, the source of *Lr41* in our NIL population. Thunderbolt was derived from a cross with KS90WGRC10 (*Lr41* donor), and TX01V5719 was derived from U1254, which likely inherited *Lr41*. Other resistant cultivars were selected from crosses using KS90WGRC39's derivative as the *Lr41* donor, but it is possible that the marker allele associated with *Lr41* was lost during its early transfer into new germplasm. Only three soft wheat accessions amplified the KS93U62 marker allele, but they do not carry *Lr41*. These accessions were IL94-1909 from Illinois USA and 117.92 and Sumai 3 from China and also amplified an additional fragment of either 250-bp or 266-bp.

Results indicate that the polymorphic level is high between resistant and susceptible accessions, albeit at a low frequency of the *Xbarc124* allele in the accessions carrying *Lr41*. Obviously, the 261-bp fragment can be a useful marker for *Lr41* if TX01V5719 and Thunderbolt are specifically used as the *Lr41* donor parents in populations undergoing MAS. If other parents listed in Table 4.2 are used as an *Lr41*

donor, the 261-bp allele cannot be detected; in this case, *Xbarc124* is not recommended for MAS.

Different from primer BARC124, GWM210 amplified two fragments in KS93U62: a monomorphic 182-bp fragment that also appeared in all 73 accessions and a 206-bp fragment that appeared in all *Lr41*-containing accessions and a few of non-*Lr41*-containing accessions. Meanwhile, almost all accessions without *Lr41* amplified a 184-bp fragment except three Oklahoma lines, therefore the 184-bp fragment can be regarded as the marker allele that was associated with susceptible genotypes. In the case of the susceptible parent, OK92G206, which amplified both the 184-bp and 206-bp fragments, the 206-bp fragment was not associated with *Lr41* resistance. Therefore if GWM210 amplifies the 206-bp fragment without accompanying of the 184-bp fragment in an accession, this accession most likely carry *Lr41*. A total of 16 accessions, including all 11 U.S. hard winter wheat accessions that carry *Lr41*, amplified the 206-bp fragment without the 184-bp fragment. The remaining five accessions that did not have *Lr41* include three Oklahoma hard winter wheat lines and two soft wheat cultivars from China and Japan. Thus, *Xgwm210* is a highly polymorphic marker among wheat accessions evaluated and can be a good marker for introgression of *Lr41* into elite wheat backgrounds.

For primer GDM35, only Thunderbolt amplified a single 182-bp fragment as in KS93U62. All other accessions that carry *Lr41* did not amplify the 182-bp fragment. Although 21 other accessions also amplified the 182-bp fragment, these accessions amplified an additional fragment of varied sizes. These 21 accessions included 18 soft red winter wheat cultivars from the USA and three soft red spring wheat accessions from

China. The specificity of marker *Xgdm35* to the resistant cultivar, Thunderbolt, indicates that *Xgdm35* can be effective for MAS only if Thunderbolt is used as the *Lr41* donor. If other resistant parents are used, one of three fragments—229-bp, 249-bp, and 265-bp—can be used as target marker alleles for GWM35.

Primer CFD36 amplified a 213-bp fragment in KS93U62. The 213-bp fragment was not amplified in any *Lr41*-containing accessions. Instead, a 215-bp fragment, which was not polymorphic with the remaining hard winter wheat accessions without *Lr41*, was identified in all 11 resistant accessions. In contrast, the 213-bp fragment was amplified in most soft wheat accessions without *Lr41*. Therefore, *Xcfd36* is not a usable marker for MAS.

In summary, *Lr41* was physically located on the distal end of chromosome 2DS. Four markers were identified tightly linked to *Lr41*. Among them, marker *Xgwm210* appears to have the greatest utility for MAS because of its high frequency in *Lr41*-containing accessions and high polymorphism in a natural population. However, this marker should be used with caution to predict the presence of *Lr41* in a natural population for its possibility of false positive. Fragments that are associated with both resistant (206 bp) and susceptible (184 bp) genotypes should be evaluated. The lines that carry *Lr41* most likely amplify the 206-bp fragment without the 184-bp fragment. For MAS, it can be scored as a codominant marker if both fragments are polymorphic between parents; or it can be scored as a dominant marker if only 184-bp fragment is polymorphic between parents. Markers *Xbarc124* and *Xgdm35* can also be used for MAS if *Lr41* donor parents have the marker alleles of KS93U62. A survey of allelic frequency in resistant accessions and polymorphism level in a natural population or association

mapping can ensure that a marker linked to a resistance gene can be widely used in MAS and may provide valuable supplemental information to facilitate quick deployment of genes in breeding programs.

Figure 4.1. ABI electrophorograms of SSR marker *Xbarc124* on chromosome 2DS showing polymorphism among KS93U62 (a, *Lr41*), OK92G206 (b, susceptible parent), resistant bulk (c), and susceptible bulk (d).

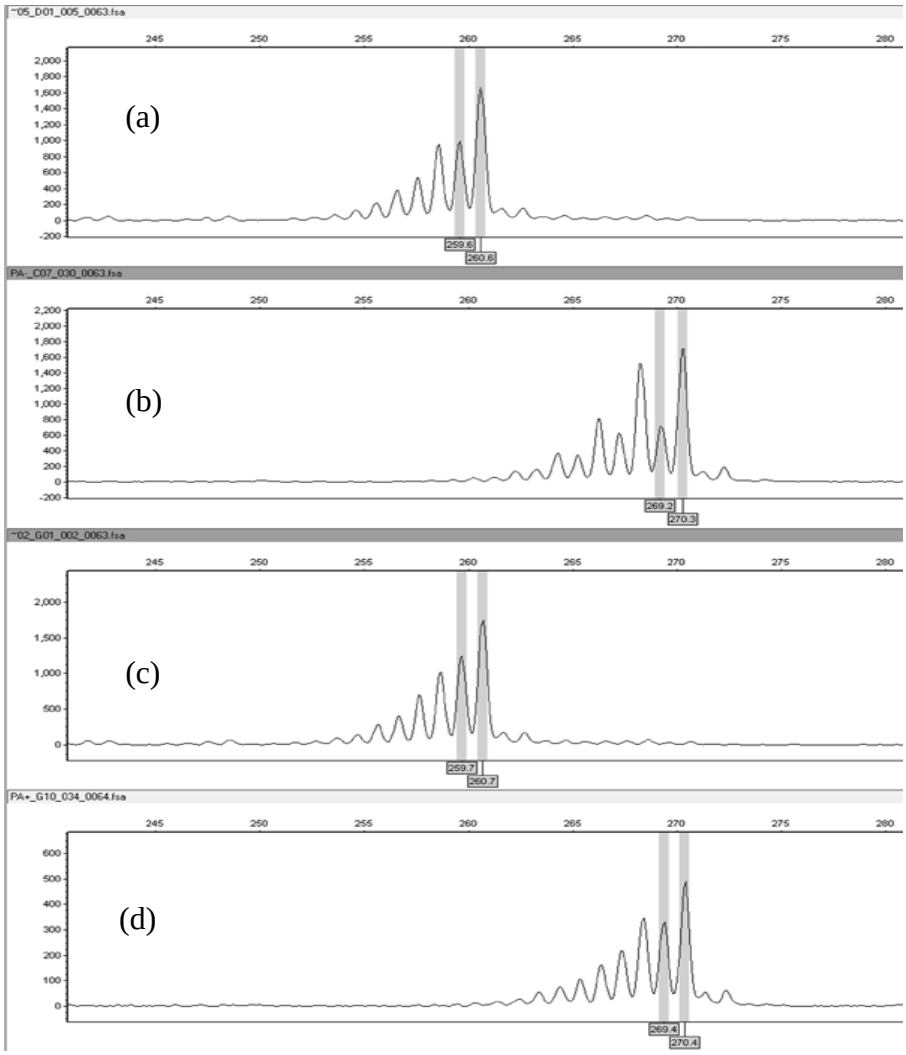
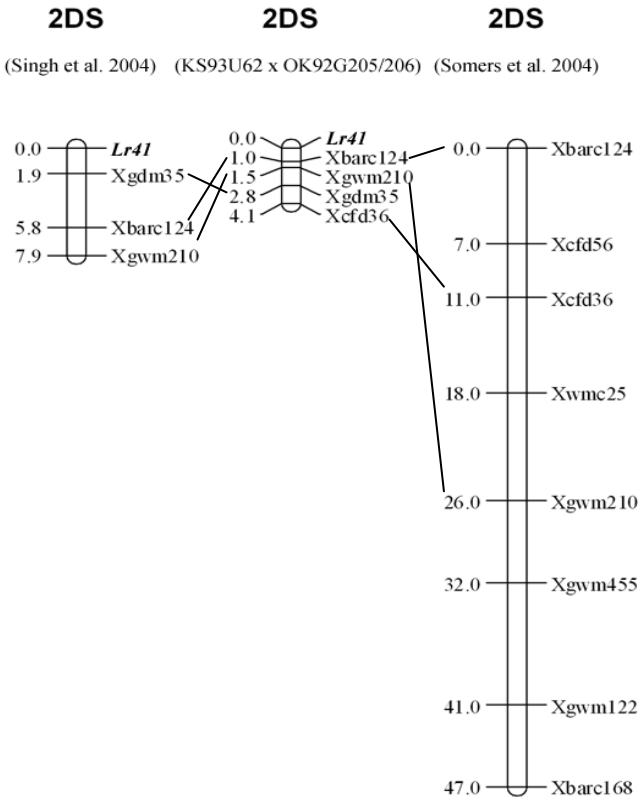


Figure 4.2. Alignment of the current *Lr41* genetic map with two other maps obtained from Somers et al. 2004 and Singh et al. 2004.
a. WGRC10/ TAM 107, b. KS93U62/ OK92U205 or OK92U206, c. Consensus map fused with four populations which are Synthetic/Opata, RL4452/AC Domain, Wuhan/Maringa and Superb/BW278. The centromere is toward bottom of the figure.



(a) (b) (c)

Figure 4.3. ABI Gel Image displaying alleles of Gwm35 (2DS) found in KS93U50 (carrying *Lr42*), KS93U62 (carrying *Lr41*), OK92G205/206 (susceptible parents), Chinese Spring, DT2DL, DT1DL, DT1DS, N2D-T2B, N2D-T2A, N1D-T1B. The allele associated with *Lr41* has the fragment of 182bp, and the fragment amplified by Chinese spring is 239bp. KS93U50 and two susceptible parents have the fragment of 265bp. Four lines across the image stand for ladder.

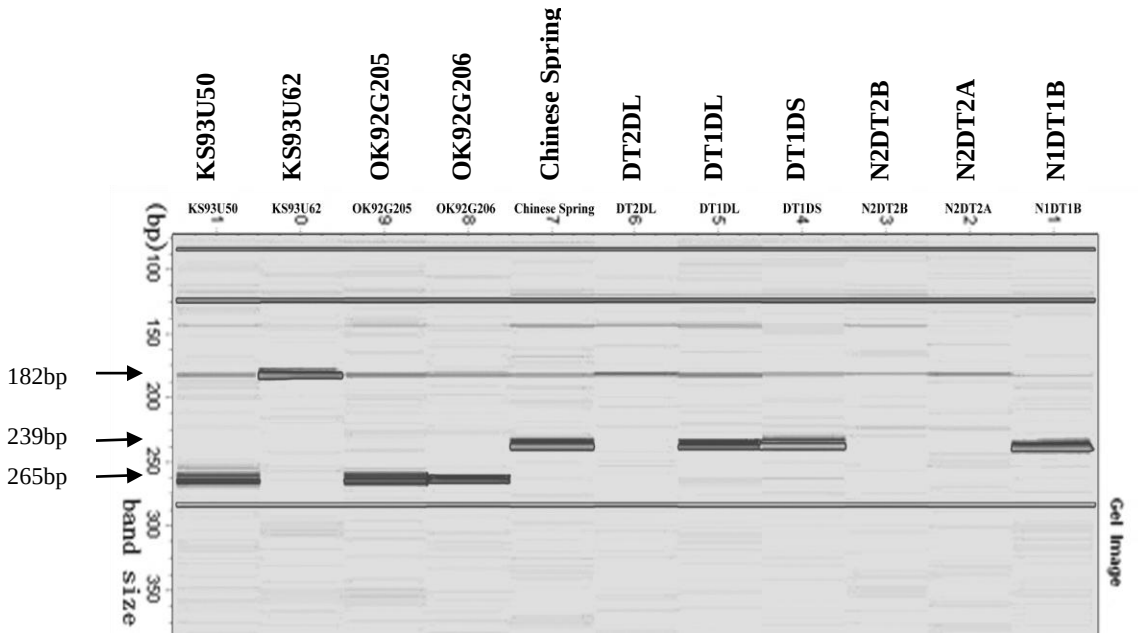


Table 4.1. Infection types exhibited by seedling and adult plants of lines containing *Lr* genes derived from *T. tauschii* and susceptible control lines inoculated with two *Puccinia triticina* cultures.

Name	PRTUS25	PRTUS35	PRTUS25[b]
KS93U62(*Lr41*)	0;[a]	3	Resistant
OK92G205	3	3	Susceptible
OK92G206	3+	3	Susceptible
TAM 110	3	3	Susceptible

[a] The seedling infection types are: 0 = no uredinia or other microscopic sign of infection, ; = no uredinia but small hypersensitive necrotic or chlorotic flecks present, 3 = medium sized uredinia with or without chlorosis, + = uredinia somewhat larger than average

[b] The adult plant reaction when it was inoculated with PRTUS25.

Table 4.2. Pedigrees and marker haplotypes of wheat cultivars and breeding lines and their reactions to leaf rust isolate PRTUS25

Name	Pedigree	Class	Origin	[a]*Lr41*	Size of Amplified Fragment (bp)			
					Xbarc124	*Xgwm210*	*Xgdm35*	*Xcfd36*
AP03T6115	Karl//Mit/Lancota/3/U1254-4-9-8-V32	HWW	USA	R	271	182/206	265	215
Bullet06ERU	KS96WGRC39/Jagger	HWW	USA	R,R’	266/271	182/206	265	215
Fuller	Bulk selection	HWW	USA	R’	271	182/206	249	215
KS93U62	Century*3/ TA2460	HWW	USA	R	261	182/206	182	213
OK Bullet	KS96WGRC39/Jagger	HWW	USA	R, R’	271	182/206	265	215
OK02522W	KS96WGRC39/Jagger	HWW	USA	R	271	182/206	265	215
OK05737W	KS96WGRC39/Jagger	HWW	USA	R	271	182/206	229	215
OK05741W	KS96WGRC39/Jagger	HWW	USA	R	271	182/206	229	215
Overley	(TAM-107 *3/TA 2460)/Heyne 'S'//Jagger	HWW	USA	R, R’	271	182/206	249	215
Postrock	Ogallala/KSU94U261//Jagger	HWW	USA	R,R’	271	182/206	265	215
Thunderbolt	Abilene/KS90WGRC10	HWW	USA	R	261	182/206	182	215
Tx01V5719	U1254-4-7-3/OGALLALA	HWW	USA	R	261	182/206	261	215
111.92	FengKang15/Cooperacion Nanihue	SWW	Argentina	S	266	182/184	252	213
113.92	FengKang15/Cooperacion Nanihue	SWW	Argentina	S	266	182/184/206	252	213
117.92	FengKang15/Cooperacion Nanihue	SWW	Argentina	S	261/266	182/184/206	182/252	213
38M.A.	Barleta 4d/Chino	SWW	Argentina	S	266	182/184	274	213
BacUp	Nuy Bay/Pioneer2375//Marshall	HSW	USA	S	271	182/184	252	213
Cardinal	Logan *2/3/Va63-5-12/Logan//Blueboy	SWW	USA	S	266	182/184/206	182/239	213

Centerfield	(TXGH12588-105*4/FS4)/2*2174	HWW	USA	S	266	182/184	229/265	215
Chinese Spring	Landrace	SSW	China	S	266	182/184/206	239	215
Chisholm	Sturdy sib / Nicoma	HWW	USA	S	266/271	182/184	261	215
Coop-Capoildo	Land race	SWW	Argentina	S	266	182/184	252	213
Deliver	(Yantar/2*Chisholm)/Karl	HWW	USA	S	266/271	182/184	229	215
Duster	W0405D/NE78488//W7469C/TX81V6187	HWW	USA	S	281	182/184	252	215
Encruzilhada	Fortaleza/Kenya Farmer	SWW	Brazil	S	266	182/184	233	235
Endurance	HBY756A/Siouxland//2180	HWW	USA	S	271	182/184	256	215
Ernie	Pike /3/ Stoddard / Blueboy // Stoddard / D1707	SWW	USA	S	266	182/184	182/233	213/235
Expert	Extrem/Mexico4040//Neuhof1/3/Extrem/HP35719	SWW	Austria	S	266	182/184/206	260	213
Extrem	Record/Br. Herrachweiten	SWW	Austria	S	266	182/184/206	260	213
Foster	Ky83-60/Tyler//KY83-75	SWW	USA	S	266	182/184	182/233	213/235
Freedom	GR876 / OH217	SWW	USA	S	266	182/184/206	182/239	213
Guymon	Intrada/Platte	HWW	USA	S	266	182/184	229	215
IL93-2283	IL84-3511/IL84-3348	SWW	USA	S	266	182/184/206	233	213/235
IL94-1549	Auburn/Ark38-1/Arther/Blueboy	SWW	USA	S	266	182/184	182/239	213
IL94-1909	Fillmore/Amigo//Tyler/Howell	SWW	USA	S	250/261	182/184	182/255	213/219
IL94-2426	Fillmore/Amigo//Tyler/Howell	SWW	USA	S	266	182/184	182/233	213/235
IL94-6280	IL87-3721/Cardinal//P808801-4-2-4-107	SWW	USA	S	271	182/184/206	245	215
IL95-1966	IL87-2834-1/IL87-6512//IL87-1968-1	SWW	USA	S	266	182/184	182/233	235
IL95-2066	IL88-7890/P7924H1-20-2-74	SWW	USA	S	266	182/184	182/239	213
IL95-2909	Freedom//IL84-2191-1/IL84-4046	SWW	USA	S	266	182/184	182/239	213

IL9634-24851	IL90-6364//IL90-9646/Ning7840	SWW	USA	S	266	182/184	182/233	213/235
Karat	Extrem/Betosfeje1	SWW	Austria	S	266	182/184/206	260	213
Kaskaskia	IL77-2933/IL77-3956//Pike/Caldwell	SWW	USA	S	266	182/184	182/256	213/219
KS93U50	Century*3/ TA2450	HWW	USA	S	250	182/184	233/265	213
Livius	Karat/Lentia	SWW	Austria	S	266	182/184/206	256	213
MO-94-193	MO 11728/Becker	SWW	USA	S	266	182/184	239	213
MO94-312	Pioneer brand 2551/Caldwell	SWW	USA	S	266	182/184	182/239	213
NTDHP	Land race from Jiangsu	SSW	China	S	271	182/184/206	239	213/219
OH552	Pur71761A4-31-5-33/MD55-286-21	SWW	USA	S	266	182/184	182/256	213
OH569	Pur 71761A4-31-5-33/MO 55-286-21	SWW	USA	S	266	182/184	182/239	213
OK03716W	Oro Blanco/OK92403 F4:11	HWW	USA	S	271	182/184	252/261	215
OK03825-5403-5	Custer*3/94M81	HWW	USA	S	271	182/184	261	215
OK04525	FFR525W/Hickok//	HWW	USA	S	281	182/206	245/261	215/235
OK05830	OK93617/Jagger	HWW	USA	S	271	182/206	229	215
OK05903C	TXGH12588-120*4/FS4//2174/3/Jagger	HWW	USA	S	271	182/206	229	215
OK05905C	TXGH12588-105*4/FS4//2174/3/Jagger	HWW	USA	S	266	182/184	229	215
OK92G205	Century*5/McNair1003	HWW	USA	S	271	182/184	233/265	213/235
OK92G206	Century*5/McNair1003	HWW	USA	S	271	182/184/206	233/265	213/235
P93D1-10-2	851423/INW9853	SWW	USA	S	266	182/184	182/239	213
PA8769-158	Titan / Caldwell	SWW	USA	S	266	182/184	182/239	213
Par-55	Unknown	SWW	China	S	266	182/184	256	219
PB2555	Coker68-16/MoW 7140//Pioneer brand W521	SWW	USA	S	266	182/184/206	245	215

PC-2	Lira's'//AU/UP301	SWW	China	S	266	182/184	239	213
Perlo	Extrem/Betosfeje1	SWW	Austria	S	250/266	182/184/206	256	213
Poncheau	Sel. from land race	SWW	France	S	266	182/184	250	215
Pontiac	Magnum/Auburn	SWW	USA	S	266	182/184	239	213
Roane	VA71-54-147/Coker68-15//IN65309C1-18-2-3-2	SWW	USA	S	266	182/184	182/229	217
Sanshukomugi	Land race from Mie	SSW	Japan	S	271	182/184/206	239	213
Shinchunaga	Land race from Mie	SSW	Japan	S	271	182/206	256	213/219
ShirasayaNo1	Land race from Mie	SSW	Japan	S	266	182/184/206	256	213/219
Spartakus	Perlo/ Extrem/Betosfeje1	SWW	Austria	S	266	182/184/206	256	215
Sumai3	Funo/Taiwan Wheat	SSW	China	S	261/266	182/206	256	213/219
Sumai49	N7922/Ning7840	SSW	China	S	266	182/184	182/256	213/219
Vilela-Sol	Land race	SWW	Argentina	S	266/271	182/184	252	213
Wangshuibai	Land race from Jiangsu	SSW	China	S	266	182/184/206	245	215
Xianmai1	Ardito/Tevere//Wannian2	SSW	China	S	266	182/184	182/265	213

[a] R refers to lines that are expected to carry *Lr41* based on pedigree and infection type of isolate PRTUS25; R' refers to lines that are expected to carry *Lr41* based on leaf rust differential test results (http://www.ars.usda.gov/main/site_main.htm?modecode=36-40-05-00); S refers lines that are not expected to carry *Lr41* based on pedigree and/or infection type.

CHAPTER 5 - Molecular Mapping of Wheat Leaf Rust Resistance Gene *Lr42*

Introduction

Lr42, a race-specific gene, was introgressed from *T. tauschii* and was located on wheat chromosome 1DS in an earlier genetic study (Cox et al. 1994). Germplasm lines containing *Lr42* have been utilized by several US and international breeding programs (Bacon et al., 2006; Singh et al., 2007). Martin et al. (2003) reported the agronomic effects of *Lr42* resistance using near-isogenic lines for the gene and concluded that *Lr42* played a significant role in increasing yield, test weight, and kernel size in Oklahoma winter wheat.

As discussed in Chapter 4, one strategy for prolonging the usefulness of race specific resistance genes is to pyramid or combine multiple resistance genes in one cultivar. Closely linked molecular markers are essential for marker-assisted selection (MAS) in breeding programs to reduce selection of false recombinants. Markers closely linked to *Lr42* have not been reported. In this study, we used NILs for *Lr42* to confirm the physical location of the gene by using molecular markers in conjunction with ditelosomic and nullitetrasomic wheat genetic stocks and identified two closely linked markers to *Lr42* in a populations developed from a cross between NILs contrasting in the gene.

Materials and methods

Plant materials and rust evaluation

Germplasm line KS91WGRC11 (Century*3/ TA2450, PI 566668) contains *Lr42* derived from *T. tauschii* accession TA2450 (Cox et al., 1994). Line KS93U50, a selection from KS91WGRC11, was crossed and backcrossed to OK92G205 (Century*5/'McNair 1003', PI 561731) and OK92G206 (Century*5/'McNair 1003', PI 561733), two Century-backcross-derived lines that do not contain *Lr42* (Carver et al., 1993). The corresponding two BC_3F_2 populations were artificially inoculated with PRTUS25 in a greenhouse to select leaf rust-resistant or susceptible lines. Plants were sprayed with a suspension of urediniospores in Soltrol 170 light mineral oil (Phillips Petroleum, Bartlesville, OK) and then incubated overnight in a dew chamber at 20-24C. Plants were subsequently grown in the greenhouse at 20-24C. About 100 resistant and 100 susceptible BC_3F_2 plants were selected from each cross, and their $BC_3F_{2:3}$ progenies were further evaluated for rust resistance at the seedling stage to identify non-segregating families homozygous for either allele at the *Lr42* locus. About 100 selected $Bc_3F_{2:4}$ and $BC_3F_{2:5}$ families were further evaluated for adult plant resistance under natural infection conditions in fields of Oklahoma in 1998 and 1999 (Martin et al., 2003). Forty-four $Bc_3F_{2:6}$ NILs (31 from KS93U50/OK92G205 and 13 from KS93U50/OK92G206) were selected on the basis of their leaf rust reactions and used in this study.

To verify the resistance of selected NILs, all 44 $Bc_3F_{2:6}$ NILs were evaluated twice for resistance as adults in March (spring) and November (fall) 2007 and for seedling resistance in spring 2008 with different isolates in the growth chamber. In the 2007 greenhouse experiments, plants were grown in Metro-Mix 360 soil mix (Hummert International, Earth City, MO, USA) in 1-liter pots. All NILs were inoculated at early anthesis with the isolate PRTUS25 (race MDB using the nomenclature of Long and

Kolmer (1989), avirulence/virulence formula: 2a, 2c, 9, 16, 26, 3ka, 11, 17, 30/1, 3, 24). Rust inoculation method was the same as described above. The experiments used a randomized-complete-block design with two replicates and five plants per replicate. Infection types of leaf rust on the leaves of adult plants were compared with both parents two weeks after inoculation and scored as either resistant or susceptible types (McIntosh et al., 1995). In the spring 2008 seedling test, six plants per NIL were planted in Metro-Mix 360 soil mix. Seedlings were inoculated with rust cultures PRTUS25, PRTUS35 (race TNR, avirulence/virulence formula: 16, 26, 17/1, 2a, 2c, 3, 9, 24, 3ka, 11, 30), and PNM (avirulence/virulence formula: 2a, 16, 26, 11, 17/1, 2c, 3, 9, 24, 3ka, 30) at the two-leaf stage. All three isolates are avirulent to *Lr42* but virulent to *Lr24*, which is present in Century (Cox et al., 1994). TAM 110 (PI 595757) was used as the susceptible check. Inoculated seedlings were kept in a dew chamber at 20±1ºC with 100% humidity for 12 h and then grown in a growth chamber for 10 days at 20±1ºC with 12 h light. Seedling infection types were scored according to McIntosh et al (1995).

Chinese Spring nullitetrasomic and ditelosomic genetic stocks, Nullisomic-1D/Tetrasomic-1B (abbreviated as N1D-T1B), N2D-T2A, N2D-T2B, Ditelosomic 1DS (abbreviated as DT1DS), DT1DL, and DT2DL (http://www.k-state.edu/wgrc/Germplasm/Stocks/stocks.html), were used to physically map the markers linked to *Lr42*. An international collection of 85 genetically diverse wheat germplasm lines from Argentina, Brazil, USA, Austria, France, China, and Japan was used to evaluate polymorphism for the new markers developed in this study. Among these lines, AR93005 and Fannin were reported to derive from *T.tauchii* accession TA2450 (Table 5.2).

Marker analysis

Seedlings from the fall 2007 experiment were used as the plant source for DNA isolation. Leaf tissue was collected in 1.1-ml strip tubes, dried in a freezer drier (Thermo Fisher, Waltham, MA) for 2 days, and ground in Mixer Mill (Retsch GmbH, Rheinische Strasse 36, Germany) to fine powder by shaking strip tubes with a 3.2-mm stainless steel bead at 25 times/sec for 5 min. Genomic DNA was extracted from parents and NILs by using the cetyltrimethyl ammonium bromide (CTAB) method (Saghai-Maroof et al. 1984). PCR amplifications were performed in a Tetrad Peltier DNA Engine (Bio-Rad Lab, Hercules, CA). A 12-µl PCR mix contained 1.2 µl of 10X NH_4 buffer (Bioline Inc. Taunton, MA), 2.5 mM of $MgCl_2$, 200 µM of each dNTP, 100 nM of forward tailed primer, 200 nM of reverse primer, 100 nM of M13 fluorescent-dye labeled primer, 1 U of *Taq* DNA polymerase, and 50 ng template DNA. A touchdown program modified from Ma et al. (2005) was used for PCR reactions. The reaction was incubated at 95ºC for 5 min then continued for five cycles of 1 min of denaturing at 96ºC, 5 min of annealing at 68ºC with a decrease of 2ºC in each of subsequent cycles, and 1 min of extension at 72ºC. For another five cycles, the annealing temperature started at 58ºC for 2 min with a decrease of 2ºC for each subsequent cycle. PCR went through an additional 25 cycles of 1 min at 96ºC, 1 min at 50ºC, and 1 min at 72ºC with a final extension at 72ºC for 5 min. PCR products were analyzed on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA).

Bulked-segregant analysis (Michelmore et al., 1991) was used to identify polymorphic SSR markers associated with *Lr42*. Equal amounts of DNA were pooled separately from five *Lr42*-resistant and five *Lr42*-susceptible NILs. In the original *Lr42* report, *Lr42* was located on 1DS with gene *Lr41* (Cox et al. 1994). However, recent

molecular mapping work relocated *Lr41* (Singh et al., 2004; Sun et al., 2009) and *Lr42* (Singh S, *unpublished*) to 2DS, not 1DS. To further confirm the physical location of *Lr42*, 60 microsatellite markers (SSR) from chromosome 1D and 55 markers from 2D (Roder et al., 1998; Somers et al., 2004) were screened between the parents and between two bulks. Polymorphic markers between the bulks were further analyzed on all the NILs for linkage analysis.

Data analysis

The data collected from the ABI DNA analyzer were further processed by using GeneMarker version 1.6 (SoftGenetics LLC. State College, PA, USA) and rechecked twice manually for accuracy. Genetic linkage among SSR makers and the leaf rust resistance locus was determined by JoinMap 3.0 (Van Ooijen and Voorrips 2001) using the Kosambi mapping function (Kosambi 1944) with a LOD threshold of 3.0.

Results

Reactions of NILs and genetically diverse accessions to leaf rust infection

OK92G205 and OK92G206 showed a fully susceptible reaction when they were inoculated with the isolate PRTUS25 at the adult growth stage. KS93U50 adult plants showed moderate resistance with an infection type (IT) of 2+. NILs ranged from fully susceptible to moderately resistant to leaf rust infection. When parents were inoculated with PRTUS25 and PRTUS35 at the seedling stage, KS93U50 showed incomplete resistance with ITs of 2C and 2+, respectively, and ITs of two susceptible parents and the check TAM 110 were 3 and 3+ (Table 5.1). The culture PNM induced ITs of 1 in KS93U50 and 3+ in OK92G205 and OK92G206. The seedling resistance classification of each individual NIL to all three isolates was consistent and agreed with results from

the adult plants with three exceptions of susceptible adults that were scored as resistant seedlings. These were interpreted as scoring errors of the adult plants.

Markers for Lr42

When 115 SSR markers from chromosome 1D and 2D were screened between parents and bulks, two SSR markers on 1DS (*Xcfd15* and *Xwmc432*) showed polymorphism between parents and between bulks. Primer CFD15 amplified a 220-bp fragment in KS93U50 and the resistant bulk (Figure 5.1a & c) and a 178-bp fragment in the susceptible parents (OK92G205 and OK92G206) and the susceptible bulk (Figure 5.1b & d). Primer WMC432 amplified two fragments, 204-bp and 211-bp, in KS93U50 as the specific banding pattern that was associated with *Lr42*. These markers were further used to analyze the 44 NILs. Linkage analysis using the two markers and rust data identified *Xwmc432* as a closely linked marker at 0.8 cM proximal to *Lr42* (Figure 5.2a). Marker *Xcfd15* was also close, about 1.6 cM proximal to *Lr42*. A distal flanking marker for *Lr42* was not identified.

To verify the physical location of *Lr42*, the proximal marker *Xwmc432* was analyzed in a set of nulli-tetrasomic lines. The primer WMC432 amplified two fragments of 204 and 211 bp in N2D-T2A and N2D-T2B but not in N1DT1B and DT1DL, suggesting that marker *Xwmc432* was on chromosome 1D (Figure 5.3). Furthermore, appearance of the marker in DT1DS confirmed that *Lr42* is on chromosome 1DS.

To evaluate the potential use of these *Lr42* markers in MAS, polymorphism of these markers in a diverse set of 85 additional wheat cultivars or breeding lines from different wheat classes and geographic areas was analyzed (Table 5.2). WMC432 amplified 9 alleles among 85 accessions with a single fragment amplified in 43

accessions and at least two fragments in 42 accessions. A 204/211-bp fragment combination was amplified by WMC432 in KS93U50 and another US line, AR93005. In addition, a single 204-bp fragment without the 211-bp fragment was amplified in a U.S. hard winter wheat, OK03716. A 211-bp fragment accompanied with an additional 202-bp fragment was amplified in the Chinese landrace, Chinese Spring. Primer CFD15 amplified four alleles across the 85 accessions. The 220-bp fragment was associated with *Lr42*-resistance as seen in KS93U50, whereas the 178-bp fragment was present in both susceptible parents and was not associated with *Lr42*. Among 85 accessions, the 220-bp fragment was only amplified in AR93005.

Discussion

In this study, we determined the location of wheat leaf rust resistance gene *Lr42* from *T. tauschii* through genetic linkage mapping and aneuploid analysis of linked markers. The small number of polymorphic markers identified in this study was expected because the populations were derived from NILs in a Century background. Genetic analysis of the population of 44 NILs showed that both marker loci, *Xwmc432* and *Xcfd15*, were tightly linked to *Lr42*. The chromosome arm containing *Lr42* was confirmed by mapping one of the two markers on 1DS ditelosomic and 1D nullitetrasomic aneuploid stocks (Figure 5.3). Based on previously published positions for the linked markers (Somers et al. 2004, Sourdille et al. 2004), *Lr42* is located near the middle of the short arm of chromosome arm 1D (Figure 5.2).

In addition to *Lr42*, two other leaf rust resistance genes were reported on 1DS. *Lr21* is located at the distal end of 1DS about 4 cM distal to marker *Gli-D1* (Huang et al, 2003). Cox et al. (1994) estimated a recombination frequency of 0.286 ± 0.023

(approximately 33 cM using the Kosambi function) between *Lr21* and *Lr42* in a compilation of several crossing experiments. The consensus map of Somers et al. (2004) indicates that *Xwmc432* and *Xcfd15* cosegregate and are approximately 20 cM proximal to *Gli-D1*, so the location of *Lr42* may be closer to *Lr21* than expected based on the results of Cox et al. (1994). Due to lack of polymorphism for *Lr21* and associated markers, we were not able to directly estimate the distance in this study. Hiebert et al. (2008) located *Lr60* 8.4 cM distal to *Xbarc149* on 1DS, which should put *Lr60* about 17 cM distal to *Lr42* according to the map of Somers et al. (2004). The location would also be near *Lr21* based on the map of Huang et al. (2003). In an allelism test, Hiebert et al. (2008) concluded that *Lr60* is 13.5 cM distal to *Lr21*, which would place *Lr60* and *Lr42* approximately 40 cM apart (Huang et al 2003, Somers et al, 2004) To confirm the relationship between *Lr60* and *Lr42*, an appropriate population needs to be developed to test genetic linkage between *Lr60* and *Lr42*.

Cox et al (1994) reported that the *Lr42* resistance phenotype varied from a hypersensitive fleck to a mixed reaction of flecks and small sporulating pustules surrounded by necrosis or chlorosis. In the present study, resistant infection types were higher and ranged from 1 (small uredinia surrounded by necrosis) to 2+ (medium uredinia surrounded by necrosis or chlorosis) (Table 5.1). This difference may be partially attributed to the utilization of different rust cultures, although one culture (PRTUS25) was used in both studies. Culture PNM gave the lowest infection type and clearly separated resistant from susceptible phenotypes in the NIL population. PNM was fully virulent on *Lr24* and was most useful for phenotyping *Lr42*.

Virulence to *Lr42* in the US was initially reported to be infrequent (Kolmer et al., 2006), but virulence has apparently increased to significant levels in the most recent surveys (Kolmer et al., 2008b). Nevertheless, line KS91WGRC11 containing *Lr42* plus *Lr24* continues to show a moderately resistant reaction in the field in Manhattan, Kansas whereas the cultivar Century containing *Lr24* is fully susceptible (D. Wilson pers. comm.) *Lr42* should be used in combinations with other leaf rust resistance genes to maximize its usefulness.

To date, *Lr42* has not been well deployed in wheat breeding programs. Among 85 accessions, only two have the *Lr42* donor, TA2450, in their pedigrees (Table 5.2). A soft red winter wheat, AR93005 derived from KSWGRC11, carried the 220-bp fragment for marker *Xcfd15* and the 204/211-bp banding pattern for marker *Xwmc432* as seen in KS93U50 (Table 5.2). However, *Xwmc432* and *Xcfd15* alleles amplified in hard red winter wheat Fannin did not match those from KS93U50 and the resistant NILs. Bacon et al. (2006) reported that AR93005 had similar resistance as KS91WGRC11 when inoculated with culture PNM (an avirulent isolate on *Lr42*), while Fannin was more susceptible than KS93U50 when they were inoculated with the same culture (Table 5.1). These data suggest that AR93005, not Fannin, carries *Lr42* and the two SSR markers, *Xcfd15* and *Xwmc432*, predicts the existence of *Lr42* derived from KSWGRC11.

Using MAS to assemble gene combinations with *Lr42* requires closely linked or flanking markers and sufficient marker polymorphism in the parental lines. The two new markers are mapped within 2 cM from *Lr42* and therefore adequate for MAS. After screening 85 genetically diverse accessions from different regions, we suggest that the polymorphism for both markers is suitable for MAS in a broad range of germplasm.

Figure 5.1. ABI electrophorograms of SSR marker *Xcfd15* on chromosome 1D showing polymorphism among (a) KS93U50 (Lr42), (b) OK92G206 (susceptible parent), (c) resistant bulk and (d) susceptible bulk.

The allele sizes in the figure were rounded off to a decimal.

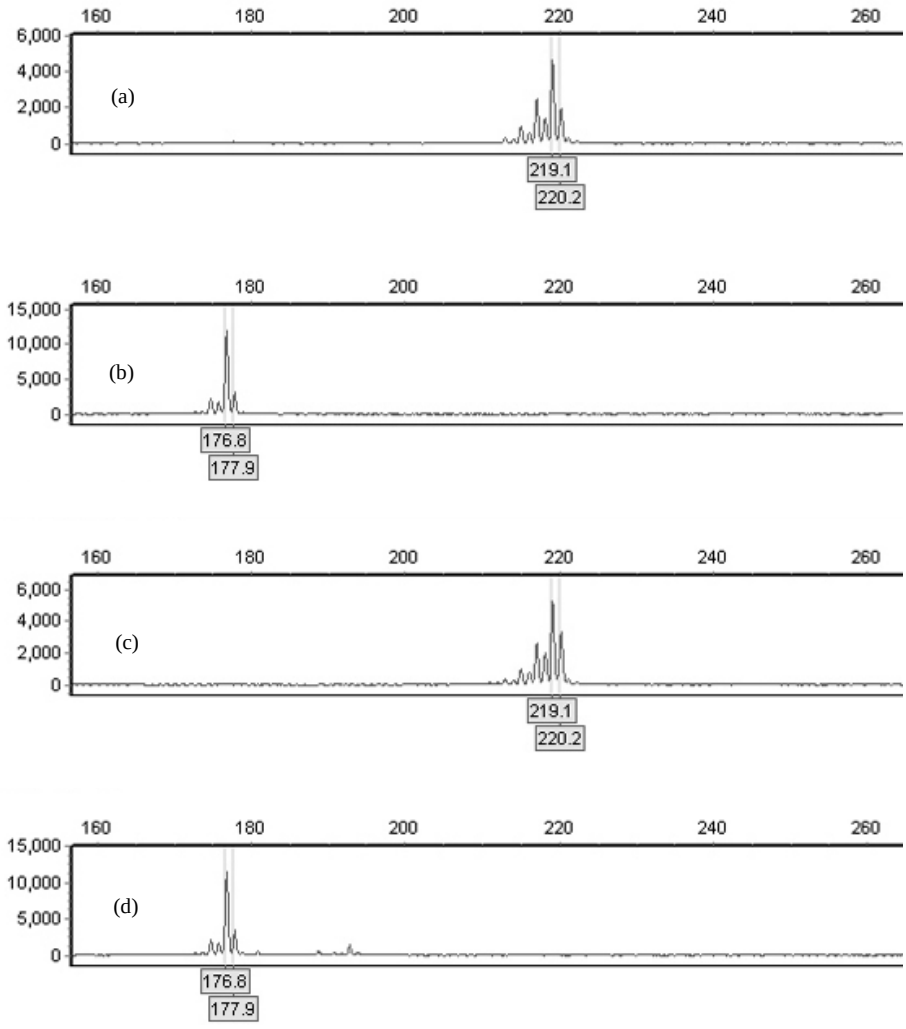


Figure 5.2. Comparison of the *Lr42* genetic map with a previously reported consensus map (Somers et al. 2004) to show location of *Lr42* in chromosome arm 1DS.

(a) The SSR genetic maps developed by Sourdille et al. (2004), (b) The map with Lr42 developed in this study, (c) The consensus map developed by Somers et al. (2004). The centromere is toward the bottom of the map. The region of gene Lr21 was estimated from Huang et al. (2003).

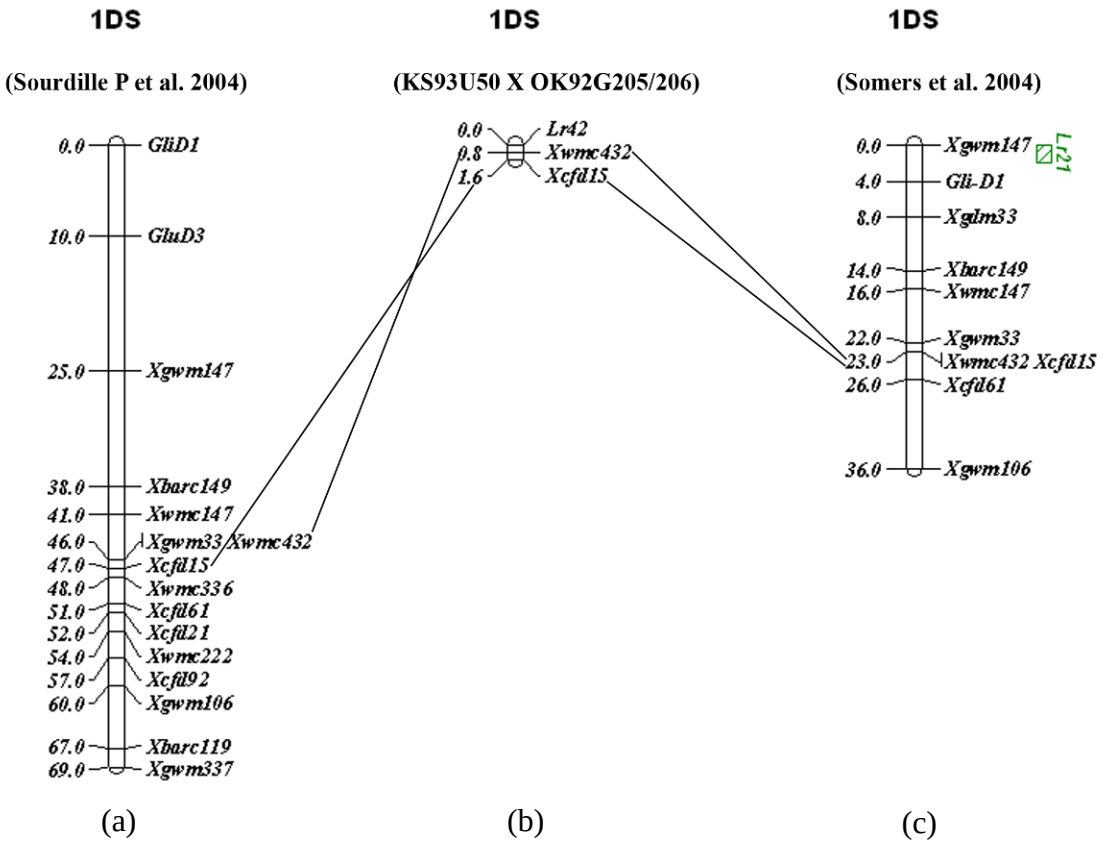


Figure 5.3. ABI gel image to show the fragments amplified by WMC432 in KS93U50 (Lr42-carrier), OK92G205 (susceptible parent), OK92G206 (susceptible parent), Chinese Spring, and Chinese Spring DT2DL, DT1DL, DT1DS, N2D-T2B, N2D-T2A, and N1D-T1B.

The fragments associated with Lr42 are 204 and 211 bp (arrow). The y-axis shows ladder size.

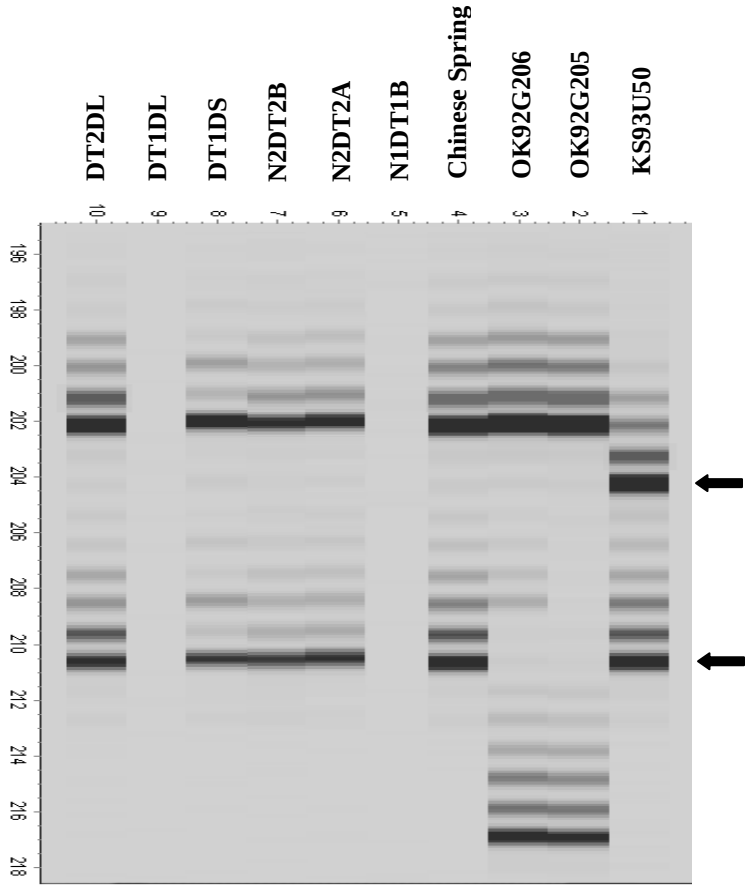


Table 5.1. Infection types evaluated by inoculating three wheat parents and controls contrasting in *Lr42* gene derived from *T. tauschii* with three *Puccinia triticina* isolates at seedling stage and one isolate at adult stages.

Name	PRTUS25[a]	PRTUS35	PNM	PRTUS25[b]
KS93U50 (*Lr42*)	2C	2+	1	Moderate Resistant
OK92G205	3	3	3+	Susceptible
OK92G206	3+	3	3+	Susceptible
FANNIN	0;	3-	2+3-	Not Done
TAM 110	3	3	3+	Susceptible

[a] The seedling infection types are: 0 = no uredinia or other microscopic sign of infection, ; = no uredinia but small hypersensitive necrotic or chlorotic flecks present, 1 = small uredinia surrounded by necrosis, 2 = small to medium uredinia surrounded by necrosis or chlorosis, 3 = medium sized uredinia with or without chlorosis, + = uredinia somewhat larger than average, C = extra chlorosis.

[b] The adult plant reaction when inoculated with leaf rust isolate PRTUS25

Table 5.2. Haplotypes of the markers linked to the *Lr42* gene in three parents of near-isogenic population and 85 additional wheat accessions collected from the USA and other countries.

Variety	Pedigree	Source	*Xcfd15*[a]	*Xwmc432*
KS93U50	Century*3/ TA2450	USA	220	204/211
OK92G205	Century*5/McNair1003	USA	178	202/217
OK92G206	Century*5/McNair1004	USA	178	202/217
Bullet06ERU	KS96WGRC39/Jagger	USA	178	202/217/236
Centerfield	(TXGH12588-105*4/FS4)/2*2174	USA	178	236
Chisholm	Sturdy sib / Nicoma	USA	178/194	202/217/238
CO02W237	98HW519(93HW91/93HW255)/96HW94	USA	178	202/213
Deliver	Yantar/2*Chisholm//Karl	USA	178	238
Duster	W0405D/NE78488//W7469C/TX81V6187	USA	178/200	202/217
Endurance	HBY756A/Siouxland//2180	USA	178	238
Fuller	Bulk selection	USA	178	238
Guymon	Intrada/Platte	USA	178/194	202/217/236
KS93U62	Century*3/ TA2460	USA	178	202/217
OK Bullet	KS96WGRC39/Jagger	USA	178	236
OK02522W	KS96WGRC39/Jagger	USA	178	236
OK03716W	Oro Blanco/OK92403 F4:11	USA	178	204
OK03825-5403-5	Custer*3/S. African BC1F2	USA	178/194	238
OK04525	FFR525W/Hickok// F4:11	USA	178	202/217
OK05737W	KS93U206//KS82W418/Stephens F8:14 HW	USA	178	236
OK05741W	KS93U206//KS82W418/Stephens F8:14 HW	USA	178	236
OK05830	OK93617/Jagger F6:12	USA	178/194	202/208/217
OK05903C	TXGH12588-120*4/FS4//2174/3/Jagger F4:10RC	USA	178/194	202/208/217/236
OK05905C	TXGH12588-105*4/FS4//2174/3/Jagger F4:10	USA	178	236

Overley	(TAM-107 *3/TA 2460)/Heyne ‘S’//Jagger	USA	178	202/208/217
Postrock	Ogallala/KSU94U261//Jagger	USA	178	202/208/217
Thunderbolt	Abilene/KS90WGRC10	USA	178	236
Tx01V5719	U1254-4-7-3/OGALLALA	USA	178/194	202/217
AP03T6115	Karl//Mit/Lancota/3/U1254-4-9-8-V32	USA	178	236
AP05T2413	(KS95U522/TX95VA0011)F1/Jagger	USA	178	236
AR93005	Wakefield/KS91WGRC11	USA	178/194/220	204/211
Fannin	TAM 105/3/NE70654/BBY//BOW"S"/4/Century*3/TA2450	USA	178/194	202/217/238
KS970187-1-10	TAM107*2/TA759//HBC197F-1/3/2145	USA	178/194	202/213
NE02558	JAGGER/ALLIANCE	USA	178	202/213
NE05496	KS95HW62-6 (=KS87H325/RIO BLANCO)/HALLAM	USA	178	202/213
OK03305	N40/OK94P455	USA	178/194	238
SD06W117	Alice/SD00W024	USA	178	238
T153	T136/T151	USA	178	238
TX03A0563	X96V107/OGALLALA	USA	178	236
TX04M410211	MASON/JAGGER//OGALLALA	USA	178	236
Bacup	Nuy Bay/Pioneer2375//Marshall	USA	178	202
Cardinal	Logan *2/3/Va63-5-12/Logan//Blueboy	USA	178/194	202/217
Ernie	Pike /3/ Stoddard / Blueboy // Stoddard / D1707	USA	178	202/208/217
Foster	Ky83-60/Tyler//KY83-75	USA	178	202
Freedom	GR876 / OH217	USA	178	202
IL93-2283	IL84-3511/IL84-3348	USA	178/200	202
IL94-1549	Auburn/Ark38-1/Arther/Blueboy	USA	178/200	202/217
IL94-1909	Fillmore/Amigo//Tyler/Howell	USA	178	202
IL94-2426	Fillmore/Amigo//Tyler/Howell	USA	178	202
IL94-6280	IL87-3721/Cardinal//P808801-4-2-4-107	USA	178/200	202/217
IL95-1966	IL87-2834-1/IL87-6512//IL87-1968-1	USA	178	202
IL95-2066	IL88-7890/P7924H1-20-2-74	USA	178/200	202

IL95-2909	Freedom//IL84-2191-1/IL84-4046	USA	178	202
IL9634-24851	IL90-6364//IL90-9646/Ning7840	USA	178/200	202
Kaskaskia	IL77-2933/IL77-3956//Pike/Caldwell	USA	178	202
MO-94-193	MO 11728/Becker	USA	178	202
MO94-312	Pioneer brand 2551/Caldwell	USA	178	202/217
OH552	Pur71761A4-31-5-33/MD55-286-21	USA	178/200	202
OH569	Pur 71761A4-31-5-33/MO 55-286-21	USA	178/200	202
P93D1-10-2	851423/INW9853	USA	178	202
PA8769-158	Titan / Caldwell	USA	178/200	202
PB2555	Coker68-16/MoW 7140//Pioneer brand W521	USA	178/200	202/217
Pontiac	Magnum/Auburn	USA	178/194/200	202/238
Roane	VA71-54-147/Coker68-15//IN65309C1-18-2-3-2	USA	178	202
Poncheau	Sel. from land race	France	178	202/208/217
Encruzilhada	Fortaleza/Kenya Farmer	Brazil	178	202
Expert	Extrem/Mexico4040//Neuhof1/3/Extrem/HP35719	Austria	178	202/208/217
Extrem	Record/Br. Herrachweiten	Austria	178	202
Karat	Extrem/Betosfeje1	Austria	178	202/236
Livius	Karat/Lentia	Austria	178	202/217
Perlo	Extrem/Betosfeje1	Austria	178	202
Spartakus	Perlo/ Extrem/Betosfeje1	Austria	178	202
111.92	FengKang15/Cooperacion Nanihue	Argentina	178	202/236
113.92	FengKang15/Cooperacion Nanihue	Argentina	178	202/236
117.92	FengKang15/Cooperacion Nanihue	Argentina	178	202/236
38M.A.	Barleta 4d/Chino	Argentina	178/194	202/217
Coop-Capoildo	Landrace	Argentina	178	202/236
Coop-Millan	Unknown	Argentina	178	240
Vilela-Sol	Landrace	Argentina	178	202/236
Chinese Spring	Landrace	China	178	202/211

NTDHP	Landrace from Jiangsu	China	178	202
Par-55	Unknown	China	178	202/236
PC-2	Lira's'//AU/UP301	China	178	202/238
Wangshuibai	Landrace from Jiangsu	China	178	202/217
Xianmai1	Ardito/Tevere//Wannian2	China	178	202/236
Sumai3	Funo/Taiwan Wheat	China	178	202/236
Sumai49	N7922/Ning7840	China	178	202/236
Sanshukomugi	Landrace from Mie	Jappan	178	202/238
Shinchunaga	Landrace from Mie	Jappan	178	202/238
ShirasayaNo1	Landrace from Mie	Jappan	178	202/238

[a] Size of amplified fragments in base pair from each wheat accession.

References

Ali, S., and L.J. Francl. 2003. Population Race Structure of *Pyrenophora tritici-repentis* Prevalent on Wheat and Noncereal Grasses in the Great Plains. Plant Dis. 87:418-422.

Ali, S., L.J. Francl, and E.D. De Wolf. 1999. First report of *Pyrenophora tritici-repentis* race 5 from North America. Plant Dis. 83:591.

Ali, S., H. Ling, S.W. Meinhardt, and L.J. Francl. 2002. A new race of *Pyrenophora tritici-repentis* that produces a putative host-selective toxin. Phytopathology 92:3.

Anderson, J.A., R.J. Effertz, J.D. Faris, L.J. Francl, S.W. Meinhardt, and B.S. Gill. 1999. Genetic Analysis of Sensitivity to a *Pyrenophora tritici-repentis* Necrosis-Inducing Toxin in Durum and Common Wheat. Phytopathology 89:293-297.

Andrie, R.M., I. Pandelova, and L.M. Ciuffetti. 2007. A Combination of Phenotypic and Genotypic Characterization Strengthens *Pyrenophora tritici-repentis* Race Identification. Phytopathology 97:694-701.

Bacon, R.K., J.T. Kelly, E.A. Milus, and C.E. Parsons. 2006. Registration of Soft Wheat Germplasm AR93005 Resistant to Leaf Rust. Crop Sci. 46:1398-1399.

Balance, G.M., and L. Lamari. 1998. Molecular aspects of host-pathogen interaction in tan spot of wheat. Can. J. Plant Pathol. 20:425-427.

Balance, G.M., L. Lamari, and B.C. C. 1989. Purification and characterization of a host selective toxin from *Pyrenophora tritici-repentis*. Physiol. Mol. Plant Pathol. 35:203-213.

Ballance, G.M., L. Lamari, R. Kowatsch, and C.C. Bernier. 1996. Cloning, expression and occurrence of the gene encoding the Ptr necrosis toxin from *Pyrenophora tritici-repentis*. Mol. Plant Pathol. On-line only: <http://www.bspp.org.uk/mppol/1996/1209ballance>.

Beckman, J.S., and J.L. Weber. 1992. Survey of human and rat microsatellites. Genomics 12:627-631.

Bockus, W.W., and M.M. Claassen. 1992. Effects of crop rotation and residue management practices on severity of tan spot of winter wheat. . Plant Dis. 76:633-636.

Bockus, W.W., and J.P. Shroyer. 1998. The impact of reduced tillage on soilborne plant pathogens. Annu Rev Phytopathol 36:485-500.

Bockus, W.W., Z. Su, K.A. Garrett, B.S. Gill, J.P. Stack, A.K. Fritz, K.L. Roozeboom, and T.J. Martin. 2007. Number of Experiments Needed to Determine Wheat Disease Phenotypes for Four Wheat Diseases. Plant Dis. 91:103-108.

Bockus, W.W., J.A. Appel, R.L. Bowden, A.K. Fritz, B.S. Gill, T.J. Martin, R.G. Sears, D.L. Seifers, G.L. Brown-Guedira, and M.G. Eversmeyer. 2001. Success stories: Breeding for wheat disease resistance in Kansas. . Plant Dis. 85:453-461.

Botstein, D., R.L. White, M. Skolnick, and R.W. Davis. 1980. Construction of genetic linkage map in man using restriction length polymorphisms. Am. J. Hum. Genet. 32:314-331.

Carver, B.F., W.E. Whitmore, and E.L. Smith. 1993. Registration of three pairs of awned vs. awnletted vear-isolines of hard red winter wheat. Crop Sci. 33:885.

Chalmers, K.J., A.W. Campbell, J. Kretschmer, A. Karakousis, P.H. Henschke, S. Pierens, N. Harker, M. Pallotta, G.B. Cornish, M.R. Shariflou, L.R. Rampling, A. McLauchlan, G. Daggard, P.J. Sharp, T.A. Holton, M.W. Sutherland, R. Appels, and P. Langridge. 2001.

Construction of three linkage maps in bread wheat, *Triticum aestivum.* Aust. J. Agric. Res. 52:1089-1119.

Chen, X., J.D. Faris, J. Hu, R. Stack, T. Adhikari, E. Elias, S. Kianian, and X. Cai. 2007. Saturation and comparative mapping of a major Fusarium head blight resistance QTL in tetraploid wheat. Molecular Breeding 19:113-124.

Cheong, J., H. Wallwork, and K.J. Williams. 2004. Identification of a major QTL for yellow leaf spot resistance in the wheat varieties Brookton and Cranbrook. Aust. J. Agric. Res. 55:315-319.

Chu, C.G., J.D. Faris, T.L. Friesen, and S.S. Xu. 2006. Molecular mapping of hybrid necrosis genes Ne1 and Ne2 in hexaploid wheat using microsatellite markers. Theor. Appl. Genet. 112:1374-81.

Churchill, G.A., and R.W. Doerge. 1994. Empirical Threshold Values for Quantitative Triat Mapping. Genetics 138:963-971.

Ciuffetti, L.M., and R.P. Tuori. 1999. Advances in the Characterization of the *Pyrenophora tritici-repentis*-Wheat Interaction. Phytopathology 89:444-9.

Ciuffetti, L.M., R.P. Tuori, and J.M. Gaventa. 1997. A Single Gene Encodes a Selective Toxin Causal to the Development of Tan Spot of Wheat. Plant Cell 9:135-144.

Cox, T.S., W.J. Raupp, and B.S. Gill. 1994. Leaf rust resistance genes *Lr41*, *Lr42*, and *Lr43* transferred from *Triticum tauschii* to common wheat. Crop Sci. 34:339-343.

Cox, T.S., W.J. Raupp, D.L. Wilson, B.S. Gill, S. Leath, W.W. Bockus, and L.E. Browder. 1992. Resistance to foliar diseases in a collection of *Tritici tauschii* germplasm. Plant Dis. 76:1061-1064.

Czembor, P.C., E. Arseniuk, A. Czaplicki, Q.J. Song, P.B. Cregan, and P.P. Ueng. 2003. QTL mapping of partial resistance in winter wheat to *Stagonospora nodorum* blotch. Genome 46:546-554.

da Luz , W.C., and R.M. Hosford, Jr. 1980. Twelve *Pyrenophora trichostoina* races for virulence to wheat in the central plains of North America. Phytopathology 70:1193-1196.

da Luz, W.C., and G.C. Bergstrom. 1986. Effect of temperature on tan spot development in spring wheat cultivars differing in resistance. Can. J. Plant Pathol. 8:451-454.

De Wolf, E.D., R.J. Effertz, S. Ali, and L.J. Francl. 1998. Vistas of tan spot research. Can. J. Plant Pathol. 20:349-444.

Duguid, S.D., and A.L. Brûlé-Babel. 2001a. Inheritance of resistance to a necrosis-and chlorosis-inducing isolate from race 1 of *Pyrenophora tritici-repentis* in spring wheat. . Can. J. Plant Sci. 81:519-525.

Duguid, S.D., and A.L. Brûlé-Babel. 2001b. Inheritance and interaction of spring wheat (*Triticum aestivum* L.) resistance to race 2 and race 3 of *Pyrenophora tritici-repentis* (Died.) Drechs. Can. J. Plant Sci. 81.

Effertz, R.J., J.A. Anderson, and L.J. Francl. 1998. QTLs associated with resistance to chlorosis induction by in adult wheat. Can. J. Plant Pathol. 20:438-439.

Effertz, R.J., J.A. Anderson, and L.J. Francl. 2001. Restriction Fragment Length Polymorphism Mapping of Resistance to Two Races of *Pyrenophora tritici-repentis* in Adult and Seedling Wheat. Phytopathology 91:572-578.

Effertz, R.J., S.W. Meinhardt, J.A. Anderson, J.G. Jordahl, and L.J. Francl. 2002. Identification of a Chlorosis-Inducing Toxin from *Pyrenophora tritici-repentis* and the Chromosomal Location of an Insensitivity Locus in Wheat. Phytopathology 92:527-533.

Elias, E., R.G. Cantrell, and R.M. Hosford, Jr. 1989. Heritability of Resistance to Tan Spot in Durum Wheat and Its Association with Other Agronomic Traits. Crop Sci. 29:299-304.
Endo, T.R., and B.S. Gill. 1996. The Deletion Stocks of Common Wheat. J. Hered. 87:295-307.
Faris, J.D., and B.S. Gill. 2002. Genomic targeting and high-resolution mapping of the domestication gene Q in wheat. Genome 45:706-718.
Faris, J.D., and T.L. Friesen. 2005. Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. Theor. Appl. Genet. 111:386-392.
Faris, J.D., J.A. Anderson, L.J. Francl, and J.G. Jordahl. 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis.* Phytopathology 86:459–463.
Faris, J.D., J.A. Anderson, L.J. Francl, and J.G. Jordahl. 1997. RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. Theor. Appl. Genet. 94:98-103.
Faris, J.D., W.L. Li, D.J. Liu, P.D. Chen, and B.S. Gill. 1999. Candidate gene analysis of quantitative disease resistance in wheat. Theor. Appl. Genet. 98:219-225.
Flor, H.H. 1947. Inheritance of reaction to rust in flax. J. Agric. Res. 74:241-262.
Friesen, T.L., and J.D. Faris. 2004. Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. Theor. Appl. Genet. 109:464-471.
Friesen, T.L., J.B. Rasmussen, C.Y. Kwon, L.J. Francl, and S.W. Meinhardt. 2002. Reaction of Ptr ToxA-Insensitive Wheat Mutants to *Pyrenophora tritici-repentis* Race 1. Phytopathology 92:38-42.
Friesen, T.L., S. Ali, S. Kianian, L.J. Francl, and J.B. Rasmussen. 2003. Role of Host Sensitivity to Ptr ToxA in Development of Tan Spot of Wheat. Phytopathology 93:397-401.
Friesen, T.L., E.H. Stukenbrock, Z. Liu, S.W. Meinhardt, H. Ling, J.D. Faris, J.B. Rasmussen, P.S. Solomon, B.A. McDonald, and R.P. Oliver. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. Nat. Genet. 38:953-956.
Fritz, A.K., T.S. Cox, B.S. Gill, and R.G. Sears. 1995. Molecular marker-facilitated analysis of introgression in winter wheat X *Triticum tauschii* populations. Crop Sci. 35:1691-1695.
Gamba, F.M., and L. Lamari. 1998. Mendelian inheritance of resistance to tan spot (*Pyrenophora tritici-repentis*) in selected genotypes of durum wheat (*Triticum turgidum*). Can. J. Plant Pathol. 20:408-414.
Gamba, F.M., L. Lamari, and A.L. Brule-Babel. 1998. Inheritance of race-specific necrotic and chlorotic reactions induced by *Pyrenophora tritici-repentis* in hexaploid wheats. Can. J. Plant Pathol. 20:401-407.
Gilchrist, S.L., S.F. Fuentes, and M.d.L.d.l.I.d. Bauer. 1984. Determination de Fuentes de Resistencia contra Helminthosporim tritici-repentis bajo conditions de campo e invernadero. Agrociencia 56:95-105.
Gill, B.S., W.J. Raupp, L.E. Browder, T.S. Cox, and R.G. Sears. 1991. Registration of KS89WGRC7 leaf rust resistant hard red winter wheat germplasm. Crop Sci. 31:246.
Gill, K.S., B.S. Gill, T.R. Endo, and T. Taylor. 1996. Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics 1444:1883-1891.
Groos, C., G. Gay, M.R. Perretant, L. Gervais, M. Bernard, F. Dedryver, and G. Charmet. 2002. Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a white×red grain bread-wheat cross. Theor. Appl. Genet. 104:39-47.
Gupta, P., H. Balyan, K. Edwards, P. Isaac, V. Korzun, M. Röder, M.F. Gautier, P. Joudrier, A. Schlatter, J. Dubcovsky, R. De la Pena, M. Khairallah, G. Penner, M. Hayden, P. Sharp,

B. Keller, R. Wang, J. Hardouin, P. Jack, and P. Leroy. 2002. Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. Theor. Appl. Genet. 105:413-422.
Guyomarc'h, H., P. Sourdille, G. Charmet, K.J. Edwards, and M. Bernard. 2002. Characterisation of polymorphic microsatellite markers from *Aegilops tauschii* and transferability to the D-genome of bread wheat. Theor. Appl. Genet. 104:1164-1172.
Hagborg, W.A.F. 1970. A device for injecting solutions and suspensions into thin leaves of plants. Can. J. Bot. 48:1135-1136.
Hiebert, C., J. Thomas, D. Somers, B. McCallum, and S. Fox. 2007. Microsatellite mapping of adult-plant leaf rust resistance gene *Lr22a* in wheat. Theor. Appl. Genet. 115:877-884.
Hiebert, C.W., J.B. Thomas, B.D. McCallum, and D.J. Somers. 2008. Genetic Mapping of the Wheat Leaf Rust Resistance Gene *Lr60* (*LrW2*). Crop Sci. 48:1020-1026.
Hosford, R.M., Jr. 1971. A form of *Pyrenphora tricosthoma* pathogenic to wheat and other grasses. Phytopathology 61:28-32.
Hosford, R.M., Jr. 1972. Propagules of *Pyrenophora trichostoma*. Phytopathology 62:627-629.
Hosford, R.M., Jr. 1982. Tan spot. p. 1-24. In R.M Hosford, Jr. ed. Tan spot of wheat and related diseases. North Dakota State University, Fargo.
Hosford, R.M., Jr., C.R. Larez, and J.J. Hammond. 1987. Interaction of wet period and temperature on *Pyrenophora tritici-repentis* infection and development in wheats of differing resistance. Phytopathology 77:1021-1027.
Huang, L., and B.S. Gill. 2001. An RGA-like marker detects all known *Lr21* leaf rust resistance gene family members in *Aegilops tauschii* and wheat. Theor. Appl. Genet. 103:1007-1013.
Huang, L., S.A. Brooks, W. L., J.P. Fellers, H. Trick, and B.S. Gill. 2003. Map based cloning of leaf rust resistance gene *Lr21* from the large and polyploidy genome of bread wheat. Genetics 164:655-664.
Hussien, T., R.L. Bowden, B.S. Gill, and T.S. Cox. 1997. Chromosomal location of leaf rust resistance gene *Lr43* from *Aegilops tauschii* in common wheat. Crop Sci. 37:1764-1766.
Kerber, E.R. 1987. Resistance to leaf rust in hexaploid wheat: *Lr32*, a third gene derived from *Triticum tauschii*. Crop Sci. 27:204-206.
Knott, D.R. 1989. The wheat rusts-breeding for resistance. Monographs on theoretical and applied genetics, vol. 12. Springer, Berlin Heidelberg New York.
Kolmer, J.A. 1996. Genetics of resistance to wheat leaf rust. Annu. Rev. Phytopathol. 34:435-455.
Kolmer, J.A. 2003. Postulation of leaf rust resistance genes in selected soft red winter wheats. Crop Sci. 43:1266-1274.
Kolmer, J.A., D.L. Long, and M.E. Hughes. 2006. Physiologic Specialization of *Puccinia triticina* on Wheat in the United States in 2004. Plant Dis. 90:1219-1224.
Kolmer, J.A., X. Chen, and Y. Jin. 2008a. Diseases which challenge global wheat production - the wheat rusts. In: Carver BF (ed) Wheat: Science and trade. Wiley-Blackwell Publishing, Ames, IA (in press).
Kolmer, J.A., D.L. Long, and M.E. Hughes. 2008b. Physiologic Specialization of *Puccinia triticina* on Wheat in the United States in 2006. Plant Dis. 92:1241-1246.
Kosambi, D.D. 1944. The estimation of map distance from recombination values. Ann. Eugen. 12:172-175.

Kwon, C.Y., J.B. Rasmussen , and S.W. Meinhardt. 1998. Activity of Ptr ToxA from *Pyrenophora tritici-repentis* requires host metabolism. Physiol. Mol. Plant Pathol. 52:201-212.

Lamari, L., and C.C. Bernier. 1989. Toxin of *Pyrenophora tritici-repentis*: Host-Specificity, Significance in Disease, and Inheritance of Host Reaction. Phytopathology 79:740-744.

Lamari, L., and C.C. Bernier. 1991. Genetics of tan necrosis and extensive chlorosis in tan spot of wheat caused by *Pyrenophora tritici-repentis*. Phytopathology 81:1092-1095.

Lamari, L., and C.C. Bernier. 1994. Temperature-induced resistance to tan spot [*Pyrenophora tritici-repentis*] of wheat. Can. J. Plant Pathol. 16:279-286.

Lamari, L., and J. Gilbert. 1998. Toward a coherent model of host-pathogen interactions in tan spot of wheat: An epilogue to the Third International Workshop on Tan Spot of Wheat. Can. J. Plant Pathol. 20:440-443.

Lamari , L., J. Gilbert, and A. Tekauz. 1998. Race differentiation in *Pyrenophora tritici-repentis* and survey of physiologic variation in western Canada. Can. J. Plant Pathol. 20:396-400.

Lamari, L., R. Sayoud, M. Boulif, and C.C. Bernier. 1995. Identification of a new race of *Pyrenophora tritici-repentis*: implications for the current pathotype classification system. Can. J. Plant Pathol. 17:312-318.

Lamari, L., S.E. Strelkov, A. Yahyaoui, J. Orabi, and R.B. Smith. 2003. The Identification of Two New Races of *Pyrenophora tritici-repentis* from the Host Center of Diversity Confirms a One-to-One Relationship in Tan Spot of Wheat. Phytopathology 93:391-6.

Lander, E.S., and D. Botstein. 1989. Mapping Mendelian Factors Underlying Quantitative Traits Using RFLP Linkage Maps. Genetics 121:185-199.

Lander, E.S., P.J. Green, A. Abrahamson, M.J. Barlow, S.E. Daly, Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

Lee, T.S., and F.J. Gough. 1984. Inheritance of Septoria leaf blotch *S. tritici.* and *Pyrenophora* tan spot (*P. tritici-repentis*) resistance in *Triticum aestivum* cv. Carifen 12. Plant Dis. 68:848-851.

Liu, Z.H., J.A. Anderson, J. Hu, T.L. Friesen, J.B. Rasmussen, and J.D. Faris. 2005. A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. Theor. Appl. Genet. 111:782-794.

Long, D.L., and J.A. Kolmer. 1989. A North American system of nomenclature for *Puccinia recondita* f.sp. *tritici*. Phytopathology 79:525-529.

Ma, H., G.R. Hughes, and W. Cao. 1998. Tan spot resistance in tetraploid and hexaploid wheat. In: Duveiller, E., Dubin, H.J., Reeves, J. and McNab, A. .Editors, Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot, CIMMYT, Mexico, DF pp. 218-222.

Ma, H.X., G.H. Bai, B.F. Carver, and L.L. Zhou. 2005. Molecular mapping of a quantitative trait locus for aluminum tolerance in wheat cultivar Atlas 66. Theor. Appl. Genet. 112:51-7.

Manning, V.A., and L.M. Ciuffetti. 2005. Localization of Ptr ToxA Produced by *Pyrenophora tritici-repentis* Reveals Protein Import into Wheat Mesophyll Cells. Plant Cell 17:3203-3212.

Manning, V.A., I. Pandelova, and L.M. Ciuffetti. 2002. A race for a novel host-selective toxin. Abstract. Phytopathology 92:51.

Martin, J.N., B.F. Carver, R.M. Hunger, and T.S. Cox. 2003. Contributions of leaf rust resistance and awns to agronomic and grain quality performance in winter wheat. Crop Sci. 43:1712-1717.

Martinez, J.P., N.W. Oesch, and L.M. Ciuffetti. 2004. Characterization of the multiple-copy host-selective toxin gene, ToxB, in pathogenic and nonpathogenic isolates of Pyrenophora tritici-repentis. Mol. Plant Microbe Interact. 17:467-74.

Martinez, J.P., S.A. Ottum, S. Ali, L.J. Franci, and L.M. Ciuffetti. 2001. Characterization of the ToxB gene from *Pyrenophora tritici-repentis*. Mol Plant Microbe Interact 14:675-7.

McIntosh, R.A., C.R. Wellings, and R.F. Park. 1995. Wheat rusts: an atlas of resistance genes. CSIRO Publications, East Melbourne, Australia.

McVey, D.V., and D.L. Long. 1993. Genes for leaf rust resistance in hard red winter wheat cultivars and parental lines. Crop Sci. 33:1373-1381.

Michelmore, R.W., I. Paran, and R.V. Kesseli. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. 88:9828-9832.

Nagle, B.J., R.C. Frohberg, and R.M. Hosford, Jr. 1982. Inheritance of resistance to tan spot of wheat. p. 40-45. In R.M Hosford, Jr. ed. Tan spot of wheat and related diseases. North Dakota State University, Fargo.

Orolaza, N.P., L. Lamari, and G.M. Ballance. 1995. Evidence of a host-specific chlorosis toxin from *Pyrenophora tritici-repenti*s, the causal agent of tan spot of wheat. Phytopathology 85:1282-1287.

Paillard, S., T. Schnurbusch, M. Winzeler, M. Messmer, P. Sourdille, O. Abderhalden, B. Keller, and G. Schachermayr. 2003. An integrative genetic linkage map of winter wheat *Triticum aestivum* L. Theor. Appl. Genet. 107:1235-1242.

Quarrie, S.A., A. Steed, C. Calestani, A. Semikhodskii, C. Lebreton, C. Chinoy, N. Steele, D. Pljevljakusić, E. Waterman, J. Weyen, J. Schondelmaier, D.Z. Habash, P. Farmer, L. Saker, D.T. Clarkson, A. Abugalieva, M. Yessimbekova, Y. Turuspekov, S. Abugalieva, R. Tuberosa, M.C. Sanguineti, P.A. Hollington, R. Aragués, A. Royo, and D. Dodig. 2005. A high-density genetic map of hexaploid wheat (Triticum aestivum L.) from the cross Chinese Spring × SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor. Appl. Genet. 110:865-880.

Raupp, W.J., S. Singh, G.L. Brown-Guedira, and B.S. Gill. 2001. Cytogenetic and molecular mapping of the leaf rust resistance gene *Lr39* in wheat. Theor. Appl. Genet. 102:347-352.

Raymond, P.J., W.W. Bockus, and B.L. Norman. 1985. Tan spot of winter wheat: Procedures to determine host response. Phytopathology 75:686-690.

Rees, R.G., G.J. Platz, and R.J. Mayer. 1988. Susceptibility of Australian wheats to *Pyrenophora tritici-repentis*. Aust. J. Agric. Res. 39:141-151.

Riede, C.R., L.J. Francl, J.A. Anderson, J.G. Jordahl, and S.W. Meinhardt. 1996. Additional Sources of Resistance to Tan Spot of Wheat. Crop Sci. 36:771-777.

Roder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.-H. Tixier, P. Leroy, and M.W. Ganal. 1998. A Microsatellite Map of Wheat. Genetics 149:2007-2023.

Rowland, G.G., and E.R. Kerber. 1974. Telocentric mapping in hexaploid wheat of genes for leaf rust resistance and other characters derived from *Aegilops squarrosa*. Can. J. Genet. Cytol. 16:137-144.

Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgensen, and R.W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. 81:8014-8.

Sandhu, D., J.A. Champoux, S.N. Bondareva, and K.S. Gill. 2001. Identification and physical localization of useful genes and markers to a major gene-rich region on wheat group 1S chromosomes. Genetics 157:1735-1747.

Schilder, A.M.C., and G.C. Bergstrom. 1992. The dispersal of conidia and ascospores of *Pyrenophora tritici-repentis*. Pages 96-99 in: Advances in Tan Spot Research. Proc. Int. Tan Spot Workshop, 2nd. L. J. Francl, J. M. Krupinsky, and M. P. McMullen, eds. N.D. Agric. Exp. Stn., Fargo.

Sears, E.R. 1954. The aneuploids of common wheat. Mo. Agric. Exp. Stn. Res. Bull. 572:1-58.

Sears, E.R. 1966. Nullisomic-tetrasomic combinations in hexaploid wheat. In Chromosome manipulations and plant genetics. Edited by R. Riley and K.R. Lewis. Oliver and Boyd, Edingburgh, Scotland. pp. 29-45.

Sears, E.R., and L.M.S. Sears. 1978. The telocentric chromosomes of common wheat. In Proc. 5th Int. Wheat Genet. Symp. 23-28 Feb. 1978. Edited by S. Ramanujam. Indian Society of Genetics and Plant Breeding, Indian Agricultural Research Institute, New Delhi. pp. 389-407.

Shabeer, A., and W.W. Bockus. 1988. Tan spot effects on yield and yield components relative to growth stage in winter wheat. Plant Dis. 72:599-602.

Singh, P., and G. Hughes. 2006. Inheritance of resistance to the chlorosis component of tan spot of wheat caused by *Pyrenophora tritici-repentis*, races 1 and 3. Euphytica 152:413-420.

Singh, P.K., and G.R. Hughes. 2005. Genetic Control of Resistance to Tan Necrosis Induced by *Pyrenophora tritici-repentis*, Races 1 and 2, in Spring and Winter Wheat Genotypes. Phytopathology 95:172-7.

Singh, P.K., J.L. Gonzalez-Hernandez, M. Mergoum, S. Ali, T.B. Adhikari, S.F. Kianian, E.M. Elias, and G.R. Hughes. 2006. Identification and Molecular Mapping of a Gene Conferring Resistance to *Pyrenophora tritici-repentis* Race 3 in Tetraploid Wheat. Phytopathology 96:885-889.

Singh, R.P., J. Huerta-Espino, R. Sharma, A.K. Joshi, and R. Trethowan. 2007. High yielding spring bread wheat germplasm for global irrigated and rainfed production systems. Euphytica 157:351-363.

Singh, S., W.W. Bockus, I. Sharma, and R.L. Bowden. 2008. A Novel Source of Resistance in Wheat to *Pyrenophora tritici-repentis* Race 1. Plant Dis. 92:91-95.

Singh, S., C.D. Franks, L. Huang, G.L. Brown-Guedira, D.S. Marshall, B.S. Gill, and A.K. Fritz. 2004. *Lr41*, *Lr39*, and a leaf rust resistance gene from *Aegilops cylindrica* may be allelic and are located on wheat chromosome 2DS. Theor. Appl. Genet. 108:586-591.

Somers, D.J., P. Isaac, and K. Edwards. 2004. A high-density microsatellite consensus map for bread wheat (Triticum aestivum L.). Theor. Appl. Genet. 109:1105-14.

Sourdille, P., T. Cadalen, H. Guyomarc'h, J. Snape, M. Perretant, G. Charmet, C. Boeuf, S. Bernard, and M. Bernard. 2003. An update of the Courtot × Chinese Spring intervarietal molecular marker linkage map for the QTL detection of agronomic traits in wheat. Theor. Appl. Genet. 106:530-538.

Sourdille, P., S. Singh, T. Cadalen, G. Brown-Guedira, G. Gay, L.L. Qi, B.S. Gill, P. Dufour, A. Murigneux, and M. Bernard. 2004. Microsatellite-based deletion bin system for the

establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct. Integr. Genomics 4:12-25.
Stock, W.S., A.L. Brule-Babel, and G.A. Penner. 1996. A gene for resistance to a necrosis-inducing isolate of *Pyrenophora tritici-repentis* located on 5BL of Triticum aestivum cv. Chinese Spring. Genome 39:598-604.
Strelkov, S.E., and L. Lamari. 2003. Host-parasite interactions in tan spot [*Pyrenophora tritici-repentis*] of wheat. Can. J. Plant Pathol. 25:339-349.
Strelkov, S.E., L. Lamari, and G.M. Ballance. 1999. Characterization of a Host-Specific Protein Toxin (Ptr ToxB) from *Pyrenophora tritici-repentis*. Mol. Plant Microbe Interact. 12:728-32.
Strelkov, S.E., L. Lamari, R. Sayoud, and R.B. Smith. 2002. Comparative virulence of chlorosis-inducing races of *Pyrenophora tritici-repentis*. Can. J. Plant Pathol. 24:29-35.
Strelkov, S.E., R.F. Kowatsch, G.M. Ballance, and L. Lamari. 2005. Characterization of the ToxB gene from North African and Canadian isolates of *Pyrenophora tritici-repentis*. Physiol. Mol. Plant Pathol. 67:164-170.
Sun, X., G. Bai, and B. Carver. 2009. Molecular markers for wheat leaf rust resistance gene *Lr41*. Molecular Breeding 23:311-321.
Sutka, J., G. Galiba, A. Vagujfalvi, B.S. Gill, and J.W. Snape. 1999. Physical mapping of the Vrn-A1 and Fr1 genes on chromosome 5A of wheat using deletion lines. Theor. Appl. Genet. 99:199-202.
Sykes, E.E., and C.C. Bernier. 1991. Qualitative inheritance of tan spot resistance in hexaploid, tetraploid, and diploid wheat. Can. J. Plant Pathol. 13:38-44.
Tadesse, W., M. Schmolke, S.L. Hsam, V. Mohler, G. Wenzel, and F.J. Zeller. 2007. Molecular mapping of resistance genes to tan spot [*Pyrenophora tritici-repentis* race 1] in synthetic wheat lines. Theor. Appl. Genet. 114:855-62.
Tomas, A., and W.W. Bockus 1987. Cultivar-Specific Toxicity of Culture Filtrates of *Pyrenophora tritici-repentis*. Phytopathology 77:1337-1366.
Tomas, A., G.H. Feng, G.R. Reeck, W.W. Bockus, and J.E. Leach. 1990. Purification of a Cultivar-Specific Toxin from *Pyrenophora tritici-repentis*, Causal Agent of Tan Spot of Wheat. Mol. Plant Microbe Interact. 3:221-24.
Torada, A., M. Koike, K. Mochida, and Y. Ogihara. 2006. SSR-based linkage map with new markers using an intraspecific population of common wheat. Theor. Appl. Genet. 112:1042-1051.
Tuori, R.P., T.J. Wolpert, and L.M. Ciuffetti. 1995. Purification and immunological characterization of toxic components from cultures of *Pyrenophora tritici-repentis*. Mol. Plant Microbe Interact. 8:41-8.
Van Ooijen, J.W., and R.E. Voorrips. 2001. JoinMap 3.0. PRI, Wageningen, Netherlands. SoftGenetics LLC.
Vos, P., R. Hogers, M. Bleeker, and M. Reijans. 1995. AFLP: a new technique for DNA fingerprinting. Nucl. Acids Res. 23:4407-4414.
Wang, S., C.J. Basten, and Z.B. Zeng. 2007. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC.
Wang, Z., J.L. Weber, G. Zhong, and S.D. Tanksley. 1994. Survey of plant short tandem DNA repeats. Theor. Appl. Genet. 88:1-6.

Wechter, W.P., M.P. Whitehead, C.E. Thomas, and R.A. Dean. 1995. Identification of a randomly amplified polymorphic DNA marker linked to the *Fom 2* Fusarium wilt resistance gene in muskmelon *MR-1*. Phytopathology 85:1245-1249.

Williams, J.G.K., A.R. Kublik, K.J. Livak, and J.A. Rafalski. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18:6531-6535.

Yu, J.B., G.H. Bai, W.C. Zhou, Y.H. Dong, and F.L. Kolb. 2008. Quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population of Wangshuibai/Wheaton. Phytopathology 98:87-94.

Zeng, Z.B. 1994. Precision Mapping of Quantitative Trait Loci. Genetics 136:1457-1468.

Zhang, H.F., L.J. Francl, J.G. Jordahl, and S.W. Meinhardt. 1997. Structural and Physical Properties of a Necrosis-Inducing Toxin from *Pyrenophora tritici-repentis*. Phytopathology 87:154-60.

Appendix A - Reaction of wheat leaves to *Pyrenophora tritici-repentis* inoculation and Ptr ToxA infiltration

Figure A.1. The development of extensive chlorosis caused by *P. tritici-repentis* race 1. (a) Initial symptom: small dark brown to black spots with very little chlorosis. (b) Disease outgrowth: oval-shaped lesions surrounded by a chlorotic halo with small dark brown spots in the center. (c) Disease climax: dark brown to black spots may or may not be distinguishable and most infected zones consist of coalescing chlorotic zones.

(a)

(b)

(c)

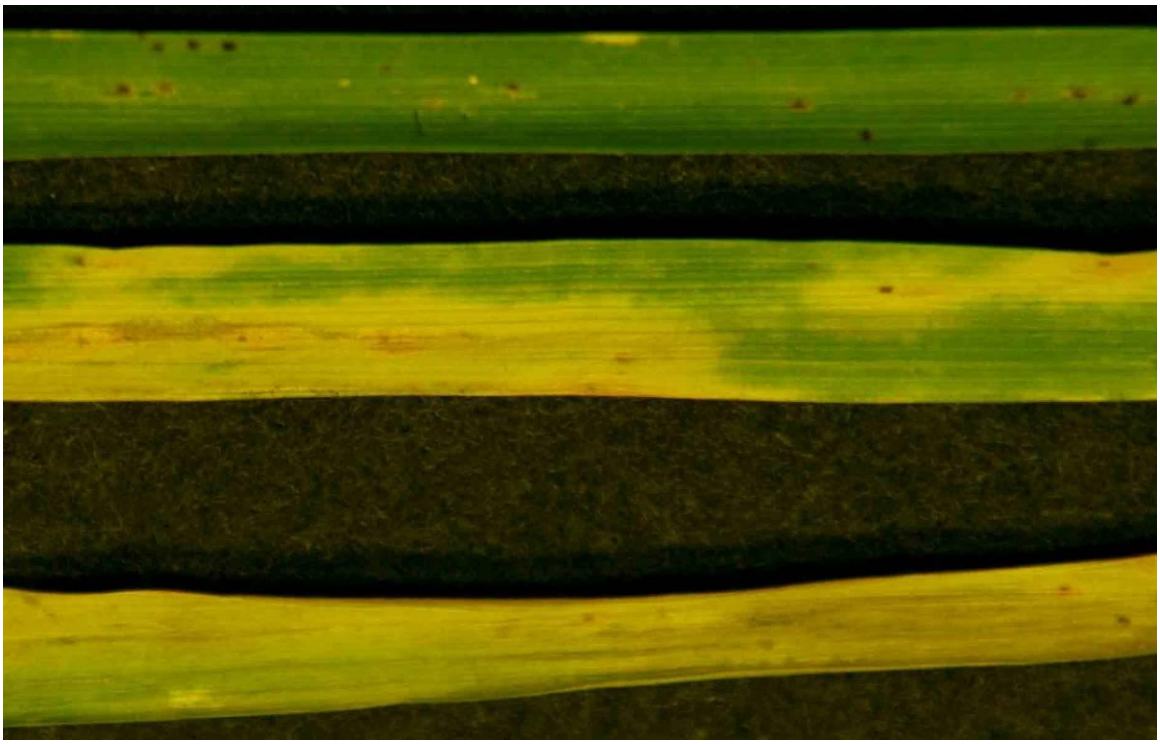


Figure A.2. Infiltration of TAM 105 (susceptible, a) and Karl 92 (resistant, b) with Ptr ToxA.

(a)

(b)

Appendix B - List of 378 worldwide wheat accessions used in Chapter 3

Table 0.1. List of 378 wheat accessions.

Accession name	Geographical source	Class	Pedgree	Developer/provider
38 M.A.	Argentina		Barleta 4d/Chino	PI 184607
Coop Capoildo	Argentina		landrace from Argentina	
Coop Millan	Argentina			
Las Rosas Inta	Argentina			
Vilela Sol	Argentina		Land race from Argentina	ACA
Capo	Austria		Pokal/Martin	
Extrem	Austria		Record/Br. Herrachweiten	USA
Livius	Austria		Karat/Lentia	
Encruzilhada	Brazil		Funo background	
Frontana	Brazil		Fronteira (=Polysu / Alfredo Chaves 6 - 21) / Mentana	
Toropi	Brazil			
Chile	Chili		JGB99-20, accession no. 26869, unknown pedigree	JIRCAS
Ai73	China		Funo background	
Anxuan2	China		Funo background	
Baisanyuehuang	China			Nanjing, China
Beiquan565	China		Funo background	
Caizihuang	China		Landrace form Jiangsu Province	Nanjing, China
Can Lao Mai	China			Nanjing, China
ChanjiBaiDongMai	China			PI447389
Chinese Spring	China		Landrace	CItr 14108
ChuShanBao	China			PI524979
DaBaiPao	China			PI592001

Dafanliuzhu	China			Nanjing, China
Dahongpao	China			Nanjing, China
DaHuangPi	China			PI502943
Dwarf Sumai3	China		Sumai 3/Tom Thumb//Tom Thumb	Jaas
Emai6	China			
F5125	China		Ning7840/Fufan904	Jaas
F60096	China		Jinzhou1/Sumai 2	Jaas
FangTouBaiMang	China			PI502938
FangTouHongMang	China			PI502939
Fengmai2	China		Funo background	
FK17	China			
Fu5114	China		LongXi 18/(Avrora/Anhui11//Sumai 3)	Jaas
Fumai3	China		Orofen/Funo	PI 447405
Fusuihuang	China			Nanjing, China
Haiyanzhong	China			Nanjing, China
HeiHangDongMai	China			PI524983
Heshangmai	China			Nanjing, China
HongHuaWu	China			PI518598
Hongjianzi	China			Nanjing, China
HongMangMai	China			PI591997
HongMongBai	China			PI518834
Hua 512	China			
Huai69-6	China		Funo background	
Huamai7	China		Funo background	
Huang Fang Zhu	China			Nanjing, China
Huangcandou	China			Nanjing, China
Hubei48130	China			
Huimaoafu	China		Funo background	
HuiShanYangMai	China			PI502930
HungGuangTou	China			PI452263

Huoshaobairimai	China			Nanjing, China
Huoshaomai	China			Nanjing, China
JG 1	China		Mayo/Armadillo//Yangmai3/Avrora/Ningmai 3	PI 531193
JG 2	China			
JiangDongMen	China			PI462138
Jinagdu1	China		Funo background	
Jingguangmai	China		Funo background	
Jingzhou 1	China			Nanjing, China
Jinmai 33	China			
KuangTuErhHsiaoMai	China			CItr7158 (PI57347)
LiangGuangTou	China			PI435109
LingHaiMao Yang Mo	China			PI445867
Linnong12	China		Funo background	
Linnong14	China		Funo background	
Luohan 2	China			
Maoyingafu-2	China		Funo background	
MeiQianWu	China			PI525074
Mengfeng8	China		Funo background	
MuTanChiang	China			CItr9018(PI70675)
Nanda 2419	China		Funo background	
Ning 8319	China			
Ning7840	China		Aurora/Anhui 11//Sumai 3	PI 531188
Ninjing 6935-8	China			
NTDHP	China		Land race from Jiangsu	PI 462149
PaiMaiTze	China			PI64285 (8349)
PC-2	China			
QiangShuiHuang	China			PI502932
Qianjiang1	China		Funo background	
Qunzhong10	China		Funo background	
SanChaHo	China			CItr9017(PI70674)

SanYueHuang	China			PI524973
ShanhaiCaiZiHuang	China			PI462150
ShuiLiZhan	China			PI502931
Siyang 936	China			
Siyang117	China		Funo background	
Sumai 3	China		Funo/Taiwan Wheat	PI 462151
Sumai 49	China		N7922/(Aurora/Anhui 11//Sumai 3)	Jaas
Sumai 1	China		Funo background	
Sumai 2	China		Funo background	
Suyang 7-2	China		Funo background	
TaFangShen	China			CItr9009(PI70666)
Taiwan	China		Funo background	
Tawanhsiaomai	China			CItr7171(PI57360)
Wan7107	China		Funo background	
WangShuiBai	China		Land race from Jiangsu Province	PI 462141
Wannian 2	China		Selection of Mentana	PI 447403
Wanya2	China		Funo background	
Wenmai 6	China			
Wumai1	China		Funo background	
Wzhhs	China		Land race from Zhejiang Province	Jaas
Xiangmai10	China		Funo background	
Xiangmai11	China		Funo background	
Xiangmai12	China		Funo background	
Xiangmai8	China		Funo background	
Xiangnong3	China		Funo background	
Xiannong68	China		Funo background	
XingHuaBaiYuHua	China			PI462154
Xuan7	China		Funo background	
Xueliqing	China			Nanjing, China
Y155(FSW)	China			

YangLaZi	China			PI502935
Yangmai1	China		Funo background	PI 447404
Yangmai158	China		Yangmai4/St 1472/506	
Yangmai2	China		Wumai/Yangmai1	Jaas
Yangmai3	China		Wumai/Yangmai1	Jaas
Yangmai4	China		Nanda2419/Triumph//Funo	Jaas
Yangmai5	China		F4 (9-16)/St 1472/ 506	Jaas
Yixi4	China		Funo background	
YouBaoMai	China			PI524987
Youyimai	China		Funo background	
YouZiMai	China			PI435124
Yunmai25	China		Funo background	
Yunmai27	China		Funo background	
Yunmai35	China		Funo background	
Zhemai6	China		Funo background	
Zhen 7495	China			Nanjing, China
Zhenghan 1	China			
Zhenmai17	China		Funo background	
Zhongliang11	China		Funo background	
Zhongshan11	China		Funo background	
Poncheau	France		Sel. from landrace	
Funo	Italy		Duecentodieci/Demiano	PI 213833
Abura	Japan		LV-FUKUOKA	PI382140
Abura Komugi	Japan		JGB99-12, accession no. 23516, unknown pedigree	Fukuoka
Aso Zairai II	Japan		JGB99-16, accession no. 23524, unknown pedigree	Kumamoto
Asotomea	Japan			Nagasaki
AsoZairai(YuubouKappu)	Japan		JGB99-18, accession no. 23521, unknown pedigree	Fukuoka
Itou Komugi	Japan		JGB99-23, accession no. 23647, unknown pedigree	Miyazaki
Kagoshima	Japan		JGB99-25, accession no. 23542, unknown pedigree	Kagoshima
Kikuchi	Japan		JGB99-28, accession no. 23546, unknown pedigree	Saga

Minamikyushu 69	Japan			PI382152
Nobeokabouzu Komugi	Japan			MiyaZaki
NobeokaBozu	Japan			PI382153
NyuBai	Japan			PI382154
Nyuubai	Japan		JGB99-36, accession no. 22957, unknown pedigree	lbaraki
Qiaomai Xiaomai	Japan			JIRCAS
Sanshukomugi	Japan		Land race from Mie	PI197130
SapporoHaruKomugiJugo	Japan			PI81791
Shanasui	Japan			
Shinchunaga	Japan		Land race from Mie	PI197128
Shirasaya No.1	Japan		Land race from Mie	PI 197129
Shiro Nankin	Japan		JGB99-58, accession no. 23277, unknown pedigree	Hyogo
Shou Komugi II	Japan		JGB99-61, accession no. 23653, unknown pedigree	Kumamoto
Soba Komugi 1B	Japan		JGB99-61, accession no. 23662, unknown pedigree	Kagoshima
Soba Komugi 1C	Japan		JGB99-61, accession no. 23665, unknown pedigree	Kagoshima
Sotome	Japan		JGB99-62, accession no. 23595, unknown pedigree	Nagasaki
Tokai66	Japan			PI382161
Zairai Yuubou	Japan		JGB99-70, accession no. 22130, unknown pedigree	JIRCAS
Chokwang	Korea			Jaas
Chukoku 81	Korea			Indiana
Suwon 92	Korea		Purdue98-3450	
Avrora (ABPOPA)	Russian		Lutescens 314H147 / Bezostaja 1	Nanjing, China
Kharkof	Ukraine	HRW	Landrace of Ukraine	check
Martin	Unknown		Clawson	
AGS 2000	USA	SRW	Pio.2555/PF84301//FL 302 (formerly GA89482E7)	Check
Antelope	USA	HWW		check
AP04T8211	USA	HRW	W98-232/KS96WGRC38	AgriPro South
AP05T2413	USA	HRW	(KS95U522/TX95VA0011)F1/Jagger	AgriPro South
AP06T3832	USA	HRW	HBK0935-29-15/KS90W077-2-2/VBF0589-1	AgriPro South
AR96077-7-2	USA	SRW	Jackson/Pio2643	Bacon

AR97044-10-2	USA	SRW	Elkhart/AR494B-2-2	Bacon
AR97124-4-3	USA	SRW	P88288C1-6-1-2/Terra SR204	Bacon
Arena exp.	USA	SRW	NASW84-345/Coker9835//OH419/OH389	Fioritto
Atlas66	USA	HRW	Frondoso // Redhart 3 / Noll 28	NCSU
B030543	USA	SRW	VA93-54-429/LA85422	Hancock
Bess	USA	SRW	MO11769/Madison (formerly MO981020)	Check
Branson	USA	SRW	Pio2737W/891-4584A (Pike/FL302) (formerly M00-3701)	Check
Centerfield	USA	HRW	TXGH12588-105*4 / FS4 // 2*2174	OSU
Century	USA	HRW		OSU
Chisholm	USA	HRW	Sturdy sib/Nicoma	OSU
Clark	USA	SRW	Beau//65256A1-8-1/67137B5-16/Sullivan/Beau//5517B8-5-3-3/Logan	PI 512337
CO02W237	USA	HWW	98HW519(93HW91/93HW255)/96HW94	CSU
CO03064	USA	HRW	CO970547/Prowers 99	CSU
CO03W043	USA	HWW	KS96HW94/CO980352	CSU
CO03W054	USA	HWW	KS96HW94//Trego/CO960293	CSU
CO03W139	USA	HWW	CO980862/Lakin	CSU
CO03W239	USA	HWW	KS01-5539/CO99W165	CSU
CO04W210	USA	HWW	NW97S343/Akron	CSU
Coker 9553	USA	SRW	89M-4035A(IL77-2656/NK79W810/Pio2580 (formerly D00*6874-2)	Check
D04*5513	USA	SRW	DK1551W/D94-50228	Hancock
D04-5012	USA	SRW	NC96BGTD1/Mason	Hancock
Deliver	USA	HRW		OSU
Duster	USA	HRW	W0405 / NE78488 // W7469C / TX81V6187	OSU
Endurance	USA	HRW	HBY756A/ Siouxland//2180	OSU
Ernie	USA	SRW	Pike/3/Stoddard/Blueboy//Stoddard/D1707	PI592001
Freedom	USA	SRW	GR876/OH217	PI592002
Fuller	USA	HRW		KAES
G41732	USA	SRW	T814/L900819	Brown
G59160	USA	SRW	T812/VA91-54-219	Brown
G61505	USA	SRW	ABI89-4584A/T814	Brown

G69202	USA	SRW	VA91-54-219/OH413	Brown
GA991209-6E33	USA	SRW	GA901146/GA96004//AGS2000	Johnson
GA991227-6A33	USA	SRW	VA97W-24/AGS2000	Johnson
GA991336-6E9	USA	SRW	GA92432//AGS2000/Pio26R61	Johnson
GA991371-6E13	USA	SRW	GA931521/2*AGS2000	Johnson
Guymon	USA	HRW		OSU
HV9W02-942R	USA	HRW	53/3/ABL/1113//K92/4/JAG/5/KS89180B	WestBred
HV9W03-539R	USA	HRW	KS94U275/1878//JAGGER	WestBred
HV9W03-696R-1	USA	HRW	N94L027/TBOLT//KS89180B	WestBred
HV9W05-881R	USA	HRW	MASON/OGALLALA-vr/Betty	WestBred
HV9W96-1271R-1	USA	HRW	HV9W00-1551WP/KS94U326	WestBred
IL00-8530	USA	SRW	IL89-1687//IL90-6364/IL93-2489	Kolb
IL02-18228	USA	SRW	Pio25R26/IL9634-24437(IL90-4813/L85-3132/Ning7840)//IL95-4162	Kolb
IL02-19463	USA	SRW	Patton/Cardinal//IL96-2550	Kolb
India exp.	USA	SRW	KY85C-35-4/Karl/Madison	Fioritto
INW0411	USA	SRW	96204A1-12//Goldfield/92823A1-11 (formerly P97397E1-11-2-4-1-1)	Check
Jerry	USA	HRW		check
KS010143K-11	USA	HRW	TAM-400/KS950301-DD-4	KSU-Manhattan
KS010379M-2	USA	HRW	KS920709-B-5-2-2/TAM-400	KSU-Manhattan
KS010514-9TM-10	USA	HRW	CM98-42/3/HBF0290/X84W063-9-39-2//ARH/4/KS940786-6-4	KSU-Manhattan
KS010957K~4	USA	HRW	2145/Karl 92//KS940786-6-11	KSU-Manhattan
KS020304K~3	USA	HRW	JAGGER/2137//KS940786-6-9	KSU-Manhattan
KS05HW121-2	USA	HWW	KS99-5-16(94HW98/91H153)//STANTON/KS98HW423(JAG/93HW242)	KSU-HAYS
KS05HW136-3	USA	HWW	KS98HW518(93HW91/93HW255)//KS98H245(IKE/TA2460//*3T200)/TREGO	KSU-HAYS
KS05HW15-2	USA	HWW	KS98HW452(KS91H153/KS93HW255)/CO960293//KS920709B-5-2(T67/X84W063-9-45//K92)	KSU-HAYS
KS07HW117	USA	HWW	KS00HW151-4(94H871//VTA/94HW301)//KS98HW151-6/00HW114-1	KSU-HAYS
KS07HW25	USA	HWW	KS025580(TREGO/CO960293)/KSO1HW152-6(TGO/BTY SIB)	KSU-HAYS
KS07HW81	USA	HWW	KS02HW25(TGO/JGR 8W)/KS00HW114-1-1(94HW117//JGR/94HW301)	KSU-HAYS
KS970093-8-9-#1	USA	HRW	HBK1064-3/KS84063-9-39-3-4W//X960103	KSU-Manhattan

KS970187-1-10	USA	HRW	TAM107*2/TA759//HBC197F-1/3/2145	KSU-Manhattan
KS980512-2-2	USA	HRW	T67/X84W063-9-45//K92/3/SNF/4/X86509-1-1/X84W063-9-39-2//K92	KSU-Manhattan
KS980554-12-~9	USA	HRW	2180*K/2163//?/3/W1062A*HVA114/W3416	KSU-Manhattan
KY96C-0769-7-3	USA	SRW	2552/Roane	Van Sanford
KY97C-0321-02-01	USA	SRW	Kristy/VA94-52-25//2540	Van Sanford
KY97C-0519-04-07	USA	SRW	SS555W/2540//2552	Van Sanford
LA01*425	USA	SRW	P2571/Y91-6B	Moreno
LA01138D-52	USA	SRW	LA841/LA422//AGS2000	Harrison
LA02-923	USA	SRW	PS8424//XY90-1B/TX851212	Moreno
LA98214D-14-1-2-B	USA	SRW	Shelby/LA87167D8-10-2(FR81-19/FL302//Coker983)	Harrison
LA99005UC-31-3-C	USA	SRW	Pio2548/Coker9835(LA90144B16-3-2)//AGS2000	Harrison
Lee	USA		Hope/Timstein	MAES
M03-3616-C	USA	SRW	Hopewell/Patton	Fogleman
M04*5109	USA	SRW	VA94-54-479/Pio2628	Fogleman
M04-4566	USA	SRW	Bradley/Roane	Fogleman
M04-4715	USA	SRW	Mason/Ernie	Fogleman
M04-4802	USA	SRW	FFR518//Elkhart/MV-18	Fogleman
MD01W233-06-1	USA	SRW	McCormick/Choptank	Costa
MD99W483-06-9	USA	SRW	VA97W358/Renwood 3260	Costa
MO011126	USA	SRW	MO94-103/Pio2552	McKendry
MO040152	USA	SRW	MO 12278/Pio2571	McKendry
MO040192	USA	SRW	IL85-2872/MO10501	McKendry
Mocha exp.	USA	SRW	OH489/OH490	Fioritto
MT0495	USA	HRW	MT9640/NB1133	MT
MT0552	USA	HRW	N95L159/CDC Clair	MT
MTS04120	USA	HRW	L'Govskaya 167/Rampart	MT
MTS0531	USA	HRW	L'Govskaya 167/Rampart//MT9409 (solid stem)	MT
N02Y5117	USA	HRW	YUMA//T-57/3/CO850034/4/4*YUMA/5/KS91H184/ARLIN S/KS91HW29//NE89526)	ARS-LNK
N98L20040-44	USA	HRW	CS/PI467024//CS/3/SXLD/4/TAM202/5/SXLD	ARS-LNK

NC03-6228	USA	SRW	A92-4452//NC96BGTD1sib/NC96BGTA6sib	Murphy
NC04-15533	USA	SRW	NC94-6275/P86958//VA96-54-234	Murphy
ND2710	USA	HSW		North Dakota
ND2928	USA	HSW	Ning7840/ND706	North Dakota
NE02533	USA	HRW	NE94458 (=GK-SAGVARI/COLT//NE86582)/JAGGER	UNL
NE02558	USA	HRW	JAGGER/ALLIANCE	UNL
NE04424	USA	HRW	KS92H363-2/COUGAR SIB(=NE85707/TBIRD)	UNL
NE04490	USA	HRW	NE95589/NE94632(=ABILENE/NORKAN//RAWHIDE)//NE95510 (=ABILENE/ARAPAHOE)	UNL
NE05426	USA	HRW	W95-091 (=KS85-663-8-9//WI81-133/THUNDERBIRD)/AKRON	UNL
NE05430	USA	HRW	IN92823A1-1-4-5/NE92458	UNL
NE05496	USA	HRW	KS95HW62-6 (=KS87H325/RIO BLANCO)/HALLAM	UNL
NE05548	USA	HRW	NE97426 (=BRIGANTINA.2*ARAPAHOE)/NE98574 (=CO850267/RAWHIDE)	UNL
NE05549	USA	HRW	NI98414 (=NE90614/NE87612//NE87612)/WESLEY	UNL
NE05569	USA	HRW	Wesley//Pronghorn/Arlin	UNL
NE06436	USA	HRW	WESLEY/OK98699 (=TAM200/HBB313//2158)	UNL
NE06472	USA	HRW	CO95043 (=HILL/PI294994//LAMAR)/KS89180B-2-1 (=KS8010-73/KS8010-1-4-2//107349/KARL)//NE98574 (=CO850267/RAWHIDE)	UNL
NE06549	USA	HRW	HALLAM/WESLEY	UNL
NE06619	USA	HRW	WESLEY/WAHOO	UNL
NI04420	USA	HRW	NE96644(=ODESSKAYA P./CODY)//PAVON/*3SCOUT66/3/WAHOO SIB	UNL
NI04427	USA	HRW	KS98HW22//W95-615W/N94L189	UNL
NW03666	USA	HRW	N94S097KS/NE93459	UNL
NW04Y2188	USA	HWW	MO8/REDLAND//KS91H184/3*RIO BLANCO	ARS-LNK
NW05M6011-6-1	USA	HWW	Nuplains/Arrowsmith	ARS-LNK
NW05M6015-25-4	USA	HWW	NW97S186/RioBlanco	ARS-LNK
NX03Y2489	USA	HWW	BaiHuo/Kanto107//Ike/3/KS91H184/3*RBL//N87V106	ARS-LNK
NX04Y2107	USA	HWW	NW98S081/99Y1442	ARS-LNK
NYCalR-L	USA	SRW	reselection out of Caledonia	Sorrells
OH02-12678	USA	SRW	Foster/Hopewell//OH581/OH569	Sneller

OH02-7217	USA	SRW	92118B4-2/OH561	Sneller
OH03-41-45	USA	SRW	IL91-14167/OH599	Sneller
OK Bullet	USA	HRW	KS96WGRC39/ Jagger	OSU
OK00514-05806	USA	HRW	KS96WGRC39/Jagger	OSU
OK01420W	USA	HRW	KS93U206/Jagger RC	OSU
OK02405	USA	HRW	Tonkawa/GK50	OSU
OK02522W	USA	HRW	OK02522W	OSU
OK03305	USA	HRW	N40/OK94P455	OSU
OK03522	USA	HRW	N566/OK94P597	OSU
OK03716W	USA	HRW	Oro Blanco/OK92403 F4:11	OSU
OK03825-5403-6	USA	HRW	(Custer*3/94M81)=STARS 0601W	OSU
OK04505	USA	HRW	OK91724/2*Jagger	OSU
OK04507	USA	HRW	OK95593/Jagger //2174	OSU
OK04525	USA	HRW	FFR525W/Hickok//Coronado F4:11	OSU
OK05122	USA	HRW	KS94U337/NE93427 F4:10	OSU
OK05128	USA	HRW	KS94U275/OK94P549 F4:10 RC	OSU
OK05134	USA	HRW	OK97411/TX91D6825 F4:10	OSU
OK05212	USA	HRW	OK95616-1/Hickok//Betty F4:10	OSU
OK05312	USA	HRW	TX93V5919/WGRC40//OK94P549/WGRC34	OSU
OK05511	USA	HRW	TAM 110/2174	OSU
OK05723W	USA	HRW	SWM866442/Betty F4:10 HW	OSU
OK05830	USA	HRW	OK93617/Jagger F6:12	OSU
OK05903C	USA	HRW	TXGH12588-120*4/FS4//2174/3/Jagger F4:10 RC	OSU
OK06210	USA	HRW	KS90175-1-2/CMSW89Y271//K92/3/ABI86*3414/X86035*-BB-34//HBC 302E RC F4:9 RC	OSU
OK06313	USA	HRW	Emma/Karl 92//2174 F4:9	OSU
OK06319	USA	HRW	Enhancer/2174 F4:9	OSU
OK06336	USA	HRW	Magvars/2174//Enhancer F4:9	OSU
OK06345	USA	HRW	FAWWON 06/2174//OK95548-26C F4:9	OSU
OK06518	USA	HRW	Palma/Hickok//2174 F4:9	OSU

OK06528	USA	HRW	Vilma/Hickok//Heyne F4:9 A-	OSU
OK06848W	USA	HRW	OK94P461/Oro Blanco F6:11	OSU
Overley	USA	HRW	TAM-107 *3/ TA 2460/ Heyne 'S'// Jagger	KSU
P02444A1-23-9	USA	SRW	981129/99793//INW0301/92145	Ohm
P03112A1-7-14	USA	SRW	INW0411//INW0315/99794	Ohm
P03207A1-7	USA	SRW	INW0304*2/RSI5//981281/3/INW0315/99794	Ohm
P04287A1-10	USA	SRW	INW0315*2/4/INW0304//9346/CS 5Am/3/91202//INW0301/INW0315	Ohm
Par-55	USA	SRW		Illinois
Pioneer Brand 26R61	USA	SRW	Omega78/S76/4/Arthur71/3/Stadler//Redcoat/Wisc1/5/Coker747/6/2555sib (formerly XW663)	Check
Roane	USA	SRW	VA71-54-147(CI17449)/C68-15//IN65309C1-18-2-3-2 (formerly VA93-54-429)	PI612958
Scout 66	USA	HRW	Composite of 85 selections from Scout, Citr 13546	UNL
SD03164-1	USA	HRW	89118RC1-X-9-3-3/TX96D2845//Expedition	SDSU
SD05118	USA	HRW	Wesley/NE93613	SDSU
SD05210	USA	HRW	SD98444/SD97060	SDSU
SD05W030	USA	HWW	SD98W302/NW97S186	SDSU
SD05W148-1	USA	HWW	SD98153/SD98W117	SDSU
SD06069	USA	HRW	Harry/Wesley//Jerry	SDSU
SD06165	USA	HRW	Wesley/SD97049	SDSU
SD06173	USA	HRW	BULK02R2B	SDSU
SD06W117	USA	HRW	Alice/SD00W024	SDSU
SD07204	USA	HRW	HARDING//SD98243/ALLIANCE	SDSU
SD07220	USA	HRW	TANDEM/Goodstreak	SDSU
SD07W041	USA	HWW	FALCON/SD99W042//TREGO	SDSU
T151	USA	HRW	T81/ KS93U206	Trio
T153	USA	HRW	T136/ T151	Trio
T154	USA	HRW	T88/2180//T811	Trio
T158	USA	HRW	KS93U206/ 2*T81	Trio
TAM 110	USA	HRW	07Kochenower	TX
TAM 107	USA	HRW		check

TN801	USA	SRW	Cardinal/FL302//AR Exp 494B-2-2/3/Fillmore/Cardinal//Jackson	West
Trego	USA	HWW	KS87H325/Rio Blanco	check
TX01V5134RC-3	USA	HRW	TAM-200/JAGGER	Texas A&M
TX02A0252	USA	HRW	TX90V6313//TX94V3724(TAM-200 BC41254-1-8-1-1/TX86V1405	Texas A&M
TX03A0148	USA	HRW	TX89A7137/TIPACNA	Texas A&M
TX03A0563	USA	HRW	X96V107/OGALLALA	Texas A&M
TX04A001246	USA	HRW	TX95V4339/TX94VT938-6	Texas A&M
TX04M410164	USA	HRW	MIT/TX93V5722//W95-301	Texas A&M
TX04M410211	USA	HRW	MASON/JAGGER//OGALLALA	Texas A&M
TX04V075080	USA	HRW	JAGGER/TX93V5722//TX95D8905	Texas A&M
TX05A001334	USA	HRW	TX87V1233-3/U1254-4-6-6//K92/3/T200*2/TA2460*2//T202	Texas A&M
TX05V5614	USA	HRW	TX96V2427/TX98U8083	Texas A&M
TX06A001084	USA	HRW	KS90WGRC10//U1275-1-11-8/TA2455/3/KS93U69/4/Ogallala/TX89V4133	Texas A&M
TX06A001239	USA	HRW	OGALLALA/KS94U275	Texas A&M
TX06A001376	USA	HRW	NE94482/TX95A1161	Texas A&M
TX06A001431	USA	HRW	T107//TX98V3620/Ctk78/3/TX87V1233/4/N87V106//TX86V1540/T200	Texas A&M
TXHT001F8-CS06/325-PRE07/75	USA	HRW	TX01M5009/Halberd	TAMU/CS
TXHT005F8-CS06/540-STA07/14	USA	HRW	Halberd/Trego	TAMU/CS
TXHT006F8-CS06/472-STA34	USA	HRW	Lockett/Halberd	TAMU/CS
TXHT023F7-CS06/607-STA07/40	USA	HRW	TX99U8544/Ogallala	TAMU/CS
U07-698-9	USA	HRW	Jagger*2/HD29	USDA-Man
USG 3555	USA	SRW	VA94-52-60/Pio2643//USG3209	Check
VA03W-412	USA	SRW	Roane/Pio2643//SS520	Griffey
VA04W-259	USA	SRW	VA97W-533 [FFR555W/Gore//Ck9803/VA87-54-636] /NC95-11612(Stella/KS85WGRC01//C8433/3/C8629/FL7927)	Griffey
VA05W-258	USA	SRW	VA98W-130(Savannah/VA87-54-558//VA88-54-328/Gore)//Coker9835/SS520	Griffey
VA05W-414	USA	SRW	Pio25W60//VA96W-606WS(FFR555W/Coker9803//Annette)/Pio2691	Griffey
VA05W-78	USA	SRW	Tribute/AGS2000	Griffey

W06-202B	USA	SRW	Ashland/Hopewell//OH546/L930605	Cisar
W98007V1	USA	SRW	F2IN82104B1-3-2(H14H15),W900003,Andy/Seneca/3/ Downy/F2IN82104B1-3-2(H14H15),Williams,IN86861-8(H18)/4/NC96BGTA6	Edge
W98008J1	USA	SRW	IN82104B1-3-2(H14H15)/Williams,IN86861-8(H18)//NC96BGTA6	Edge
Wesley	USA	HRW	Plainsman V / Odesskaya 51 // Colt / Cody	PI 605742
Wheaton	USA	HRW	CRIM(CI-13465)/2*(CI-13986)ERA//BUITRE/GALLO	PI 469271