MOLECULAR CHARACTERIZATION OF DURABLE YELLOW AND LEAF RUST RESISTANCE IN TWO WHEAT POPULATIONS

A Dissertation

by

BHOJA R. BASNET

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2012

Major Subject: Plant Breeding

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Approved by:

Co-Chairs of Committee,	Amir Ibrahim
	Ravi Singh
Committee Members,	Jackie Rudd
	Dirk Hays
	Wayne Smith
Head of Department,	David Baltensperger

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ABSTRACT

Molecular Characterization of Durable Yellow and Leaf Rust Resistance in Two Wheat Populations. (May 2012)

Bhoja R. Basnet, B.Sc. (Ag), Tribhuwan University; M.S., South Dakota State University

Co-Chairs of Advisory Committee: Dr. Amir Ibrahim
Dr. Ravi Singh

Wheat (*Triticum aestivum* L.) is one of the most important food crops, comprising the largest source of daily calorie and protein intake of human beings worldwide. Among the several diseases of wheat, Yellow Rust (YR; caused by *Puccinia striiformis* Westend. f. sp. *tritici*) and Leaf Rust (LR; caused by *Puccinia triticina* Erikss. & Henn.) have always been major production constraints since the domestication of wheat. For the last few decades, scientists have invested large efforts to identify, characterize and utilize Adult Plant Resistance (APR), a.k.a. slow rusting resistance, in wheat germplasm to promote durability of resistance against rust. The objectives of this study were to 1) understand the genetics of APR to YR and/or LR present in two potential wheat lines 'Quaiu 3' and 'TAM 111', and 2) map the putative Quantitative Trait Loci (QTL) associated with YR and LR resistance using DNA-based molecular markers. Two Recombinant Inbred Line (RIL) populations were subjected to YR and LR disease evaluation experiments in multiple years and locations. Visual evaluation of Disease severity (DS) and Infection Type (IT) score in both RIL populations showed that

APR to YR and LR were highly heritable quantitative traits with significant correlation among experiments.

In spring wheat population, composite interval mapping consistently detected four and three large effect QTL for YR and LR resistance, respectively. Among those QTLs, 1B, 3B and 1D QTL were found to be associated with previously characterized genes *Lr46/Yr29*, *Sr2/Yr30* and *Lr42*, respectively. However, QTLs *QYr.tam-3D* and *QYr.tam-2D* were potentially novel. The largest YR QTL *QYr.tam-2D* was located on long arm of chromosome 2D explaining about 48 to 61% of the total phenotypic variation.

Similarly, in winter wheat population, apart from three environment-specific QTL on chromosomes 1A, 2A and 7D, the QTL on chromosome 2B (*QYr.tam-2B*) was found to express consistently in multiple environments explaining about 23 to 63% of total phenotypic variation.

This study has further elucidated the inheritance mechanism of APR to YR and LR present in two different wheat lines, Quaiu 3 and TAM 111, and resulted in the successful mapping and characterization of the genetic loci associated with corresponding disease resistance traits. These findings should be very useful to isolate the novel APR genes and/or directly use in wheat breeding programs to enhance durable rust resistance in diverse wheat germplasm and cultivars in the future.

DEDICATION

To my parents, wife, and all family members

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First of all, I would like to express my sincere gratitude to my major advisor Dr. Amir M.H. Ibrahim for providing me this great opportunity to pursue my Ph.D. in Plant Breeding under his supervision. I am always grateful for his excellent guidance, eminent support and practical training during the course of my study and research at Texas A&M University (TAMU).

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NOMENCLATURE

RIL	Recombinant Inbred Lines
APR	Adult Plant Resistance
QTL	Quantitative Trait Loci
SSR	Simple Sequence Repeats
DArT	Diversity Arrays Technology
YR/LR	Yellow Rust/Leaf Rust
DS	Disease Severity
IT	Infection Type
COI	Coefficient of Infection
AUDPC	Area Under Disease Progress Curve
LOD	Logarithm Of Odds
MAS/B	Marker Assisted Selection/Backcrossing
CIM	Composite Interval Mapping
ICIM	Inclusive Composite Interval Mapping

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Wheat (*Triticum aestivum* L.) rust (caused by *Puccinia* spp.), one of the most common diseases of wheat, has been a major production constraint in the majority of wheat growing regions worldwide. Host plant resistance is the most economic and environmentally safe method of controlling this disease; however, race-specific major genes are quite often defeated by newly evolving virulent races of the pathogen. For few decades, wheat scientists have been striving to breed for durable resistance to yellow (stripe) rust (YR) and leaf (brown) rust (LR), caused by *Puccinia striiformis* f. sp. *tritici* and *P. triticina*, respectively. YR is known to cause regular regional crop losses ranging from 0.1 to 5% that in some instances can be from 5 to 25% (Wellings, 2011). Similarly, the losses due to LR range from 5 to 15% depending on the stage of crop development when the initial infection occurs (Samborski and Dyck., 1976). The durability of resistance to YR and LR is considered to be associated with the slow rusting, adult-plant resistance (APR) genes that are inherited quantitatively and often conditioned by the accumulated effects of several genes. Thus, slow rusting or durable resistance genes are also often called APR genes (or minor genes) which interact with a broad range of pathogen races providing partial resistance in the adult plant stage despite seedling susceptibility.

This dissertation follows the style of *Crop Science*.

In addition to main slow rusting genes *Lr34/Yr18* (Dyck, 1987; Krattinger et al., 2009), *Lr46/Yr29* (Singh et al., 1998; William et al., 2003) and *Lr67/Yr46* (Herrera-Foessel et al., 2011; Hiebert et al., 2011) different studies have reported the presence of several additive minor genes in wheat germplasm (Singh et al., 2000b; Navabi et al., 2003; Navabi et al., 2004; Santra et al., 2008). As minor genes may not provide enough protection when used alone, experimental evidences have shown that combining at least four to five slow rusting genes can produce near-immunity for long periods of time (Singh et al., 2000a). Hence, identification and characterization of novel durable resistance genes, using molecular markers, will provide powerful tools for dissecting their complex mechanism and true genetic effects which will be useful to develop new resistant cultivars via Marker Aided Selection (MAS) and/or gene pyramiding approaches. Here, we propose two different studies to characterize the novel durable resistance genes to leaf and YR at the molecular level using Recombinant Inbred Lines (RIL) derived from hexaploid winter and spring wheat lines 'TAM111' and 'Quaiu 3', respectively.

Study I: Molecular mapping of Stripe rust adult plant resistance in a TAM 111 X TAM 112 mapping population

'TAM 111', marketed by AgriPro Wheat, is currently Texas AgriLife Research most popular variety and is widely grown in the central and southern Great Plains. Its popularity is attributed to its excellent yield records and its APR to YR. Subsequent

observations for the last ten years have revealed that its resistance reaction starts early on during plant development (J. Rudd, personal communication, 2011), making it a unique source of APR to YR. Early infection of YR in susceptible varieties can cause up to 40% yield loss (Bowden, 2001). So, YR resistance in TAM 111 is novel, yet has not been mapped to date. Tagging this resistance will improve our gain from selection, especially if combined with our existing markers for *Gb3*, *1RS.1AL*, *WSMV*, and anticipated markers for *Ug*99 and minor genes for LR resistance.

Thus, the objectives of this study is to understand the mode of inheritance of adult plant resistance to stripe conferred by the wheat line TAM111, and map the associated Quantitative Trait Loci (QTL)/gene using molecular markers.

Study II: Molecular characterization of durable resistance to stripe and leaf rusts in a spring wheat cultivar Quaiu 3

Quaiu3 (Pedigree: Babax/*Lr42*//Babax*2/3/Vivitsi) is a spring wheat line that contains major resistance genes *Lr42* and *SrTMP* for LR and stem rust (SR, caused by *P. graminis*) respectively (Singh et al., 2009b). It also contains *Sr2* slow rusting resistant gene for stem rust and 2-3 unknown minor genes providing leaf and yellow rust durable resistance (R.P. Singh, personal communication, 2011). Several phenotypic screenings have shown that durable resistance provided by Quaiu3 seems to be unique and promising, which, after characterization of its molecular control for both rust types, can be combined with other sources to achieve higher level of durable resistance in the future.

Thus, the objectives of this study are: (1) to understand the mode of inheritance and estimate the number of genes responsible for durable rust resistance in the spring wheat line Quaiu3, and (2) map the associated QTL present in Quaiu3 using molecular markers.

Review of Literature

Wheat rust: a brief overview

Scientists believe that cereal rusts have been present and evolving during the crop domestication process and concurrent human civilization (Schafer et al., 1984). Evidences from earlier records have shown that wheat was affected by maladies, called blight, blasting and mildew, which, now scientists believe should be, in part, by rust fungi (Roelfs et al., 1992). Kislev (1982) reported the recovery of two rust-infected tetraploid wheat (*Triticum parvicoccum*) lemma fragments harboring uredospores of stem rust fungus from the late Bronze Age II (1400-1200 BC) at Tel Batash, Israel. However, the persons to understand and recognize the "rust" as a parasitic fungus were G. Targioni Tozzetti and Felice Fontana who independently published a detailed description of the rust fungus in 1767 (Schafer et al., 1984). According to Chester (1946), Christiaan H. Persoon was the first person to recognize the rust as a distinct group of fungi, and named it *Puccinia graminis*, in 1794. However, de Candolle was the first person to distinguish the LR pathogen, thus named *Uredo rubigo-vera*, from Persoon's *Puccinia graminis* in 1815 (Chester, 1946). The literature shows that Eriksson and Henning were the first to claim that YR was caused by a separate pathogen, thus

named *Puccinia glumarum*, in 1896 (Roelfs et al., 1992). However, Hylander et al. (1953, cited in Roelfs et al., 1992) revised the name to *P. striiformis*.

The understanding the biology, host ranges, and epidemiology of cereal rust pathogens became more comprehensible in several landmark studies by plant pathologist and geneticists in the 20th century (McIntosh et al., 1995). The currently accepted names for the three different rust pathogens of wheat are *Puccinia triticina* Erikss. & Henn. for leaf rust (LR, also called brown rust), *Puccinia graminis* Pers. f. sp. *tritici* for stem rust (SR, also called black rust) and *Puccinia striiformis* Westend. f. sp. *tritici* for stripe rust (YR, also called yellow rust). The wheat stem rust pathogen is macrocyclic heteroecious fungus whose primary hosts are wheat, triticale and barley, and alternate hosts are several species of the *Barberries* and *Mahonia* (Ahrendt, 1961, cited in Roelfs, 1985; Roelfs et al., 1992). Similarly, the wheat LR pathogen is also a macrocyclic heteroecious fungus whose primary hosts are wheat and triticale, and alternate hosts are several species of *Thalictrum, Anchusa, Clematis and Isopyrum* (Jackson and Mains, 1921, cited in Samborski, 1985; Roelfs et al., 1992). On the other hand, the YR pathogen fungus has wheat and triticale as primary hosts and *Barberries* spp. as alternate hosts (Roelfs et al., 1992; Jin et al., 2010).

LR is the most common of all rust diseases of wheat and is omnipresent in wheat growing areas around the world (Roelfs et al., 1992). Normally, the LR infection occurs on the upper surface of leaf and yield loss caused by it ranges from 10-30% (Roelfs et al., 1992), but under severe epidemics the yield loss can be up to 50% (Roelfs, 1978). Stem rust, which is more regional in nature and needs higher humidity and warmer

temperature, causes infection on leaf (rust pustules can be seen on both sides of leaf) and stem. The yield loss in stem rust epidemics may range from moderate (50%) to high (up to 100%) depending upon the environmental conditions and genetic architecture of host plants (Roelfs et al., 1992; Roelfs, 1978). The YR, which was once considered to be prevalent but sporadic rust disease adapted in temperate region, has now become more nuisance in a wider geographic region causing serious economic damage to wheat growers (Wellings, 2011). It causes infection on leaf blades, sheaths and even it moves towards to spike under high inoculum pressure conditions. A detailed review on historical assessment of grain yield loss and economic impact caused by YR epidemics around the globe bas been presented by Wellings (2011). Based on his review, YR caused yield losses from 11 to 80% depending on the time and place of infection. Some studies have reported a 100% of crop loss under extreme conditions (Roelfs et al., 1992).

Genetics of host-pathogen interaction

Throughout the history of rust research, studies on genetics of virulence in rust pathogen have always been preceded by studies on genetics of resistance in host plants as the tradition of selecting disease-free plants was an older practice in crop improvement (Roelfs and Bushnell, 1985). Eriksson in 1894, was the first person to show that SR possesses different biological races that are undistinguishable morphologically but differ in their pathogenicity to infect their cereal hosts (Agrios, 1997). In fact, the gene-for-gene hypothesis proposed by H.H. Flor in 1942 was one of the biggest landmarks in the genetics of host plant-pathogen interaction. According to

Flor (1942), the gene-for-gene theory can be stated as: “for each gene determining resistance in the host plant, there is a specific and related gene determining pathogenicity in the pathogen”. Though Flor’s theory was based on a work in the Flax-rust system, it was equally applicable to any host-pathogen system in which resistance was inherited in a Mendelian fashion. This theory clearly demonstrated the idea that host-pathogen interaction is a two-way system where, the host plant can resist disease (incompatible interaction) only when there is a mutual recognition between the resistance gene in the host and the avirulence gene in the pathogen. In spite of some unrealistic assumptions (Roelfs et al., 1992), the gene-for-gene hypothesis became the basis for identifying physiological races of pathogens by observing their interaction with a set of host varieties termed “differentials”, in which, ideally each member possesses a single unique resistance gene (Flor, 1971). By using a standard set of differentials, rust pathotype nomenclature and race designation have been routinely practiced since 1930s, which resulted in the identification of a number of rust resistance genes in different wheat germplasm and their deployment in developing rust-resistant varieties (McIntosh et al., 1995).

Race specific and non-specific resistance

In the host-pathogen system, host resistance has been defined as the ability of the host plant to hold back the growth and/or development of the pathogen inside its tissue (Parlevliet, 1985). Classification of disease resistance into distinct categories has always been a matter of debate among plant pathologists and geneticists for long (Vanderplank,

1968; Nelson, 1978; Ellingboe, 1975; Parlevliet, 1985). Though there are plenty of definitions of terminologies that relate to the mechanisms of disease resistance in plants, most of them revolve around the specificity of pathogen to make a compatible interaction with corresponding host plant resulting into disease of various magnitude. As a simplistic definition, race-specific, a.k.a. vertical, resistance means resistance to some specific pathogen isolates but not to others; whereas non-race-specific, a.k.a, horizontal, resistance refers to the resistance to all isolates of the pathogen (Dyck and Kerber, 1985). Furthermore, race-specific resistance is considered to be inherited in a simple Mendelian fashion as opposed to the non-race-specific resistance which is inherited quantitatively or in a polygenic fashion. Nelson (1978) defined the term horizontal resistance as an infection rate reducing resistance which is conditioned by more than one gene. However, he denied the existence of different classes of genes for vertical or horizontal resistance i.e., the same gene may be horizontal or vertical depending on the epidemiological situation and genetic background of host plants. Similarly, Ellingboe (1975) defined non-specific resistance as "field, horizontal or generalized resistance which has not yet been shown to be specific".

In general, race-specific resistance is caused by the interaction of specific genes in the host with corresponding genes in the pathogen (Dyck and Kerber, 1985). The fundamental genetic principle behind race-specific resistance is the classical gene-for-gene theory developed by Flor (1942). In fact race-specific resistance system has given birth to the co-evolution of host-plant- pathogen continuum in nature, assisted by man guided evolution where pathogen develops adaptability over time to overcome the

resistance of new host plant (Dyck and Kerber, 1985). Different variants of specific resistance suggested by Dyck and Karber (1985) includes hypersensitivity, immunity, moderate and adult plant resistance whose expressions are influenced by environmental conditions (temperature sensitivity, for example), interactions with other genes, inhibitory effects by non-allelic genes, background genetic effects and multiple allelism.

Parlevliet (1985) argued that the variation present in both plants and pathogen is relative to one another, which may be either specific in which the rust races can be identified with differential cultivars and vice versa, or non-specific whereby the rust races cannot be recognized by using host cultivars and vice-versa. This is because recognition of races is based on gene-for-gene interaction between host and pathogen genotypes. Further, Parlevliet (1985) believes that non-race-specific resistance is based on two genetic systems: "when the host and the pathogen genes have small effects and operate on a gene-for-gene basis and when the host and pathogen genes, whether small or large in effect, do not operate on a gene-for-gene basis".

Partial/slow rusting/adult plant and durable resistance

According to Parlevliet (1985), a partial resistance is a form of incomplete resistance with some degree of spore production, where susceptible infection type individual lesions are visible. Slow rusting is another term first used by Caldwell et al. (1970) who observed some spring wheat cultivars (slow rusting cultivars) which remained free of severe LR in pure stand before the senescence started as compared to fast rusting cultivars. Though partial resistance and slow rusting resistance have been

used interchangeably by many scientists and authors in the literature, Parlevliet (1979) has cautioned that qualitatively inherited race-specific resistance can also be expressed as slow rusting. Similarly, the resistance that is first expressed in older plants is called adult plant resistance (APR, also called mature plant or post-seedling resistance) (Dyck and Kerber, 1985). Johnson (1978; 1984) introduced the term durable resistance which was defined as a resistance that remains effective during its prolonged and widespread use in disease favorable environment. In spite of these different terminologies, the most essential feature of genes that every plant scientists want to have is its durability. Generally, non-race-specific partial or slow rusting resistance, which results into longer latent period with fewer number of small uredinia formation (Ohm and Shanner, 1976; Kuhn et al., 1978), is assumed to be durable. However, numerous examples of single gene durable resistance have also been reviewed by Johnson (1984). Similarly, contrary to the general consensus that adult-plant resistance is of horizontal nature (Robinson, 1976), which is expected to be effective against a wide spectrum of races, several exceptions have been reported about the race specificity of many adult plant resistance genes including *Lr12*, *Lr13* and *Lr22* (Dyck and Kerber, 1985). Recent findings from molecular cloning of an adult plant resistance gene *Lr34*, which has been durable and effective for the last 60 years, revealed that its protein is similar to an ABC transporter protein of pleiotropic drug resistance sub family (Krattinger et al., 2009). This gene is expressed strictly at the adult plant stage by stimulating senescence-like processes and providing resistance to multiple diseases including LR, YR and powdery mildew.

Distribution and importance of leaf rust and yellow rust

Out of the three rusts of wheat, LR is the most common and widely distributed disease worldwide (Kolmer et al., 2009). This disease has been widely distributed in almost all the continents with specific epidemiological regions including Canada, USA, Mexico, West Asia, East Asia, Central Europe, Eastern Europe, Middle East, Southern Africa, Northern Africa and Australia (German et al., 2007; Roelfs et al., 1992, Kolmer et al., 2009; Huerta-Espino et al., 2011). In the United States, LR is commonly found on different classes of wheat including hard red winter wheat grown in Southern Great Plains, hard red winter wheat in Northern Great Plains, soft red winter wheat in the Southeastern States and Ohio Valley, and to some extent, spring or winter wheat in California and the Pacific Northwest (Kolmer et al., 2009). Southern Texas and the Golf Coast region are usually considered to favor overwintering of *P. triticina* spores which infect the nearly grown wheat crops as early as February and reach the highest severity levels in March and April (Roelfs, 1989). Thus, Texas is considered to be the forefront of the *Puccinia* pathways whereby the windblown urediniospores are carried to the northern and eastern states including Oklahoma, Kansas and Verginia where the LR severities reaches the maximum during April and mid-May (Kolmer et al., 2009). Similarly, spring wheat gets infected by LR in Minnesota, South Dakota, and North Dakota during mid-June to mid-July.

Though earlier researchers believed that LR caused a little or no damage to wheat as compared to other rusts, past records have shown that it is causing significant yield and quality loss continuously for decades, including some historical epidemics in

different geographical regions in the world (Kolmer et al., 2009; Dubin and Brennan, 2009). Yield loss due to LR infection in different geographical regions of the US and Canada ranged from 10 to 28% in resistant cultivars, whereas it ranged from 25 to 95% in susceptible cultivars of soft red winter, hard red winter and hard red spring wheat (Mains, 1930; Caldwell et al., 1934; Chester, 1939; Peturson et al., 1945; Johnston, 1931). In the US, losses due to LR during the 2002 to 2004 crop seasons were estimated over 3 million tones, which is worth over $350 million, (Huerta-Espino et al., 2011). Kansas, the largest wheat growing state in the US, suffered a big yield loss of about 14% in 2007, whereas it dropped down to 1% in 2010 with twenty-year average of 3.8% (Appel et al., 2010). A trend of yield loss of winter wheat cultivars caused by LR epidemics from 2002 to 2011 in some selected US states is shown in Figure 1.1. A historical LR epidemic that developed in northern Mexico resulting into more than 115,200 ha of wheat being sprayed with systemic fungicides reduced the yield loss to only 15% as compared to 40% in unsprayed field (Dubin and Torres, 1981). However, for the last 20 years the LR situation has been observed to be stable in Mexico because of the deployment of many slow using genes in wheat cultivars (Huerta-Espino, 2011). Similarly, the southern cone of South America (especially Argentina, Brazil, Chile, Paraguay, Bolivia, and Uruguay) has been affected by yield loss in wheat due to the emergence of new virulence of LR pathogen after 1995 which resulted into a loss of US$172 million in 10 years (German et al., 2004). Hanson et al. (1982) have summarized the yield loss of up to 50% with an average of 15-20% in developing

countries including Mexico, India, Pakistan, Bangladesh and China where around 90% of wheat growing area was under epidemics.

YR has been reported in more than 60 countries in Africa, Asia, Australia, New Zealand, Europe, North America and South America, where significant yield loss has been observed over the years (Stubbs, 1985; Chen, 2005). A record of literature by Eriksson and Henning (1896, cited in Chen, 2005) shows that wheat YR was first described by Gadd in 1777. The YR pathogen prefers relatively cooler temperature, so it usually occurs in temperate climates or high altitudes in tropical areas (Chen, 2005). In the US, YR has been a big problem west of the Rocky Mountains for the last 50 years (Chen, 2005). However, in recent years this disease has been causing significant yield loss in California, Oregon, Washington, Idaho, Montana, Colorado, Texas, Oklahoma, Kansas, Nebraska, South Dakota, Louisiana, Arkansas, Missouri, Alabama, and Georgia (Kolmer et al., 2009). As the Pacific Northwest of the US and the nearby areas of Canada usually provide favorable environments to YR pathogen to survive throughout the year; therefore, urediniospores can be found at almost any time of year in this region (Kolmer et al., 2009). The YR pathogen was found to overwinter in California where it can survive the mild summer on wheat and grasses at high elevations leading to early epidemics (Tollenaar and Houston, 1966; Line, 1976). Late planted wheat in northern Mexico has been found to harbor the YR pathogen and produce urediniospores (the sole source of inoculums) which moves northward and infect early wheat in the Southern US Plains including Texas, Louisiana, Mississippi and Arkansas (Kolmer et al., 2009). The spores produced in the Southern States due to infection in the late fall or early spring

provide the inoculums source for northern states including Oklahoma, Kansas, Nebraska, Georgia etc.

Yield loss caused by wheat YR has been observed to be more severe than that caused by LR, most probably because this pathogen starts infection at an early growth stage whereby a single infection spreads along the leaf forming long stripes of spores. In high inoculum pressure the YR pathogen infects the wheat heads as well. Studies using susceptible spring wheat cultivars showed a yield loss of up to 60% due to the YR (Bever, 1937, cited in Wellings, 2011). In the US, several historical epidemiological evidences caused by YR have been reported after 1950s (Kolmer et al., 2009). Severe epidemics of YR were observed in the Pacific Northwest, California, Idaho and Montana during 1960 to 1961 with estimated yield loss of 28-56% in California (Hendrix, 1994; Shaner and Powelson, 1971; Pope et al., 1963; Tollenaar and Houston, 1966). Similarly, from 1974 to 1978, another YR epidemic occurred in California, Washington, Oregon, and Idaho with estimated yield loss ranging from 8 to 17% (Kolmer et al., 2009). Another big hit by YR was observed in the Pacific Northwest in 1980 and 1981 with estimated yield loss ranging from 5-13% across three states of Washington, Oregon and Idaho. A trend of yield loss of winter wheat cultivars in YR affected US states from 2002 to 2011 is shown in Figure 1.2. However, the yield loss estimates were relatively lower as compared to previous epidemics because of the heavy use of fungicides (Kolmer et al., 2009). Though YR was considered to be a problem of the Pacific Northwest prior to 2000, recently this disease is becoming equally important in the

South Eastern States and the Central Great Plains of US (Chen, 2005). A map of distribution of strip rust epidemics from 2003 to 2005 in the US is given in Figure 1.3.

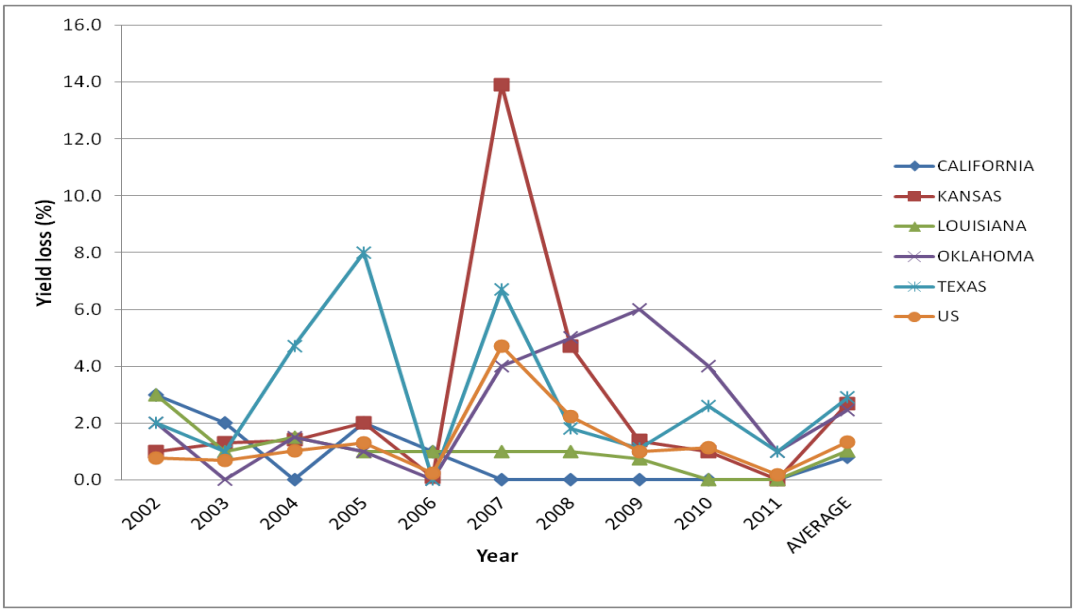

Figure 1.1 Trend of yield loss percentage in winter wheat caused by leaf rust infection from 2002 to 2011 in some selected US states and the average of the whole country (data source: USDA-ARS, URL: http://www.ars.usda.gov/Main/docs.htm?docid=10123)

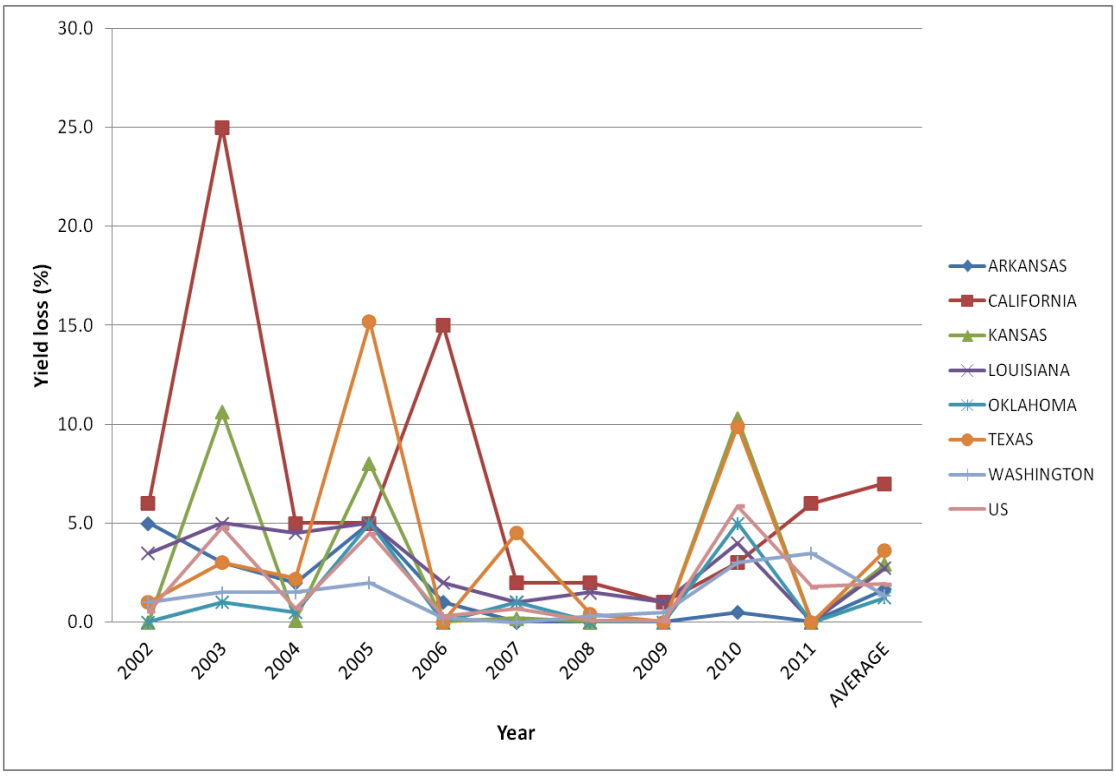

Figure 1.2 Trend of yield loss percentage in winter wheat caused by yellow rust infection from 2002 to 2011 in some selected US states and the average across the country (data source: USDA-ARS, URL: http://www.ars.usda.gov/Main/docs.htm?docid=10123)

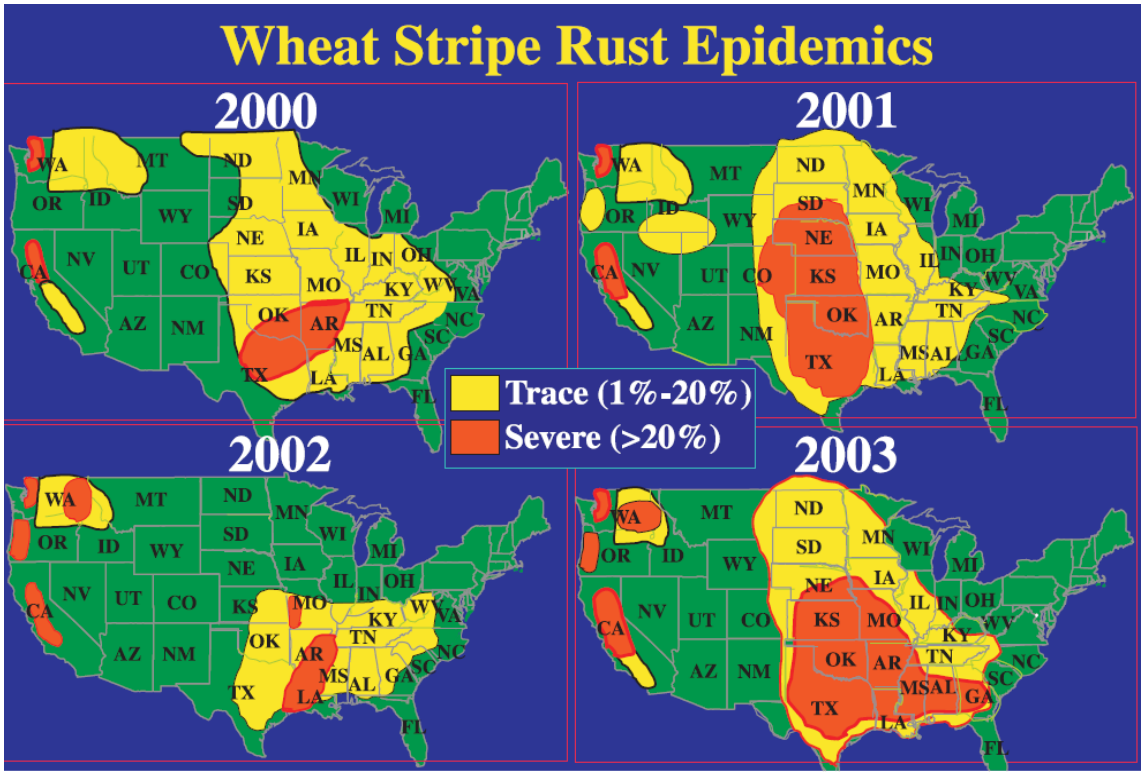

Figure 1.3 Distribution of stripe rust epidemics from 2000 to 2003 in the US (Source: Chen, 2005)

Characterization of non-race-specific resistance to leaf and yellow rusts in wheat

Resistance durability depends on the genes that confer non-race-specific resistance or combination of genes that have been found to be effective for long periods of time in spite of prevalent disease pressure (Kolmer et al., 2009). The high rate of asexual and sexual multiplication of rust pathogen followed by selection pressure imposed by deploying a single major gene in widely grown cultivars have resulted into a rapid growth of virulent races in wheat growing regions. As a result, the single race-specific gene becomes ineffective against newly evolved races of rust pathogen. This is one of the reasons why plant breeders and plant pathologists have been trying hard to achieve durable resistance by using combination non-race-specific genes in newly developed germplasm. To this date, more than 90 LR resistance genes (including standard designation from *Lr1* to *Lr68*) and 89 YR resistance genes (including standard designation from *Yr1* to *Yr49*) have been identified in different wheat germplasm (McIntosh et al., 2010). However, most of the earlier designated *Lr* and *Yr* genes are race-specific major genes. The emergence of virulence against many *Lr* genes such as *Lr2a*, *Lr3*, *Lr9*, *Lr11*, *Lr14a*, *Lr16*, *Lr17*, *Lr18*, *Lr24*, *Lr26*, *Lr28*, *Lr41* in different time periods have been reported (Kolmer et al., 2007; Long et al., 2000; Kolmer et al., 2009). Similarly, studies have reported the existence of virulent races to most of the race specific *Yr* genes, except *Yr5*, *Yr15*, *Yr26* and *Yr40* which are still effective against identified races the in US (Chen, 2005; Kuraparthy et al., 2007). In recent decades, long term experiences in breeding for rust resistance and rapid advancement in molecular technologies have facilitated the identification and characterization of non-race-specific

resistance (slow rusting resistance) genes specially expressed at the adult plant stage. To this date, several non-race-specific APR genes, which have been observed to be effective against a broad range of races of LR and YR pathogens for long periods of time, have been successfully characterized at the molecular level and are being used in national and international breeding programs to enhance durable resistance to wheat rust worldwide.

Probably, *Lr34* is an example of one of the most common and well characterized non race-specific genes, which has remained effective for the last 60 years (Dyck, 1987; Krattinger et al., 2009). Though the origin of *Lr34* has been traced back to the Italian variety 'Mentana', it has been reported to be present in South American varieties such as 'Frontana' and its derivatives, and many landraces of Chinese origin such as 'Chinese Spring' (Borghi, 2001; Kolmer et al., 2008). Recently, this gene has been sequenced on chromosomal arm 7DS (Krattinger et al., 2009). Co-segregation of *Lr34* gene with adult plant YR resistance gene *Yr18* (McIntosh, 1992; Singh 1992) and partial resistance to powdery mildew gene (pm38) (Spielmeyer et al., 2005; Lillemo et al., 2008) has proven that *Lr34/Yr18/Pm38* is multi-pathogenic in nature providing general resistance to different fungal pathogens. A detail survey on the distribution of *Lr34/Yr18* gene in different wheat germplasm worldwide has been done by Kolmer et al. (2008). It has been reported that the frequency of *csLV34b*, an allele associated with *Lr34* gene, in soft red and hard red winter wheat cultivars of North America is extremely low. However, this gene is distributed more frequently (about 50% of total studied) in Hard Red Spring Wheat (HRSW) cultivars. Similarly, high frequency *Lr34* associated allele was observed in Australian and CIMMYT derived cultivars and germplasm, but the frequency was low

in Western European varieties. The sequence analysis of ABC transporter in different wheat germplasm revealed the presence of five different haplotypes in *Lr34* coding region (Lagudah et al., 2009; Dakouri et al., 2010). A unique haplotype in the winter wheat cultivar 'Jagger' showed that the ABC transporter contains a G/T SNP in exon 22 resulting in premature stop codon which enhances a truncation in gene product rendering it non-functional (Lagudah et al., 2009; Cao et al., 2010). It has been suggested that specifically designated markers should be used to select against the non-functional haplotype, *Lr34/Yr18d*, in Jagger derived population (Lagudah et al., 2009).

Similarly, the second most studied non race-specific adult plant resistance gene is *Lr46* which was first identified in cultivar 'Pavon 76' (Singh et al., 1998), and was believed to be different than *Lr34*. This gene was mapped on the 1BL chromosomal region by using monosomics of susceptible cultivar 'Lalbahadur' and later by using DNA markers (Singh et al., 1998; William et al., 2003). Similar to *Lr34* gene, *Lr46* has been found to be pleiotropic to YR resistance gene *Yr29* (Singh et al., 1998; William et al., 2003) and powdery mildew partial resistance gene *pm39* (Lillemo et al., 2008). Also, it co-segregates with leaf tip necrosis gene *Ltn2* (Rosewarne et al., 2006). Though both *Lr34* and *Lr46* genes appear to have similar mechanisms of slow rusting i.e., prolonged latent period, early abortion of colonies, reduced colony size and lower disease severity (Martinez et al., 2001), there is not any evidence of gene or chromosomal segment duplication nearby these genes (Lagudah, 2011). This gene is widely distributed in bread wheat worldwide specifically in CIMMYT derived germplasm (Singh et al., 2011).

Recently, a new slow rusting gene *Lr67/Yr46* has been identified in the Canadian wheat genotype 'RL6077' and mapped on 4DL chromosomal region (Herrera-Foessel et al., 2011; Hiebert et al., 2011). Though the slow rusting gene on RL6077 was thought to be Lr34 previously (Dyck et al., 1994), haplotype analysis revealed that it is different from *Lr34* (Kolmer et al., 2008; Lagudah et al., 2009). Similarly, past studies have shown that a spring wheat cultivar 'Parula' contains slow rusting resistance for leaf, stripe and stem rusts (Singh and Rajaram, 1992; Singh et al., 2011a). Herrera-Foessel et al. (2012) have successfully tagged a new slow rusting gene *Lr68* of Parula (previously designated as *LrP*) on 7BL chromosomal location at 0.6cM proximal from SSR marker *Xgwm146*. This gene is expected to be widely distributed in spring wheat germplasm worldwide (Singh et al., 2011a).

A high temperature adult plant (HTAP) gene *Yr36*, which confers adult plant slow rusting resistance against YR when exposed to relatively higher temperature, has been sequenced on the 6BS chromosomal region (Uauy et al., 2005, Fu et al., 2009). The molecular mechanism of slow rusting gene *Yr36*, which encodes a protein with an N-terminal kinase domain and a C-terminal START (Steroidogenic Acute Regulatory Protein-Related Lipid Transfer) domain, has been found to be different than that of ABC transporter of *Lr34* (Fu et al., 2009; Krattinger et al., 2009).

Apart from these well-known APR genes *Lr34/Yr18*, *Lr46/Yr29*, *Lr67/Yr46*, *Lr68* and *Yr36*, several minor effect additive genes are expected to be present in CIMMYT derived and other germplasm worldwide (Singh et al., 2011a). With the advancement of molecular marker technology and sophisticated genetic mapping

software, the process of tagging disease resistance gene/QTL in plants has been growing rapidly for the last fifteen years. Probably, molecular mapping of non race-specific adult plant rust resistance is one of the most studied fields in wheat Genetics. To this date, almost all the chromosomes of hexaploid wheat have been reported to carry at least one QTL associated with slow rusting for both LR and YR (Table 1.1 and 1.2). However, challenges still exist in the application process of these findings in crop improvement. Validation of QTL through independently repeated studies and developing universal markers to track the resistance are the most important steps we need to have before implementing marker aided selection (MAS). Similarly, assessment of true breeding values of small effect QTL always remains elusive in small size mapping populations and low density genetic maps. As plant breeding is all about increasing yield and improving end-use in a sustainable manner, we cannot merely concentrate on one trait while keeping thousands of other important traits in jeopardy. So, combining many slow rusting genes into one elite line possessing high yield potential and wide range of adaptability has always been more than a challenge for wheat scientists.

Table 1.1 A list of some selected QTL mapping studies carried out for non race-specific adult plant resistance to **stripe rust** in wheat.

Chr	Resistance source	Reference
1A	Kariega, Stephens, Kariega	Ramburan et al. (2004), Dolores Vazquez et al. (2012), Prins et al. (2011)
1B	Alcedo, Guardian, Pavon 76, Attila, CD87, Pavon 76, Kukri, Pavon 76 and Parula, Guardian	Jagger et al. (2011), Melichar et al. (2008), William et al. (2003), Roswarne et al. (2008), Bariana et al. (2001), William et al. (2006), Bariana et al. (2010), Singh et al. (2005), Melichar et al. (2008)
1D	Stephens	Dolores Vazquez et al. (2012)
2A	PI610750, Recital, Camp Remy, *T. monococcum,* Pioneer 26R61, Stephens, Camp Remy	Lowe et al. (2011), Dedryver et al. (2009), Mallard et al. (2005), Chhuneja et al. (2008), Hao et al. (2011), Dolores Vazquez et al. (2012), Boukhatem et al. (2002)
2B	UC1110, Pingyuan 50, Renan, Camp Remy, Luke, Attila/Avocet-S, Kariega, Stephens , Camp Remy, Kariega	Lowe et al. (2011), Lan et al. (2010), Dedryver et al. (2009), Mallard et al. (2005), Guo et al. (2008), Roswarne et al. (2008), Ramburan et al. (2004), Dolores Vazquez et al. (2012), Boukhatem et al. (2002), Prins et al. (2011)
2D	Alcedo, Guardian, Camp Remy, Kategwa, Guardian	Jagger et al. (2011), Melichar et al. (2008), Mallard et al. (2005), Bariana et al. (2001) , Melichar et al. (2008)
3A	Stephens	Dolores Vazquez et al. (2012)
3B	Oligoculum, UC1110, Opata85, Renan, AGS 2000, Pavon 76, Kukri, Pavon 76 and Parula,	Suenaga et al. (2003), Lowe et al. (2011), Singh et al. (2000), Dedryver et al. (2009), Hao et al. (2011), William et al. (2006), Bariana et al. (2010), Singh et al. (2005)
3D	Opata85, Recital, Opata 85	Singh et al. (2000), Dedryver et al. (2009), Boukhatem et al. (2002),
4A	Kariega, Avocet-S, Stephens, Avocet-S,	Ramburan et al. (2004), Dolores Vazquez et al. (2012), Prins et al. (2011)
4B	Oligoculum, Alcedo, Guardian, Stephens/Platte, Avocet-S, Pavon 76, Guardian	Suenaga et al. (2003), Jagger et al. (2011), Melichar et al. (2008), Dolores Vazquez et al. (2012), William et al. (2006), Singh et al. (2005), Melichar et al. (2008)
4D	Oligoculum, RL6077	Suenaga et al. (2003), Herrera-Foessel et al. (2011), Hiebert et al. (2011)
5A	Alcedo, PI610750, Pingyuan 50, *T. boeoticum,* Opata 85,	Jagger et al. (2011), Lowe et al. (2011), Lan et al. (2010), Chhuneja et al. (2008), Boukhatem et al. (2002),
5B	Oligoculum, Camp Remy, AGS 2000, Janz,	Suenaga et al. (2003), Mallard et al. (2005), Hao et al. (2011), Bariana et al. (2010)
5D	Opata85	Singh et al. (2000)
6A	Pioneer 26R61, Platte, Avocet-S, Avocet-S, Avocet-S	Hao et al. (2011), Dolores Vazquez et al. (2012), William et al. (2006), Prins et al. (2011), Singh et al. (2005)
6B	Oligoculum, Pingyuan 50, Renan, Stephens, Pavon 76, *T. turgidum*, Janz, Kariega	Suenaga et al. (2003), Lan et al. (2010), Dedryver et al. (2009), Santra et al. (2008), William et al. (2006), Uauy et al. (2005), Bariana et al. (2010), Prins et al. (2011)
6D	Opata 85	Boukhatem et al. (2002),
7A	Kariega, Stephens, Kariega	Ramburan et al. (2004), Dolores Vazquez et al. (2012), Prins et al. (2011)
7B	Oligoculum, Alpowa, Attila, Stephens, Kukri	Suenaga et al. (2003), Lin and Chen, (2007), Roswarne et al. (2008), Dolores Vazquez et al. (2012), Bariana et al. (2010)
7D	Fukuho-komugi, Opata85, CD87, Janz, Kariega, Parula	Suenaga et al. (2003), Singh et al. (2000), Boukhatem et al. (2002), Bariana et al. (2001), Bariana et al. (2010), Prins et al. (2011), Singh et al. (2005)

Column from left to right: the chromosome on which putative QTL have been detected, wheat genotype that contributed the resistance, and list of references for corresponding studies.

Table 1.2 A list of some selected QTL mapping studies carried out for non race-specific adult plant resistance to **leaf rust** in wheat.

Chr	Resistance Source	Reference
1A	Oligoculum, Beaver	Suenaga et al. (2003), Singh et al. (2009b),
1B	Forno, Pavon 76, Forno, Attila, Parula, Pavon 76, Beaver, Pavon 76 and Parula	Schnurbusch et al. (2004), William et al. (2003), Messmer et al. (2000), Roswarne et al. (2008), William et al. (1997), William et al. (2006), Singh et al. (2009b)
1D	Parula	William et al. (1997)
2A	Forno	Schnurbusch et al. (2004)
2B	Oberkulmer, Attila, CI13227	Messmer et al. (2000), Roswarne et al. (2008), Xu et al. (2005)
2D	Forno/Arina, CI13227	Schnurbusch et al. (2004), Xu et al. (2005)
3A	Forno, TA4152-60	Messmer et al. (2000), Chu et al. (2009)
3B	TA4152-60, Beaver,	Chu et al. (2009), Singh et al. (2009)
3D	Beaver	Singh et al. (2009b)
4A	Opata, Beaver	Faris et al. (1999), Singh et al. (2009b)
4B	Forno, Avocet-S, Beaver,	Messmer et al. (2000), William et al. (2006), Singh et al. (2009)
4D	Forno, ND495, Beaver, RL6077	Messmer et al. (2000), Chu et al. (2009), Singh et al. (2009b) Herrera-Foessel et al. (2011), Hiebert et al. (2011)
5A	Beaver	Singh et al. (2009b)
5B	TA4152-60	Chu et al. (2009)
5D	Oberkulmer	Messmer et al. (2000)
6A	Avocet-S, Avocet-S	William et al. (2006)
6B	Pavon-76, TA4152-60	William et al. (2006), Chu et al. (2009)
7B	Forno, Attila, Parula, Opata, CI132227, Parula	Messmer et al. (2000), Roswarne et al. (2008), William et al. (1997), Faris et al. (1999), Xu et al. (2005), Herrera-Foessel et al. (2012)
7D	Fukuho-komugi, Forno, Opata, Opata,	Suenaga et al. (2003), Schnurbusch et al. (2004), Nelson et al. (1997) Faris et al. (1999)

Column from left to right: the chromosome on which putative QTL have been detected, wheat genotype that contributed the resistance, and list of references for corresponding studies.

CHAPTER II

GENETIC ANALYSIS OF ADULT PLANT RESISTANCE TO YELLOW AND LEAF RUSTS IN COMMON SPRING WHEAT QUAIU 3

Introduction

Yellow (stripe) rust and leaf (brown) rust diseases of wheat, caused by *Puccinia striformis* f. sp. *tritici* and *P. triticina*, respectively, have been a significant threat in the majority of the world's wheat (*Triticum aestivum* L.) growing regions. Yellow rust (YR) is known to cause regular regional crop losses ranging from 0.1 to 5% to in some instances as much as 5 to 25% (Wellings et al., 2011). Similarly, the losses due to leaf rust (LR) range from 5 to 15% depending on the stage of crop development when the initial infection occurs (Sambroski and Dick, 1976). Though the use of host resistance is the most economical and environmentally friendly method to control rusts in wheat, evolution of new races of pathogens can easily overcome the resistance conferred by the existing race-specific genes if deployed as the sole source of resistance. The YR epidemics reported in various periods in the past were attributed to the failures of resistance genes like *Yr2*, *Yr9*, *YrA, Yr17, Yr27*, etc. deployed in predominant varieties (McIntosh, 2009; Singh et al., 2004; Wellings et al., 2009; Wellings et al., 1988). However, slow rusting resistance genes are considered to be more durable and their expression in the field and greenhouse is conditioned by slow disease development, longer latent period, fewer and smaller uredinia and lower spore production (Allard, 1960). Durable rust resistance is more likely expressed in the adult-plant stage and is

often conditioned by the accumulated effects of several genes; moreover, it is not associated with gene-for-gene interactions leading to hypersensitive response (McIntosh, 1992). Thus, slow rusting durable resistance genes are often called Adult-Plant Resistance (APR) genes which interact with a broad range of pathogen races providing partial resistance in the adult plant stage despite seedling susceptibility (Bariana and McIntosh, 1995; Johnson, 1980; Singh and Rajaram, 1993). Due to the need of durable rust resistance for sustainable increases in wheat production, breeding for APR has been one of the primary objectives for national and international wheat research programs in recent years.

Though a single APR gene may not provide enough protection against rust, especially under high disease pressure, studies have revealed that combinations of 3 to 5 slow rusting genes usually result in "near-immunity" comparable to a high level of resistance (Singh et al., 2000a). One of the most studied and utilized APR genes that has provided protection against a broad range of LR and YR races for the last 60 years is *Lr34/Yr18* located on chromosome arm 7DS (Dyck, 1987; Singh, 1992). This gene has recently been cloned (Krattinger et al., 2009), and is now being introgressed into elite lines in many countries using gene specific DNA markers. Similarly, *Lr46/Yr29* is another important APR gene located in the 1BL chromosomal region (Singh et al., 1998; William et al., 2003), initially identified in a CIMMYT-derived Mexican variety 'Pavon 76'. It is considered to be widely distributed in CIMMYT-derived germplasm worldwide (Singh et al., 2011a). Recently, a new and promising APR gene, located on chromosomal arm 4DL, was identified in a Canadian wheat line 'RL6077' and

designated as *Lr67/Yr46* (Herrera-Foessel et al., 2011; Hiebert et al., 2011). Though the presence of this gene in diverse wheat germplasm is still to be investigated (Singh et al., 2011a), it could be combined effectively with other APR genes to achieve a higher level of durable resistance to LR and YR in the future. In an effort to meet the challenges of breeding for durable rust resistance, genetic characterization of additional APR genes is desirable. This will aid breeders in enhancing genetic diversity for APR in their germplasm (Zhang et al., 2008).

The first step in breeding for durable rust resistance is to extensively evaluate a set of diverse germplasm for adult-plant resistance, and to understand the genetics underlying any potential sources. As slow rusting resistance is quantitatively inherited, understanding the inheritance behavior, such as heritability of traits, segregation pattern, and the expected number of genes conferring the resistance, is important for their successful utilization by breeding programs. The knowledge of heritability and the number of genes controlling slow rusting helps breeders to decide the right time to start selection, and to choose the optimum population sizes to be grown in various segregating generations (Das et al., 2004). Previous studies have suggested the presence of a few to several minor genes (usually 2 to 21) responsible for slow rusting resistance within different wheat lines in phenotype-based quantitative and qualitative approaches of gene number estimates under different mating schemes (Bjarko and Line, 1988; Das et al., 2004; Navabi et al., 2003; 2004; Singh and Huerta-Espino, 1995; Singh and Rajaram, 1993; Singh et al., 1995; Zhang, 2008). Recent molecular mapping studies have identified two to eight quantitative trait loci (QTLs) associated with slow rusting

resistance of YR and LR in different wheat genotypes (Chhuneja et al., 2008; Dedryver et al., 2009; Lan et al., 2010; Mallard et al., 2005; Messmer et al., 2000; Ramburan et al., 2004; William et al., 2006). Similarly, few QTLs/genes associated with durable High Temperature Adult Plant Resistance (HTAPR) to yellow rust were identified in some wheat lines under different mapping experiments (Guo et al., 2008; Lin and Chen, 2007; Navabi et al., 2005; Santra et al., 2008).

Quaiu 3 is a high yielding spring wheat line developed at CIMMYT that shows immunity to both YR and LR in the adult plant stage during field trials. It shows a susceptible reaction to yellow rust at the seedling stage and does not have any effective characterized race-specific resistance genes for yellow rust. Race-specific resistance gene *Lr42*, transferred to wheat from *Aegilops tauschii*, present in Quaiu 3 provides intermediate seedling reactions and moderate levels of resistance to LR in the field. Therefore, we, hypothesized that Quaiu 3 should possess additional resistance genes, most likely slow rusting, that contribute to the immunity against both LR and yellow rust. This study was conducted to analyze the inheritance of adult plant resistance to both LR and yellow rust, and to estimate the minimum number of genes present in spring wheat line Quaiu 3 using recombinant inbred lines population developed by crossing it with the susceptible parent Avocet-*YrA*.

Materials and Methods

Plant materials

A set of 198 randomly advanced F_5 recombinant inbred lines (RILs) derived from the cross Avocet-YrA/Quaiu 3 was used in this study. Quaiu 3 (pedigree: Babax/*Lr42*//Babax*2/3/Vivitsi) is a high yielding CIMMYT line that has shown high levels of adult-plant resistance to leaf and yellow rust in field conditions based on international data. The source parent for resistance gene *Lr42* was a winter wheat germplasm line developed by the UDSA-ARS Germplasm Program at Kansas State University and was kindly provided by Stan Cox. Unfortunately, the exact pedigree of this line is unknown. The susceptible parent Avocet-*YrA* is a reselection from the original heterogeneous Australian cultivar that lacks the race-specific resistance gene *YrA* and is also known as 'Avocet S'. For simplicity, the reselection will be designated as Avocet throughout the paper and Quaiu 3 as Quaiu. To develop the population, a single spike from each F_2 plants, generated from three different F_1 plants, were randomly harvested under fungicide application and advanced to F_5 generation by harvesting and growing a single spike in each subsequent generation. The F_5 plots, derived from single F_4 spikes, were then harvested as bulk to obtain sufficient seed of the F_5 recombinant inbred lines (RILs).

Seedling testing

Greenhouse seedling testing of parents for yellow rust, and whole population for LR was carried out in a greenhouse at CIMMYT headquarters in 2010. For yellow rust,

12 seeds of parental lines and a differential set comprised of Avocet near-isogenic lines (NILs) and other testers were planted as hills in 6 rows x 8 columns planting geometry on trays of size 20 cm x 15 cm with each entry replicated twice. Similarly, for LR, 8-10 seeds of all the lines and a set of LR differentials comprised of 48 entries available in CIMMYT were planted as hills. The young but fully expanded first leaves (about 10 days after planting) of three experimental sets of materials were inoculated with the *P. triticina* race MBJ/SP and *P. striiformis* isolates Mex96.11 and Mex08.13, respectively, by spraying urediniospores suspended in Soltrol 170 oil using an atomizer. The post inoculation environmental condition for both rusts was maintained as described by Herrera-Foessel et al. (2011). The LR infection type for each entry was recorded 11 days after inoculation based on a 0-4 Scale (Roelfs et al., 1992), whereas the yellow rust response was recorded based on the 0-9 Scale (McNeal et al., 1971) at 20 days post inoculation.

Field experiments and disease evaluation

Field experiments were carried out in Mexico during the 2008-2009 and 2009-2010 crop seasons for LR and 2009 and 2010 crop seasons for yellow rust at CIMMYT research stations near Ciudad Obregon, recently named Norman E. Borlaug Experimental Station, CENEB, and Toluca, respectively. Toluca (State of Mexico) research station is located in the highlands of central Mexico (18°N, 2640 meters above sea level), whereas, CENEB in the state of Sonora is located in northwestern Mexico (28° N, 39 meters above sea level). The environment in Toluca, which averages monthly

maximum temperatures of 19 to 23°C and averages monthly precipitation from 65 to 145 mm during the growing season, is very conducive to high yellow rust development. Similarly, the CENEB is considered to be a highly suitable place for LR development during the crop season with average monthly maximum temperatures of 24 to 31°C and average monthly precipitation of 15 to 22 millimeters. In Toluca, planting begins in mid May and the season ends in early October, whereas, in CENEB, planting begins in late November and the season ends in early April.

About 4-5 g seeds (expected 60-70 mature plants) of the parents and 198 RILs were hand sown in 1-m paired-rows, spaced 10 cm apart, on top of 80 cm wide raised beds. The CENEB experiments were planted in the third week of November during the 2008-2009 season and in the first week of December during 2009-2010. To create homogenous rust epidemics, spreader rows of the highly susceptible cultivar Morocco were planted around the experimental area and at one side of each plot in the middle of the 0.5m-wide pathways. Artificial inoculations were carried out twice, about 8 weeks after sowing, with an equal mixture of prevalent Mexican *P. triticina* races: MBJ/SP and MCJ/SP using the method as described by Herrera-Foessel et al. (2011). Similarly, the Toluca experiments were planted in the fourth week of May during both growing seasons. In Toluca, the spreaders consisted of a mixture of six susceptible wheat lines that possessed the defeated race-specific resistance gene *Yr27* and were derived from the cross Avocet × 'Attila'. The varying maturities of these lines ensure a continuous production of inoculum during the critical crop-growing period. An artificial epidemic was created by inoculating the spreaders with Mexican isolates MEX96.11 and

MEX08.13 of *P. striiformis* f. sp. *tritici* at least three times four to five weeks after planting. The avirulence/virulence characteristics of the races used in our studies were previously described by Herrera-Foessel et al. (2011).

Disease severity (DS) scores were recorded following the 0-100% visual ratings based on the modified Cobb's Scale (Peterson et al., 1948). For both LR and YR, the first disease severity readings were taken when the susceptible parent, Avocet, showed at least 70% disease severity followed by second and/or third readings at weekly intervals. Similarly, the host reaction/infection type data were recorded based on the visual criteria (Irfaq et al., 2009; Roelfs et al., 1992; Singh and Rajaram, 1993) with some modification on Scale, where R= resistant (necrotic tissue or yellow stripes with or without tiny uredinia), R-MR=resistant to moderately resistant (necrotic tissues or stripes with few small uredinia), MR= moderately resistant (necrotic or chlorotic tissues or stripes with smaller to medium sized uredinia), M (or MRMS)= moderately resistant to moderately susceptible (necrotic or chlorotic tissues or stripes with medium sized uredinia and intermediate sporulation), MS= moderately susceptible (medium sized uredinia or stripes without chlorosis and necrosis and abundant sporulation), MS-S= moderately susceptible to susceptible (medium to large sized uredinia or stripes without chlorosis and necrosis and abundant sporulation), and S= susceptible (large sized uredinia or stripes without chlorosis and necrosis and abundant sporulation). These reaction type readings were then assigned with numeric response value as R=0.2, R-MR=0.3, MR=0.4, M=0.6, MS=0.8, MS-S=0.9, and S=1.0. Coefficients of infection (CI) values for each line were calculated by using CIMMYT's adopted method i.e. by multiplying disease severity scores with

reaction type values. Furthermore, for the repeated measurements, the Area Under Disease Progress Curve (AUDPC) values for disease severity and CI were calculated by using the following equation (Bjarko and Line, 1988):

$$\text{AUDPC}=\sum_{i=1}^{n-1}\left(\frac{x_i + x_{i+1}}{2}\right)t_i$$

where x_i=the rust severity scores on date i; t_i=time intervals in days between date i and date i+1; n=number of readings.

Molecular marker analysis for selected rust resistance genes

The parents, Quaiu and Avocet, were analyzed with 10 molecular markers (*csGS, Xgwm533, Xgwm192, VPM, Xwmc432, Sr24, csLV46G22, csLV34, +Lr34sp and –Lr34sp*) for 10 well-known rust resistance genes in bread wheat (Table 2.1). The markers *csLV46G22*, *Xgwm533, and Xwmc432* for the *Lr46/Yr29*, *Sr2/Yr30 and Lr42* genes were further analyzed in all the RILs in addition to the parental lines. The source genotypes or well-validated lines for the resistance genes were used as positive controls in the marker analysis. Among the genes analyzed, *Lr34/Yr18* and *Lr46/Yr29* are considered to be the best characterized and most utilized APR genes for both leaf and yellow rust in wheat. The young leaf tissue from parents and RILs was harvested from 10 randomly selected plants of each line planted in the Toluca field station. The DNA was extracted using the CTAB method, and subsequently, PCR reactions, gel electrophoresis and visualization of amplified products were performed using the standard CIMMYT laboratory protocol (CIMMYT, 2005).

Table 2.1 Molecular marker analysis of wheat parents Avocet and Quaiu for selected rust resistance genes

Gene	Marker	Marker analysis†		Positive control	Reference
		Avocet	Quaiu		
Lr68	*csGS*	-	-	Avocet/Prl	(Herrera-Foessel et al., 2012)
Sr2/Yr30	*Xgwm533*	-	+	Parula	(Spielmeyer et al., 2003)
Lr67/Yr46	*Xgwm192*	-	-	RL6077	(Herrera-Foessel et al., 2011)
Yr17/Sr38	*VPM*	-	-	*Sr38*	(Robert et al., 1999)
Lr24/Sr24	*Sr24*	-	-	Krichauf	(Mago et al., 2005)
Lr42	*Xwmc432*	-	+	*Lr42*	(Sun et al., 2010)
Lr46/Yr29	*csLV46G22*	-	+	Parula	E. Lagudah, Pers. Comm., 2011
Lr34/Yr18	*csLV34*	-	-	Parula	(Lagudah et al., 2006)
Lr34/Yr18	*+Lr34sp*	-	-	Parula	(Krattinger et al., 2009)
Lr34/Yr18	*-Lr34sp*	-	-	Parula	(Krattinger et al., 2009)

† Presence (+) or absence (-) of the genes in two parents based on corresponding marker alleles. Avocet is the negative control for all the markers under study.

Statistical analysis

All the statistical analyses including phenotypic distribution, correlation coefficient, Analysis of Variance (ANOVA), and marker-phenotype regression were performed using SAS 9.2 (SAS Institute, Cary, NC). Analysis of Variance was carried out to determine the differences in disease severity scores among 198 recombinant inbred lines based on first evaluation of LR and YR. As the two experiments were treated as replications, the interaction effect between experiment and genotype could not be tested (Bernardo, 2010). However, the F-test for genotypes can be effectively carried out with interaction mean square as an error term. As the disease severity scores were highly correlated between two years of experiments, the interaction effect was relatively

unimportant (J. Crossa, CIMMYT, pers. comm.). Calculation of narrow sense heritability (h^2) was carried out using the variance component method as: $h^2=\sigma^2_g/\sigma^2_p$; where, $\sigma^2_g= (MS_g-MS_{ge})/r$, and $\sigma^2_p= \sigma^2_g+ \sigma^2_{ge}/r$; in this formulae, σ^2_g= genetic variance, σ^2_p= phenotypic variance, σ^2_{ge}=variance attributed to interaction between experiments and genotypes (equivalently, total error variance in this analysis), r= number of replications (equivalently, number of experiments in this analysis), MS_g =Mean square of lines, and MS_{ge} = Mean square of lines & experiment interaction . The exact 90% confidence interval of heritability was estimated by using the equation given by Knapp et al. (Knapp et al., 1985). This calculation of heritability is considered narrow sense heritability because the $F_{4:5}$ recombinant inbred lines are very close to complete homozygosis that results in the dominance variance being close to zero, and additive-by-additive interactions can be accounted as additive components of variance (Lillemo et al., 2006).

Gene number estimates

A qualitative approach to gene number estimation was carried out using the expected and observed F_5 segregation ratio. All the 198 recombinant inbred lines were classified into three phenotypic classes based on disease severity responses: homozygous for parental type resistant (HPTR), homozygous for parental type susceptible (HPTS) and intermediate types (Others) as described by Singh and Rajaram (1993). In this classification, all the lines in HPTR and HPTS are assumed to have resistant and susceptible alleles in the homozygous state, respectively. Though classification is a

subjective approach, in this study a consistent classification rule was used based on parental disease scores, where the lines with disease scores equal to that of the resistant parent or less were classified as HPTR. Similarly, the lines with disease scores equal to that of the susceptible parent or more were classified as HPTS, and the remaining intermediate types were classified as Others. Based on this qualitative classification of recombinant inbred lines, chi-square tests were carried out for 2, 3 or 4 gene segregation ratios.

Similarly, a quantitative assessment of the minimum number of genes controlling APR to LR and YR in wheat was carried out using Wright's formula (Wright, 1968) with some modification to correct the level of inbreeding (Bjarko and Line, 1988; Cockerham, 1983; Herrera-Foessel et al., 2008; Mulitze and Baker, 1985); $N= (GR)^2/ 4.57\sigma^2_g$, where, N= minimum number of genes present in the segregating population, GR= The genotypic range of lines at a given generation, σ^2_g= genetic variance of the population. Generally, the genotypic range is estimated by subtracting phenotypic means of two parents or two extreme observations in the segregating population. When two parents are used to calculate the genotypic range, the assumptions, such as no dominance, linkage, or epistasis, equal effect of all loci, and no transgressive segregation should hold true. Moreover, it is assumed that all the contributing alleles are from one parent. Whereas, when two extreme observations were used to calculate the genotypic range, the assumption of no transgressive segregation is ruled out allowing the possibility of the contributing alleles coming from both parents. In this study, the gene number estimate was performed by using both types of genotypic range. Additionally,

another genotypic range was calculated as the phenotypic range between two parents multiplied by corresponding heritability estimates. This third approach, which gives better estimates of genotypic range since it accounts for the environmental influence (Mulitz and Baker, 1985), has been effectively used to estimate the stable number of gene estimates in previous studies (Das et al., 2004; Lillemo et al., 2006; Singh et al., 1995).

Results

Seedling studies

Both parents, Avocet and Quaiu, showed a susceptible infection type in seedling tests in the greenhouse with the two *P. striiformis* Mex96.11 and Mex08.13 isolates (with seedling infection types of 8-9 and 7-8 on 0-9 Scale for Avocet and Quaiu, respectively). In contrast, only Avocet seedlings were susceptible to *P. triticina* race MBJ/SP with seedling score of 3+. Quaiu seedlings were scored 1+ on 0-4 infection type Scale. This observation indicated that Quaiu possibly possessed a race-specific resistance gene. This was verified by χ^2 test for single gene segregation of RILs in Avocet/Quaiu population where the observed segregation ratio, i.e., 71 resistant: 97 susceptible: 21 segregating lines conformed to the expected single gene segregation ratio of 0.44:0.44:0.12 (P= 0.104). By using a molecular marker assay (SSR marker *Xwmc432*) (Sun et al., 2010), we found that Quaiu possessed race-specific resistance gene *Lr42* that conferred seedling resistance reaction in the population (Table 2.1).

Field studies

Excellent yellow rust development occurred in trials at Toluca during both seasons and the susceptible parent Avocet displayed 70 to 90% severity at flowering stage when the first evaluations were made. Average YR severities of RILs ranged from 41.3 to 62.3% for two experiments at different stages of evaluation. The distribution of RILs for YR severity, CI and AUDPC were continuous but not normal across experiments (Figure 2.1). Relatively higher disease pressure was observed during the 2010 season resulting in a higher average disease severity of RILs compared to 2009. Similarly, LR development was also excellent during both seasons at Cd. Obregon and the susceptible parent displayed 100% severity in both experiments at early grain filling stage. Average LR severities of RILs were less than YR severities and ranged from 22.1 to 33.6% across two experiments and different stages of evaluation. The distribution of RILs for LR severity scores was significantly skewed towards the resistant side (Figure 2.1).

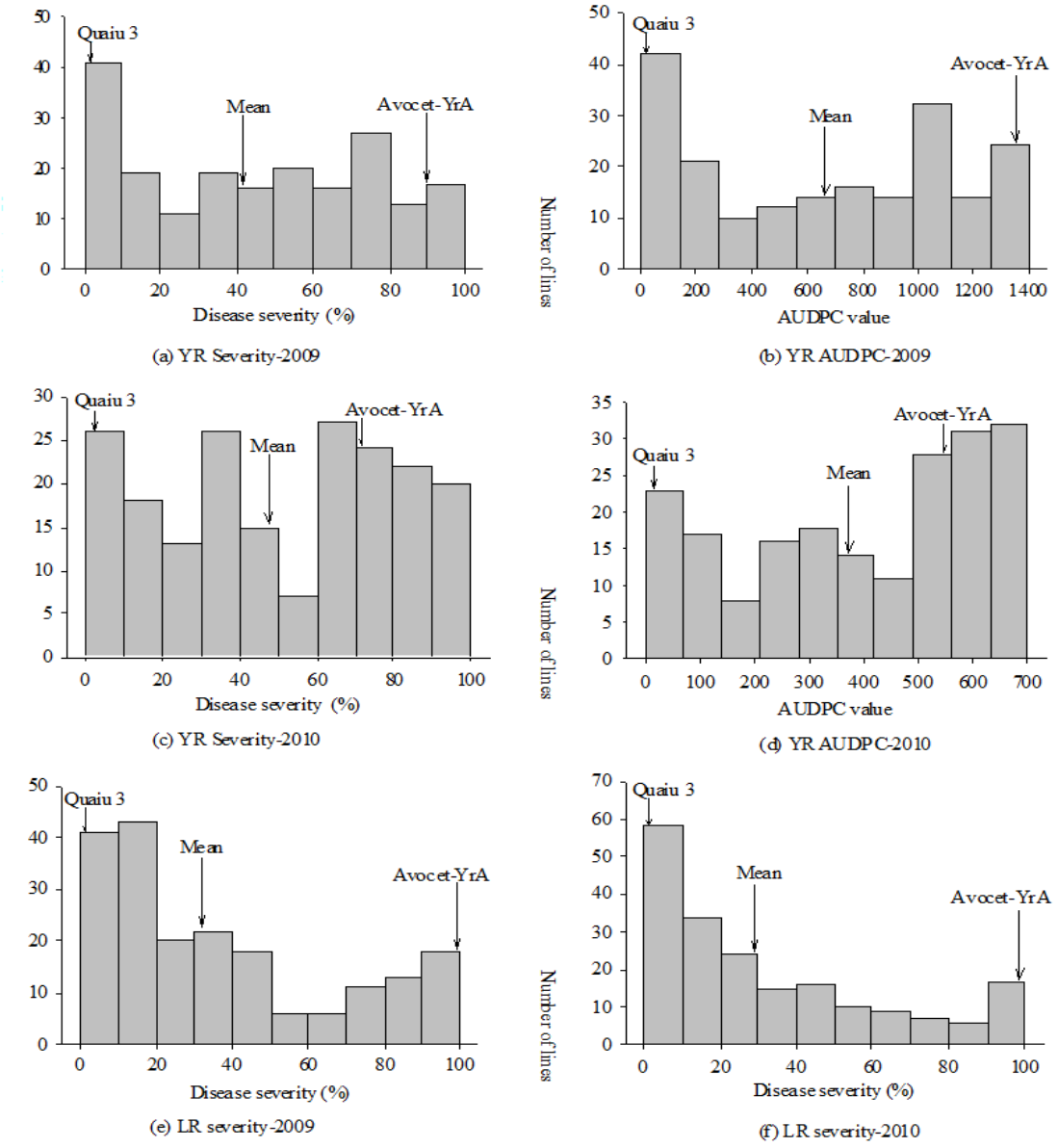

Figure 2.1 Phenotypic distributions of Avocet x Quaiu F_5 RILs for yellow rust severities (a, c) and AUDPC values (b, d), and leaf rust severity (e, f) in 2009 and 2010 field experiments.

The Pearson's correlation coefficients (r) between experiments and diseases were highly significant (Table 2.2). For yellow rust, it ranged from 0.88 to 0.90, whereas for LR it ranged from 0.86 to 0.88 between the two experiments for all types of disease scores measured. The correlation coefficients between YR and LR ranged from 0.21 to 0.38 across experiments and scores. Analysis of variance showed that both the experiments and genotypes had highly significant effects on disease severity scores (Table 2.3). The narrow sense heritability estimates (with a 90% confidence interval) for YR and LR severity scores were 0.95 (0.96, 0.93) and 0.92 (0.94, 0.90), respectively. Similarly, after removing the lines with the *Lr42* gene, the heritability estimate for LR severity scores was 0.91 (0.93, 0.87). These high levels of heritability estimates indicate high stability of resistance and/or less environmental influence on disease severities and reactions of RILs.

Table 2.2 Pair-wise correlation coefficients among yellow rust (YR) and leaf rust (LR) disease scores (first evaluation) of Avocet x Quaiu F_5 RILs for two years.

Exp†	Disease Scores[b]	YR2009				YR2010				LR2009	LR2010
		DS	CI	AUDPC1	AUDPC2	DS	CI	AUDPC1	AUDPC2	DS	DS
YR2009	CI	0.94									
	AUDPC1	0.98	0.90								
	AUDPC2	0.94	0.96	0.94							
YR2010	DS	0.90	0.81	0.91	0.84						
	CI	0.88	0.85	0.88	0.86	0.95					
	AUDPC1	0.90	0.80	0.92	0.82	0.99	0.92				
	AUDPC2	0.89	0.85	0.90	0.87	0.95	0.99	0.94			
LR2009	DS	0.29	0.31	0.28	0.33	0.22	0.24	0.21	0.24		
LR2010	DS	0.32	0.36	0.31	0.38	0.23	0.26	0.22	0.26	0.86	
	AUDPC1	0.32	0.36	0.31	0.37	0.23	0.27	0.23	0.26	0.88	0.99

† Year of Experiments for diseases (YR = yellow rust, or LR = leaf rust) evaluation, [b]DS=Disease severity %, CI= Coefficient of infection, AUDPC1 and 2= Area under disease progress curve scores for DS and CI respectively. Note: All the coefficient of correlation values (r) are significant at P =0.01

Table 2.3 ANOVA and computation of genetic variance and heritability (90% confidence interval) for leaf rust and yellow rust severity scores for Avocet x Quaiu RILs in the first evaluation across two years of experiments

Components	YR		LR		LR-I‡	
	d.f.	Estimate	d.f.	Estimate	d.f.	Estimate
MS Year (E)	1	2465.0**	1	13377.8**	1	11512.8**
MS Lines (G)	197	1731.7**	197	1651.2**	94	1999.4**
MS Error (GXE)	197	89.9	195	127.9	94	183.8
Genetic variance (σ^2_g)	820.9		761.7		907.8	
Heritability (90% CI) †	0.95(0.96, 0.93)		0.92 (0.94, 0.90)		0.91(0.93, 0.87)	

* Significant F-test at p = 0.01
†Variance component based heritability estimates with exact 90% confidence interval
‡ Estimates for RILs which showed seedling susceptible reaction for leaf rust, i.e. lacking *Lr42*.

Gene number estimates

The qualitative approach of gene number estimates suggested that at least 3 to 4 genes segregated in the RIL population for YR in the 2009 experiment (Table 2.4). However, in the 2010 experiment, all the χ^2 tests for 4 gene segregation were found to be significant, indicating that observed phenotypic classes best represent a 3 gene segregation ratio, except for one test which agreed with a 2 gene segregation. The reason for lower gene number estimates in the 2010 experiment may be due to a large number of transgressive segregants which showed higher disease severity than the susceptible parent Avocet resulting in a large number of lines in the HPTS category. On the other hand, in the 2009 experiment, the numbers of lines in resistant and susceptible categories were close to each other resulting in a consistent χ^2 tests for all three response groupings

conforming to a 3 gene segregation ratio. Similarly, a smaller number of HPTR lines in the 2009 experiment agreed with the predicted 4 genes segregation ratio.

Table 2.4 Classification of Avocet x Quaiu F_5 RILs into different phenotypic classes based on disease scores, and χ^2 test for Mendelian segregation for different number of genes.

Disease scores†	No. of RILs§			χ^2- probability value testing segregating ratio for¶					
				2-Genes		3-Genes		4-Genes	
	HPTR	HPTR	Others	*P* value#	Exp ratio	*P* value#	Exp ratio	*P* value#	Exp ratio
Yellow rust evaluation									
DS1_09	12	16	168	…	…	0.51,0.26,0.92	I,II,III	0.07	II
CI1_09	12	14	170	…	…	0.40,0.26,0.54	I,II,III	0.07	II
DS2_09	12	22	162	…	…	0.21,0.26,0.15	I,II,III	0.07	II
CI2_09	12	21	163	…	…	0.29,0.26,0.24	I,II,III	0.07	II
DS3_09	9	24	163	…	…	0.06,0.06	II,III	0.49	II
CI3_09	9	24	163	…	…	0.06,0.06	II,III	0.49	II
AUDPC1_09	8	13	175	…	…	0.06,0.38	I,III	0.08,0.75	I,II
AUDPC2_09	8	12	176	…	…	0.26	III	0.17,0.75,0.07	I,II,III
DS1_10	17	65	114	…	…	0.89	II	…	…
CI1_10	17	22	157	…	…	0.34,0.89,0.15	I,II,III	…	…
DS2_10	14	75	107	…	…	0.54	II	…	…
CI2_10	14	27	155	0.06	III	0.54	II	…	…
AUDPC1_10	14	62	120	…	…	0.54	II	…	…
AUDPC2_10	14	20	162	…	…	0.56,0.54,0.35	I,II,III	…	…
Leaf rust evaluation									
DS_09	10	14	172	…	…	0.19,0.10,0.54	I,II,III	0.28	II
DS_09‡	2	13	82	0.15	III	0.07	III	0.39	II
DS1_10	42	10	142	0.37	II	0.13	III	0.27	III
DS1_10‡	15	9	73	0.36	II	0.75	III	…	…
DS2_10	29	11	154	0.14	II	0.18	III	0.27	III
DS2_10‡	6	10	81	…	…	0.61,0.44,0.49	I,II,III	0.19	II
AUDPC_10	29	10	155	0.14	II	0.11	III	0.27	III
AUDPC_10‡	5	5	87	…	…	0.24,0.25,0.25	I,II,III	0.53,0.43,0.43	I,II,III

†DS1, DS2, DS3= Disease severity scores for 1st, 2nd and 3rd evaluation dates in given growing seasons; CI1, CI2, CI3=Coefficient of infection for 1st, 2nd and 3rd evaluation dates; AUDPC1 and AUDPC2=Area under disease progress curve for DS and CI respectively

‡Disease scores of RILs after excluding the *Lr42* gene

§HPTR=Homozygous parental type resistant (within the boundary of Resistant parent score or less); HPTS= Homozygous parental type susceptible (within the boundary of Susceptible parent score or more); Others= Lines with Intermediate type of disease scores

¶ For Chi-square test, expected segregation ratio (Exp ratio) for:
(1) 2 genes (II) HPTR:(HPTS+Others)=0.1914:0.8086, (III) HTPS:(HPTR+Others)=0.1914:0.8086,
(2) 3 genes (I) HPTR:HPTS:Others=0.0837:0.0837:0.832, (II) HPTR:(HPTS+Others)=0.0837:0.9163,
(III) HPTS:(HPTR+Others)=0.0837:0.9163, and
(3) 4 genes (I) HPTR:HPTS:Others=0.03664:0.03664:0.92672, (II) HPTR:(HPTS+Others)= 0.03664:0.96336,
(III) HPTS:(HPTR+Others)= 0.03664:0.96336

#The chi-square probability values (*P*-value) are provided for the tests which have p> 0.05

For LR, the gene number estimates ranged from 2 to 4 with or without *Lr42* containing lines (Table 2.4). We observed fairly equal numbers of resistant and susceptible type lines for LR severity in 2009, whereas, significantly more resistant lines were observed in the 2010 experiment. The large number of resistant lines, thus, causing lower gene number estimates, may be due to relatively larger effect of *Lr42* and *Lr46* as compared to unknown APR genes in the RILs. However, based on AUDPC scores, an equal number of lines in HPTR and HPTS category was observed in the 2010 experiment with perfect agreement to 3 and 4 gene number estimates. Similarly, the gene number estimates based on a quantitative approach was found to be consistent with qualitative estimates, i.e., 2 to 3 genes are segregating in the population for both LR and YR (Table 2.5).

Table 2.5 Quantitative approach of gene number estimation in Avocet x Quaiu F_5 RIL population using modified Wright's formula

	Estimated number of genes†			
Experiment	Method-I	Method-II	Method-III	Method-IV
YR2009	2.16	2.66	1.95	2.41
YR2010	1.27	2.66	1.14	1.97
LR2009	2.87	2.87	2.43	2.87
LR2010	2.87	2.87	2.43	2.87
LR2009I‡	2.41	2.41	1.99	2.41
LR2010I‡	2.41	2.41	1.99	2.41

† In Method I: genotypic range (GR) is the difference of disease severity between two parents, Method II: the genotypic range is the difference of disease severity between two extreme observations, Method III: the genotypic range is between two parents multiplied by respective heritability estimates, and Method IV: average number of gene number estimates from method I and II. Gene number calculation is based on Wright's formula adjusted for F_5 generation: $(GR)^2/4.57*\sigma^2_G$

‡Leaf rust severity field data for the seedling susceptible lines only

Effect of known genes

Interestingly, it was observed that many seedling susceptible lines were resistant in the adult-plant stage for LR. Gene number estimates on the seedling susceptible lines revealed that at least 2 to 4 genes are present in the population for LR resistance in the adult plant stage (Table 2.4, 2.5). Similarly, Quaiu showed a positive allele for the gene *Lr46/Yr29* (with marker *csLV46G22*) and *Sr2/Yr30* (with marker *gwm533*), but showed negative results for other genes tested (Table 2.1). Furthermore, the marker *csLV46G22* was used to screen the whole population and regressed with disease severity data for both leaf and yellow rust. The marker-phenotype regression showed a significant intercept and slope effect for both YR and LR severities in all experiments (Table 2.6). The slope effect simply represents the additive effect of the *Lr46/Yr29* gene on disease severities. The additive effects of *Lr46/Yr29* on YR severities were relatively small as compared to its effects on LR severities. Similarly, the variance explained by the gene ranged from 8.04 to 8.13% for YR, whereas it ranged from 21.89 to 22.56% for LR. After excluding the lines with the *Lr42* gene, the additive effects of *Lr46/Yr29* on LR severities increased significantly and it explained up to 46.85% of total phenotypic variation in the regression model. The phenotypic distributions of RILs with and without *Lr42* and *Lr46/Yr29* genes are given in Fig 2.2. As expected, the lines with *Lr42* showed a high degree of resistance in the adult-plant stage, ranging from 0 to 40% of disease severity indicating that the gene *Lr42* alone confers only a moderate level of resistance with disease severity reaching to 40%. Further, the presence of additional slow rusting genes in conjunction with *Lr42* imparted near-immunity to LR because lines without

Lr42 showed a wide range of disease severities ranging from 10-100%. After removing the lines that carried *Lr46/Yr29* and *Lr42* genes, the distribution curve shifted more towards the susceptible side. However, we could still observe lines with moderate resistance levels indicating that there were additional slow rusting APR genes in the population.

Similarly, for the YR reaction, the *Lr46/Yr29* gene did not make any significant changes in the distribution pattern of RILs for average severity scores indicating that more unknown APR genes/QTLs are present in the population. Based on the marker-phenotype regression analysis, *Sr2/Yr30* gene also showed significant effect on yellow rust severity (Table 2.6). Average yellow rust severity among the lines with *Sr2/Yr30* alone was found to be significantly different from the lines without *Sr2/Yr30* and *Lr46/Yr29* genes in the 2009 experiment (Figure 2.3). Similarly, the lines with both *Sr2/Yr30* and *Lr46/Yr29* showed significantly lower disease severity than those with *Sr2/Yr30* only. Average yellow rust severity among the lines with *Lr46/Yr29*, which was found to have a significant association with yellow rust severity scores based on marker phenotype regression, was found to be significantly different from the lines without *Sr2/Yr30* and *Lr46/Yr29* in both the experiments. However, RILs with both *Sr2/Yr30* and *Lr46/Yr29* were not found to reduce the disease significantly as compared to the RILs with *Lr46/Yr29* only. These observations indicate that, in addition to *Yr29* and *Yr30*, additional unknown genes should be present in the population

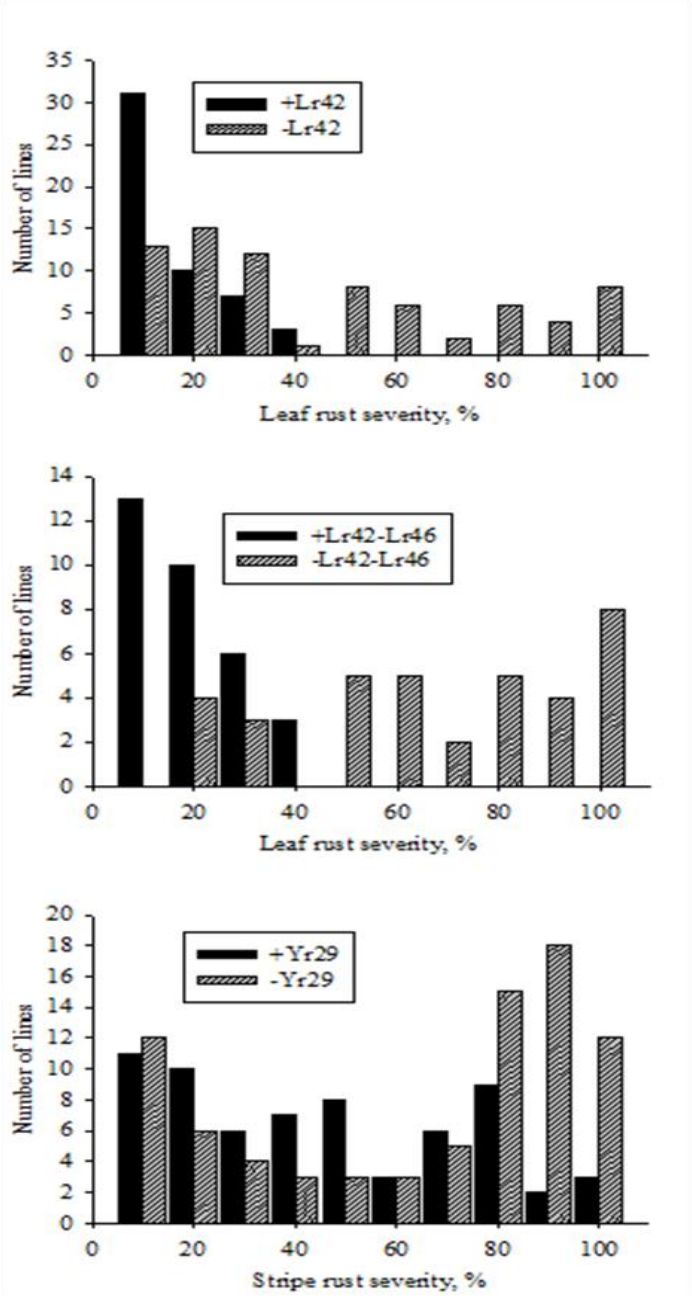

Figure 2.2 Phenotypic distributions of Avocet x Quaiu F_5 RILs for average disease severity across experiments for the recombinant inbred lines with different gene combinations: leaf rust severity with and without *Lr42* gene (top), leaf rust severity with and without *Lr42* genes after excluding *Lr46* containing lines (middle), and yellow rust severity in the lines with and without *Yr29* gene (bottom).

Table 2.6 Marker-phenotype regression for *Lr46/Yr29* and *Sr2/Yr30* on disease severity scores (first evaluation) of Avocet x Quaiu F_5 RILs in different experiments

Gene	Experiment	Intercept		Slope[b]		R^2 (%)[c]
		Estimate (±SE)	pr>t	Estimate (±SE)	pr>t	
Lr46/Yr29	YR2009	50.76±3.4	<.0001	-17.95±5.1	0.0005	8.04
	YR2010	55.16±3.3	<.0001	-17.58±4.9	0.0005	8.13
	LR2009	47.58±3.1	<.0001	-29.02±4.6	<.0001	21.89
	LR2010	35.58±3.0	<.0001	-28.65±4.5	<.0001	22.56
	LR2009[a]	69.31±4.1	<.0001	-40.33±5.6	<.0001	41.17
	LR2010[a]	58.89±4.3	<.0001	-47.32±5.9	<.0001	46.85
Sr2/Yr30	YR2009	53.68±3.9	<.0001	-19.91±5.4	0.0003	9.69
	YR2010	53.98±3.9	<.0001	-11.72±5.5	0.0336	3.56

[a] Recombinant inbred lines excluding *Lr42* gene.
[b] Regression coefficients (equivalent to additive effects of *Lr46/Yr29* gene) on Marker-phenotype regression. The negative sign represents the effects from resistant parent (Quaiu3) allele.
[c] Variation explained by the model (in this case, percent of total phenotypic variance explained by *Lr46/Yr29* and *Sr2/Yr30* genes).

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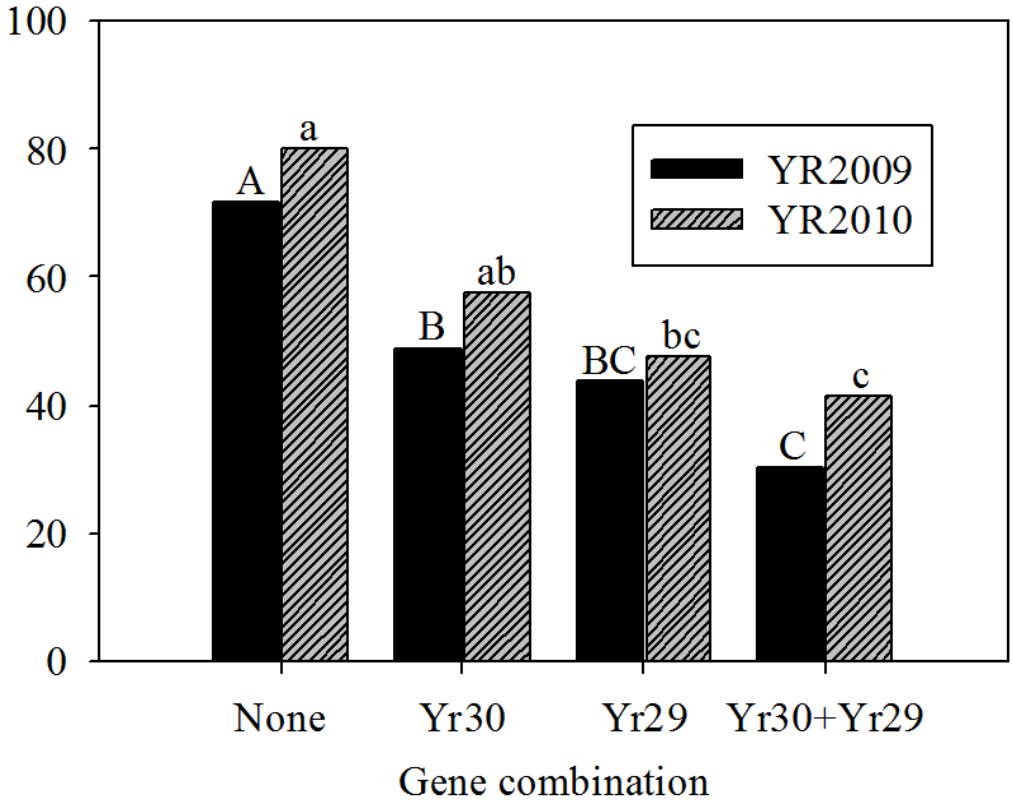

Figure 2.3 Average yellow rust severities of Avocet x Quaiu F5 RILs with *Lr46/Yr29* and *Sr2/Yr30* genes and their interaction. The bars not connected by the same letter are significantly different.

Discussion

The presence of the moderately effective race-specific resistance gene *Lr42* in the resistant Quaiu was confirmed based on the seedling greenhouse reactions of parents and RILs with *P. triticina* race MBJ/SP and co-segregation with the linked molecular marker *gwm432*. The segregation ratio for resistant, susceptible and heterozygous RILs conformed to the single gene segregation ratio. Seedlings of both parents displayed susceptible reactions to the two *P. striiformis* used in our studies indicating Quaiu possessed adult-plant resistance to yellow rust.

RILs were continuously distributed for LR and YR severities in field trials; however, the normality assumption was not met in all experiments for both diseases. Although the lack of the normal distribution may be due to the presence of epistasis or linkage between rust resistance genes (Bjarko and Line, 1988), it is often caused by the unequal and larger effects on disease resistance by one or more segregating resistance genes. Normality cannot simply rule-out the quantitative inheritance in the segregating population, as some evidence has shown continuous variation, rather than discrete classes, without enough evidence of normality for slow rusting resistance (Bjarko and Line, 1988; Kuhn et al., 1980; Lillemo et al., 2006; Navabi et al., 2003; 2004; Singh and Rajaram, 1993; Zhang et al., 2008). Additionally, normal distribution of a trait can be better observed in the presence of a large number of genes, each with relatively smaller effect, within a particular bi-parental RIL population, indicating low amount of variances thus giving lower heritability estimates (Allard, 1960; Skovmand et al., 1978). Transgressive segregation towards higher susceptibility was evident during both years

for yellow rust disease considering the first evaluation severities and coefficient of infection, or the AUDPC values. This indicates that the susceptible parent Avocet also contributed some level of slow rusting resistance in the RIL population. This observation is further supported by a QTL mapping study by William et al. (2006), who has reported two Avocet derived resistance QTL that explained 5.8-13.1 and 2.4-6.1% of total phenotypic variation for YR and LR severities, respectively. Mid-parental (MP) disease severity values were comparable to that of population averages (PA) for yellow rust (MP=45%, PA=41.34% and MP=35.5%, PA=46.52% in the 2009 and 2010 growing seasons, respectively). However, larger differences were observed in case of LR severities (MP=50%, PA=33.6% and MP=50%, PA=22.07% in the 2009 and 2010 growing seasons, respectively). These observations support the additive gene actions for adult-plant resistance to yellow rust. Presence of the moderately effective race-specific resistance gene *Lr42* could have caused lower PA values of RILs for LR in field trials. In our study, the heritability estimates of LR and YR severity scores were very high which are in close agreement with previous studies, especially when associated with low estimates of gene numbers (Bjarko and Line, 1988; Gavinlertvatana and Wilcoxson, 1978; Navabi et al., 2003; 2004; Skovmand et al., 1978). The highly significant correlation coefficients among the experiments suggest that the experimental conditions were well controlled and consistent with little experiment-by-genotype interaction. Similarly, relatively smaller but significant correlations between LR and YR scores suggest the possibility that some slow rusting genes are effective against both rust diseases or were closely linked.

By exploring all possible segregation patterns for different expected numbers of genes, χ^2 tests were carried out for disease severity scores, coefficient of infection and AUDPC values to determine the best fit between observed and expected number of lines with different phenotypic classes. Though one test provided evidence of 2 gene segregation for yellow rust, most of the tests agree with a minimum of 3 to 4 genes segregating in the population. Similarly, for LR, the χ^2 tests supported 2 to 4 genes segregating in the population. The gene number estimates did not differ significantly with or without excluding lines containing *Lr42*. This may be attributed to the relatively larger effect of *Lr42* gene, which violates the assumption of equal effect genes, resulting in underestimation of gene number. Consistent results on gene number estimates were observed based on four different methods of quantitative approach. Thus, we can conclude that this segregating population contains a minimum of 3 to 4 genes for slow rusting resistance to both yellow and LR. Based on the distribution of disease scores among RILs, all LR resistance genes were contributed by the resistant parent; however, it is likely that a small-effect gene from Avocet also contributed to yellow rust resistance. The gene number estimates in this study seem very reasonable based on phenotypic distribution pattern, heritability estimates, and previous studies on gene number estimates for slow rusting resistance in different wheat crosses (Das et al., 2004; Gavinlertvatana and Wilcoxson, 1978; Khanna et al., 2005; Milus and Line, 1986; Navabi et al., 2003; 2004; Singh and Huerta-Espino, 1995; Singh and Rajaram, 1993; Skovmand et al., 1978; Zhang et al., 2001; 2008). Most molecular mapping studies have shown more QTLs/genes present in a segregating population than the corresponding

gene number estimates based on phenotypic segregation ratios and quantitative methods (Li-Jun et al., 2009; Lillemo et al., 2006; 2008; Navabi et al., 2003).

The presence of the pleiotropic slow rusting resistance gene *Lr46/Yr29* in Quaiu contributed a relatively large proportion of phenotypic variation for adult-plant LR severity after excluding RILs carrying *Lr42*. However, a broad range of LR severities could still be observed for RILs that lacked both *Lr42* and *Lr46/Yr29,* indicating that there were additional slow rusting genes conferring resistance to LR at the adult plant stage. On the other hand, *Lr46/Yr29* had a relatively smaller effect on slow rusting resistance in yellow rust as compared to its effect on LR. This observation was consistent with a previous study by Lillemo et al. (2008). The distribution pattern did not differ significantly with or without the exclusion of *Lr46/Yr29*-containing lines. Similarly, the *Sr2/Yr30* gene was observed to have a significant impact on disease reduction. However, based on closely linked markers, it explained a smaller proportion of the variation in the 2010 experiment as compared to 2009 (Table 2.6). All the analyses suggest that at least 2 to 3 APR genes are present in the resistant parent Quaiu for both rusts.

Because visual disease severity scores were highly heritable in RILs for both LR and YR, transferring resistance from Quaiu to other genetic backgrounds should be relatively simple through visual selection in segregating populations grown under high disease pressures. Because virulence to the moderately effective race-specific resistance gene *Lr42*, transferred to hexaploid wheat from *T. tauschii*, is not known in North American *P. triticina* populations, its utilization in conjunction with *Lr46/Yr29* and other

unidentified slow rusting resistance genes is a more attractive strategy for achieving near-immunity to LR. The near-immunity to yellow rust in Quaiu, based on the presence of at least 3 APR genes, further increases its breeding value as a parent for achieving durable rust resistance. Further molecular mapping studies will help to determine the genomic locations of the uncharacterized resistance genes and to realize the goal of marker assisted breeding for durable leaf and yellow rust resistance.

CHAPTER III

QTL MAPPING OF ADULT PLANT RESISTANCE TO YELLOW AND LEAF RUSTS IN AVOCET-YRAXQUAIU 3 POPULATIONS

Introduction

Leaf rust (LR), a.k.a. brown rust, and yellow rust (YR), a.k.a. stripe rust, caused by *Puccinia triticina* and *Puccinia striiformis* f.sp. *tritici*, respectively, are the most common foliar diseases of wheat (*Triticum aestivum* L.). It has been more than a century since scientists started to understand the genetics behind rust resistance in wheat (Boyd, 2005). To this date more than 92 LR and 89 YR resistance genes originating from different *Triticum* and allied species have been designated (McIntosh et al., 2010). Due to the rapid evolution of new rust races defeating the existing major rust resistance genes, in recent decades, wheat scientists are more interested in identification and deployment of novel Adult Plant Resistance (APR) genes in diverse wheat germplasm to prolong the durability of resistance. It is hard to establish a clear relationship between number of APR genes required to achieve a high level of protection in field conditions as each slow rusting gene produces different levels of phenotypic effects based on environmental condition and other genes present in a wheat line (Singh et al., 2011a). However, long term experience of breeding for durable rust resistance, using various sources, has suggested that a combination of four to five APR genes give more stable expression across different environments with near-immune response to the rust disease (Sing et al., 2000a). Though some prominent APR genes, Lr34/Yr18 (Dyck,1987;

McIntosh 1992, Singh 1992b, Spielmeyer et al., 2005, Krattinger et al., 2009), Lr46/Yr18 (Singh et al., 1998; William et al., 2003) and Lr67/Yr46 (Herrera-Foessel et al., 2011; Hiebert et al., 2011) have already been characterized and are being utilized in wheat breeding programs, there is still a need for identification and molecular characterization of more novel sources of APR in order to achieve the goal of durable resistance in a global context.

Quaiu 3, a high yielding spring wheat line developed by the International Maize and Wheat Improvement Center (CIMMYT), provides high level of protection against LR and YR, and moderate level of protection against stem rust (SR, caused by *P. graminis*) race *Ug99* (Singh et al., 2011b) under natural field conditions. Apparently, Quaiu 3 does not seem to carry any major genes for YR resistance as it gives very high infection type readings (>6 in 0-9 scale) in seedling screenings. However, it possesses a moderately effective race-specific LR resistance gene *Lr42*, which was confirmed by seedling test and using diagnostic SSR marker *Xwmc432* (Sun et al., 2010). In addition to the *Lr42* gene, some APR genes are expected to be present in Quaiu 3 as many seedling susceptible lines showed a high level of resistance reaction in the field (Basnet et al., 2012, Unpublished data). Combination of high yield potential and prominent rust resistance genes *Sr2*, *SrTmp*, *Lr42* and at least three unknown slow rusting genes for LR and YR has made Quaiu3 a very promising CIMMYT line which can be released as a cultivar or used as a source of a number of valuable genes in crossing blocks. Thus, molecular mapping of APR in Quaiu 3 helps to identify molecular makers linked to corresponding putative QTL, which can easily be transferred to other germplasm to

enhance durable resistance via marker-assisted selection (MAS) or gene pyramiding approaches in the future. The objective of this study was to construct an integrated genetic linkage map with DArT and SSR markers, and identify the association of those markers with LR and YR disease severity scores in Avocet-YrA/Quaiu 3 recombinant inbred lines (RILs).

Materials and Methods

Plant materials

A set of 180 randomly advanced F_5 RILs derived from the cross Avocet-YrA/Quaiu 3 was used in this study. Quaiu 3 (pedigree: 'Babax'/*Lr42*//'Babax'*2/3/'Vivitsi') is a high yielding CIMMYT line that has shown high levels of adult-plant resistance to leaf and YR in field conditions based on international data. The source parent for resistance gene *Lr42* was a winter wheat germplasm line developed by the UDSA-ARS Germplasm Program at Kansas State University and was kindly provided by Stan Cox. Unfortunately, the exact pedigree of this line is unknown. The susceptible parent Avocet-*YrA* is a reselection from the original heterogeneous Australian cultivar that lacks the race-specific resistance gene *YrA* and is also known as 'Avocet S'. For simplicity, the reselection will be designated as Avocet throughout the paper and Quaiu 3 as Quaiu. To develop the population, a single spike from each F_2 plant, generated from three different F_1 plants, were randomly harvested under fungicide application and advanced to F_5 generation by harvesting and

growing a single spike in each subsequent generation. The F_5 plots, derived from single F_4 spikes, were then harvested as bulk to obtain sufficient seed of the F_5 RILs.

Field experiments and disease evaluation

Field experiments were carried out in Mexico during the 2008-2009 and 2009-2010 crop seasons for LR, and 2009 and 2010 crop seasons for YR evaluation at CIMMYT research stations near Ciudad Obregon, recently named Norman E. Borlaug Experimental Station, CENEB, and Toluca, respectively. Toluca (State of Mexico) research station is located in the highlands of central Mexico (18°N, 2640 meters above sea level), whereas CENEB is located in the state of Sonora in northwestern Mexico (28° N, 39 meters above sea level). The environment in Toluca, which has average monthly temperatures of 19 to 23°C and average monthly precipitation of 65 to 145 mm during the growing season, is very conducive to high YR development. Similarly, the CENEB is considered to be a highly suitable place for LR development during the crop season with average monthly maximum temperatures of 24 to 31°C and average monthly precipitation of 15 to 22 millimeters. In Toluca, planting begins in mid May and the season ends in early October, whereas in CENEB, planting begins in late November and the season ends in early April.

About 4-5 g seeds (expected 60-70 mature plants) of the parents and 198 RILs were hand sown in 1-m paired-rows, spaced 10 cm apart, on top of 80 cm wide raised beds. The CENEB experiments were planted in the third week of November during the 2008-2009 season and in the first week of December during 2009-2010. To create

homogenous rust epidemics, spreader rows of the highly susceptible cultivar 'Morocco' were planted around the experimental area and at one side of each plot in the middle of the 0.5m-wide pathways. Artificial inoculations were carried out twice, about 8 weeks after sowing, with an equal mixture of prevalent Mexican *P. triticina* races: MBJ/SP and MCJ/SP using the method described by Herrera-Foessel et al. (2011). Similarly, the Toluca experiments were planted in the fourth week of May during both growing seasons. In Toluca, the spreaders consisted of a mixture of six susceptible wheat lines that possessed the defeated race-specific resistance gene *Yr27* and were derived from the cross Avocet × 'Attila'. The varying maturities of these lines ensure a continuous production of inoculum during the critical crop-growing period. An artificial epidemic was created by inoculating the spreaders with Mexican isolates MEX96.11 and MEX08.13 of *P. striiformis* f. sp. *tritici* at least three times four to five weeks after planting. The avirulence/virulence characteristics of the races used in our studies were previously described by Herrera-Foessel et al. (2011).

Disease severity scores were recorded following the 0-100% visual ratings based on the modified Cobb's Scale (Peterson et al., 1948). For both LR and YR, the first disease severity readings were taken when the susceptible parent, Avocet, showed at least 70% disease severity followed by second and/or third readings at weekly intervals. Similarly, the host reaction/infection type data were recorded based on the visual criteria (Irfaq et al., 2009; Roelfs et al., 1992; Singh and Rajaram, 1993) with some modification on Scale, where R= resistant (necrotic tissue or yellow stripes with or without tiny uredinia), R-MR=resistant to moderately resistant (necrotic tissues or stripes with few

small uredinia), MR= moderately resistant (necrotic or chlorotic tissues or stripes with smaller to medium sized uredinia), M (or MRMS)= moderately resistant to moderately susceptible (necrotic or chlorotic tissues or stripes with medium sized uredinia and intermediate sporulation), MS= moderately susceptible (medium sized uredinia or stripes without chlorosis and necrosis and abundant sporulation), MS-S= moderately susceptible to susceptible (medium to large sized uredinia or stripes without chlorosis and necrosis and abundant sporulation), and S= susceptible (large sized uredinia or stripes without chlorosis and necrosis and abundant sporulation). These reaction type readings were then converted to numeric response value as R=0.2, R-MR=0.3, MR=0.4, M=0.6, MS=0.8, MS-S=0.9, and S=1.0. Coefficients of infection (CI) values for each line were calculated by using CIMMYT's adopted method, i.e., by multiplying disease severity scores with reaction type values. Furthermore, for the repeated measurements, the Area Under Disease Progress Curve (AUDPC) values for disease severity and CI were calculated by using the following equation (Bjarko and Line, 1988):

$$\text{AUDPC}=\sum_{i=1}^{n-1}\left(\frac{x_i + x_{i+1}}{2}\right)t_i$$

where x_i=the rust severity scores on date i; t_i=time intervals in days between date i and date i+1; n=number of readings.

Statistical analysis

All the statistical analyses including phenotypic distribution, correlation coefficient, Analysis of Variance (ANOVA), and marker-phenotype regression were performed using SAS 9.2 (SAS Institute, Cary, NC). Analysis of Variance was carried

out to determine the differences in disease severity scores among 182 recombinant inbred lines based on first evaluation of LR and YR. Similarly, the variation and mean differences of disease scores among different QTL genotypes was analyzed using one-way ANOVA and t-test comparisons in SAS9.2.

Molecular marker analysis and genetic linkage map construction

DNA from the two parental lines, Avocet and Quaiu, was used to screen for 450 SSR markers (Rodder et al., 1998; Pestsova et al., Sourdille et al., 2001; Song et al., 2002; Somer et al., 2004) as a polymorphism survey. The identified polymorphic SSR markers were used to genotype the whole mapping population of 182 recombinant inbred lines. Following polymerase chain reaction (PCR), high resolution allele separation was performed using Applied Biosystems (Foster City, CA) 3130xl genetic analyzer followed by allele size determination with GeneMapper v3.7 software (Applied Biosystems). Similarly, from parents and RILs 30ul of DNA samples (concentration of 50ng/µl) were sent to Triticarte Pyt. Ltd., Yarrallumla, Australia for Diversity Arrays Technology (DArT) genotyping. An assay of Wheat PstI(TaqI) v2.3 was used to screen for polymorphic DArT marker along the genome in whole mapping population (Jaccoud et al., 2001; Akbari et al., 2006). Combining both marker types, genetic map construction was performed using QTL ICiMapping software, abbreviated as ICIM, (Li et al., 2008). A minimum LOD score of 3.5 and maximum recombination frequency of 0.40 was set to make the linkage group, where Kosambi mapping function (Kosambi, 1944) was used to convert the recombination frequency into map distance. To finalize

the linkage map, marker ordering and rippling were performed by using RECORD and COUNT algorithms available in the ICIM software.

QTL analysis of yellow rust and leaf rust resistance

QTL analysis of disease severity and AUDPC values for both YR and LR were performed using QTL ICiMapping (Li et al., 2008) and WinQTLCart v2.5 (Wang et al., 2011) mapping softwares. Inclusive Composite Interval Mapping (ICIM) program with probability of stepwise regression 0.001 was employed in QTL ICiMapping; whereas, composite interval mapping with backward and forward regression was used in WinQTLCart. In both cases, LOD threshold was calculated by 1000 permutation tests. All the lines were grouped into different QTL genotype classes based on closest significant markers for all the significant QTLs, and their mean performance and variances were analyzed using one-way ANOVA and pair-wise t-tests.

Results

Disease evaluation

Substantial and uniform YR development occurred in the trials at Toluca during both seasons and the susceptible parent Avocet displayed 70 to 90% severity at the flowering stage when the first evaluation was made. Average YR severities of RILs ranged from 41.3 to 62.3% in the two experiments at different evaluation times. The distribution of RILs for YR severity, CI and AUDPC was continuous but not normal across experiments. Relatively higher disease pressure was observed during the 2010

season resulting in a higher average disease severity of the RILs compared to 2009. Similarly, LR development was also substantial and uniform during both seasons at Cd. Obregon and the susceptible parent displayed 100% severity in both experiments during the early grain filling stage. Average LR severities of RILs were lower than those of YR and ranged from 22.1 to 33.6% across two experiments and two stages of evaluation. The distribution of RILs for LR severity scores was significantly skewed towards resistance. The Pearson's correlation coefficients (r) among experiments and diseases were highly significant. These coefficients ranged from 0.88 to 0.90 for YR and 0.86 to 0.88 for LR across the two experiments and evaluation times. The correlation coefficients between YR and LR ranged from 0.21 to 0.38 across experiments and evaluation times. Analysis of variance showed that both the experiments and genotypes had highly significant effects on disease severity scores. The narrow sense heritability estimates (with a 90% confidence interval) for YR and LR severity scores were 0.95 (0.96, 0.93) and 0.92 (0.94, 0.90), respectively. Similarly, after removing the lines with the *Lr42* gene, the heritability estimate for LR severity scores was 0.91 (0.93, 0.87). These high levels of heritability estimates indicate high stability of resistance and/or less environmental influence on disease severities and reactions of the RILs.

Genetic linkage mapping and QTL analyses

Out of 650 DArT and 130 SSR polymorphic markers, a total of 495 markers (389 DArT and 106 SSR) were placed in the final linkage map. Markers with high level of redundancy, segregation distortion and missing values were deleted. A total of 22

linkage groups were developed representing all 21 chromosomes of hexaploid wheat (Figure 3.1). The total genetic distance covered by all linkage groups was 2763cM with an average distance of 5.6cM per marker. The largest and smallest chromosomes in terms of map distance were 1B and 4D with corresponding map distances of 213 and 26cM respectively. Similarly, chromosomes 2D and 6B linkage groups had densely covered 40 markers within a total distance of 143 and 127cM respectively.

For both YR and LR disease scores, QTL analyses were performed using this final linkage map information. In all QTL analyses, the results given by both mapping software i.e., QTL ICiMapping and WinQTLCart, were very consistent. For consistency throughout this manuscript, all the QTL effects presented in this document were obtained from composite interval mapping of WinQTLCart program. The LOD threshold value obtained from 1000 permutation tests ranged from 2.9 to 3.8 for different traits. As some minor QTL, mostly derived from the susceptible parent Avocet, were detected below the LOD threshold level, they were reported and discussed separately.

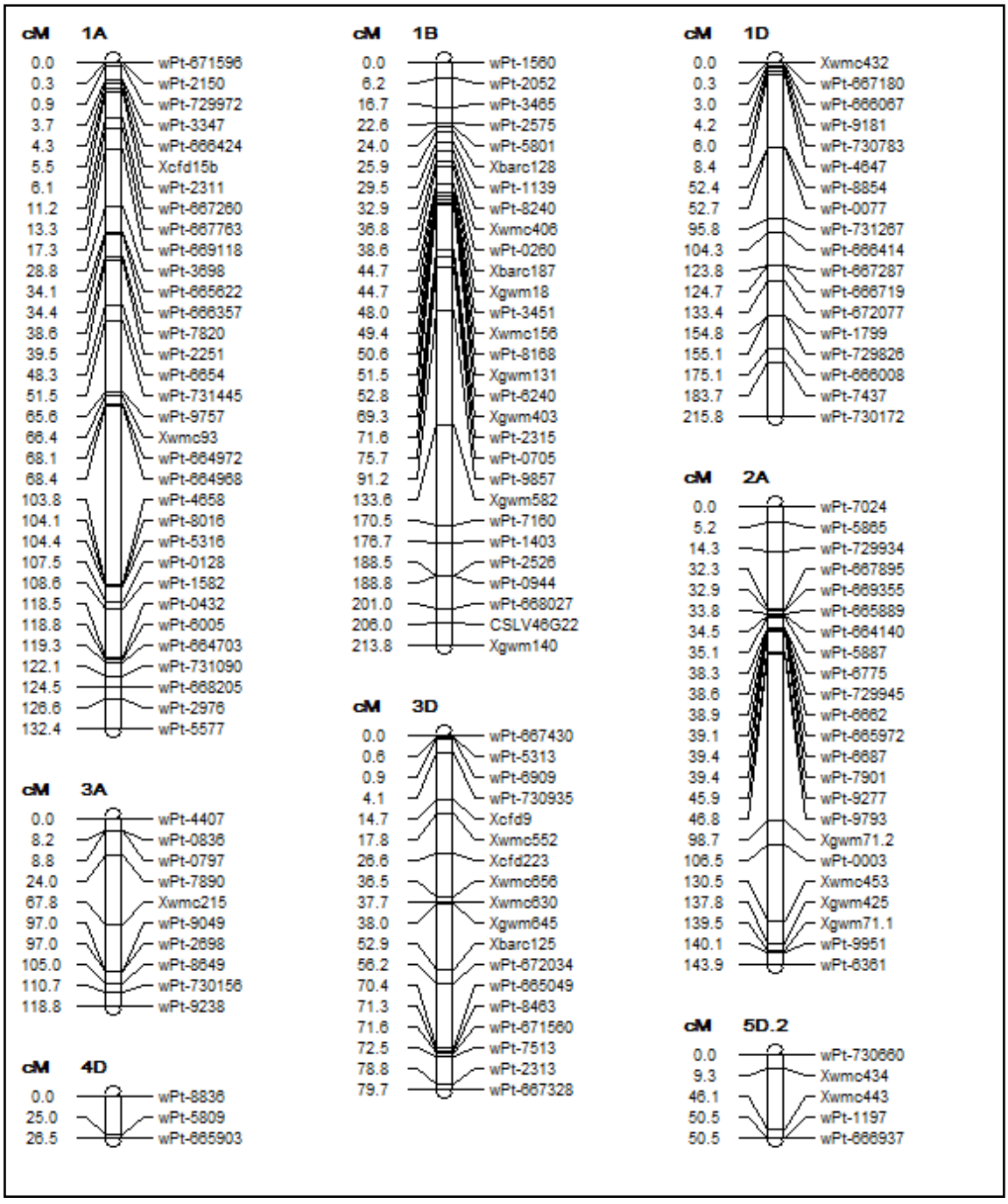

Figure 3.1 Genetic linkage map of DArT and SSR markers in Avocet x Quaiu recombinant inbred lines.

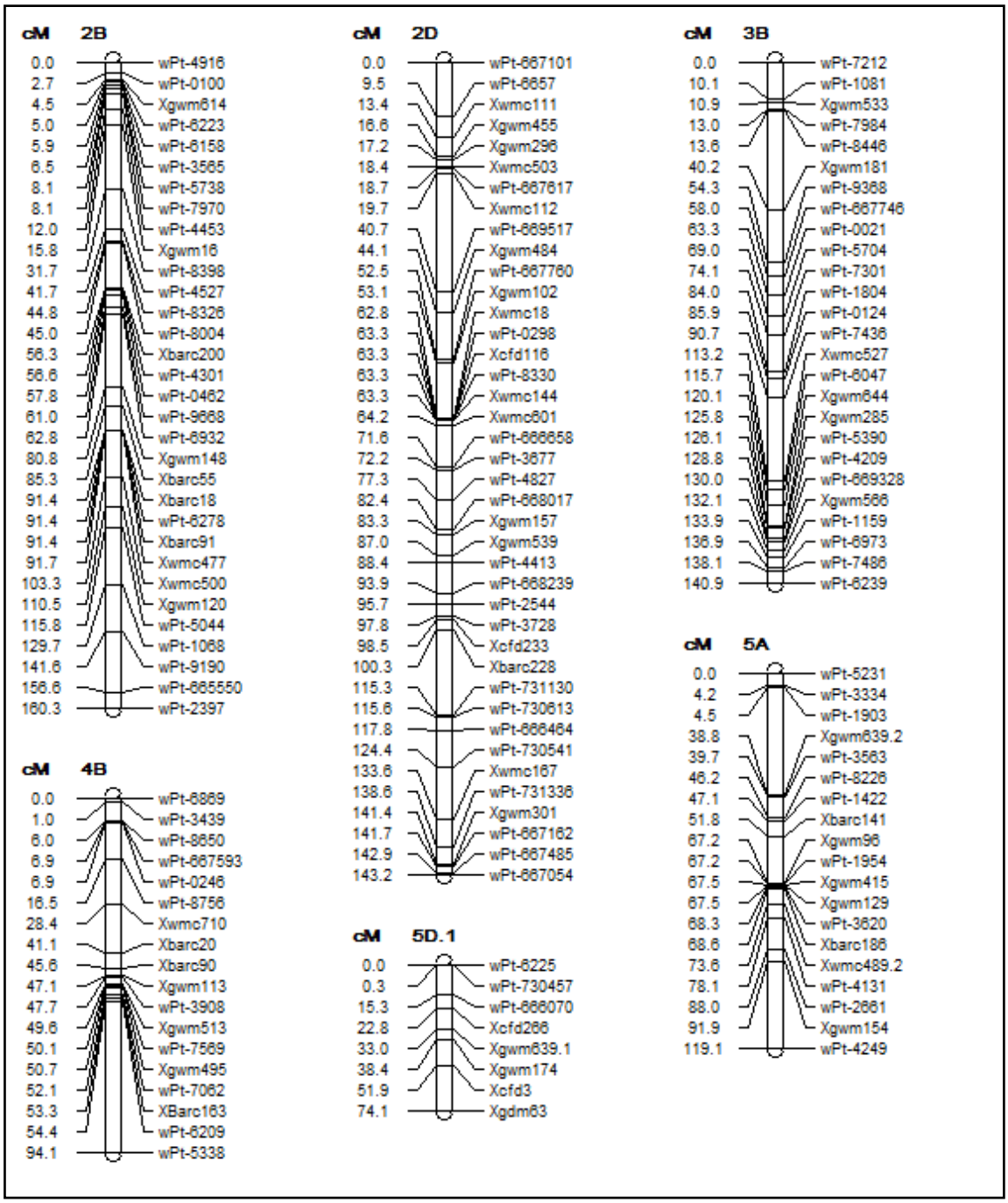

Figure3.1 Continued.

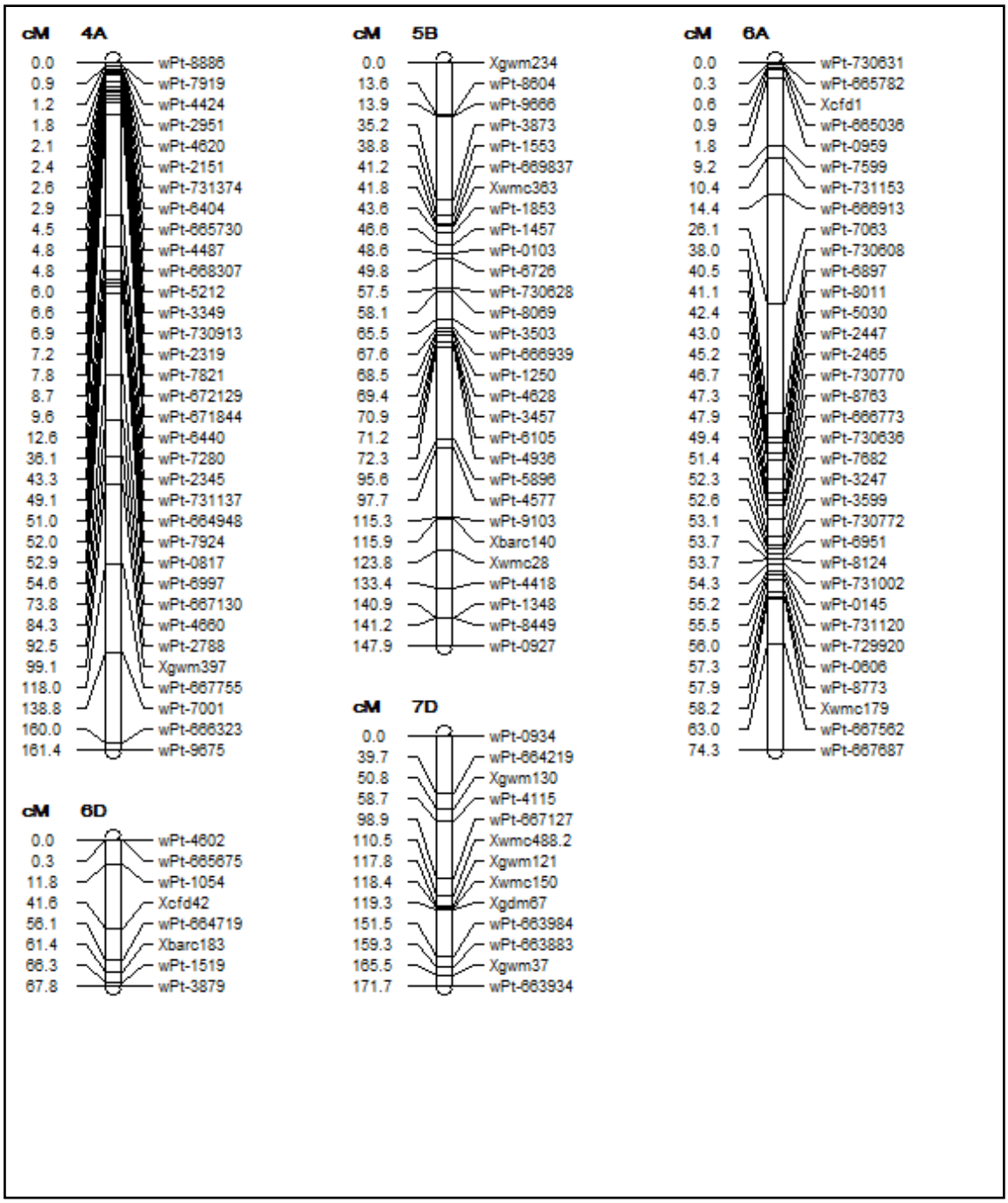

Figure3.1 Continued.

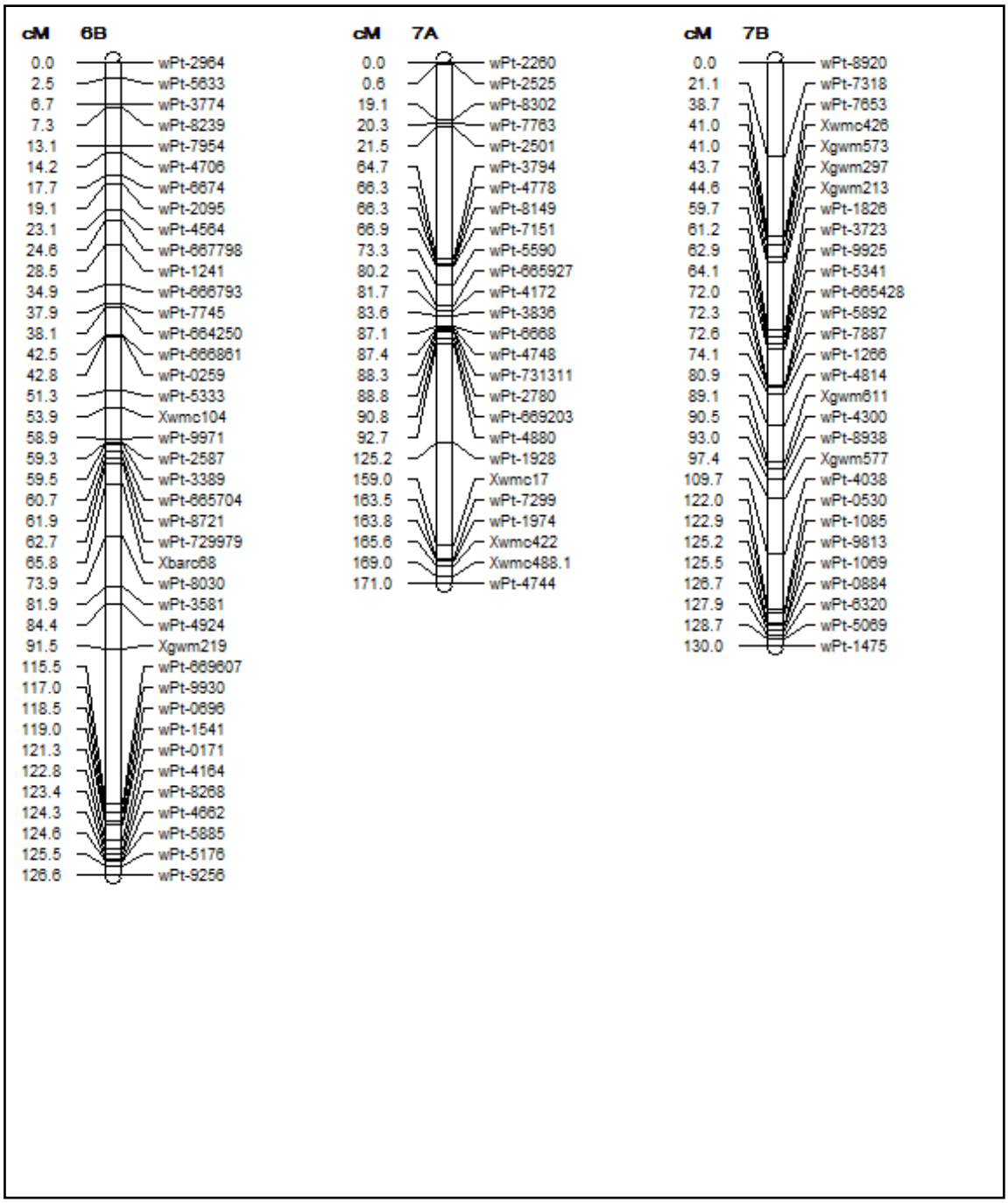

Figure3.1 Continued.

Using composite interval mapping, four major QTL were identified (hereafter, QTL term is used as synonymous to significant homozygous loci contributing disease resistance) for YR resistance as measured by severity and AUDPC values, in both 2009 and 2010 (Table 3.1). The first QTL (*QYr.tam-1B*), which explained 5.7 to 7.2% of the total phenotypic variation, was detected on the 1BL chromosomal location flanked by the markers *wpt-668027* and *cSLV46G22*. Similarly, another QTL (*QYr.tam-3B*) identified on the 3BS chromosomal region was flanked by the markers *Xgwm533* and *wpt-7984*. This QTL explained 2.5 to 9.9% of total phenotypic variation. The third QTL (*QYr.tam-3D*), which explained 3.2 to 4.3% of the total phenotypic variation, was found on the 3DL chromosomal location, flanked by the *Xbarc125/wpt-665049* and *wpt-672034* markers. Similarly, a QTL on the 2DL chromosomal region (*QYr.tam-2D*) was found to have the largest effect on the total phenotypic variation. It was detected near the distal end of chromosome 2D within 1cM distance from marker *Xgwm301*, and was flanked by markers *wpt-667162* and *wpt-667485*. This QTL explained 49 to 61% of total phenotypic variation. The LOD score graphs for all QTLs associated with YR resistance are presented in Figure 3.2.

Similarly, we found three major QTL for LR resistance as measured by severity and AUDPC values, for both 2009 and 2010 experiments (Table 3.1). The first QTL (*QLr.tam-1B*), which explained about 31 to 35% of total phenotypic variation, was detected on the 1BL chromosomal region, flanked by the markers *wpt-668027* and *cSLV44G22*. The second QTL (*QLr.tam-1D*) was detected on the 1DS chromosomal region within 1cM distance from the marker *Xwmc432*, and was flanked by the markers

wpt-667180 and *wpt-666067*. This QTL explained 29 to 39% of total phenotypic variation across experiments. The third QTL (*QLr.tam-3D*) for LR resistance was detected on the 3D chromosome and explained 3 to 6% of total phenotypic variation. This QTL was flanked by the markers *Xbar125* and *wpt-672034*. Interestingly, we found an overlap between two YR and LR resistance QTLs, *QYr.tam-3D* and *QLr.tam-3D* on chromosome 3D. Though the exact location of the QTL associated with both LR and YR resistance varied, all of them resided within 53 to 66cM distances in the linkage map, and were flanked by two of the three neighboring markers *Xbar125*, *wpt-672034* and *wPt-665049*.

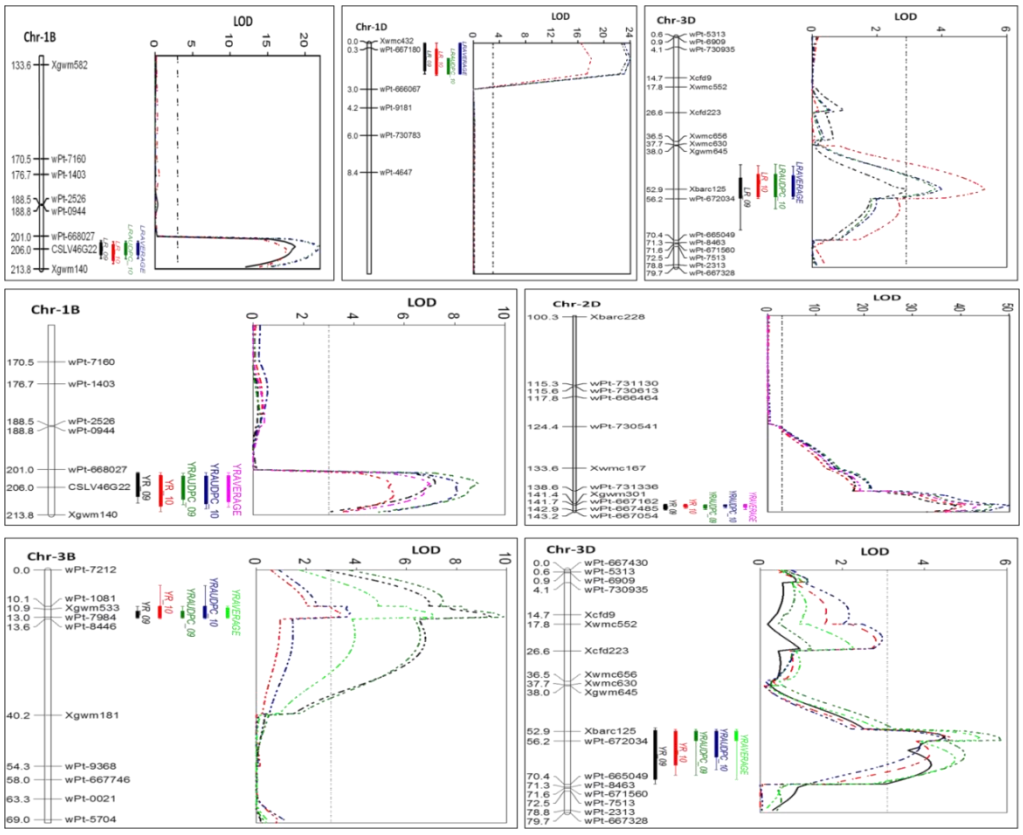

Figure 3.2 LOD profile of significant Quantitative Trait Loci associated with Leaf rust and yellow rust disease severity scores measured in an Avocet x Quaiu population.

Table 3.1 Characteristics of Quantitative Trait Loci detected with Composite interval mapping for leaf rust and yellow rust severity scores and their AUDPC values in Obregon 2009/2010 and Toluca 2009/2010 experiments respectively.

Trait†	QTL‡	Chr§	Position¶	L-Marker#	R-Marker#	LOD††	PVE(%)‡‡	Parent§§
YR_09	*QYr.tam-1B*	1BL	205	wPt-668027	CSLV46G22	7.2	6.1	Quaiu
YR_09	*QYr.tam-2D*	2DL	142	wPt-667162	wPt-667485	39.0	48.9	Quaiu
YR_09	*QYr.tam-3B*	3BS	13	Xgwm533	wPt-7984	12.1	9.9	Quaiu
YR_09	*QYr.tam-3D*	3D	66	wPt-672034	wPt-665049	4.5	4.3	Quaiu
YRAUDPC_09	*QYr.tam-1B*	1BL	205	wPt-668027	CSLV46G22	8.9	7.2	Quaiu
YRAUDPC_09	*QYr.tam-2D*	2DL	142	wPt-667162	wPt-667485	46.7	54.1	Quaiu
YRAUDPC_09	*QYr.tam-3B*	3BS	13	Xgwm533	wPt-7984	10.8	7.8	Quaiu
YRAUDPC_09	*QYr.tam-3D*	3D	56	Xbarc125	wPt-672034	4.8	3.2	Quaiu
YR_10	*QYr.tam-1B*	1BL	208	CSLV46G22	Xgwm140	5.6	6.1	Quaiu
YR_10	*QYr.tam-2D*	2DL	142	wPt-667162	wPt-667485	40.9	54.0	Quaiu
YR_10	*QYr.tam-3B*	3BS	12	Xgwm533	wPt-7984	3.4	3.1	Quaiu
YR_10	*QYr.tam-3D*	3D	56	Xbarc125	wPt-672034	4.4	3.7	Quaiu
YRAUDPC_10	*QYr.tam-1B*	1BL	205	wPt-668027	CSLV46G22	8.1	5.7	Quaiu
YRAUDPC_10	*QYr.tam-2D*	2DL	142	wPt-667162	wPt-667485	50.1	61.4	Quaiu
YRAUDPC_10	*QYr.tam-3B*	3BS	13	Xgwm533	wPt-7984	4.4	2.5	Quaiu
YRAUDPC_10	*QYr.tam-3D*	3D	56	Xbarc125	wPt-672034	6.1	3.6	Quaiu
YRAVERAGE	*QYr.tam-1B*	1BL	205	wPt-668027	CSLV46G22	7.0	6.3	Quaiu
YRAVERAGE	*QYr.tam-2D*	2DL	142	wPt-667162	wPt-667485	43.8	53.8	Quaiu
YRAVERAGE	*QYr.tam-3B*	3BS	13	Xgwm533	wPt-7984	7.3	5.8	Quaiu
YRAVERAGE	*QYr.tam-3D*	3D	55	Xbarc125	wPt-672034	4.4	3.5	Quaiu
LR_09	*QLr.tam-1B*	1BL	205	wPt-668027	CSLV46G22	18.7	31.8	Quaiu
LR_09	*QLr.tam-1D*	1DS	1	wPt-667180	wPt-666067	23.6	38.5	Quaiu
LR_09	*QLr.tam-3D*	3D	53	Xbarc125	wPt-672034	3.0	3.1	Quaiu
LR_10	*QLr.tam-1B*	1BL	206	wPt-668027	CSLV46G22	17.5	30.7	Quaiu
LR_10	*QLr.tam-1D*	1DS	1	wPt-667180	wPt-666067	18.1	28.8	Quaiu
LR_10	*QLr.tam-3D*	3D	53	Xbarc125	wPt-672034	4.4	6.1	Quaiu
LRAUDPC_10	*QLr.tam-1B*	1BL	205	wPt-668027	CSLV46G22	21.7	34.8	Quaiu
LRAUDPC_10	*QLr.tam-1D*	1DS	1	wPt-667180	wPt-666067	24.1	36.7	Quaiu
LRAUDPC_10	*QLr.tam-3D*	3D	53	Xbarc125	wPt-672034	3.1	3.7	Quaiu
LRAVERAGE	*QLr.tam-1B*	1BL	205	wPt-668027	CSLV46G22	22.0	35.2	Quaiu
LRAVERAGE	*QLr.tam-1D*	1DS	1	wPt-667180	wPt-666067	24.0	36.3	Quaiu
LRAVERAGE	*QLr.tam-3D*	3D	53	Xbarc125	wPt-672034	3.3	3.9	Quaiu

† Yellow rust (YR) and Leaf rust (LR) disease severity and AUDPC scores for 2009 and 2010 experiments
‡ Temporary designation of disease resistance QTL,
§ Chromosome where QTL are residing,
¶ Map position of QTL in cM from the first marker.
# Left and Right flanking markers of QTL
†† Logarithm of odd (LOD) score of QTL peak
‡‡ Percent of total phenotypic variation explained by the QTL (R^2)
§§ Source parent contributing resistance allele of QTL

Table 3.2 Characteristics of minor effect Quantitative Trait Loci detected with Composite interval mapping for leaf rust and yellow rust severity scores and their AUDPC values in Toluca 2009 and 2010 experiments respectively.

Trait†	QTL‡	Chr§	Position¶	L-Marker#	R-Marker#	LOD††	PVE(%)‡‡	Parent§§
YR_09	*QYr.tam-5A*	5A	5	wPt-1903	Xgwm639.2	3.5	2.88	Quaiu
YR_10	*QYr.tam-1A*	1A	0	wPt-671596	wPt-2150	2.6	1.62	Avocet-YrA
YR_10	*QYr.tam-3D.1*	3D	24	Xfcd552	Xcfd223	2.8	2.20	Avocet-YrA
YR_10	*QYr.tam-4A*	4A	3	wPt-6404	wPt-665730	2.8	1.80	Avocet-YrA
YR_10	*QYr.tam-6A*	6A	49	wPt-666773	wPt-730636	3.0	1.96	Avocet-YrA
YRAUDPC_10	*QYr.tam-1A*	1A	0	wPt-671596	wPt-2150	2.6	1.35	Avocet-YrA
YRAUDPC_10	*QYr.tam-3D.1*	3D	23	Xfcd552	Xcfd223	3.0	1.86	Avocet-YrA
YRAUDPC_10	*QYr.tam-6A*	6A	49	wPt-666773	wPt-730636	2.8	1.52	Avocet-YrA
LR_10	*QLr.tam-5A*	5A	51	wPt-1422	Xbarc141	2.6	3.75	Avocet-YrA
LRAUDPC_10	*QLr.tam-5A*	5A	51	wPt-1422	Xbarc141	2.5	2.74	Avocet-YrA
LRAVERAGE	*QLr.tam-5A*	5A	51	wPt-1422	Xbarc141	2.5	2.68	Avocet-YrA

† Yellow rust (YR) and Leaf rust (LR) disease severity and AUDPC scores for 2009 and 2010 experiments
‡ Temporary designation of disease resistance QTL,
§ Chromosome where QTL are residing,
¶ Map position of QTL in cM from the first marker.
# Left and Right flanking markers of QTL
†† Logarithm of odd (LOD) score of QTL peak
‡‡ Percent of total phenotypic variation explained by the QTL (R^2)
§§ Source parent contributing resistance allele of QTL

In order to identify minor effect QTL, we performed composite interval mapping analyses by setting the LOD threshold to 2.5. Many minor effect QTLs were detected with LOD values ranging from 2.5 to 3.5, which were, in most cases, below the LOD threshold obtained by 1000 permutation tests. Most of the minor effect QTL were detected in early-stage disease scoring, and were inconsistent across experiments. For YR resistance, 5 minor QTLs were detected (Table 3.2). Among them, only the QTL on chromosome 5A (*QYr.tam-5A*, R^2=2.9) was contributed by the parent Quaiu, and was detected in 2009 experiment only. Other QTLs on chromosomes 1A (*QYr.tam-1A*), 3D (*QYr.tam-3D.1*) and 6A (*QYr.tam-6A*) with R^2 ranging from 1.5-2.2%, were detected in both experiments where the resistant alleles were contributed by the susceptible parent

Avocet. Similarly, one minor QTL (*QYr.tam-4A*) derived from Avocet was detected on chromosome 4A (R^2=1.8%) in the 2010 experiment. Similarly, for LR resistance, a minor QTL on 5A (*QLr.tam-5A* with R^2=2.7 to 3.8%) was detected in both experiments. The resistant allele of this 5A QTL was also derived from Avocet.

Mapping for additive by additive epistatic interaction

For mapping additive by additive epistatic interaction among different chromosomal loci, we used ICI-EPI program of QTL ICiMapping and 2D-genome scanning of QTLNetwork2.0 (Yang et al., 2007). Epistasis mapping program of ICIM is considered to be efficient and powerful to detect epistatic QTL networks along the genome irrespective of significant additive main effect of QTLs (Li and Wang, 2008). Similarly, QTLNetworks2.0 uses a mixed model approach with a very strong algorithm for 2D genome scan for different types and levels of interaction among chromosomal loci (Yang et al., 2008). For YR disease severity and AUDPC scores, small epistatic effects were detected between different loci across genome with LOD score of less than 4. Most of the epistatic interactions were found between significant QTL on chromosomes 1B and 2D, which explained up-to 2% of total phenotypic variation (Figure 3.4). However, large effect epistatic interactions were evident on LR severity scores among different loci across genome (Table 3.3, Figure 3.3). Two significant QTL on chromosome 1B and 1D showed a high level of additive by additive epistatic interaction which explained 9 to 21% of total phenotypic variation (Figure 3.4).

Table 3.3 Additive by additive epistatic interaction between different marker loci across the genome for leaf rust severity and AUDPC scores in Obregon 2009 and 2010 experiments.

Trait†	CHR1‡	POS1§	Marker1¶	CHR2‡	POS2§	Marker2¶	LOD#	PVE(%)††	AXA‡‡
LR_09	1D	0	Xwmc432	1D	30	wPt-4647	4.13	10.57	8.82
LR_09	1B	210	CSLV46G22	1D	0	Xwmc432	9.37	21.27	10.94
LR_10	1D	0	Xwmc432	7D	75	wPt-4115	4.07	8.76	6.74
LR_10	3A	30	wPt-7890	5A	5	wPt-1903	4.84	8.83	-8.04
LR_10	1B	210	CSLV46G22	1D	70	wPt-0077	4.94	11.39	-6.39
LR_10	1D	0	Xwmc432	7D	135	Xgdm67	5.08	11.76	-8.60
LR_10	1B	210	CSLV46G22	6D	30	wPt-1054	5.74	9.47	7.84
LR_10	1B	210	CSLV46G22	1D	0	Xwmc432	15.98	20.01	12.67
LRAUDPC_10	3B	15	wPt-8446	6D	65	Xbarc183	4.45	4.57	41.62
LRAUDPC_10	2D	100	Xcfd233	5B	125	Xwmc28	5.34	4.33	41.37
LRAUDPC_10	1B	210	CSLV46G22	1D	0	Xwmc432	13.08	15.71	72.72

† Leaf rust (LR) disease severity and AUDPC scores for 2009 and 2010 experiments
‡ Chromosomes with significant epistatic interaction,
§ Map positions (cM) of significant epistatic interaction
¶ Markers at chromosome 1 and 2 with significant interaction
# LOD score for significant interaction
†† Percent of total phenotypic variation explained by the epistatic interaction
‡‡ Additive by additive interaction effect

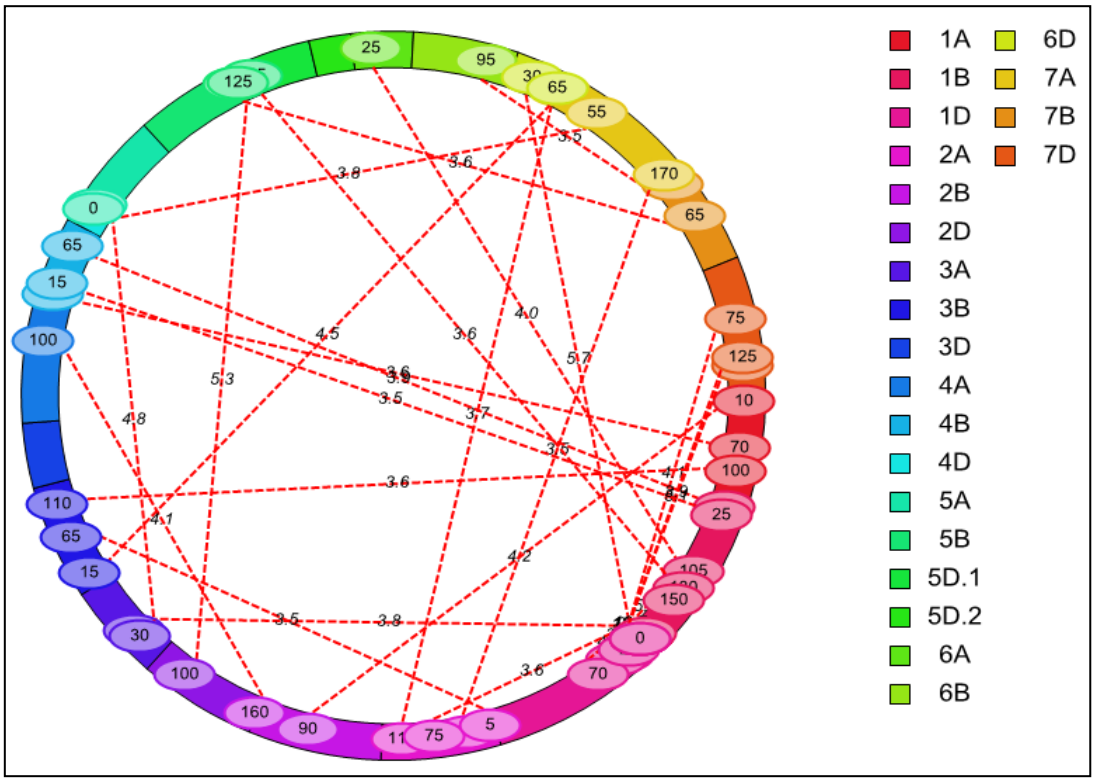

Figure 3.3 An epistatic interaction network among markers at 22 linkage groups obtained by 2D-genome scanning with ICI-EPI program of QTL ICiMapping software. The different color segments in the circular ring represent different linkage groups. The markers with significant interaction are connected with dotted lines with their corresponding LOD values

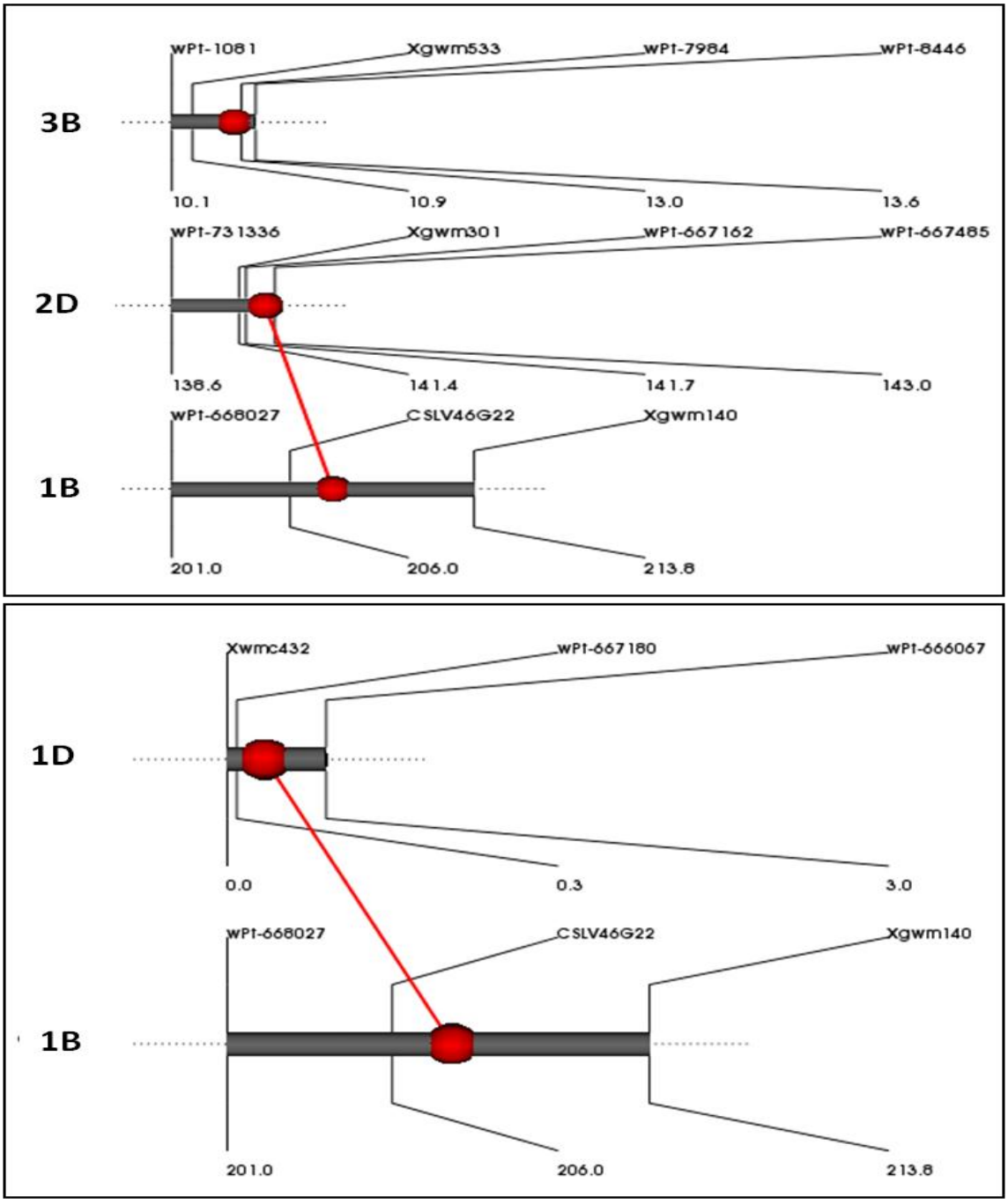

Figure 3.4 Epistatic interaction between significant QTL for yellow rust (top) and leaf rust resistance (bottom) obtained by QTLNetwork2.0.

Effect of QTL combination on disease resistance

All the recombinant inbred lines were classified into 16 and 8 classes of genotypes based on the presence of the major QTL alleles for YR and LR severity percentage in each line respectively. The QTLs were represented by a parental allele of closest marker contributing to disease severity reduction. Using the QTL genotypes as predictor variable, one way ANOVA was carried out on disease severity scores to determine the significance of QTL genotypes. Then, the Student's t-test was carried out on least square means for pair-wise mean comparison of each QTL genotypes (Tables 3.4, 3.5). For YR, *QYr.tam-1B* and *QYr.tam-3D* were not found to be significant in reducing disease as compared to the non-QTL control group (i.e., genotypes without any QTL). However, when present alone, *QYr.tam-3B* and *QYr.tam-2D* significantly reduced the disease severity by 29 and 71% respectively as compared to the non-QTL group. Most of the small-effect QTL were found to be very effective in reducing disease when they were combined with other QTL. When combined, *QYr.tam-1B* and *QYr.tam-3D* were very effective reducing YR severity by up to 37% as compared to non-QTL group. Similarly, *QYr.tam-3B* and *QYr.tam-2D* were found to combine best among all two QTL combinations. When all four QTLs were combined, host reaction was near-immunity. Bar graphs of mean YR severity percentage associated with different QTL genotypes are shown in Figure 3.5.

For LR, *QLr.tam-3D* significantly reduced the disease severity in recombinant inbred lines by about 12-43% as compared to non-QTL group. Similarly, *QLr.tam-1B* and *QLr.tam-1D* were also very effective in reducing the disease severity by more than

60% as compared to non-QTL group when present alone. It was observed that the QTL were more effective when combined. When all the three QTLs were combined, the host reaction was near-immunity (i.e., <1% disease severity was observed in the lines) in RILs. Bar graphs of mean LR severity percentage associated with different QTL genotypes are shown in Figure 3.6.

Table 3.4 Least square mean comparison between different QTL genotypes for yellow rust disease severity scores.

QTL Genotype†	YR_09		YR_10		YRAVERAGE	
	LS-Mean	t-group‡	LS-Mean	t-group‡	LS-Mean	t-group‡
No-QTL	81.9	a	81.9	a	81.9	a
1B	75.7	a	77.1	ab	76.7	ab
3D	80.0	a	73.3	ab	76.4	ab
3B+3D	61.0	b	72.0	ab	66.5	b
3B	58.0	b	70.0	b	64.0	bc
1B+3D	52.0	bc	48.0	cd	50.0	cd
1B+3B	41.5	cd	57.0	c	49.3	d
1B+3B+3D	30.9	de	34.5	de	32.7	e
2D	23.3	ef	25.8	ef	24.6	ef
1B+2D+3D	10.2	fg	13.5	fg	11.8	fg
1B+2D	10.0	fg	16.0	fg	13.0	fg
2D+3D	8.2	fg	17.0	fg	12.6	fg
2D+3B	2.8	g	11.7	fg	7.3	g
2D+3B+3D	3.5	g	7.0	g	5.3	g
1B+2D+3B	2.8	g	5.5	g	4.1	g
1B+2D+3B+3D	1.8	g	3.3	g	2.6	g

† Group of recombinant inbred lines with resistance allele combination for *QYr.tam-1B*, *QYr.tam-2D*, *QYr.tam-3B*, *QYr.tam-3D*

‡ Pair-wise mean comparison using student's t-test. QTL genotypes connected with same alphabet are not significantly different at P=0.05

Table 3.5 Least square mean comparison between different QTL genotypes for leaf rust disease severity scores.

QTL	LR_09		LR_10		LR_AVERAGE	
Genotype†	LS-Mean	t-group‡	LS-Mean	t-group‡	LS-Mean	t-group‡
None	81.5	a	88.5	a	85.0	a
3D	71.7	b	50.0	b	60.8	b
1D	32.9	c	16.7	c	24.8	c
1B	33.8	c	14.4	c	24.1	c
1B+3D	19.7	d	6.9	d	12.6	d
1D+3D	13.4	de	5.5	d	10.2	d
1B+1D	6.6	ef	2.8	d	4.7	de
1B+1D+3D	0.3	f	0.0	d	0.2	e

† Group of recombinant inbred lines with resistance allele combination for *QLr.tam-1B*, *QLr.tam-1D*, *QLr.tam-3D*

‡ Pair-wise mean comparison using student's t-test. QTL genotypes connected with same alphabet are not significantly different at P=0.05

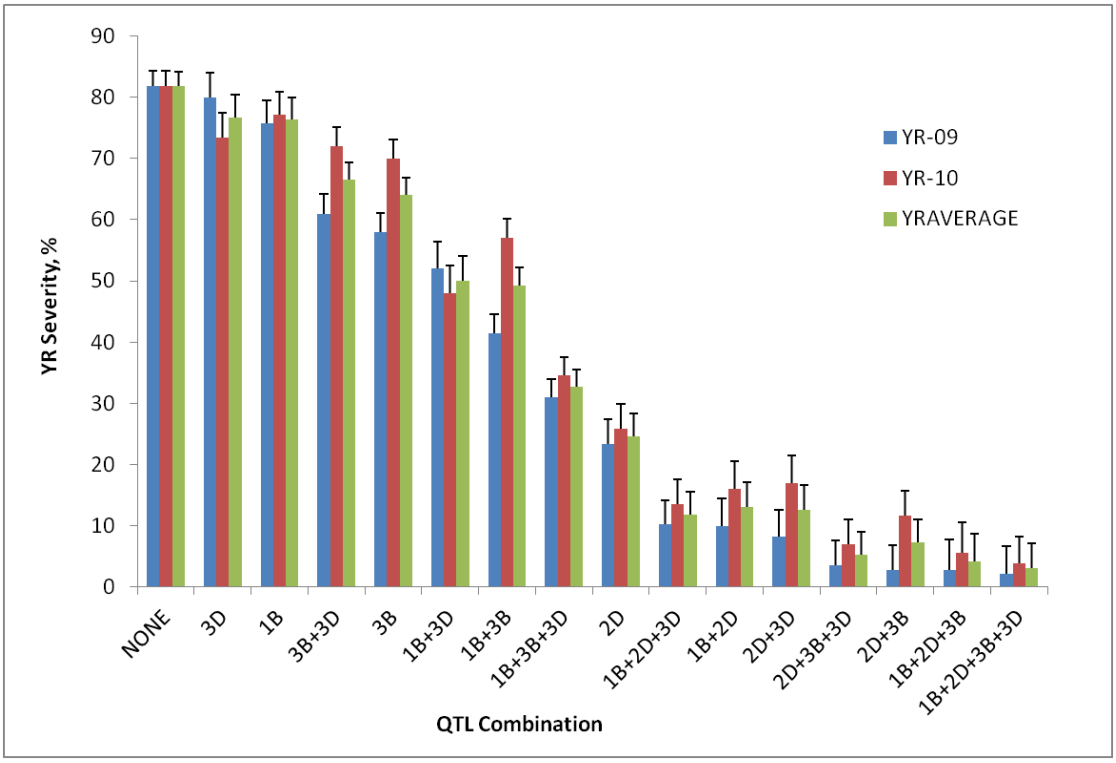

Figure 3.5 Effect of individual QTL, and their combinations on yellow rust severity scores in Toluca 2009 and 2010 experiments.

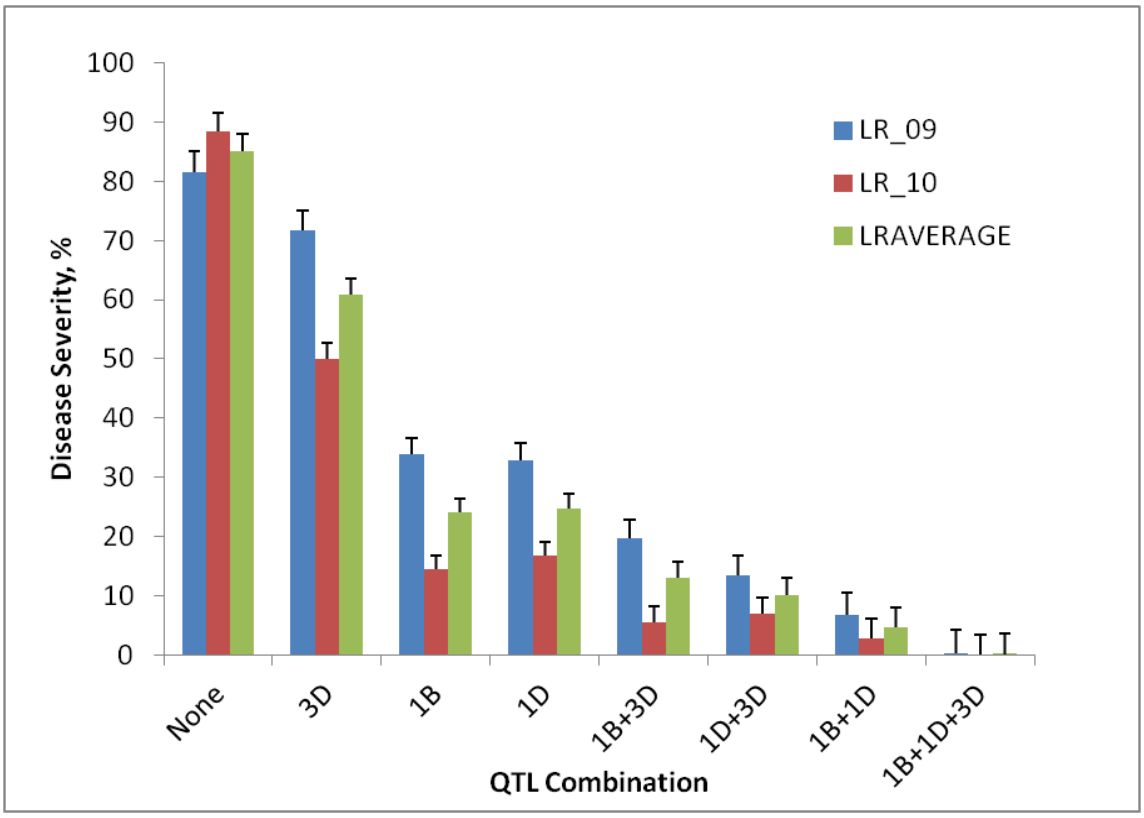

Figure 3.6 Effect of individual QTL and their combinations on leaf rust severity scores in Obregon 2009 and 2010 experiments.

Discussion

Our genetic linkage map, with integrated SSR and DArT markers, spanned around 2,763cM covering all the 21 chromosomes which was comparable with previous maps of 2,569cM (Somers et al., 2004) and 2,937cM (Akbari et al., 2006) for hexaploid wheat. As the number of markers in chromosome 5D were relatively low, this chromosome was broken down in to two linkage groups. High quality final genetic linkage map was constructed with 495 markers by removing markers with high level of segregation distortion and possible double cross over points.

The YR and LR resistance QTL i.e., *QYr.tam-1B* and *QLr.tam-1B*, present on the 1BL chromosomal region is expected to be associated with *Lr46/Yr29* gene (William et al., 2003). In our study, *QYr.tam-1B* and *QLr.tam-1B* were mapped at 1cM distance from the marker *csLV46G22* which is 8cM away from the distal marker *Xgwm140*. Thus, presence of *Lr46/Yr29* gene in Quaiu became evident as the *QYr.tam-1B* and *QLr.tam-1B* were closely linked with the marker csLV46G22 (a *Lr46/Yr29* diagnostic marker developed by E. Lagudah, pers. comm.) which was successfully used by Lillemo et al. (2011) to assess the effects of *Lr46* with various additive YR resistance genes in Avocet/Saar mapping population.

Similarly, *QYr.tam-3B* was detected at 1-2cM distance from marker *Xgwm533* which is also considered to be closely linked with pleiotropic gene *Sr2/Yr30* (Sing et al., 2000b; Suenaga et al., 2003; Spielmeyer et al., 2003). Moreover, the Quaiu allele fragment amplified with marker *Xgwm533* was found to be similar to that of 'Parula' which has been reported to carry *Sr2* gene for stem rust resistance (Lillemo et al., 2011).

Based on these evidences, we assume that *QYr.tam-3B,* detected in our population, is same as previously designated gene *Sr2/Yr30*. However, Lowe et al. (2011) and Bansal et al., (2010) have cautioned that a YR resistance 3BS QTL closely linked with *Xgwm533.1* might also be associated with a gene different from *Sr2/Yr30*. So, at this point, we cannot conclude that *QYr.tam-3B* is associated with *Sr2/Yr30* unless further evidences are gathered through allelism tests and other validation procedures.

Based on pedigree information, it is expected that the race-specific and moderately effective LR resistance gene *Lr42* is present in Quaiu (pedigree: Babax/*Lr42*//Babax*2/3 /Vivitsi). It has been further supported by a resistance reaction of Quaiu in seedling tests with *P. triticina* race MBJ/SP in the greenhouse. Furthermore, a *Lr42* diagnostic marker *Xwmc432*, which has been mapped at 0.8cM distance from *Lr42* gene on the short arm of chromosome 1D (Sun et al., 2010), was used to screen the whole mapping population. A perfect co-segregation was observed between R-type reaction in seedling tests and *Lr42* allele of marker *Xwmc432*. Similarly, QTL mapping results showed a highly significant QTL peak within less than 1cM distance from marker *Xwmc432*. Thus, we confirmed that *QLr.tam-1D* detected in our study is associated with the race-specific gene *Lr42*.

In this study, we found a very large effect QTL (*QYr.tam-2D*) on the long arm of chromosome 2D associated with YR severity reduction in all experiments. In the map, *QYr.tam-2D* is located at <1cM distance from the SSR marker *Xgwm301*, and it is flanked by the DArT markers *wpt-667162* and *wpt-667485*. This QTL showed a peak at LOD score range of 39 to 50 and explained up to 61% of the total phenotypic variation.

In addition to the 23 DArT markers, the relative map positions of 17 SSR markers aligned long chromosome 2D are comparable to microsatellite consensus map developed by Somers et al. (2004). Thus we can conclude that this QTL is located at the distal end of 2D chromosomal arm.

Previous studies have reported the presence of resistance gene *Yr16* derived from 'Cappelle Desprez' (Worland and Law, 1986) and some other QTL responsible for YR resistance in a different location of chromosome 2D. Mallard et al. (2005) reported a YR resistance QTL derived from cultivar 'Camp Remy' near the centromeric region of 2D chromosome flanked by markers *Xgwm102* and *Xgwm539*. It has been suggested that this QTL might be associated with *Yr16*, a YR adult plant resistance gene derived from Cappelle Desprez (Worland and Law, 1986), which has been mapped at 9cM distance from the centromere (Hart et al., 1993). Similarly, Melichar et al. (2008) have also reported the presence of a QTL near a SSR marker *Xgwm539* which was responsible for significant reduction of infection caused by YR pathogen in cultivars 'Guardian' and 'Claire' respectively. In our study, we did not find any QTL signal near markers *Xgwm102* and *Xgwm539*, though they were polymorphic between the two parents, Avocet and Quaiu, in our mapping population. The marker *Xgwm301*, which is one of the closest markers from the Quaiu-derived *QYr.tam-2D*, is 53cM distal from marker the *Xgwm539*. Thus, *QYr.tam-2D* detected in our mapping population is different than those reported in in the aforementioned studies. However, consistent with our findings, Jagger et al. (2011) reported a resistance QTL derived from the cultivar 'Alcedo' on the 2D chromosome near the markers *Xgwm320* and *Xgwm301*. This QTL explained about 30 to

50% of total phenotypic variation for disease severity percentage and infection type scores for stripe rust. Similarly, Suenaga et al. (2003) have also reported stripe rust resistance QTL on 2DL chromosomal region closer to the *markers Xgwm320*, *Xgwm349* and *Xgwm301*. We believe that all of these reported YR QTL on distal end of 2DL chromosomal region will probably represent the same YR adult plant resistance gene derived from a common ancestor. Lillemo et al. (2012) and Bougot et al. (2006) reported a QTL responsible for partial resistance to powdery mildew in the 2DL genomic region similar to that of *QYr.tam-2D*. These observations suggest that possibly a common pleiotropic gene might be responsible for partial resistance to both YR and powdery mildew.

Similarly, a region on chromosome 3D was found to be associated with both LR and YR severity reduction. Both *QYr.tam-3D* and *QLr.tam-3D* were flanked by the markers *Xbarc125* and *wpt-672034* from 53 to 56cM distance from the first marker in linkage group except for YR-2009 experiment, where the QTL was detected at 66cM from the first marker. For both traits, these QTL explained about 3 to 4% of total phenotypic variation. Though Chhuneja et al. (2006) and Dedryver et al. (2009) have reported the same QTL responsible for YR resistance in the cultivars 'Opata' and 'Renan', there is no similar reports on this QTL for LR resistance. Thus, we believe that *QYr.tam-3D* and *QLr.tam-3D* might be associated with a pleiotropic gene responsible for both YR and LR severity reduction.

Interestingly, several minor QTL have been detected with LOD score of 2.5 to 3.5 and R^2 values ranging from 1.35 to 3.75%. A QTL derived from resistant parent

Quaiu, *QYr.tam-5A*, was detected on short arm of chromosome 5A for YR resistance in the 2009 experiment only. This QTL might be similar to that of Jagger et al. (2011) who reported a QTL on the short arm of 5A that responsible for YR resistance. All the other minor effect QTL were derived from the susceptible parent Avocet. Some minor effect QTLs from Avocet were expected as we observed very good level of transgressive segregation for YR and LR resistance during the 2010 experiments. A resistance allele of a minor QTL (*QYr.tam-1A*), on short arm chromosome 1A and flanked by markers *wpt-671596* and *wpt-2150*, that was detected for YR severity in 2010 experiment, was contributed by the susceptible parent Avocet. However, Ramburan et al. (2004) and Prins et al. (2011) have reported a minor QTL on the short arm of 1A chromosome associated with YR resistance which was contributed by the parent 'Kariega' rather than Avocet. The QTL report by Prins et al. (2011) showed that the 1A QTL is located close to the DArT marker *wpt-3698* which lies about 28cM distal from the marker *wpt-2150*. Thus we can infer that our 1A QTL might be different than the previously reported one by Prins et al (2011). Similarly, *QYr.tam-4A* and *QYr.tam-6A* alleles detected in the YR 2010 experiment were derived from the susceptible parent Avocet, and were also reported in previous studies (Singh et al., 2001; Ramburan et al., 2004; Prins et al., 2011). However, the Avocet-derived *QYr.tam-3D.1* detected in our study seems to be novel. Similarly, an Avocet-derived *QLr.tam-5A* seems to be a unique QTL associated with reducing LR severity and AUDPC values. These findings indicate that the susceptible cultivar Avocet also contains some minor additive genes for YR and LR disease resistance.

It has been observed that epistatic interaction effects are not given much importance by researcher in estimating QTL effects. However, our study shows that genomic loci always interact with each other to produce some significant phenotypic effects even though their additive main effects are not significant. However, detailed analysis on epistatic interaction among the marker loci and their significance on improved phenotypic expression is beyond the scope of this study. The additive-by-additive interactions between significant QTL loci were also found to have significant impact on phenotypic effects. The QTLXQTL interaction for stripe rust resistance was found to have relatively smaller effect i.e. R^2<2%, than the interaction effect between *Lr42* (*QLr.tam-1D*) and *Lr46* (*QLr.tam-1B*) for LR resistance (R^2 =9%).

For both LR and YR, resistance level was found to be significantly improved through combination of different QTL together. Our study showed that by combining two APR QTL with one major gene *Lr42* conferred near-immune response to LR. Similarly, combination of four APR QTL substantially improved the level of YR resistance. Thus, our findings suggest that durable resistance can be enhanced either by accumulating multiple APR genes into a new cultivar or by adding new gene into a cultivar which is known to possesses some moderately effective race specific genes. Among the novel QTL identified in this study, *QYr.tam-2D* found to be very effective against YR when present alone or in combination with other APR genes such as *Lr46*. Similarly, *QYr.tam-3D* and *QLr.tam-3D* on chromosome 3D are most probably associated with a dual APR gene which is effective against both rust types. As these QTL have been mapped within less than 5cM of their corresponding flanking markers,

MAS can be effectively implemented in breeding programs to transfer these QTL into new lines.

CHAPTER IV

MOLECULAR MAPPING OF POST-SEEDLING RESISTANCE TO YELLOW RUST IN THE WINTER WHEAT CULTIVAR TAM 111

Introduction

Yellow, a.k.a. stripe, rust (YR), caused by *Puccinia striiformis* Westend. f. sp. *tritici*., is a major disease of wheat (*Triticum aestivum* L.) worldwide. Though YR was considered to have sporadic prevalence in temperate regions in the past, recent surveys have shown that it has become prevalent in the majority of the wheat growing regions worldwide, which includes but is not limited to the US, East Asia, South Asia, East Africa and Western Europe (Wellings, 2011). In the US, YR epidemics are more prevalent in the Pacific Northwest region due to mild summer and winter climates. However, recent studies have shown that new races of YR are becoming more problematic in the South-central Great Plains and South Eastern regions of the US (Chen, 2005). In recent years, severe YR epidemics in the US caused significant yield losses in different wheat growing states, including California (2003, 2006 and 2011 causing an estimated yield loss of 25, 15 and 6% yield loss respectively), Texas (2005, 2007 and 2010 causing an estimated yield loss of 15, 5 and 10% respectively) and Kansas (in 2003, 2005 and 2010 causing an estimated yield loss of 11, 8 and 10% respectively) in spite of heavy fungicide use (USDA-ARS, URL: http://www.ars.usda. gov/Main/docs. htm?docid=10123).

Though genetic resistance is the most reliable and environmental friendly means of YR management, the combination of newly developed and previously existing virulent races of the rust pathogen can easily circumvent the resistance genes in grown cultivars resulting in frequent epidemics (Chen, 2007). Though a virulent race Pst-78 was first discovered to overcome the resistance gene *Yr8* and *Yr9* in 2000 (Chen, 2002), it became more prevalent and subsequently overcame the resistance present in *Yr6* and *Yr7* genes (Chen, 2007). In the US, a number of YR races have been reported (Pst-1 to Pst-121 by 2005), which are virulent to most of the major genes present in US cultivars (Chen, 2007; Kolmer et al., 2009). Even though the life span of a single major gene is very short, combination of many genes from different sources has been proven to be durable against a broad range of pathogen races. A multiline cultivar 'Rely', which was developed by combining 10 YR resistance genes from different sources, has been effective against YR in the Pacific Northwest for more than 20 years (Allan et al., 1993; Chen, 2005; 2007). Adult Plant Resistance (APR), which is characterized by non-hypersensitive reaction, is generally considered to be effective against a broad range of races for longer durations of time. Some race non-specific APR genes have been identified and used in breeding programs for many years. Some of the most utilized APR genes include Lr34/*Yr18*, Lr46/*Yr29* and Lr67/*Yr46* located on the 7DS, 1BL and 4DL chromosomal regions, respectively (Dyck, 1987; Krattinger, 2009; Singh et al., 1998; William et al., 2003; Herrera-Foessel et al., 2011; Hiebert et al., 2011). In the US, many sources of High Temperature Adult Plant Resistance (HTAPR), an APR which confers resistance at relatively higher temperature, have been identified and used in breeding

program to enhance durable resistance (Kolmer et al., 2009). The most studied HTAPR gene *Yr36,* which has been sequenced recently (Fu et al., 2009), confers non-specific resistance to YR at an optimal temperature of 25-35^{0}C during the adult plant stage (Uauy et al., 2005). With the advancement of genomics, recently many race specific and non-specific YR resistance genes have been tagged with molecular markers which can be effectively used in Marker Assisted Breeding (MAB).

'TAM 111', a hard red winter wheat (HRW) cultivar developed by Texas AgriLife Research in 2002, is very popular cultivar in the South-Central States of the US. Its popularity among growers is attributed to its high yield potential, good milling and baking quality, high level of resistance to biotic stresses such as YR and stem rust (SR), caused by *P. graminis*, and moderate resistance to Barley Yellow Dwarf Virus (BYDV) and Wheat Streak Mosaic Virus (WSMV) (Lazar et al., 2004). The YR resistance of TAM 111 has remained effective in the South and Central Great Plains of the US for more than 12 years (J. Rudd, personal communication, 2011). Previously, YR resistance in TAM 111 was thought to be similar to that of 'Jagger' HRW (Sears et al., 1997; Lazar et al., 2004) which became susceptible to new races that caused severe YR epidemics in 2010. Among the very few cultivars which remained highly resistant to immune during the 2010 epidemics was TAM 111. The resistance present in TAM 111 is expressed at the post-seedling stages and lacks any common race-specific seedling resistance genes (R. Bowden, personal communication, 2011). So, it seems that TAM 111 resistance is associated with a unique APR gene which has remained effective against YR for many years. Thus, investigating the genetic control of YR resistance in

TAM 111 and its molecular mapping will further facilitates the development of new cultivars by combining other important traits such as greenbug (*Schizaphis graminum* Rondani) and leaf rust (LR, caused by *P. triticina*) resistance, and heat and drought tolerance using MAB. Our objectives in this study were to 1) investigate the genetic control of YR resistance in TAM 111 and 2) map the associated candidate gene/QTL using DNA-based molecular makers.

Materials and Methods

Plant Materials

In this study, we used a population of 124 Recombinant Inbred Lines (RILs) developed by crossing the two winter wheat cultivars TAM 111 and 'TAM 112', and advancing the progeny to the F_6 generation using the single seed descent method. Though both parents are popular HRW cultivars developed by Texas AgriLife Research, in contrast with TAM 111, TAM 112 lacks APR resistance to YR. The RILs and parents were used in all phenotypic evaluations and molecular marker analysis throughout this study. Also, chaff color was used as phenotypic marker as TAM 111 and TAM 112 are white- and red-chaffed cultivars, respectively.

Field evaluation and analysis of yellow rust resistance

The parents and RILs were evaluated for YR resistance during the 2009-2010, 2010-2011, and 2011-2012 growing seasons at six locations of US: Castroville, TX (2009-2010 and 2011-2012; abbreviated as CAS10/12), Pullman and Mt. Vernon, WA

(2009-2010 and 2010-2011; abbreviated as PULL10/11 and MtV10/11), Yuma, AZ (2009-2010; abbreviated as YUMA10), Manhattan, KS and Fayetteville, AR (2011-2012; abbreviated as MAN12 and FTV12). In all the experiments, about 4 grams seed of each line was planted as 1 meter long head-row in four-row plots. All the experiments in 2010-2011 and 2011-2012 were laid out as Randomized Complete Block Design (RCBD) with two replications. The 2009-2010 experiments were planted in a single replication. Field reaction to YR on the RILs and parents was evaluated under natural infection conditions in Castroville, Pullman, Mt. Vernon and Yuma; whereas the Manhattan and Fayetteville nurseries were artificially inoculated with prevalent *P. striiformis* races.

Disease severity (DS) scores were recorded using 0-100% visual ratings based on the modified Cobb's Scale (Peterson et al., 1948). First disease severity readings were taken when the susceptible check showed at least 60-70% disease severity followed by second and/or third readings until the plants became necrotic. Similarly, in Pullman and Mt. Vernon experiments, the Infection Type (IT) data were recorded on a 0-9 visual scale, where 0 and 9 correspond to absolutely resistant and susceptible types, respectively. For the remaining experiments, the host reaction/infection type data were recorded based on the visual criteria given by Roelfs et al. (1992), with some modification in the scale, where R= resistant, RMR=resistant to moderately resistant, MR= moderately resistant, MR/MS = moderately resistant to moderately susceptible, MS= moderately susceptible, MSS= Moderately susceptible to susceptible, and S= Susceptible. These reaction type readings were then converted to numeric response

values (R=0.2, R-MR=0.3, MR=0.4, M=0.6, MS=0.8, MS-S=0.9, and S=1.0). Coefficients of infection (COI) values for each line were calculated by using CIMMYT's adopted method in which disease severity scores were multiplied by the reaction type values (COI=DS*IT/10). All the statistical analyses including descriptive statistics, phenotypic distribution, normality tests and Pearson's correlation coefficient of disease severity and infection type data for all the experiments were performed using SAS 9.2 (SAS Institute, Cary, NC).

Molecular marker analysis and genetic mapping

DNA samples from the two parents and randomly selected 92 RILs were sent to Triticarte Pyt. Ltd., Yarrallumla, Australia for Diversity Arrays Technology (DArT) genotyping. An assay of Wheat *PstI(TaqI)v3* was used to screen for polymorphic DArT markers along the genome in whole mapping population (Jaccoud et al., 2001; Akbari et al., 2006). A genetic linkage map was constructed by using QTL ICiMapping software, abbreviated as ICIM, (Li et al., 2008). A minimum LOD score of 3.5, and maximum recombination frequency of 0.35 was set to make the linkage group, whereas Kosambi mapping function (Kosambi, 1944) was used to convert the recombination frequency into map distance. To finalize the linkage map, marker ordering and rippling were performed using RECORD and COUNT algorithms available in the ICIM software, respectively.

QTL analysis for yellow rust resistance

QTL analysis on DS, IT and COI values for YR was performed using QTL ICiMapping (Li et al., 2008) and WinQTLCart v2.5 (Wang et al., 2011) mapping software. Inclusive Composite Interval Mapping (ICIM) program with probability of stepwise regression 0.001 was employed in QTL ICiMapping; whereas, composite interval mapping with backward and forward regression was used in WinQTLCart. In both cases, LOD threshold was calculated by 1000 permutation tests. The significant QTL were reported with their characteristics i.e., map position, LOD score, flanking markers, QTL heritability and source parent of resistance allele. Graphical representation of linkage groups with their corresponding QTL positions was developed by using MapChart v2.2 (Voorrips, 2002).

Results

Disease evaluation

There was a good level of YR infection in the CAS10 experiment. The parent TAM 111 expressed highly resistant reaction with no visible rust spores on the vegetative parts of the plants. However, the parent TAM 112 expressed a susceptible reaction with nearly 60% of disease severity on flag leaves during flowering and post-flowering stages of development. Similarly, a very high disease pressure was observed in the PULL10 experiment. The average disease severity score was 54% with a range of 20 to 95% among RILs. The parent TAM 112 showed a highly susceptible reaction with a disease severity of 70 to 80%, whereas TAM 111 expressed moderate level of infection

with a disease severity ranging from 40-70%. In MtV10 experiment, the YR severity ranged from 5 to 80% with a population average of 23%. TAM 111 showed a resistance reaction with disease severity ranging from 5 to 10%, whereas TAM 112 showed a susceptible reaction with disease severity score of 60 to 80%. In the MtV2011 experiment disease occurrence was negligible and both parents showed a resistance reaction with very low range of disease severity scores among the RILs. Therefore, this experiment was excluded from analysis due to lack of variability among lines and insignificant YR pressure. On the other hand, the PULL11 experiment had a very good level of YR infection. Though the disease severity on TAM 111 and TAM 112 was 40 and 60% respectively, both parents had moderately resistant reaction. However, transgressive segregation was evident in the population where disease severity scores ranged from 15 to 85% among the RILs. In YUMA10, YR infection was moderate, where two parents, TAM 111 and TAM 112, showed a resistance and susceptible reaction, respectively. Pearson's correlation coefficients for YR disease scores among different experiments were highly significant except between PULL10 and MtV10 experiments (Table 4.1).

Table 4.1 Pearson pair-wise correlation coefficients of yellow rust disease severity and coefficient of infection scores among different experiments.

	DS1_CAS10	DS2_CAS10	DS_PULL10	DS_MtV10	COI_PULL10	COI_MtV10
DS2_CAS10	0.85**					
DS_PULL10	0.34**	0.38**				
DS_MtV10	0.73**	0.69**	0.10^{ns}			
COI_PULL10	0.39**	0.40**	0.97**	0.14^{ns}		
COI_MtV10	0.74**	0.69**	0.12^{ns}	0.96**	0.15^{ns}	
DS_PULL11	0.44**	0.40**	0.41**	0.46**	0.46**	0.45**

** Significant at P=0.01, ^{ns} Not significant at P=0.05
Abbreviations: DS= Disease severity, COI=Coefficient of infection, CAS10=Castroville 2010 experiment, PULL10/11= Pullman 2010/2011 experiment, MtV10= Mt. Vernon 2010 experiment.

Genetic Linkage Mapping

Linkage map construction was carried out using QTL ICIMapping software (Li et al., 2008). Out of total 879 polymorphic DArT markers, the final linkage map retained only 335 markers. A large number of markers with high level of redundancy and severe segregation distortions were discarded from the analysis. A total of 26 linkage groups, which represented 20 chromosomes of hexaploid wheat and spanned about 2350 cM of the genetic distance, were formed. The average map density was about 7cM per marker. The largest linkage group was formed on chromosome 1B with 34 markers, whereas the smallest linkage groups were formed on chromosomes 4A, 4D and 5A with 2 markers each. We did not find any polymorphic markers on chromosome 5D.

QTL/Gene mapping

QTL mapping for YR DS, IT and/or COI scores was carried out by using both QTL ICiMapping (Li et al., 2008) and WinQTLCart v2.5 (Wang et al., 2011) software.

Some additional traits i.e., glume color (GC), greenbug (GB) resistance and partial resistance to powdery mildew (PMD, caused by *Erysiphe graminis*), were also used as phenotypic markers in our analyses. The LOD threshold wad calculated using 1000 permutation test. An account of QTL detected for YR resistance and other additional traits and their characteristics are presented in table 4.2.

A large effect QTL on the long arm of chromosome 2B (temporarily designated as *QYr.tam-2B*) was consistently detected for YR DS, IT and COI scores in three experiments i.e., CAS10, MtV10 and YUMA10 (Table 4.2, Figure 4.1). In all the experiments, it was flanked by *wpt-6242* and *wpt-6471* except in the second evaluation of DS in CAS2010 where this QTL was flanked by markers *wpt-5044* and *wpt-6242*. Across experiments, *QYr.tam-2B* was found to reside 2 to 5 cM away from the closest marker explaining about 18 to 64% of total phenotypic variation. The resistance allele of this QTL was derived from parent TAM 111 (hereafter, the term 'QTL' for any disease traits will be used to refer the QTL with resistance alleles per se).

Similarly, another QTL on the short arm of chromosome 1A (temporarily designated as *QYr.tam-1A.1*) was detected for DS and COI scores in the PULL10 and PULL11 experiments. This QTL explained 10 to 21% of total phenotypic variation, and was flanked by the markers *wpt8105* and *wpt-7074*. The *QYr.tam-1A.1*allele was contributed by TAM 111. As this QTL was not detected in other environments, it probably represents a YR resistance gene that is more environment and race specific. Similarly, two other environment specific QTL for YR resistance were detected on chromosome 1A (*QYr.tam-1A.2*) and 2A (*QYr.tam-2A*) in PULL11 and YUMA10

experiments, respectively. These two QTL, *QYr.tam-1A.2* and *QYr.tam-2A* explained about 15% and 21% of the total phenotypic variation respectively.

Table 4.2. Characteristics of QTL/Genes detected for different yellow rust resistance and other traits in a TAM 111 x TAM 112 population by using Inclusive Composite Interval Mapping program of QTL ICIMapping software.

Exp†	Trait‡	Designation§	Chr¶	Map#	L-Marker[f]	R-Marker††	LOD‡‡	PVE(%)§§	Parent¶¶
Castroville 2010 (CAS10)	YR-DS1	*QYr.tam-2B*	2B	111	wPt-6242	wPt-6471	3.13	22.78	TAM111
	YR-DS2	*QYr.tam-2B*	2B	100	wPt-5044	wPt-6242	3.34	26.56	TAM111
	PM-DS	*QPm.tam-1A*	1A	17	wPt-672158	wPt-7784	5.03	32.14	TAM112
Pullman 2010 (PULL10)	YR-DS	*QYr.tam-1A.1*	1A	12	wPt-8105	wPt-7074	3.14	21.23	TAM111
	YR-COI	*QYr.tam-1A.1*	1A	12	wPt-8105	wPt-7074	4.10	26.79	TAM111
Mt. Vernon 2010 (MtV10)	YR-DS	*QYr.tam-2B*	2B	110	wPt-6242	wPt-6471	11.83	63.82	TAM111
	YR-COI	*QYr.tam-2B*	2B	110	wPt-6242	wPt-6471	6.92	43.50	TAM111
Yuma 2010 (YUMA10)	YR-IT	*QYr.tam-2A*	2A	85	wPt-6431	wPt-0277	5.11	24.02	TAM111
	YR-IT	*QYr.tam-2B*	2B	112	wPt-6242	wPt-6471	4.77	17.60	TAM111
Pullman 2011 (PULL11)	YR-DS	*QYr.tam-1A.1*	1A	12	wPt-8105	wPt-7074	3.31	9.54	TAM111
	YR-DS	*QYr.tam-1A.2*	1A	90	wPt-2976	wPt-732591	4.84	14.64	TAM112
	YR-DS	*QYr.tam-2B*	2B	107	wPt-6242	wPt-6471	7.07	22.73	TAM111
	YR-DS	*QYr.tam-7D*	7D	8	wPt-730455	wPt-732048	3.44	12.58	TAM111
Bushland 2011 (BUSH11)	GB (%)	*Gb3*	7D	18	wPt-732048	wPt-664264	9.51	39.89	TAM112
	GC (R/W)	*Rg1*	1B	12	wpt-7034	wpt-3477	6.85	-	-

† Experiments conducted in different location and year for yellow rust and additional traits evaluation
‡Evaluated traits in the experiments: YR= Yellow rust, DS (1,2)= Disease severity scores (first, second evaluation), IT=Infection Type scores, COI= Coefficient of Infection (DS*IT/10), GB (%)= Greenbug resistance (%), GC =Glume Color (Red/White), PM= Powdery Mildew
§ Temporary Designation of unknown QTL and name of known genes
¶ Chromosomes in which significant QTL were detected
# Map position of Gene or putative QTL detected in the analysis (in cM)
††Left and right flanking markers of significant QTL/Gene
‡‡ Odd of likelihoods expressed in logarithmic scale
§§ Percent of total phenotypic variation explained by the QTL (also called R^2 or heritability of QTL)
¶¶ Source parent for resistant allele of significant QTL

In addition to YR resistance traits, QTL/Gene mapping was carried out for some other traits, namely disease severity scores for partial resistance to PMD, glume color and green bug resistance. For PMD disease severity scores, a QTL was detected on the short arm of chromosome 1A (temporarily designated as *QPm.tam-1A*). It was flanked by the markers *wpt-672158 and wpt-7784* explaining about 32% of the total phenotypic variation. The *QPm.tam-1A* allele was contributed by TAM 112. Similarly, a locus on chromosome 7D, contributed by TAM 112, was found to be significantly associated with green bug resistance and explained about 40% of the total phenotypic variation. As a phenotypic marker, glume color was also map using the same RILs. Being the binary trait, glume color data was combined with marker genotype to identify the appropriate linkage group. Thus, this glume color locus was found to have nearly perfect association with DArT markers of chromosome 1A. A genetic linkage map of chromosomes 1A, 2A, 1B, 2B and 7D with significant QTL/Gene position for different traits has been presented in Figure 4.2.

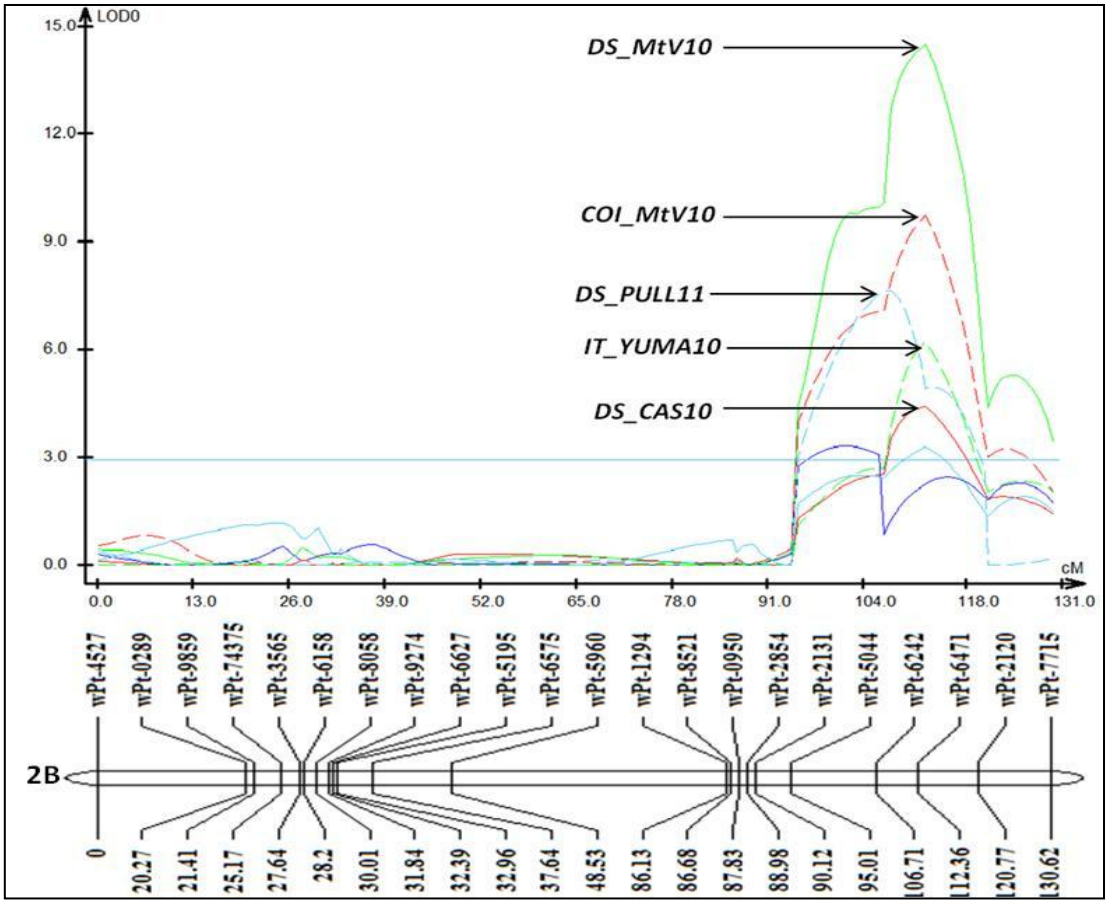

Figure 4.1 LOD profiles of quantitative trait loci peaks detected for yellow rust disease severity (DS), infection type (IT) and coefficient of infection (COI) scores in a TAM 111 x TAM 112 population evaluated in Mt. Vernon, WA 2010, Pullman, WA 2011, Yuma, AZ 2010 and Castroville, TX 2010 experiments.

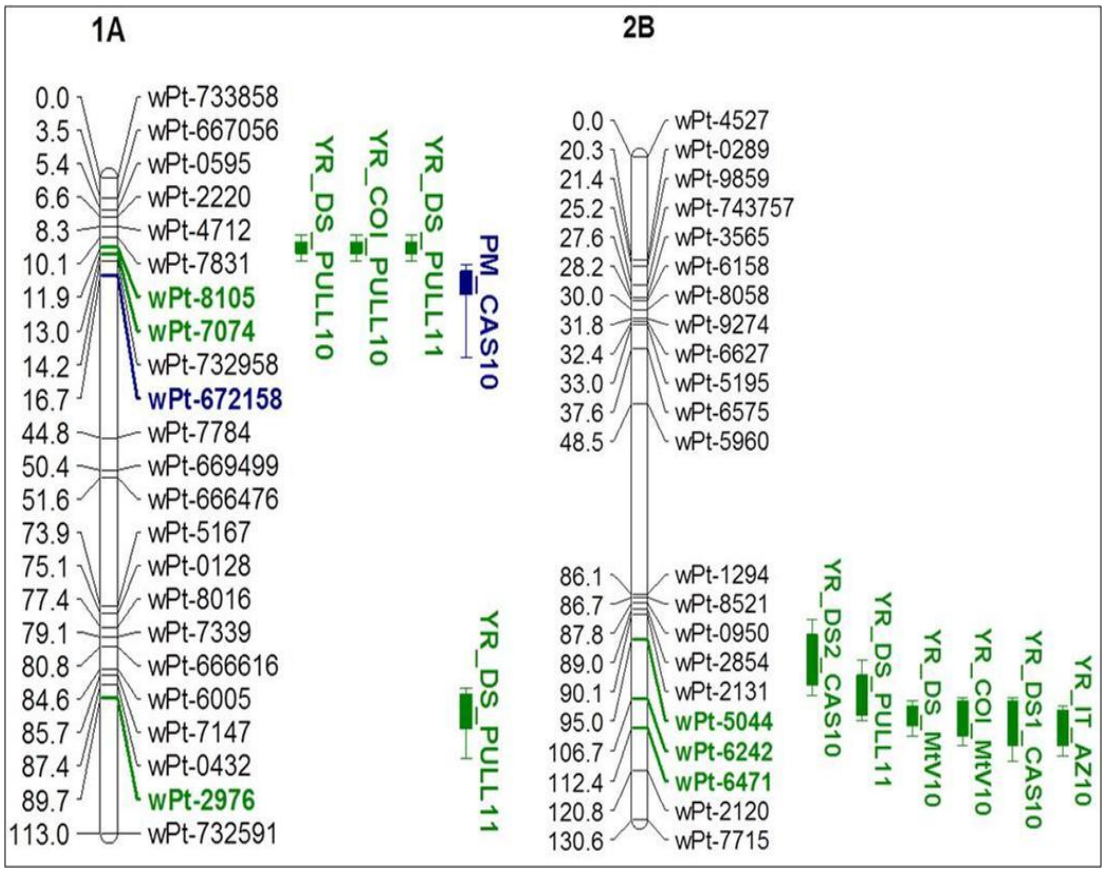

Figure 4.2 Genetic linkage maps of chromosomes 1A, 2B, 2A, 1B and 7D with corresponding positions of significant QTL/Gene for different traits and experiments in a TAM 111 x TAM 112 population.

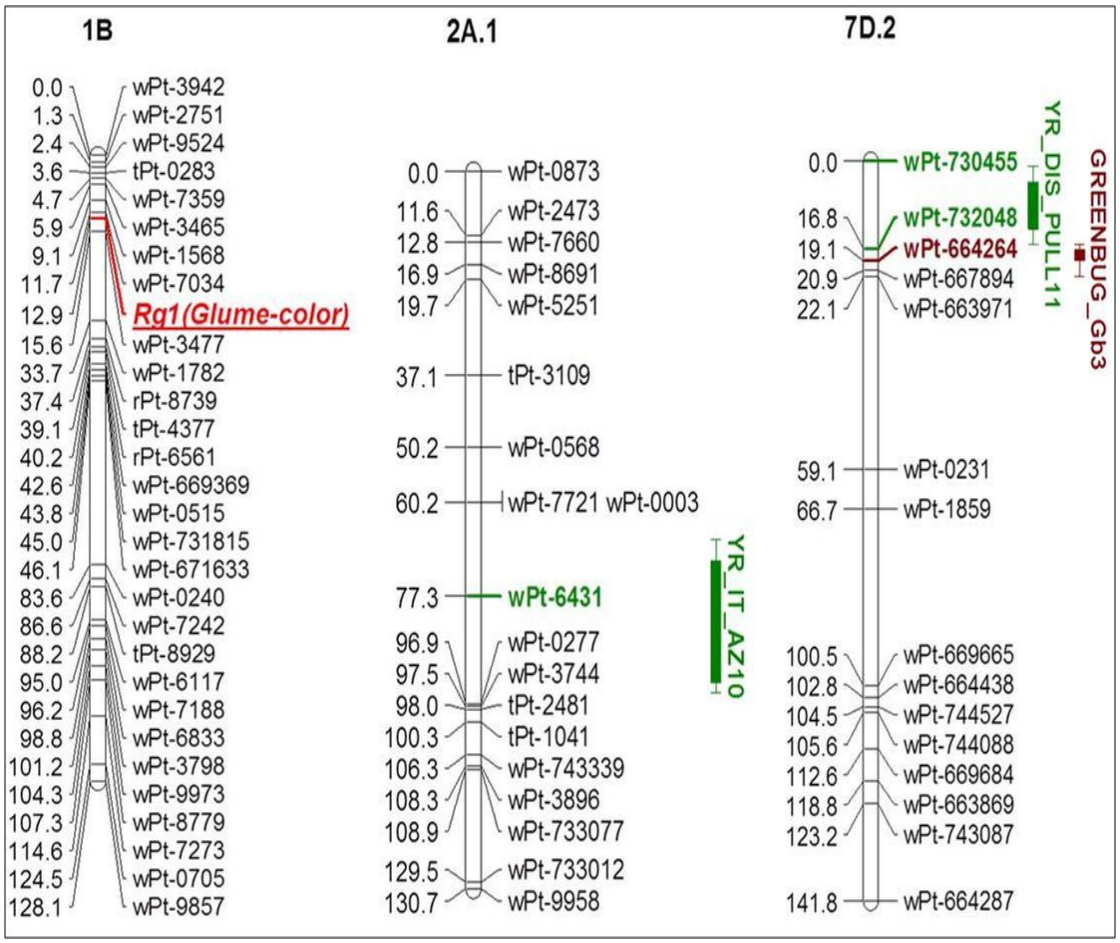

Figure 4.2 Continued.

Discussion

The RILs showed a varied level of YR development in the different experiments. In most of the experiments, TAM 111 was resistant to YR except in PULL10 where it had a moderately susceptible infection. These observations indicate that the resistance present in TAM 111 might be associated with race-specificity. As the disease severity scores in PULL10 and MtV10 do not correlate, high degree of variation in *P. striiformis* races can be speculated even within Washington State where neither TAM 111 nor TAM 112 are grown. The YR severity scores among RILs were not normally distributed in any of the experiments, indicating that specific resistance should be present in the parents.

A high quality linkage map was constructed with a total of 335 polymorphic DArT markers. To refine the genetic map, redundant markers within <0.5cM distance were removed. Similarly, segregation distortion for each marker was measured using the chi-square test. Markers with significant segregation distortion ($P<0.001$) and extreme allele frequency (<0.3 or >0.7) were also removed from the linkage map. The total genetic distance covered by our map was slightly lower as compared to previous maps for hexaploid wheat (Somers et al., 2004; Akbari et al., 2006).

In our study, a QTL on the long arm of chromosome 2B (*QYr.tam-2B*) was consistently detected for YR DS and COI scores in CAS10, YUMA10, MtV10 and PULL11 experiments. Previous studies have reported the presence of YR resistance gene *Yr5* (Hart et al., 1993) and *Yr7* (Macer, 1966; Law, 1976) in the 2BL region. Bariana et al. (2001) mapped the *Yr7* gene at the 2BL chromosomal region, about 15 to 30 cM distal from the centromere, in two different populations. Similarly, Yan et al. (2003)

found some Resistance Gene Analog (RGA) markers co-segregating with *Yr5* gene on the long arm of chromosome 1B. Whereas, Smith et al. (2007) mapped the *Yr5* gene on the 1BL arm, 20 cM distal from the centromere. As both *Yr7* and *Yr5* genes are found to reside on the same region, it has been suggested that these two genes are probably allelic (Mallard et al., 2005). Similarly, some YR resistance QTL on the 1BL chromosomal region have been reported in several studies (Crossa et al., 2007; Roswarne et al., 2008; Mallard et al., 2005; Dolores Vazquez et al., 2012). Boukhatem et al. (2002) have found an APR QTL on 2BL, which coincided with the seedling resistance gene *Yr7* in winter wheat cultivar 'Camp Remy' which provided a good level of field resistance to YR for more than 20 years. Based on the evidences of association between APR loci and seedling resistance genes, it has been hypothesized that seedling-expressed, race specific resistance genes may still confer residual resistance at the post-seedling stage (Boyd, 2005). However, virulent races to *Yr7* has been already reported in Australia and most of the wheat growing areas in North America (McIntosh et al., 1995; Singh et al., 2008). Thus, the *QYr.tam-2B* QTL detected in TAM 111 HRW possibly represents *Yr5* gene or nearby 1BL QTL which provides post-seedling residual resistance to prevalent YR races similar to the findings of Boukhatem et al. (2002).

In our study, another YR QTL *QYr.tam-1A.1* was detected on the short arm of chromosome 1A. As *QYr.tam-1A.1* was detected in PULL10 and PULL11 experiments only, it probably represents environment- or race-specific genes for YR resistance. Though not any designated Yr genes are reported to reside in this region, few studies have reported some YR resistance QTL in this location (Crossa et al., 2007; Ramburan

et al., 2004). On the long arm of 1A, another QTL (*QYr.tam-1A.2*) was also detected in the PULL11 experiment. Contrary to other QTL, *QYr.tam-1A.2* allele was derived from the parent TAM 112. This QTL is probably the same as reported in previous studies (Crossa et al., 2007; Chen et al., 1995; Dolores Vazquez et al., 2012). Two other QTL, *QYr.tam-2A* and *QYr.tam-7D*, were detected in single experiments i.e., YUMA10 and PULL11, respectively. Chromosome 2A has been reported to possess *Yr17* (Barianna and McIntosh, 1993) and *Yr32* (Eriksen et al., 2004) genes and many other YR resistance QTL (Crossa et al., 2007; Dolores Vazquez et al., 2012), whereas chromosome 7D contains *Yr18* gene and some YR resistance QTL (Singh et al., 2000b; Bariana et al., 2001; Crossa et al., 2007).

In addition to YR, this population was screened for some of the well known traits possessed by the parents, TAM 111 and TAM 112. TAM 112 possesses PMD resistance (on chromosome 1A) inherited from 1AL/1RS translocation from 'Amigo' (Heun et al., 1990), and green bug resistance gene *Gb3* (on chromosome 7D) inherited from 'Largo' (Weng et al., 2005). Our mapping analysis also detected the TAM 112-derived PMD and green bug resistance loci on 1AS and 7DL chromosomal locations, respectively. Similarly, TAM 111 and TAM 112 possess white and red color glume (chaff), respectively. In our analysis, the glume color trait was mapped on chromosome 1B, possibly similar to that of glume color locus *Rg1* reported in a previous study (Khlestkina et al., 2006). Mapping of these additional phenotypic traits in expected chromosomal locations has further validated our overall QTL mapping results for YR resistance in the TAM 111 x TAM 112 population.

CHAPTER V

CONCLUSIONS

This study showed that APR to LR and YR possessed by Quaiu 3 is highly heritable quantitative trait. Molecular marker analysis and seedling test results revealed that Quaiu 3 carries known APR genes *Lr46/Yr29* and *Sr2/Yr30* for YR resistance, and moderately effective seedling resistance gene *Lr42* for LR resistance. In addition to these known genes, many other QTL were detected for YR and LR resistance. Among them, a potentially novel QTL on long arm of chromosome 2D, *QYr.tam-2D*, was found to have the largest effect on reducing the YR disease in the field. Similarly, QTLs *QYr.tam-3D* and *QLr.tam-3D* most probably represent a common genetic locus on chromosome 3D with dual APR resistance. This might be useful to improve YR and LR resistance simultaneously in wheat genotypes. Significant additive by additive epistatic interactions revealed that appropriate combination of two or more resistance genes/QTLs is very important to achieve higher level of resistance. Susceptible parent Avocet-YrA also possesses few minor QTLs which are often overshadowed by large effect QTL present in background genotypes.

Similarly, a TAM 111 QTL, *QYr.tam-2B*, associated with APR to YR was mapped on long arm of chromosome 2B. Though *QYr.tam-2B* was detected in multiple environments in post-seedling stage, it was found to be highly affected by growing environments and their race structures. Molecular mapping of some additional traits i.e.,

chaff color, partial resistance to powdery mildew and greenbug resistance further validated our mapping results in TAM 111 derived winter wheat population.

Our results can be successfully applied in improving durable resistance to leaf and yellow rust resistance in wheat through conventional as well as molecular breeding approaches. Both the wheat genotypes, Quaiu 3 and TAM 111, are great sources of durable rust resistance which can be deployed in combination with other known sources via marker assisted or conventional selection approaches in the breeding programs.

REFERENCES

Agrios, G.N. 1997. Plant Pathology. Fourth ed. Academic Press, San Diego.

Akbari, M., P. Wenzl, V. Caig, J. Carling, L. Xia, S. Yang, G. Uszynski, V. Mohler, A. Lehmensiek, H. Kuchel, M. Hayden, N. Howes, P. Sharp, P. Vaughan, B. Rathmell, E. Huttner, and A. Kilian. 2006. Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theor. Appl. Genet. 113:1409-1420.

Allan, R.E., G.L. Rubenthaler, C.F. Morris, and R.F. Line. 1993. Registration of three soft white winter wheat germplasm lines resistant or tolerant to strawbreaker foot rot. Crop Sci. 33:1111-1112.

Allard, R.W. 1960. Principles of Plant Breeding. John Wiley and Sons, N.Y.

Appel, J.A., E. DeWolf, W.W. Bockus, T. Todd, and R. Bowden. 2010. Kansas Cooperative Plant Disease Survey Report: Preliminary 2010 Kansas wheat disease loss estimates. Kansas State University, URL: http://www.ksda.gov /includes/document_center/plant_ protection /Plant_Disease_Reports/ 2010KSWheatDiseaseLossEstimates.pdf, Accessed 15 March 2012.

Bansal, U.K., H.S. Bariana, M.B. Gill, and M.J. Hayden. 2010. Chromosomal location of an uncharacterised stripe rust resistance gene in wheat. Euphytica 171:121-127.

Bariana, H., U. Bansal, A. Schmidt, A. Lehmensiek, J. Kaur, H. Miah, N. Howes, and C. McIntyre. 2010. Molecular mapping of adult plant stripe rust resistance in wheat and identification of pyramided QTL genotypes. Euphytica 176:251-260.

Bariana, H.S., and R.A. McIntosh. 1993. Cytogenetic studies in wheat XV. Location of rust resistance genes in VPM1 and their genetic linkage with other resistance genes in chromosome 2A. Genome 36:476-482.

Bariana, H.S., and R.A. McIntosh. 1995. Genetics of adult plant stripe rust resistance in four Australian wheats and the French cultivar 'Hybride-de-Bersee'. Plant Breed. 114:485-491.

Bariana, H.S., M.J. Hayden, N.U. Ahmed, J.A. Bell, P.J. Sharp, and R.A. McIntosh. 2001. Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. Aust. J. Agric. Res. 52:1247-1255.

Bernardo, R. 2010. Breeding for Quantitative Traits in Plants. Second ed. Stemma Press, Woodbury, Minnesota.

Bjarko, M.E., and R.F. Line. 1988. Heritability and number of genes controlling leaf rust resistance in four cultivars of wheat. Phytopathology 78:457-461.

Borghi, B. 2001. Italian wheat pool. In A.P. Bonjean and W.J. Angus (ed.), The world wheat book: A history of wheat breeding. Intercept, London. p. 289–309

Boukhatem, N., J.M. Jacquemin, D. Mingeot, and P.V. Baret. 2002. Quantitative trait loci for resistance against Yellow rust in two wheat-derived recombinant inbred line populations. Theor. Appl. Genet. 104:111-118.

Bowden, R.L. 2001. Wheat stripe rust. Department of Plant Pathology, Kansas State University. (Online resource, Verified in 04/09/2009, URL: http://www.oznet.ksu.edu/path-ext/factSheets/ Wheat/Wheat%20Stripe%20 Rust.asp).

Boyd, L.A. 2005. Can Robigus defeat an old enemy? Yellow rust of wheat. J. Agric. Sci. 143:233-243.

Caldwell, R.M., J.J. Roberts, and Z. Eyal. 1970. General resistance ("slow rusting") in *Puccinia recondita* f.sp. *tritici* in winter and spring wheats. Phytopathology 60:1287 (Abstr.).

Cao, S., B. Carver, X. Zhu, T. Fang, Y. Chen, R. Hunger, and L. Yan. 2010. A single-nucleotide polymorphism that accounts for allelic variation in the Lr34 gene and leaf rust reaction in hard winter wheat. Theor. Appl. Genet. 121:385-392.

Carldwell, R.M., H.R. Karybill, J.T. Sullivan, and L.E. Compton. 1934. Effect of leaf rust (*Puccinia triticina*) on yield, physical characters, and composition of winter wheats. J. Agric. Res. 48:1049-1071.

Chen, X., M. Moore, E.A. Milus, D.L. Long, R.F. Line, D. Marshall, and L. Jackson. 2002. Wheat Stripe Rust Epidemics and Races of *Puccinia striiformis* f. sp. *tritici* in the United States in 2000. Plant Dis. 86:39-46.

Chen, X.M. 2005. Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. Can. J. Plant Pathol. 27:314-337.

Chen, X.M. 2007. Challenges and solutions for stripe rust control in the United States. Aust. J. Agric. Res. 58:648-655.

Chen, X.M., R.F. Line, and S.S. Jones. 1995. Chromosomal Location of Genes for Resistance to Puccinia striiformis in Winter Wheat Cultivars Heines VII, Clement, Moro, Tyee, Tres, and Daws. Phytopathology 85:1362-1367.

Chester, K.S. 1939. The 1938 wheat leaf-rust epiphytotic in Oklahoma. Plant Dis. Rep. 112:-1-19.

Chester, K.S. 1946. The Nature and Prevention of the Cereal Rusts as Exemplified in the Leaf Rust of Wheat, Waltham, Massachusetts, USA.

Chhuneja, P., S. Kaur, R.K. Goel, and H.S. Dhaliwal. 2006. Mapping of leaf rust and stripe rust resistance QTLs in bread wheat x synthetic RIL population under Indian field conditions. Indian J. Crop Sci. 1: 49-54

Chhuneja, P., S. Kaur, T. Garg, M. Ghai, S. Kaur, M. Prashar, N.S. Bains, R.K. Goel, B. Keller, H.S. Dhaliwal, and K. Singh. 2008. Mapping of adult plant stripe rust resistance genes in diploid A genome wheat species and their transfer to bread wheat. Theor. Appl. Genet. 116:313-24.

Chu, C.G., T. Friesen, S. Xu, J. Faris, and J. Kolmer. 2009. Identification of novel QTLs for seedling and adult plant leaf rust resistance in a wheat doubled haploid population. Theor. Appl. Genet. 119:263-269.

CIMMYT. 2005. Laboratory protocols: CIMMYT applied molecular genetics laboratory, 3rd edn., CIMMYT, Mexico.

Cockerham, C. C. 1983. Covariances of Relatives from Self-Fertilization1. Crop Sci. 23:1177-1180.

Crossa, J., J. Burgueno, S. Dreisigacker, M. Vargas, S.A. Herrera-Foessel, M. Lillemo, R.P. Singh, R. Trethowan, M. Warburton, J. Franco, M. Reynolds, J.H. Crouch, and R. Ortiz. 2007. Association Analysis of Historical Bread Wheat Germplasm Using Additive Genetic Covariance of Relatives and Population Structure. Genetics 177:1889-1913.

Dakouri, A., B. McCallum, A. Walichnowski, and S. Cloutier. 2010. Fine-mapping of the leaf rus Lr34 locus in Triticum aestivum (L.) and characterization of large germplasm collections support the ABC transporter as essential for gene function. Theor. Appl. Genet. 121:373-384.

Das, M.K., W.E. Kronstad, C.C. Mundt, and S. Rajaram. 2004. Number of genes controlling slow rusting resistance to leaf rust in five spring wheat cultivars. Ann. Appl. Biol. 145:91-94.

Dedryver, F., S. Paillard, S. Mallard, O. Robert, M. Trottet, S. Nègre, G. Verplancke, and J. Jahier. 2009. Characterization of Genetic Components Involved in Durable Resistance to Stripe Rust in the Bread Wheat 'Renan'. Phytopathology 99:968-973.

Dolores Vazquez, M., C. James Peterson, O. Riera-Lizarazu, X. Chen, A. Heesacker, K. Ammar, J. Crossa, and C. Mundt. 2012. Genetic analysis of adult plant, quantitative resistance to stripe rust in wheat cultivar 'Stephens' in multi-environment trials. Theor. Appl. Genet. 124:1-11.

Dubin, H.J., and E. Torres. 1981. Causes and consequences of the 1976-77 wehat leaf rust epidemic in Northwest Mexico. Annu. Rev. Phytopathol. 19:41-49.

Dubin, H.J., and J.P. Brennan. 2009. Combating stem and leaf rust of wheat: Historical perspective, impacts, and lessons learned. IFPRI Disscussion Paper. International Food Policy Research Institute, Washington, D.C.

Dyck, P.L. 1987. The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. Genome 29:467-469.

Dyck, P.L., and E.R. Kerber. 1985. Resistance of the Race-Specific Type. In: A. P. Roelfs and W. R. Bushnell, eds. The Cereal Rusts, Volume II; Diseases, Distribution, Epidemiology, and Control. Academic Press, Orlando, FL. p. 469-500.

Dyck, P.L., E.R. Kerber, and T. Aung. 1994. An interchromosomal reciprocal translocation in wheat involving leaf rust resistance gene Lr34. Genome 37:556-559.

Ellingboe, A.H. 1975. Horizontal resistance: An artifact of experimental procedure? Aust. Plant Pathol. Soc. Newsl. 4:44-46.

Eriksen, L., F. Afshari, M.J. Christiansen, R.A. McIntosh, A. Jahoor, and C.R. Wellings. 2004. Yr32 for resistance to stripe (yellow) rust present in the wheat cultivar Carstens V. Theor. Appl. Genet. 108:567-575.

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. 1942. Inheritance of paghogenicity of *Melampsora lini*. Phytopathology 32:653-669.

Flor, H.H. 1971. Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. 9:275-296.

Fu, D., C. Uauy, A. Distelfeld, A. Blechl, L. Epstein, X. Chen, H. Sela, T. Fahima, and J. Dubcovsky. 2009. A Kinase-START Gene Confers Temperature-Dependent Resistance to Wheat Stripe Rust. Science 323:1357-1360.

Gavinlertvatana, S., and R.D. Wilcoxson. 1978. Inheritance of slow rusting of spring wheat by *Puccinia recondita* f.sp. *tritici* and host parasite relationships. Trans. Br. Mycol. Soc. 71:413-418.

German S., M. Kohli, M. Chaves, A. Barcellos et al. 2004. Breakdown of resistance of wheat cultivars and estimated losses caused by recent changes in the leaf rust population in South America. In: Proceedings of 11th international cereal rusts and powdery mildews conference abstract, John Innes Center, Norwich, UK. pp. A2.21.

German, S., A. Barcellos, M. Chaves, M. Kohli, P. Campos, and L. de Viedma. 2007. The situation of common wheat rusts in the Southern Cone of America and perspectives for control. Aust. J. Agric. Res. 58:620-630.

Guo, Q., Z.J. Zhang, Y.B. Xu, G.H. Li, J. Feng, and Y. Zhou. 2008. Quantitative Trait Loci for High-Temperature Adult-Plant and Slow-Rusting Resistance to *Puccinia striiformis* f. sp. *tritici* in Wheat Cultivars. Phytopathology 98:803-809.

Hanson, H., N.E. Borlaug, and R.G. Anderson. 1982. Wheat in the Third World. Westview Press, Boulder, Colorado, USA.

Hao, Y., Z. Chen, Y. Wang, D. Bland, J. Buck, G. Brown-Guedira, and J. Johnson. 2011. Characterization of a major QTL for adult plant resistance to stripe rust in US soft red winter wheat. Theor. Appl. Genet. 123:1401-1411.

Hart, G.E., M.D. Gale, and R.A. McIntosh. 1993. Linkage mas of *Triticum aestivum* (hexaploid wheat, 2n=42, genomes A, B,and D) and *T. tauschii* (2n=14, genome D). In: S. J. O'Brien, ed. Genetic maps: locus maps of complex genomes. Cold Spring Harbor Laboratory Press, Cold Spring Harbor. p. 6.204-6.219.

Hendrix, J.W. 1994. Stripe rust, what it is and what to do about it. Wash. State Univ. Stn. Circ. 424:1-6.

Herrera-Foessel, S. A., R.P. Singh, J. Huerta-Espino, J. Crossa, A. Djurle, and J. Yuen. 2008. Genetic Analysis of Slow-Rusting Resistance to Leaf Rust in Durum Wheat. Crop Sci. 48:2132-2140.

Herrera-Foessel, S., E. Lagudah, J. Huerta-Espino, M. Hayden, H. Bariana, D. Singh, and R. Singh. 2011. New slow-rusting leaf rust and stripe rust resistance genes *Lr67* and *Yr46* in wheat are pleiotropic or closely linked. Theor. Appl. Genet. 122:239-249.

Herrera-Foessel, S., R. Singh, J. Huerta-Espino, G. Rosewarne, S. Periyannan, L. Viccars, V. Calvo-Salazar, C. Lan, and E. Lagudah. 2012. Lr68: a new gene conferring slow rusting resistance to leaf rust in wheat. Theor. Appl. Genet. DOI: 10.1007/s00122-012-1802-1

Heun, M., B. Friebe, and W. Bushuk. 1990. Chromosomal Location of the Powdery Mildew Resistance Gene of Amigo Wheat. Phytopathology 80:1129-1133.

Hiebert, C.W., J.B. Thomas, B.D. McCallum, D.G. Humphreys, R.M. Depauw, M.J. Hayden, R. Mago, W. Schnippenkoetter, and W. Spielmeyer. 2011. An introgression on wheat chromosome 4DL in RL6077 (Thatcher*6/PI 250413) confers adult plant resistance to stripe rust and leaf rust (Lr67). Theor. Appl. Genet. 121:1083-91.

Huerta-Espino, J., R. Singh, Germ, S. n, B. McCallum, R. Park, W. Chen, S. Bhardwaj, and H. Goyeau. 2011. Global status of wheat leaf rust caused by *Puccinia triticina*. Euphytica 179:143-160.

Irfaq, M., T. Mohammad, S.J.A. Shah, M. Ajab, and G.S.S. Khattak. 2009. Genetic Behavior of Controlling Area Under Disease Progress Curve for Stripe Rust (Puccinia striiformis f. sp. tritici) in Two Wheat (*Triticum aestivum*) Crosses. Phytopathology 99.

Jaccoud, D., K. Peng, D. Feinstein, and A. Kilian. 2001. Diversity arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res. 29: e25

Jagger, L., C. Newell, S. Berry, R. MacCormack, and L. Boyd. 2011. The genetic characterisation of stripe rust resistance in the German wheat cultivar Alcedo. Theor. Appl. Genet. 122:723-733.

Jin, Y., M. Carson, and L.J. Szabo. 2010. Century-Old Mystery of Puccinia striiformis Life History Solved with the Identification of Berberis as an Alternate Host. Phytopathology 100:432-435.

Johnson, R. 1978. Practical breeding for durable resistance to rust diseases in self-pollinating cereals. Euphytica 27:529-540.

Johnson, R. 1980. Genetics of adult plant resistance to stripe rust in winter wheat cultivars. In: Proc. 5th Eur. and Mediter. Cereal Rusts Conf., Bari and Rome, Italy, p59-63.

Johnson, R. 1984. A Critical Analysis of Durable Resistance. Annu. Rev. Phytopathol. 22:309-330.

Johnston, C.O. 1931. Effect of leaf rust infection on yield of certain varieties of wheat. J. Am. Soc. Agron. 23:1-12.

Khanna, R., U.K. Bansal, and R.G. Saini. 2005. Genetics of adult plant stripe rust resistance in CSP44, a selection from Australian wheat. J. Genet. 84:337-340.

Khlestkina, E., T. Pshenichnikova, M. Röder, E. Salina, V. Arbuzova, and A. Börner. 2006. Comparative mapping of genes for glume colouration and pubescence in hexaploid wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 113:801-807.

Kislev, M.E. 1982. Stem rust of wheat 3300 years old found in Israel. Science 216:993-994.

Knapp, S. J., W. W. Stroup and W. M. Ross. 1985. Exact Confidence Intervals for Heritability on a Progeny Mean Basis. Crop Sci. 25:192-194.

Knapp, S.J., W.W. Stroup, and W.M. Ross. 1985. Exact Confidence Intervals for Heritability on a Progeny Mean Basis1. Crop Sci. 25:192-194.

Kolmer, J.A., D.L. Long, and M.E. Hughes. 2007. Physiological specialization specialization of Puccinia triticina on wheat in the United States in 2005. Plant Dis. 91:979-984.

Kolmer, J.A., R.P. Singh, D.F. Garvin, L. Viccars, H.M. William, J. Huerta-Espino, F.C. Ogbonnaya, H. Raman, S. Orford, H.S. Bariana, and E.S. Lagudah. 2008. Analysis of the Rust Resistance Region in Wheat Germplasm. Crop Sci. 48:1841-1852.

Kolmer, J.A., X. Chen, and Y. Jin. 2009. Diseases which challenge global wheat production-the wheat rust. In: B. F. Carver, ed. Wheat Science and Trade. Wiley-Blackwell, Ames, Iowa. p. 89-124.

Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugen. 12:172-175

Krattinger, S.G., E.S. Lagudah, W. Spielmeyer, R.P. Singh, J. Huerta-Espino, H. McFadden, E. Bossolini, L.L. Selter, and B. Keller. 2009. A Putative ABC Transporter Confers Durable Resistance to Multiple Fungal Pathogens in Wheat. Science 323:1360-1363.

Kuhn, R.C., G.E. Shaner, and H.W. Ohm. 1978. Slow leaf rusting resistance in wheat against twenty two isolates of *Puccinia recondita*. Phytopathology 61: 651-656.

Kuhn, R.C., H.W. Ohm, and G. Shaner. 1980. Inheritance of Slow Leaf-rusting Resistance in Suwon 85 Wheat. Crop Sci. 20:655-659.

Kuraparthy, V., P. Chhuneja, H. Dhaliwal, S. Kaur, R. Bowden, and B. Gill. 2007. Characterization and mapping of cryptic alien introgression from *Aegilops*

geniculata with new leaf rust and stripe rust resistance genes Lr57 and Yr40 in wheat. Theor. Appl. Genet. 114:1379-1389.

Lagudah, E. 2011. Molecular genetics of race non-specific rust resistance in wheat. Euphytica 179:81-91.

Lagudah, E., S. Krattinger, S. Herrera-Foessel, R. Singh, J. Huerta-Espino, W. Spielmeyer, G. Brown-Guedira, L. Selter, and B. Keller. 2009. Gene-specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance to multiple fungal pathogens. Theor. Appl. Genet. 119:889-898.

Lagudah, E.S., H.S. Bariana, W. Spielmeyer, J. Huerta-Espino, H. McFadden, and R.P. Singh. 2006. Molecular genetic characterization of the Lr34/Yr18 slow rusting resistance gene region in wheat . Theor. Appl. Genet. 114:21-30.

Lan, C.X., S.S. Liang, X.C. Zhou, G. Zhou, Q.L. Lu, X.C. Xia, and Z.H. He. 2010. Identification of Genomic Regions Controlling Adult-Plant Stripe Rust Resistance in Chinese Landrace Pingyuan 50 Through Bulked Segregant Analysis. Phytopathology 100:313-318.

Law, C.N. 1976. Genetic control of yellow rust resistance in *T. spelta album*. Plant Breeding Institute, Cambridge. Annu. Rep. 1975. p. 108–109.

Lazar, M.D., W.D. Worrall, G.L. Peterson, A.K. Fritz, D. Marshall, L.R. Nelson, and L.W. Rooney. 2004. Registration of 'TAM 111' Wheat. Crop Sci. 44:355-356.

Li, H., J.-M. Ribaut, Z. Li, and J. Wang. 2008. Inclusive composite interval mapping (ICIM) for digenic epistasis of quantitative traits in biparental populations. Theor. Appl. Genet. 116:243-260.

LiJun Z., Z., Lillemo, M., XianChun X., DaQun L., WenXiang Y., JiaChuan L., Wang HaiYan W. 2009. QTL mapping for adult-plant resistance to leaf rust in CIMMYT wheat cultivar Saar. Sci. Agric. Sin. 42:388-397.

Lillemo, M., B. Asalf, R. Singh, J. Huerta-Espino, X. Chen, Z. He, and Å. Bjørnstad. 2008. The adult plant rust resistance loci Lr34/Yr18 and Lr46/Yr29 are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor. Appl. Genet. 116:1155-1166.

Lillemo, M., M.v. Ginkel, R.P. Singh, and H. Skinnes. 2006. Genetic analysis of partial resistance to powdery mildew in bread wheat line Saar. Plant Dis. 90:225-228.

Lillemo, M., R. P. Singh, M. William, S. Herrera-Foessel, J. Huerta-Espino, S. German, P. Campos, M. Chaves, R. Madriaga, X. C. Xia, S. S. Liang, D. Liu, Z. F. Li, and E. S. Lagudah. 2011. Multiple rust resistance and gene additivity in wheat: Lessons from multi-location case studies in cultivars Parula and Saar. In: R. McIntosh, ed., Proceedings-Borlaug Global Rust Initiative 2011 Technical Workshop, June 13-16, Saint Paul, Minnesota, U.S.A. p. 11-120.

Lillemo, M., S. Bjørnstad, H. Skinnes. 2012. Molecular mapping of partial resistance to powdery mildew in winter wheat cultivar Folke. Euphytica, DOI 10.1007/s10681-011-0620-x

Lin, F., and X.M. Chen. 2007. Genetics and molecular mapping of genes for race-specific all-stage resistance and non-race-specific high-temperature adult-plant resistance to stripe rust in spring wheat cultivar Alpowa. Theor. Appl. Genet. 114:1277-1287.

Line, R.F. 1976. Factors contributing to an epidemic of rstripe rust on wheat in the Sacramento Valley of Californina in 1974. Plant Dis. Rep. 60:312-316.

Long, D.L., K.J. Leonard, and M.E. Hughes. 2000. Virulence of Puccinia triticina on wheat in the Univted States from 1996 to 1998. Plant Dis. 84:1334-1341.

Lowe, I., L. Jankuloski, S. Chao, X. Chen, D. See, and J. Dubcovsky. 2011. Mapping and validation of QTL which confer partial resistance to broadly virulent post-2000 North American races of stripe rust in hexaploid wheat. Theor. Appl. Genet. 123:143-157.

Macer, R. 1966. The formal and monosomic genetic analysis of stripe rust (*Puccinia striiformis*) resistance in wheat. Hereditas [Suppl 2]:127-142.

Mago, R., G.J. Lawrence, A.J. Pryor, J.G. Ellis, H.S. Bariana, I.S. Dundas, and W. Spielmeyer. 2005. Development of PCR markers for the selection of wheat stem rust resistance genes Sr24 and Sr26 in diverse wheat germplasm. Theor. Appl. Genet. 111:496-504.

Maines, E.B. 1930. Effect of leaf rust (*Puccinia tritician* Eriks.) on yield of wheat. J. Agric. Res. 40:417-446.

Mallard, S., D. Gaudet, A. Aldeia, C. Abelard, A.L. Besnard, P. Sourdille, and F. Dedryver. 2005. Genetic analysis of durable resistance to yellow rust in bread wheat. Theor. Appl. Genet. 110:1401-1409.

Martínez, F., R.E. Niks, R.P. Singh, and D. Rubiales. 2001. Characterization of Lr46, a Gene Conferring Partial Resistance to Wheat Leaf Rust. Hereditas 135:111-114.

McIntosh, R. A. 2009. History and status of the wheat rusts. In: R.A. McIntosh, ed., Proceeding of Borlaug Global Rust Initiative (BGRI) 2009 Technical Workshop, Cd Obregon, Mexico, pp11-23.

McIntosh, R.A. (ed.) 2009. Proceeding of Borlaug Global Rust Initiative (BGRI) 2009 Technical Workshop, Cd Obregon, Mexico.

Mcintosh, R.A. 1992. Close Genetic-Linkage of Genes Conferring Adult-Plant Resistance to Leaf Rust and Stripe Rust in Wheat. Plant Pathol. 41:523-527.

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

McIntosh, R.A., Y.Yamazaki, J.Dubcovsky, J.Rogers, C.Morris, D.J.Somers, R.Appels and K.M.Devos. 2010. MacGene-2010: Catalogue of Gene Symbols for Wheat. Avaialable at Wheat Genetic Resource Databases/KOMUGI, URL: http://www.shigen.nig.ac.jp/ wheat/komugi/genes/download.jsp (accessed on 18 Mar. 2012).

McNeal F.H., K.C.F., Smith E.P., Tate W.S., Russel T.S. 1971. A uniform system for recording cereal research data. USDA-ARS Bull.

Melichar, J.P.E., R. MacCormack, L.A. Boyd, S. Berry, and C. Newell. 2008. QTL identification and microphenotype characterisation of the developmentally regulated yellow rust resistance in the UK wheat cultivar Guardian. Theor. Appl. Genet. 117:391-399.

Messmer, M.M., R. Seyfarth, M. Keller, G. Schachermayr, M. Winzeler, S. Zanetti, C. Feuillet, and B. Keller. 2000. Genetic analysis of durable leaf rust resistance in winter wheat. Theor. Appl. Genet. 100:419-431.

Milus, E.A., and R.F. Line. 1986. Number of Genes-Controlling High-Temperature, Adult-Plant Resistance to Stripe Rust in Wheat. Phytopathology 76:93-96.

Mulitze, D.K., and R.J. Baker. 1985. Genotype Assay and Method of Moments Analyses of Five Quantitative Traits in a Spring Wheat Cross. Crop Sci. 25:162-167.

Navabi, A., J.P. Tewari, R.P. Singh, B. McCallum, A. Laroche, and K.G. Briggs. 2005. Inheritance and QTL analysis of durable resistance to stripe and leaf rusts in an Australian cultivar, *Triticum aestivum* 'Cook'. Genome 48:97-107.

Navabi, A., K.G. Briggs, J.P. Tewari, and R.P. Singh. 2003. Genetic analysis of adult-plant resistance to leaf rust in five spring wheat genotypes. Plant Dis. 87:1522-1529.

Navabi, A., R.P. Singh, J.P. Tewari, and K.G. Briggs. 2004. Inheritance of high levels of adult-plant resistance to stripe rust in five spring wheat genotypes. Crop Sci. 44:1156-1162.

Nelson, J.C., R.P. Singh, J.E. Autrique, and M.E. Sorrells. 1997. Mapping Genes Conferring and Suppressing Leaf Rust Resistance in Wheat. Crop Sci. 37:1928-1935.

Nelson, R.R. 1978. Genetics of Horizontal Resistance to Plant Diseases. Annu. Rev. Phytopathol. 16:359-378.

Ohm, H.W., and G.E. Shaner. 1976. Three components of slow leaf rusting at different growth stages in wheat. Phytopathology 66:1356-1360.

Parlevliet, J.E. 1979. Components of resistance that redue the rate of epidemic development. Annu. Rev. Phytopathol. 17:203-222.

Parlevliet, J.E. 1985. Resistance of the Non-Race-Specific Type. In: A. P. Roelfs and W. R. Bushnell, eds., The Cereal Rusts, Volume II; Diseases, Distribution, Epidemiology, and Control. Academic Press, Orlando. p. 501-525.

Pestsova, E., M.W. Ganal, and M.S. Roder. 2000. Isolation and mapping of microsatellite markers specific for the D genome of bread. Wheat Genome 43:689-697

Peterson, R.F., A.B. Campbell, and A.E. Hannah. 1948. A diagrammatic scale for estimating rust severity on leaves and stems of cereals. Can. J. Res. C. 26:496-500.

Peturson, B., M. Newton, and A.G.O. Whiteside. 1945. The effect of leaf rust on the yield and quality of wheat. Can. J. Res. 23:105-114.

Pope, W.K., E.L. Sharp, and H.S. Fenwick. 1963. Stripe rust of wheat in the pacific Northwest in 1962. Plant Dis. Rep. 47:554-555.

Prins, R., Z. Pretorius, C. Bender, and A. Lehmensiek. 2011. QTL mapping of stripe, leaf and stem rust resistance genes in a Kariega × Avocet S doubled haploid wheat population. Mol. Breed. 27:259-270.

Ramanujam, S. (ed.). 1976. 5th International Wheat Genetic Symposium, New Delhi, India. Indian Society of Genetics and Plant Breeding.

Ramburan, V.P., Z.A. Pretorius, J.H. Louw, L.A. Boyd, P.H. Smith, W.H. Boshoff, and R. Prins. 2004. A genetic analysis of adult plant resistance to stripe rust in the wheat cultivar Kariega. Theor. Appl. Genet. 108:1426-33.

Robert, O., C. Abelard, and F. Dedryver. 1999. Identification of molecular markers for the detection of the yellow rust resistance gene Yr17 in wheat. Mol. Breed. 5:167-175.

Robinson, R.A. 1976. Plant Pathosystems Springer-Verlag, Berlin and New York.

Röder, 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

Roelfs, A.P., and W.R. Bushnell (eds.). 1985. The Cereal Rusts, Vol. II: Diseases, Distribution, Epidemiology, and Control. Academic Press, Orlando.

Roelfs, A.P., R.P. Singh, Saari E.E. 1992. Rust Disease of Wheat: Concepts and methods of disease management. CIMMYT, Mexico, DF.

Roelfs, A.P. 1978. Estimated losses caused by rust in small grain cereals in the United States-1918-1976. Misc. Pub. U.S. Dept. Agric. 1363:1-85.

Roelfs, A.P. 1985. Wheat and rye stem rsut. In: A. P. Roelfs and W. R. Bushnell, eds., The Cereal Rusts, Volume II; Diseases, Distribution, Epidemiology, and Control. Academic Press, Orlando.

Roelfs, A.P. 1989. Epidemiology of the cereal rusts in North America. Can. J. Plant Pathol. 11:86-90.

Rosewarne, G., R. Singh, J. Huerta-Espino, and G. Rebetzke. 2008. Quantitative trait loci for slow-rusting resistance in wheat to leaf rust and stripe rust identified with multi-environment analysis. Theor. Appl. Genet. 116:1027-1034.

Rosewarne, G.M., S. Cloutier, H. McFadden, E.S. Lagudah, S. Bouchet, R.P. Singh, J. Huerta-Espino, and H.M. William. 2006. Leaf tip necrosis, molecular markers and beta1-proteasome subunits associated with the slow rusting resistance genes Lr46/Yr29. Theor. Appl. Genet. 112:500-508.

Samborski, D.J. 1985. Wheat leaf rust. In: A. P. Roelfs and W. R. Bushnell, eds., The Cereal Rusts, Volume II; Diseases, Distribution, Epidemiology, and Control. Academic Press, Orlando.

Samborski, D.J., and P.L. Dyck. 1976. Inheritance of virulence in *Puccinia recondita* on six backcross lines of wheat with single genes for resistance to leaf rust. Can. J. Bot. 54:1666-1671.

Santra, D.K., K.G. Campbell, K.K. Kidwell, X.M. Chen, and M. Santra. 2008. Identification and mapping QTL for high-temperature adult-plant resistance to stripe rust in winter wheat (*Triticum aestivum* L.) cultivar 'Stephens'. Theor. Appl. Genet. 117:793-802.

Schafer, J.F., A.P. Roelfs, and W.R. Bushnell. 1984. Contributions of early scientists to knoweledge of cereal rusts. In: W. R. Bushnell and A. P. Roelfs, eds., The Cereal Rusts, Volume II; Origins, Specificity, Structure, and Physiology. Academic Press, Orlando.

Schnurbusch, T., B. Keller, M. Messmer, and E. Bossolini. 2004. Tagging and validation of a major quantitative trait locus for leaf rust resistance and leaf tip necrosis in winter wheat cultivar forno. Phytopathology 94:1036-1041.

Sears, R.G., J.M. Moffatt, J.M. Martin, T.S. Cox, R.K. Bequette, S.P. Curran, O.K. Chung, W.F. Heer, J.H. Long, and M.D. Witt. 1997. Jagger wheat. Crop Sci. 37:1010.

Shaner, G., and R.L. Powelson. 1971. Epidemiology of stripe rustof wheat, 1961-1968. Tech. Bull. 117. Agricultural Experiment Station, Oregon State University, Oregon.

Singh, D., H.S. Bariana, J.W. Snape, J. Simmonds, and R.F. Park. 2009a. Inheritance and QTL mapping of leaf rust resistance in the European winter wheat cultivar 'Beaver'. Euphytica 169:253-261.

Singh, D., R.F. Park, R.A. McIntosh and H.S. Bariana. 2008. Characterization of stem rust and stripe rust seedling resistance genes in selected wheat cultivars from the United Kingdom. J. Plant Pathol. 90:553-562.

Singh, R. P., A. Mujeeb-Kazi and J. Huerta-Espino. 1998. *Lr46*: A Gene Conferring Slow-Rusting Resistance to Leaf Rust in Wheat. Phytopathology 88:890-894.

Singh, R.P. 1992. Genetic Association of Leaf Rust Resistance Gene Lr34 with Adult-Plant Resistance to Stripe Rust in Bread Wheat. Phytopathology 82:835-838.

Singh, R.P., A. Mujeeb-Kazi, and J. Huerta-Espino. 1998. Lr46: A Gene Conferring Slow-Rusting Resistance to Leaf Rust in Wheat. Phytopathology 88:890-894.

Singh, R.P., and J. Huerta-Espino. 1995. Inheritance of seedling and adult plant resistance to leaf rust in wheat cultivars Ciano 79 and Papago 86. Plant Dis. 79:35-38.

Singh, R.P., and S. Rajaram. 1992. Genetics of Adult-Plant Resistance of Leaf Rust in Frontana and 3 Cimmyt Wheats. Genome 35:24-31.

Singh, R.P., and S. Rajaram. 1993. Genetics of Adult-Plant Resistance to Stripe Rust in 10 Spring Bread Wheats. Euphytica 72:1-7.

Singh, R.P., D.P. Hodson, J. Huerta-Espino, Y. Jin, S. Bhavani, P. Njau, S. Herrera-Foessel, P.K. Singh, S. Singh, and V. Govindan. 2011b. The Emergence of Ug99 Races of the Stem Rust Fungus is a Threat to World Wheat Production. Annu. Rev. Phytopathol. 49:465-481

Singh, R.P., H.M. William, J. Huerta-Espino, G. Rosewarne. 2004. Wheat rust in Asia: meeting the challlenges with old and new technologies. In: 4th Int Crop Science Cong, 26 Sep- 1Oct 2004, Brisbane, Australia. p.1-13. URL: http://www.cropscience.org.au/icsc2004/ symposia/3/7/141_singhrp.htm

Singh, R.P., J. Huerta-Espino, and H.M. William. 2005. Genetics and Breeding for Durable Resistance to Leaf and Stripe Rusts in Wheat. Turk. J. Agric. For. 29:121-127.

Singh, R.P., J. Huerta-Espino, and M. William. 2001. Slow rusting gene based resistance to leaf and yellow rusts in wheat: genetics and breeding at CIMMYT. In: 10th Assembly proceedings of the Wheat Breeding Society of Australia Inc., Mildura, Australia. 16th-21st September 2001. p.103–108

Singh, R.P., J. Huerta-Espino, and S. Rajaram. 2000a. Achieving near-immunity to leaf and stripe rusts in wheat by combining slow rusting resistance genes. Acta Phytopathol. Entomol. Hung. 35:133-139

Singh, R.P., J. Huerta-Espino, S. Bhavani, D. Singh, P.K. Singh, S.A. Herrera-Foessel, P. Njau, R. Wanyera, and Y. Jin. 2009b. Breeding for minor gene-based adult plant resistance to stem rust in wheat. Paper presented at 2009 Technical Workshop: Borlaug Rust Initiative, Cd. Obregon, Sonora, Mexico, March 17-20, 2009.

Singh, R.P., J. Huerta-Espino, S. Bhavani, S. Herrera-Foessel, D. Singh, P. Singh, G. Velu, R. Mason, Y. Jin, P. Njau, and J. Crossa. 2011. Race non-specific resistance to rust diseases in CIMMYT spring wheats. Euphytica 179:175-186.

Singh, R.P., M.E. Sorrells, and J.C. Nelson. 2000b. Mapping of Yr28 and other genes for resistance to stripe rust in wheat. Crop Sci. 40:1148-1155.

Singh, R.P., S. Rajaram, and H. Ma. 1995. Genetic analysis of resistance to scab in spring wheat cultivar Frontana. Plant Dis. 79:238-240.

Skovmand, B., R.D. Wilcoxson, B.L. Shearer, and R.E. Stucker. 1978. Inheritance of slow rusting to stem rust in wheat. Euphytica 27:95-107.

Smith, P.H., J. Hadfield, N.J. Hart, R.M.D. Koebner, and L.A. Boyd. 2007. STS markers for the wheat yellow rust resistance gene Yr5 suggest a NBS-LRR-type resistance gene cluster. Genome 50:259-265.

Somers, D., P. Isaac, and K. Edwards. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 109:1105-1114.

Song, Q.J., E.W. Fickus, P.B. Cregan. 2002. Characterizationof trinucleotide SSR motifs in wheat. Theor. Appl. Genet. 104:286–293

Sourdille, P., M. Tavaud, G. Charmet, M. Bernard. 2001. Transferability of wheat microsatellite to diploid Triticeae species carrying the A, B and D genomes. Theor. Appl. Genet. 103:346–352

Spielmeyer, W., E.S. Lagudah, and P.J. Sharp. 2003. Identification and validation of markers linked to broad-spectrum stem rust resistance gene Sr2 in wheat (*Triticum aestivum* L.). Crop Sci. 43:333-336.

Spielmeyer, W., R.A. McIntosh, J. Kolmer, and E.S. Lagudah. 2005. Powdery mildew resistance and Lr34/Yr18 genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. Theor. Appl. Genet. 111:731-735.

Stubbs, R.W. 1985. Stripe rust. In: A. P. Roelfs and W. R. Bushnell, eds., The Cereal Rusts. Vol. II. Academic Press, Orlando, Florida.

Suenaga, K., H.M. William, J. Huerta-Espino, and R.P. Singh. 2003. Microsatellite markers for genes Lr34/Yr18 and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93:881-890.

Sun, X., R. Bowden, B.F. Carver, and G. Bai. 2010. Molecular Mapping of Wheat Leaf Rust Resistance Gene Lr42. Crop Sci. 50:59-66.

Ti-Lin, F., C. Ying, L. Gen-Qua, X. Shi-Chang, X. Chao-Jie, Y. Ming-Shan. 2008. Molecular Characterization of a Stripe Rust Resistance Gene from Wheat Line S2199 and Its Allelism with Yr5. Acta Agron. Sin. 34:355-360.

Tollenaar, H., and B.R. Houston. 1966. Effect of temperature during uredospore production and of light on in vitro germination of uredospores from *Puccinia striiformis*. Phytopathology 56:787-789.

Uauy, C., J.C. Brevis, X.M. Chen, I. Khan, L. Jackson, O. Chicaiza, A. Distelfeld, T. Fahima, and J. Dubcovsky. 2005. High-temperature adult-plant (HTAP) stripe rust resistance gene Yr36 from Triticum turgidum ssp dicoccoides is closely linked to the grain protein content locus Gpc-B1. Theor. Appl. Genet. 112:97-105.

Vanderplank, J.E. 1968. Disease Resistance in Plants. Academic Presss, New York.

Voorrips, R.E. 2002. MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs. J. Hered. 93:77-78.

Wang, S., C. J. Basten, and Z.-B. Zeng. 2011. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Wellings, C. 2011. Global status of stripe rust: a review of historical and current threats. Euphytica 179:129-141.

Wellings, C.R., R.A. McIntosh, and M. Hussain. 1988. A New Source of Resistance to *Puccinia striiformis* f. sp. *tritici* in Spring Wheats (*Triticum aestivum*). Plant Breed. 100:88-96.

Weng, Y., W. Li, R.N. Devkota, and J.C. Rudd. 2005. Microsatellite markers associated with two Aegilops tauschii-derived greenbug resistance loci in wheat. Theor. Appl. Genet. 110:462-469.

William, H.M., D. Hoisington, R.P. Singh, and D. González-de-León. 1997. Detection of quantitative trait loci associated with leaf rust resistance in bread wheat. Genome 40:253-260.

William, H.M., R.P. Singh, J. Huerta-Espino, G. Palacios, and K. Suenaga. 2006. Characterization of genetic loci conferring adult plant resistance to leaf rust and stripe rust in spring wheat. Genome 49:977-990.

William, M., R. P. Singh, J. Huerta-Espino, S. O. Islas and D. Hoisington. 2003. Molecular Marker Mapping of Leaf Rust Resistance Gene *Lr46* and Its Association with Stripe Rust Resistance Gene *Yr29* in Wheat. Phytopathology 93:153-159.

Worland, A.J., and C.N. Law. 1986. Genetic Analysis of Chromosome 2D of Wheat I. The Location of Genes Affecting Height, Day-Length Insesitivity, Hybrid Dwarfism and Yellow-Rust Resistance. Z. Pflanzenzuchtg 96:331-345.

Wright, S. 1968. Evolution and genetics of populations Genetic and Biometric foundations. University of Chicago press, Chicago.

Xu, X., G. Bai, B.F. Carver, G.E. Shaner, and R.M. Hunger. 2005. Molecular Characterization of Slow Leaf-Rusting Resistance in Wheat. Crop Sci. 45:758-765.

Yan, G.Y., X.C. Chen, R.L. Line, and C.W. Wellings. 2003. Resistance gene-analog polymorphism markers co-segregating with the *YR5* gene for resistance to wheat stripe rust. Theor. Appl. Genet. 106:636-643.

Zhang, J.X., R.P. Singh, J.A. Kohner, J. Huerta-Espino, Y. Jin, and J.A. Anderson. 2008. Genetics of leaf rust resistance in brambling wheat. Plant Dis. 92:1111-1118.

Zhang, Z.J., S.L. Jin, X.B. Yang, G.H. Yang, and G.H. Li. 2001. Transgressive segregation, heritability, and number of genes controlling durable resistance to stripe rust in one Chinese and two Italian wheat cultivars. Phytopathology 91:680-686.

VITA

Name:	Bhoja R. Basnet
Country of Origin:	Nepal
Address (Nepal):	Damak-2, Jhapa, Nepal
Address (USA):	415 College Main # 7, College Station, TX, 77840, USA
Email Address:	bbasnet@ag.tamu.edu; br_basnet38@hotmail.com
Education:	B.Sc., Agriculture, Tribhuwan University, Nepal, 2004 M.S., Plant Science, South Dakota State University, 2008 Ph.D., Plant Breeding, Texas A&M University, 2012
Interests:	Plant Breeding and Genetics International Agriculture Research and Development