AN ABSTRACT OF THE THESIS OF

Maria Dolores Vazquez for the degree of Master of Science in Crop Science presented on February 25, 2010.

Title: Genetic Factors that Provide Adult Plant Resistance Against *Puccinia striiformis* f. sp. *tritici* to Wheat Cultivar 'Stephens' in a Multilocation Analysis.

Abstract approved:

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C. James Peterson

Stripe rust *(Puccinia striiformis* f. sp. *tritici)* has been receiving increased attention in the USA since the appearance of more virulent races detected in the past decade. These races caused yield losses even in areas where the disease previously was rarely detected. Host plant resistance is the most cost effective and environmentally friendly means of controlling stripe rust. Deployment of single, major genes for resistance has been compromised due to the genetic variability of the pathogen, emphasizing the need to breed for durable resistance. 'Stephens' wheat (*Triticum aestivum* L.) has been

grown commercially in the Pacific Northwest region of the USA for 30 years and shows resistance to *P. striiformis* f. sp. *tritici* “old” and “new” races. The durable resistance of Stephens to stripe rust is believed to be due to a combination of seedling and adult plant resistance genes. Multilocation field trials and molecular genotyping were used to study the genetics of the resistance response in ‘Stephens’. Disease severity, based on the percentage of leaf area infected in field plots, was recorded in eight locations: five in 2008 and three in 2009. Locations could be divided into two mega-environments based on time of appearance of disease and QTL identified. Quantitative trait loci (QTL) analysis identified 11 chromosomal regions associated with resistance to stripe rust in the resistant parent (‘Stephens’) and two regions derived from the susceptible parent (‘Platte’). Many QTL locations coincided to those of previous reports. Furthermore, this study suggests that a combination of additive resistance genes acting at different plant stages is responsible for the durable resistance of ‘Stephens’. Significant QTL x environment interactions were found, suggesting that specificity to plant stage, race, and/or temperature are responsible for different disease responses.

Genetic Factors that Provide Adult Plant Resistance Against *Puccinia striiformis* f. sp. *tritici* to Wheat Cultivar 'Stephens' in a Multilocation Analysis

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Maria Dolores Vazquez, Author

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GENERAL INTRODUCTION

CHAPTER ONE

1.1 Wheat Production

Cereals are world's most important sources of food for direct human consumption and livestock feed .The Food and Agriculture Organization of the United Nations (FAO) urges an increase in food production to feed a human population that is expected to reach 9 billion by 2050 (FAO, 2002). Wheat is the second greatest staple crop in the world, surpassed only by maize (Global Crop Diversity Trust, 2006). In spite of its importance, wheat acreage is declining in the United States (USA). According to the United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS), the area seeded to winter wheat for 2009 was 6.5% less than in 2008, while total corn and soybean acres were on par or even up from 2008 (USDA, NASS, 2009).

In the USA, wheat is a fundamental food crop for the security and economy of the country. The global total of wheat production in 2008 and 2009 was 25 billion bushels on average (FAOSTAT, 2009). Total wheat production in the United States in 2008 was 2.50 billion bushels and 2.21 billion bushels in 2009, with 1.5 billion bushels produced from winter wheat plantings (USDA, ERS, 2009b). The United States produces 10% of the global wheat production and is the world's biggest wheat exporter (USDA, ERS, 2009a). Winter wheat farm-gate value in 2008 was approximately 11 billion dollars at a price of 6.0 dollars per bushel, but water and land constraints, market volatility and low returns relative to other crops make wheat less competitive compared to corn and soybeans (USDA, ERS, 2009a).

1.2 Wheat (*Triticum sp*)

The wide adaptability of wheat is achieved through adjustments to its life cycle to suit local seasonal climatic conditions. Appropriate adjustments to the different phases can avoid winter damage from cold temperatures, and escape drought and high temperatures in the warmer summer months. The major components of the wheat life cycle are the time between planting and emergence; the period after emergence to before the onset of floral initiation (vegetative stage); the length of the period of floral initiation to terminal spikelet; the period between terminal spikelet and heading; and finally, the time of flowering through grain filling to maturity. Although the timing of planting is environmentally determined, most of the other components vary between different varieties, and, hence, are under genetic control (Snape et al., 2001).

A complex group of genes in any wheat genotype influences flower initiation, which may vary from seven weeks to almost a complete year. Those gene groups are vernalization (Vrn genes), photoperiod response (Ppd genes) and earliness per se (Eps genes). Based on vernalization response, wheat varieties can be broadly divided into spring, winter and an intermediate group known as facultative (Worland and Snape, 2000). Vernalization is the exposure to low, non-freezing temperatures, either in natural winter or in artificial cold treatment; it is a survival mechanism to tolerate low temperatures and is required for winter wheat to enter the reproductive stage by inducing flowering (Streck et al., 2003).

Spring type alleles are dominant and are insensitive to cold treatment, which means they will initiate flowering without regard to cold treatment; the recessive winter alleles normally require at least six weeks of vernalization under an optimal temperature of 4°C. Facultative wheat, compared to true winter wheat, is generally less cold tolerant, a shorter vernalization period is required, and flowering starts earlier (Braun and Sãulescu, 2002). Photoperiod and earliness per se affect winter wheat. Photoperiod-sensitive wheat will remain vegetative until day lengths increase in the spring to satisfy photoperiod requirements and enable the plant to initiate flowering. Earliness genes influence flowering time independently of environmental responses (Worland and Snape, 2000).

Wheat is a polyploid with two main species in commercial production. Durum wheat (*Triticum turgidum* L. var durum) is tetraploid wheat with 28 chromosomes and genomes designated AB. It evolved from the ancestral wild emmer *Triticum dicoccoides*, which originated from *Triticum urartu* as donor of A genome (14 chromosomes) and a species related to *Triticum speltoides* that donated the B genome (14 chromosomes). Durum wheat has high protein content; its most important commercial end-use is pasta (Feldman, 2000).

Bread wheat (*Triticum aestivum*), also known as common wheat, is the most widely cultivated wheat species. It is a hexaploid, with 42 chromosomes and genomes designated as ABD. Bread wheat is considered to have originated in northwest Iran or northeast Turkey as a result of a hybridization of tetraploid wheat *Triticum turgidum*, (AB genome) and diploid *Aegilops tauschii*, the donor of the D genome (Feldman, 2000; Bernardo, 2002).

1.3 Wheat (*Triticum sp*) and Rust Diseases (*Puccinia sp*)

Rusts have been a problem for small grains cereals probably since domestication. According to the USDA Agricultural Research Service (ARS), wheat rusts are the most common diseases in the USA and worldwide, causing millions of dollars in losses annually in all wheat market classes (McIntosh, 2009; USDA, ARS, 2009). Rust pathogens adapt to many different types of environments, evolve rapidly and the airborne spores spread quickly over long distances (Hovmøller, 2001). Rust fungi are known as specialized pathogens; each rust species is divided into specialized forms having a specific host genotype to attack under particular environmental conditions (McIntosh, 2009).

The genus *Puccinia* includes three important species of rust fungi that attack wheat. The rust *Puccinia graminis* f. sp. *tritici* causes the stem (black) rust that tends to occur in the warmer, moister regions; *Puccinia triticina* causes the leaf (brown) rust disease that occurs in all wheat-growing areas with moister climates, and *Puccinia striiformis* f. sp. *tritici* causes stripe (yellow) rust that occurs in high rainfall, cooler regions (McIntosh, 1998). Taxonomically, wheat rusts belong to the family Pucciniaceae, order Uredinales, class Urediniomycetes, phylum Basidiomycota

(Bolton et al., 2008). All three rust fungi are obligate parasites, which mean they are completely dependent on living tissue for reproduction and survive by the production of huge amounts of wind-dispersed spores known as urediniospores (Brown and Hovmøller, 2002).

These three rust diseases of wheat occur in the USA and cause millions of dollars annually in yield losses (Kolmer et al., 2009). The rust pathogens of wheat have similarities but also clear differences in terms of life cycle, alternate host, reproduction and biologic forms. Yield losses due to wheat rusts can be substantial depending on the crop development stage, the level of resistance as well the environmental conditions (McIntosh, 2009).

1.3.1 Leaf (brown) Rust (*Puccinia triticina*)

Leaf rust caused by *Puccinia triticina* Eriks is also known as *P. recondita* Roberge ex Desmaz, f. sp. *tritici* or simply brown rust. It occurs nearly wherever wheat is grown and is the most widely distributed disease of wheat in the USA, mainly where temperatures are mild and dew formation is frequent during jointing through the flowering wheat stage. It is disseminated by wind-blown urediniospores produced by uredinia, orange-brown circular pustules seen on both upper and lower leaf surfaces. Temperatures of 15-20°C at night with adequate moisture for dew formation allow urediniospores to germinate. Penetration is through the stomata. Once infection occurs, temperatures of 10-25°C are required for eight to14 days (latent period) until new urediniospores are released to start new infections in susceptible host (Kolmer et al., 2007).

Puccinia triticina is heteroecious, meaning it has a known alternate host. It requires a host (usually wheat) for the telial/uredinial stages and an alternative host (*Thalictrum speciosissimum* or *Isopyrum fumaroides*) for the pycnial/aecial stages, to complete the full life cycle (Bolton et al., 2008). The alternate host contributes to the evolution of leaf races by sexual stage recombinations, but the *Thalictrum* and *Isopyrum* species native to North America are resistant to leaf rust infections and thus in the USA, the alternate host does not play an important role in the origin of the new races. Mutations provide the basic variation that occurs in *P. triticina* in the USA,

since the pathogen persists through reproduction from asexual urediniospores. A single mutation of an avirulent allele would be sufficient for the isolate to gain virulence (Bolton et al., 2008; Kolmer et al., 2007).

The uredinial infections of leaf rust that survive the summer on volunteer wheat in the USA farm fields and roadside ditches are the source of inoculum for infections that become established on autumn-planted wheat in the southern USA. Leaf rust infections on winter wheat can often be found along the southern Gulf Coast and Atlantic seaboard in February. By mid May, leaf rust is usually widespread throughout the soft red winter wheat of the southeastern States and in the hard red winter wheat of the southern Great Plains. Initial infections of leaf rust on winter wheat in the northern Great Plains of Minnesota and the Dakotas are usually detected in the latter half of May (Bolton et al., 2008; Kolmer et al., 2007).

Greater yield losses result when the initial infections occur early in the growing season, especially before the jointing and tillering stages. Most of the losses are due to a reduction in the number of kernels per head and a reduction in the kernel weight. Current yield losses due to leaf rust in the USA, on winter wheat were 4 and 2% for 2007 and 2008, respectively (Long, 2009). In the USA many different leaf rust-resistance genes are present in the various market classes of wheat. The continual release of wheat cultivars with different resistance genes has placed constant selection pressure on the *P.triticina* populations. A compilation of wheat leaf rust genes can be seen in at the USDA-ARS Cereal Disease Laboratory website.

1.3.2 Stem (black) Rust (*Puccinia graminis* Pers. sp.f. *tritici*)

Stem (black) rust occurs worldwide wherever wheat is grown. The damage caused by wheat stem rust can be more impressive than any other cereal disease. Epidemics that occurred in North America in the 1910's and Australia in the 1970's showed the potential destructiveness of wheat stem rust devastating entire fields (Roelfs, 1985).

Puccinia graminis is heteroecius with barberry (*Berberis vulgaris*) as its alternate host. Teliospores germinate producing basidiospores that infect barberry. Five to 10 days later, aeciospores are released; historically these spores were an

important source of inoculum in northern North America and Europe (Kolmer et al., 2007).

The eradication of the barberry in the USA eliminated an important source of inoculum; genetic variation in the fungal population also was reduced by eliminating the sexual cycle leaving only urediniospores to maintain the fungus as the sole inoculum source in the USA. Mutation or asexual recombination in an avirulent isolate is the main means to change from an avirulent to a virulent isolate (Leonard and Szabo, 2005).

Urediniospores overwinter in wheat fields in the southern USA and northern Mexico and are then airborne northward. If the weather is favorable for stem rust development in the South, urediniospores probably will cause epidemics in northern wheat-growing areas. Stem rust is more important where dew is frequent during and after the heading stage and temperatures are warm, 18-30°C. The latent period is favored by hot days 25-30°C and mild nights 15-20°C with adequate moisture help urediniospores to germinate (Leonard and Szabo, 2005).

Damage is greatest when the disease becomes severe before the grain is completely formed. Grain is shriveled due the damage of the conducting tissue, resulting in fewer nutrients being transported to the grain. Severe disease can cause straw breakage, which results in a loss of spikes with combine harvesting. Losses are often severe (50-70%); large areas and individual fields can be destroyed. More than 50% of the wheat yield in North Dakota and Minnesota was lost to stem rust in the worst epidemic in 1935 (Leonard, 2005). Although there has not been a significant wheat stem rust epidemic in the USA since 1974, the pathogen is still present and dangerous. The recently detection of the race Ug99 in Uganda in 1998 challenged the misconception that stem-rust was a conquered disease; up to 80% of world's wheat is now considered stem rust susceptible (Hodson et al., 2009).

1.3.3 Stripe (yellow) Rust (*Puccinia striiformis* f. sp. *tritici*)

Recent evidence suggests that *Puccinia striiformis* f. sp. *tritici* is heteroecius with *Berberis* spp as its alternated host (Yue Jin, Les Szabo, Marty Carson in press).comprised a life cycle where uredial and telial stages are produced on wheat

while pycnia and aecia stages are produced in *Berberis* spp. The fungus can develop virulent pathotypes rapidly to infect wheat cultivars with new sources of resistance (Hovmøller, 2001). The mechanisms by which new variations are created in *P.striiformis* f. sp. *tritici* are not fully understood, but recent discover of sexual recombination and mutation from avirulence to virulence could be considered to occur (Hovmøller et al., 2008).

Puccinia striiformis f. sp. *tritici* prefers cooler and highly humid climates compared to leaf or stem rust disease (McIntosh, 1998), but recently a new strain is proliferating in warmer and drier areas (Hovmøller et al., 2008; Milus et al., 2009).The characteristic symptom is the development of yellow uredia along upper leaf veins with the appearance of yellow stripes. These uredia release urediniospores that can be wind blown over long distances. Stripe rust, compared with many other diseases, depends even more on very specific weather conditions when urediniospores and susceptible host plants are present; night temperature plays a more critical role. Shrunken seeds result from stripe rust infected plants (Chen, 2005).

Once a urediniospore has reached a susceptible host, at least 3 hours of continuous moisture are required on the plant surfaces to germinate and infect plants under an ideal temperature of 12-18°C (Milus et al., 2006). Germination results in a short primary germ tube that recognizes the host surface and subsequently penetrates stomata with a fungal hypha (Mallard et al., 2008). Penetration takes place in darkness through closed stomata (de Vallavieille-Pope et al., 2002). Successful penetration leads to the formation of the first haustorium or intracellular hypha, a specialized rust structure that the fungus forms in host cells to colonize and derive nutrients for spore formation and the subsequence spore release (Niks and Rubiales, 2002). Under optimal conditions, symptoms appear in about one week and sporulation appears after two weeks (Chen, 2005).

In the United States, *P.striiformis* f. sp. *tritici* can overwinter and oversummer in the region of eastern Washington, northern Idaho, and northeastern Oregon. This region has its own local inoculum, but is also influenced by inoculum from outside of the region. The central and northern areas of the Great Plains (Kansas, Nebraska,

South Dakota and North Dakota) usually receive inoculum of stripe rust from the southern Great Plains (Texas and Louisiana). The timing, type, and direction of winds determine the earliness, scale, and development rate of epidemics of stripe rust (Chen, 2005).

Stripe rust or yellow rust is an economically important wheat disease on all continents where wheat is grown (Markell and Milus, 2008). It has been detected in more than 60 countries around the world (Chen, 2005). Severe epidemics have been reported in North America (Chen et al., 2002), Europe (Hovmøller, 2001); South Africa (Boshoff et al., 2002); Midle East (Yahyaoui et al., 2002); Australia (Wellings, 2007) and China (Wan et al., 2007; Zeng and Luo, 2008). Recent epidemics have appeared in warm areas, for example, in the eastern USA where stripe rust was considered a non-significant disease in wheat (Chen, 2005; Milus et al., 2006) or even considered absent as in Western Australia (Wellings et al., 2003).

1.4 Genetics of Pathogen-Host Resistance

Two types of resistance have been recognized in plant-pathogen interactions, first by van der Plank and then by Pavlevliet. These are designated as vertical or race-specific resistance and horizontal or non-race specific resistance (Parlevliet, 2002). Recently, rust pathogens have been described that share similar and complex genetics interaction with their respective hosts (Collins et al., 2007; Dodds et al., 2007).

Resistance for rust pathogens has been divided into seedling or all-stage resistance, which is often race-specific, and in adult plant or post-seedling resistance, which is often non-race-specific. Some sources of adult plant resistance may be race-specific, however (Collins et al., 2007; McIntosh, 2009).Mallard et al. (2008) demonstrated in a molecular study on the expression of wheat cultivar “Camp Remy” with durable resistance to stripe rust, that resistance-related and defense- related genes are differently expressed at the adult plant stage as compared to the seedling stage.

1.4.1 Seedling Resistance

Seedling, or all stage resistance, also known a race-specific resistance, expresses a complete or nearly complete resistance in the host. This type of resistance corresponds to single genes with major effects that are simply inherited and follow the

"gene-for-gene" hypothesis (Flor, 1971) in which an interaction between a dominant resistance allele (R gene) in the host plant and a dominant avirulance allele (Avr gene) of the pathogen induces a rapid activation of defense mechanism often called the hypersensitive response (Parvlevliet, 2002). The hypersensitive response (HR) is characterized by a necrosis or elimination of the penetrated cell or cluster of cells surrounding the penetration site (Collins et al., 2007).

In this system, a resistance gene in a plant genotype is expressed only in the presence of a matching pathogen avirulence gene. The pathogen usually produces elicitors that are recognized by receptors in the host (Bariana et al., 2007). These elicitors or avirulence factors are proteins often containing a leucine-rich repeat domain (LRR) (Dodds et al., 2007). The hypersensitive response of plant tissue typically occurs after the pathogen has penetrated the plant cell wall, and has started to produce a haustorium or an intracellular hypha (Niks and Rubiales, 2002).

Just one mutation may cause a pathogen to become virulent on a host with the matching resistance gene. Virulence in the pathogen is based on a few loci that are under intense selection pressure by resistance genes deployed in cultivars (Markell and Milus, 2008). This simple loss-of-function mutation in the pathogen's avirulence gene would result in "failure" to elicit the HR and therefore the mutant pathogen would successfully infect the host plant (Hovmøller et al., 2008). The mutation of *P. striiformis* f. sp. *tritici* from avirulent to virulent can be either a complete deletion of the avirulence gene; a point mutation resulting in a frame shift and truncation of the gene or substitution of an aminoacid affecting the essential feature of the gene product (Parvlevliet, 2002). Thus it is relatively easy for the pathogen to cause disease in a race-specific cultivar by mutation in a single locus (Hovmøller et al., 2008; Niks and Rubiales, 2002). These resistance genes have been widely used in released cultivars in the past years, but they rarely remain effective in controlling the disease when used for several years over large areas (Hovmøller, 2001; Kaur et al., 2008).

1.4.2 Adult Plant Resistance

Adult plant resistance (APR) or post-seedling resistance is known to be ineffective during seedling stages, but increase in effectiveness with plant age. It is

reported to be non-race-specific and more durable than seedling race-specific resistance against rust pathogen populations. Durable indicates that a cultivar possessing this type of resistance could be widely cultivated and while remaining effective against the pathogen over several years (Parvlevliet, 2002). Adult plant resistance to wheat stripe rust has been described as being temperature-sensitive, thus being called high temperature adult plant resistance (HTAPR) (Chen and Line, 1995a). McIntosh (2009), suggested all APR to stripe rust as temperature-sensitive, with any increases or decreases in sporulation post-flowering caused by changes in the weather.

In plants expressing APR, there are releases of toxic metabolites that cause local inhibition of haustorium formation and hyphal branching. Individual resistant elements of APR produce plant phenotypes with different levels of disease severity, which slow down the rate of disease development (Bariana et al., 2007). The components of slow rusting include a longer latent period, low infection frequency, smaller uredial size, and reduced duration and quantity of spore production due to the frequent failure of haustorium formation (Li et al., 2006; Niks and Rubiales, 2002). Sporulation has been considered to be a key factor of stripe rust epidemiology and as the main component of pathogen aggressiveness, the capacity for a higher epidemic development rate. Sporulation along with latent period, and infection efficiency are components of aggressiveness of *P.striiformis* f. sp. *tritici* (Milus et al., 2006).

Adult plant resistance is based on the additive effects of some to several genes with small effects. This resistance is believed to be of different nature than the hypersensitive reaction, meaning APR does not follow Flor's gene–for–gene model (McIntosh, 1998; Parvlevliet, 2002). APR is believed to be composed in part of constitutive and inducible resistance mechanisms relative to features of the plant where fungal growth is typically terminated before fungi start the process of cell wall penetration. Some of these changes may represent mounting physical or chemical barriers to avoid pathogen entry (Collins et al., 2007). Crop architecture such as upright leaves may affect humidity, leaf wettability and aeration in the crop and, hence, reduce the chances for successful infection by the pathogen. Stomata in some accessions of *Hordeum chilense* are extensively covered by cuticular wax that

prevents rust fungal germ tubes from perceiving the stomata, resulting in failure of penetration of the pathogen into the leaf (Niks and Rubiales, 2002).

Other changes associated with attempted entry of cells and the host responses are described as systemic acquired resistance (SAR) and induced systemic resistance (ISR) as a general phenomenon where chemical signals and local compounds such as opal silica, phenolic compounds, and hydrogen peroxide are produced. Proteins that inhibit fungal enzymes or degrade fungal cell walls also may be involved in APR (Métraux et al., 2001; Oostendorp et al., 2001).

The prehaustorial or pre-cell-wall penetration resistance mechanism has recently been described as the failure of haustorium formation and may lead to slower development rates (longer latency period) and lower spore production. The only reaction visible at the light microscopic level is the local apposition of cell wall material, called a papilla. This papilla may be instrumental in blocking the penetration attempt, but it also may be a reaction to repair the cell wall after the abortion of the penetration attempt by other means (Collins et al., 2007, Nicks and Rubiales, 2002).However, the contributions these biochemical changes and compounds might make to resistance, and the molecular machinery underlying them, are not well known (Collins et al., 2007; Mallard et al., 2008; Tuzun, 2001).

Recent studies in map-based cloning on genes related to partial resistance ("slow rusting") Yr36 and Yr18/Lr34 reported different proteins and resistance mechanism involve. Evidence suggests gene Yr18/Lr34encodes a protein that belongs to the pleiotropic drug resistance subfamily of ABC transporters. Pleiotropic drug resistance transporters are known to confer resistance to various drugs. Current model suggests that PEN3 may be involved in translocating toxic compounds derived from glucosinolates into the apoplast. For the case of gene Yr36 includes a kinase and a START lipid-binding domain probably related to cascade signaling initiation (Fu et al., 2009; Krattinger et al., 2009)

1.5 Stripe Rust History in the United States and Recent Epidemics

Stripe rust *Puccinia striiformis* f. sp. *tritici* was first reported in the United States in Sacaton, Arizona in 1915 by the USDA's visitor scientist Kolpin Ravn from

Denmark. In June of that year, an abundant amount of stripe rust in wheat was found in Oregon and Washington (Carleton, 1915). In subsequent years during the 1920's to 1930's, research was done on occurrence of the disease, life cycle, geographic distribution and host specificity (Line, 2002).

During the 1930's to late 1950's, no severe rust epidemics were reported, therefore stripe rust was considered not to be an important disease in the USA. According to Chen and Line (1995a), stripe rust was diminished because most cultivars grown in the stripe rust locations were resistant (mainly the Pacific Northwest). In the late 1950's, stripe rust was reported in the Great Plains and by early 1960's severe stripe rust epidemics devastated production in California with yield losses estimated at 28-56%; Oregon and Washington with losses close to 25% of total production (7.5 to 15 million bushels); and Idaho and Montana with approximately 1% (Line, 2002; Long, 2009). At that time, stripe rust became the most important disease of wheat in the Pacific Northwest and California but with infrequent occurrences in states east of the Rocky Mountains (Milus et al., 2006).

It is cited that the epidemics in the early 1960's were caused by a change in cultivar, where race-specific resistant cultivars began to be widely used, as well as changes in pathogenic races, crop management and weather (Chen and Line, 1995a; Line, 2002). During late the 1960's and 1970's, breeding for stripe rust resistance was recognized as important and consolidated in many universities. By the early 1980's to 1990's, stripe rust became increasingly important in the south-central states of Arkansas, Louisiana, Kansas, Oklahoma, and Texas (Line, 2002; Line and Qayoum, 1991; Milus et al., 2006).

While stripe rust was considered to occur most in western states of the USA, in 2000 stripe rust became an important problem in the south-central states and Great Plains (Chen et al., 2007). The occurrences after 2000 were in at least 20 states, from the Pacific Northwest and California to Virginia; and from Texas to North Dakota. Yield losses due to the disease were most severe in the south-central USA. Frequent epidemics with measurable yield loss in states east of the Rocky Mountains were rare prior to 2000 (Markell and Milus, 2008). The outbreak from 2000 to 2005 was the

most wide-spread epidemic of stripe rust in the United States in areas where stripe rust previously was not recorded (Chen et al., 2002; Milus et al., 2006; Milus et al., 2009). In 2002 and 2005, stripe rust caused losses close to 5% of the total wheat production (Long, 2009). Recently, with fungicide applications, stripe rust represents less than 1% of the total wheat production losses.

Most information about diversity of *P.striiformis* f. sp. *tritici* in the United States depends on virulence tests. According to Line and Qayoum (1991), the pathogen is considered avirulent when there are no symptoms or there are necrotic or chlorotic flecks or blotches without sporulation or with only a trace of to slight sporulation. The pathogen is considered to be virulent if it results in moderate to abundant sporulation with or without chlorosis or necrosis. A standard set of 20 differentials (cultivars possessing different resistance genes) is used for determining diversity of races of *P.striiformis* f. sp. *tritici* in the USA at seedling stage (Chen, 2005; Chen, 2007; Markell and Milus, 2008). A list of identified *P.striiformis* f. sp. *tritici* pathotypes and host-resistance genes can be found on the USDA Cereal Disease Laboratory website. Many of the resistance genes to stripe rust (Yr genes) that have been identified to correspond to race-specific resistance. It is well known that this resistance can be easily overcome by the pathogen.

The stripe rust races identified after 2000 (new races) east of the Rocky Mountains are virulent to the key genes Yr8 and Yr9 (Markell and Milus, 2008).The more virulence genes a race has, the more cultivars it is capable of infecting, which may increases its frequency in the pathogen population (Chen, 2005). Isolates representing this new population of *P.striiformis* f. sp *tritici* show high spore production, shorter latent periods and faster spore germination at warmer temperatures (18°C), while 12°C is considered the ideal temperature (Chen, 2005; Milus et al., 2006).

According to the stabilizing selection concept (Parlevliet, 1981), races with wide unnecessary virulence, not subject to selection by host-resistance genes, should have lower fitness and be less competitive than races with only necessary virulence. In the new isolates identified after 2000, this concept seems not to apply. These new

isolates have shown an increased aggressiveness, new unnecessary virulence, and better fitness, (the ability to survive and reproduce). They have a demonstrated enhanced ability to survive over summer when it has been considered too hot for *P.striiformis*. f. sp *tritici*.(Milus et al., 2006; Milus et al., 2009).

The differences in virulence, fitness, and aggressiveness among new isolates and those collected before 2000 suggest that the contemporary *P.striiformis* f. sp. *tritici* population in the eastern USA is different from the population that existed before 2000. It is considered that this new race is an exotic introduction with an unknown origin (Markell and Milus, 2008); this hypothesis is supported by an extensive study done by Hovmøller et al. (2008) who suggest that this new introduction resulted from human-mediated travel and commerce.

As stated by Milus et al. (2006), these new isolates with an increased aggressiveness and wide virulence could cause severe and widespread disease. The use of major resistance genes may not be highly effective; rather it seems necessary to focus on slow minor rusting genes that appear to be durable. Many cultivars with durable resistance to stripe rust have been released over the past 60 years (Stuthman et al., 2007). One of those cultivars is ‘Stephens’, widely planted over 30 years with a well-known stripe rust resistance even to new races. It is an important task in the Pacific Northwest to identify and incorporate those resistance genes into new cultivars.

1.6 Breeding for Durable Resistance

Breeding for durable resistance has been a main goal in crop improvement programs. Breeding and releasing cultivars with effective genetic resistance is the most sustainable, environmentally sound, and economical means of control. Sufficiently high resistance to fungal pathogens is an ongoing challenge for wheat breeding. Various aspects contribute to the development of new wheat cultivars with increased resistance to diseases, such as understanding pathogen biology, characterization of pathogen avirulence, identification of plant disease resistance genes as resistance sources, and obtaining information on wheat genetic diversity and

relationships among elite experimental lines and cultivars (Kaur et al., 2008; Mahmood et al., 2004).

The use of molecular approaches, particularly molecular markers, has allowed better characterization of the genetic diversity in wheat germplasm. The availability of high-throughput molecular markers linked to resistance genes and their genetic location could make the selection process faster and more cost effective. In addition, different genes can be combined in a pyramiding strategy for resistance breeding. Despite these efforts, at this point high-throughput molecular marker technology is lacking, the genetic base of disease resistance in wheat remains narrow, and the continually evolving of pathogens is a continuing threat that challenges the resistance of existing and future elite material (Kaur et al., 2008).

1.6.1 Molecular Markers

Molecular markers are known to be useful in the process of identification of disease resistance genes. Those markers are based on differences in the DNA sequence of individuals and provide guide points that are useful to pinpointing the location of specific genes. They follow mendelian inheritance patterns, so the relative positions of the marker along the genome is estimated by the observed recombinant events (Bernardo, 2002; Weising et al., 2005). The degree of genetic variation in wheat (*Triticum sp*.) has been assessed with different types of DNA molecular markers. Restriction fragment length polymorphism (RFLP) markers were first used in the 1990's in wheat (Prasad et al., 2000), but for the purpose of this research, only two types of markers will be discussed: Diversity Array Technology (DArT) markers and Simple sequence repeat (SSR).

Diversity Array Technology (DArT) it is a low-cost high-throughput, robust system with minimal DNA sample requirements capable of providing comprehensive genome coverage without any DNA sequence information needed. DArT markers are based on a microarray hybridization technology that detects the presence versus absence of individual DNA fragments in a genomic representation of an organism or a population of an organism (Akbari et al., 2006; Jaccoud et al., 2001). This technology consists in an array that stands on a micro-scale solid support (eg. nylon membrane,

nitrocellulose), where DNA fragments or polymerase chain reaction (PCR) products are spotted in an ordered pattern. Those DNA fragments are labelled with green or red fluorescent dye, mixed, and hybridized. The ratio of green:red signal intensity is measured at each array spot. Polymorphic spots are identified by binary distributions of signal ratios among the samples (Jaccoud et al., 2001). The DArT markers are useful to create a medium density skeleton map, which provides anchor points for extended mapping using markers as microsatellites (Weising et al., 2005).

Simple sequence repeat (SSR), or microsatellite markers rely on the use of PCR, a technique used to amplify a sequence piece of DNA, generating as a product millions copies of that specific DNA piece. These types of markers work by identifying primers that flank a tandem repeat. A tandem repeat is a repetitive DNA sequence made up of very short motifs with a size of 1 to 6 base pairs (Weising et al., 2005). Tandem repeat sequences constitute 80% of the wheat genome, and are widely distributed across the genome, and therefore highly abundant.

Simple sequence repeats have been the markers of choice in wheat because of their codominant inheritance, i.e., they can discriminate between homozygous and heterozygous alleles; high abundance based on tandem repeated sequence; ease of assessing and identification by size. PCR amplification products are placed in a matrix gel and separated in different band sizes by an electrical current. The bands are stained with ethidium bromide and visualized under ultraviolet light (Leonard et al., 2008). The presence of polymorphism can be interpreted as differences in SSR fragment length, which implies the presence of different alleles (Prasad et al., 2000; Weising et al., 2005). One important disadvantage of these markers is the necessity of sequence information for primer design although the increasing availability of molecular wheat maps based on SSR markers favors the identification and cloning of important genes (Kaur et al., 2008).

1.6.2 Quantitative Trait Loci

A quantitative trait is known as a trait influenced by both multiple genetic and environmental factors and its phenotypic values follow a continuous or normal distribution. Examples of quantitative traits are height, yield, and some disease

resistance. Qualitative traits are identified when phenotype values fall into distinct categories well defined by one or few genes and can be measured by phenotypic ratios (Bernardo, 2002; Xu, 2002).

During the 1940's, it was established that quantitative traits are determined by multiple genes with potentially small individual effects, sometimes called "polygenes." This led to the definition of quantitative trait loci (QTL), as genetic loci in which different alleles segregate, causing effects on a quantitative trait (Mauricio, 2001). Studies on quantitative traits have shown that genes with a major effect do exist and can be experimentally mapped on chromosomes by evaluating the correlation between the quantitative trait value and the allelic states at linked molecular markers (Salvi and Tuberosa, 2005). Understanding the genetic basis of such complex quantitative traits requires a combination of modern molecular genetic techniques and powerful statistical methods. Quantitative resistance is characterized by a more-or-less continuous transition from susceptible to resistant genotypes in a segregating population, while clear groups of resistant and susceptible lines can be defined for qualitative genes. Quantitative resistance is often assumed to be more durable than single-gene resistance or qualitative resistance (Jahoor et al., 2004; Salvi and Tuberosa, 2005). According to McIntosh (2009), there are now hints that certain QTL for stripe rust resistance are race-specific and it is necessary to be careful with the widespread assumption that QTL represent non-specificity and therefore durable resistance.

1.6.3 Quantitative Trait Loci (QTL) Mapping

In 1923, Karl Sax mapped a QTL for seed size in the bean *Phaseolus vulgaris* by statistically associating it with a mendelian locus for seed pigmentation (Mauricio, 2001). Quantitative trait loci mapping is defined as "the study for the genetic architecture of quantitative traits using molecular markers" (Xu, 2002). At its most basic level, QTL mapping simply involves finding an association between a genetic marker and a phenotype that one can measure and does not require previous knowledge of the gene function and sequence (Mauricio, 2001). Mapping of QTL is of increasing importance for disease resistant breeding in research and breeding

programs for several crops because it can help to understand the respective roles of specific resistance loci versus partial resistance genes and the interactions between the genes and the environment. It also is expected to serve breeder as a tool for marker-assisted selection of complex disease resistance traits (Kaur et al., 2008; Salvi and Tuberosa, 2005).

Quantitative trait loci mapping in plants involves a few basic steps. The primary requirement is that two parental lines must differ in the alleles that affect variation in a trait. The parental lines are crossed, creating a large segregating population, preferably more than 100 individuals (Vales et al., 2005), in which the phenotype and the genotype of each individual are measured. The genotype of the population is measured by a polymorphic genetic map (based on molecular markers) that allows the two parental lines to be distinguished genetically. Both phenotypic and genotypic data are statisticaly analysized to reveal all possible marker loci where allelic variation correlates with the phenotype (Mauricio, 2001). The information resulting from this analysis consists of number and location of QTL; its effect and magnitude is expressed as a percentage of phenotypic variance (Xu, 2002).

Several crossing schemes are used to generate a population for QTL mapping. In all of these schemes, the parents are mated to generate an F1 population. In one approach, recombinant inbred lines (RIL) can be created by selfing (self-fertilizing) each of the F1 progeny for several (usually six to eight) generations to reduce heterozygousity. In an ‘F2 design’, mating the F1 progeny to each other generates the mapping population. In a ‘backcross design’, crossing the F1 progeny to either one or both parents generates the mapping population. Several variations on these crossing schemes have been designed to maximize the recombination of parental alleles (Keurentjes et al., 2007; Weising et al., 2005).

The genetic or linkage map used for QTL mapping is based on recombination of polymorphic alleles. The more detailed the genetic map (that is, the greater the number of markers), the better the genetic mapping resolution and accuracy of the positioning of the QTL. A genetic or linkage map is a graph representing the relative positions of markers in linkage groups. Markers or loci close together on the same

chromosome have fewer recombinations than do loci farther apart on the same chromosome (Bernardo, 2002; Weising et al., 2005). Genetic mapping is used to determine the linear order of molecular markers or loci along the DNA (Weising et al., 2005).

Genetic maps are measured in centimorgans (cM), the unit of recombination frequency for measuring genetic linkage. The statistical criterion for linkage between two markers is based on the logarithm of the odds (to the base 10), which tests the null hypothesis that no linkage exists between two loci. The decimal logarithm of the odds (LOD score) is conventionally reported. A LOD score of 3 (odds ratio of 1000:1) is normally accepted as a lower threshold to assets linkage because it represents the least acceptable probability that two loci are linked (Weising et al., 2005).

Because the above procedure of QTL mapping only allows for an approximate positioning of the QTL, it is usually referred to as primary (or coarse) QTL mapping. A primary QTL mapping locates a QTL within a chromosome in an interval of 10 to 30 cM. This does not give the exact location of the gene or genes regulating the trait nor does it explain the function, but it can be useful for a marker-assisted breeding program to target regions of interest (Mauricio, 2001; Salvi and Tuberosa, 2005).

1.6.4 Genotype x Environment Interaction

Along with the estimation of QTL, it is important to calculate heritability and genotype x environment interaction; without those estimates, the information is incomplete. Heritability is defined as the proportion of the phenotype variance among individuals in a population that is due to heritable genetic effects (Holland et al., 2003). Because the evaluation of a population should be done under multiple locations, the estimation of the genotype x environment (GE) effect is important. The additive main effects and multiplicative interaction (AMMI) model and the genotype main effects and genotype × environment interaction effects (GGE) model [also called site regression (SREG) model], are two cases of general lineal-bilinear models (GLBM).GLBM models include the additive effects (lineal main effects) of analysis of variance and the multiplicative effects (bilinear, interaction effects) of principal component analysis (PCA) (Crossa and Cornelius 1997; Zobel et al., 1988). A quick

visualization of results from those models is done by using biplot graphs that serve as a descriptive graphic to look at relationships among genotypes, among environments, or interactions between genotypes and environments. It is constructed using the first two principal components (PC1 and PC2) derived from singular value decomposition of environment-centered multi-environment trials data. As a result, a polygon is dividend in sectors by perpendicular lines and its interpretation is based on geometrical principles (Yan et al., 2000).

1.7 Thesis Objectives

The main objective of this research is to identify the genetics of durable resistance to stripe rust in the cultivar ‘Stephens’, which has been durable for more than 30 years in the Pacific Northwest of the USA. The cultivar ‘Stephens’ is present in the background of many cultivars around the world. It is important to understand the genetics of this resistance in the presence of the new aggressive races as well as the role of environment interactions with disease response. Many publications describe QTL for durable resistance that has been stable in different regions.

Specific objectives for this project are:

- To determine the inheritance of durable resistance against stripe rust in ‘Stephens’ wheat cultivar.
- To determine important genotype x environment interactions in the expression of durable resistance in ‘Stephens’ wheat cultivar.
- To provide tools for future application of marker assisted selection that may speed up the breeding process to achieve resistance in new cultivars.

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Genetic Factors that Provide Adult Plant Resistance Against *Puccinia striiformis* f. sp. *tritici* to Wheat Cultivar 'Stephens' in a Multilocation Analysis

CHAPTER 2

2.1 Abstract

'Stephens' wheat (*Triticum aestivum* L.) has been grown commercially in the USA Pacific Northwest for 30 years. It has durable resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*), believed to be due to a combination of minor and major genes to provide adult plant resistance. To better understand the genetic basis of stripe rust resistance, diversity arrays technology (DArT) and simple sequence repeat (SSR) markers were used to identify quantitative trait loci. A linkage map was constructed, based on 156 recombinant inbred lines (RILs) derived from a cross of 'Stephens' with the cultivar 'Platte'. RILs were assessed for stripe rust response in eight locations/years: five in 2008 and three in 2009. Locations were associated in two mega-environments, each showing different disease-patterns. Data were then analyzed in two separate sets: Mt. Vernon vs all others; and as individual locations/years within each set. Composite interval mapping (CIM) from Mt. Vernon datasets identified three QTL, QYr.orr-1AL, QYr.orr-4BS, QYr.orr-6AL, which accounted for 12, 11, and 6% of the phenotypic variance, respectively. CIM across six locations, when excluding Mt. Vernon, identified four main QTL. Two QTL,QYr.orr-2BS.2 and QYr.orr-7AS, were significant in five of six locations/years, accounting for 11 and 15% of the phenotypic variance, respectively. The QTL QYr.orr-2AS andQYr.orr-4BL were significant across four and three of six locations/years respectively, accounting for 19 and 9% of the phenotypic variance, respectively. The susceptible parent 'Platte' contributed QTL for resistance on 4BL and 6AL. For each location/year, additional QTL were significant, each accounting for 6 to 10% of the phenotypic variance at that site. Additive QTL with moderate levels of resistance were identified in both 'Stephens' and 'Platte'. Significant QTL x environment interactions were identified, which suggests specificity to plant stage, pathogen genotype, and/or temperature of some resistance QTL.

2.2 Introduction

Adult plant quantitative resistance is often known to be more durable than qualitative seedling resistance or all-stage resistance (Parvlevliet, 2002). This type of resistance to rusts diseases is associated with reduced rates of disease development resulting from a longer latent period, low infection frequency, smaller uredial size, and reduced duration and quantity of spore production due to the frequent failure of hastorium formation (Li et al., 2006; Milus et al., 2006; Niks and Rubiales, 2002). Breeding for durable resistance against stripe rust is receiving increasingly international attention due the appearance of virulent races of *Puccinia striiformis* f.sp *tritici* in the past decade. These races are more threatening to wheat worldwide than before the year 2000 (Chen, 2005; Milus et al., 2006; Milus et al., 2009; Hovmøller et al., 2008).

The soft white winter wheat cultivar ‘Stephens’ (Kronstad et al., 1978) has been widely planted for 30 years in the Pacific Northwest (Santra et al., 2008) where new stripe rust races are present (X. Chen personal communication, 2009). Markell and Milus (2008) have reported that ‘Stephens’ maintains moderate levels of infection type in the presence of those new races, which are of great concern for their ability to cause high yield losses on wheat even in places that were previously reported as unfavorable environments (Milus et al., 2009). This situation represents a problem for new varieties that may not carry durable resistance as ‘Stephens’ does, even when many new released varieties have this cultivar in their background.

Screening, identifying and understanding the sources of disease resistance with molecular markers as tools is becoming a routine task in breeding programs in a effort to design more targeted breeding schemes. This source of information could aid in the decision-making process for performing crosses and maintaining lines for further evaluation.

Our goal in this experiment is to identify QTL underlying genetic variability for disease resistance across locations in a recombinant inbred line (RIL) mapping population developed from a cross between ‘Stephens’ and a stripe rust susceptible line (‘Platte’).

2.3 Materials and Methods

2.3.1 Plant Material and Field Analysis

The population for this study consisted of 156 F_6- derived recombinant inbred lines (RILs) developed by single seed descent from a cross between 'Stephens' (a cultivar with moderate to high levels of adult plant resistance to stripe rust) and 'Platte' (a cultivar highly susceptible to stripe rust). 'Platte' was released in 1999 by HybriTech Seed International and has the pedigree Tesia79/Chatt'S//Abilene (USDA-AMS, 2009). 'Stephens' (CI 017596) is a cultivar released in 1978 in the Pacific Northwest (Kronstad et al., 1978) and has shown moderate to high levels of adult plant resistance to stripe rust. The pedigree of 'Stephens' is Pullman101/Nord-Desprez (Wheat Pedigree On Line, 2009).

The F_6- derived bulk harvested from the greenhouse, was used to establish plots in the field. The parents and the RIL progeny were evaluated in the field in randomized complete blocks with two replications at five locations in 2008 and three locations in 2009. Locations for 2008 were: Toluca, Mexico (MX); Corvallis, Oregon (OR); Pendleton, OR; Whitlow, Washington (WA) and Mt. Vernon (WA). In 2009 the locations were: Mt. Vernon WA, Corvallis OR and Toluca MX.

Plots consisted of two rows, 1 meter long. The percent rust severity for each plot was evaluated on an adult plant basis and according to the modified Cobb Scale (Roelfs et al., 1992). Several notes for rust severity were recorded on multiple dates, one to two weeks after the susceptible parent showed around 70% severity at adult stage, except at Whitlow and Mt. Vernon, where initial notes were taken at seedling stage.

For all locations except Toluca, rust was established by natural inoculation. Artificial inoculation in Toluca was initiated about 4 weeks after planting by inoculating susceptible spreader rows. Two spreader rows were located at the beginning and end of each block. The suspension of rust urediniospores was in lightweight mineral oil, Sotrol 170 (Chevron Phillips Chemical Company, The Woodlands, TX, USA) with the stripe rust strain known as MX-94.11 (J. Huerta-Espino personal communication). Predominant races in Corvallis and Pendleton were

PST-100, PST-114 and PST-116. In Mt. Vernon and Whitlow, the natural population was a mixture of old races, PST-12, PST-17, PST-43, PST-45 and new races, PST-100; PST-114; PST-116 (X. Chen and A. Wan, personal communication, 2009).

2.3.2 Molecular Analysis and Map Construction

Parental and F_6 progeny DNA from young leaves were extracted using the DNeasy Plant DNA extraction kit (QIAGEN), sending 15 ng to Triticarte Pty. Ltd Canberrra Australia to be genotyped with DArT (Diversity Array Tecnhnologies) markers (Akbari et al., 2006). Additional simple sequence repeat (SSR) markers were screened for polymorphism between 'Platte' and 'Stephens' using approximately 50 ng genomic DNA extracted from young leaves (Riera-Lizarazu et al., 2000). PCR amplifications were done using the recommended annealing temperature for the respective SSR markers. Visualization of the amplified SSR products was done using agarose gel electrophoresis (3%) stained with ethidium bromide (Leonard et al., 2008). Once loci associated with the resistant parent were identified, additional SSR markers in the vicinity were selected using linkage maps available in the database GrainGenes 2.0 (2009). DNA concentration was tested using NanoDrop ND-1000 UV-Vis Spectrophotometer.

Genotypic data from the 156 RILs were used to create the genetic linkage map with the software JoinMap v. 4.0 (Van Ooijen and Voorrips, 2001). The original map was constructed using a total of 735 markers (681 DArTs and 54 SSRs), from which a subset of 161 markers (7 SSR and 154 DArT), spaced every 10 cM, were used to construct the 32 linkage groups, each representing chromosomal areas from all chromosomes. Genetic distances were calculated using the Haldane function (Haldane, 1919). For each linkage group, the best marker loci order was determined using the maximum likelihood in Join Map 4.0.

2.3.3 Statistical and QTL analysis

The last date-note of disease severity for each location/year was used to perform all statistical and QTL analysis (APPENDIX A). The PROC MIXED procedure in SAS software was used to calculate least square means (LSM) and family heritability. RIL effects were considered to be random and replications to be fixed.

Family narrow-sense heritability calculation on plot basis is based on the general formula $h^2 = \sigma^2 g / \sigma^2 p = \sigma^2 f / (\sigma^2 f + \sigma^2 fe/e + \sigma^2 e/er)$, where the variance components are $\sigma^2 g$, genetic variance; $\sigma^2 p$, phenotypic variance, $\sigma^2 f$, family variance; $\sigma^2 fe$, family by environment interaction variance; $\sigma^2 e$, error variance; e, number of environments and r, number of replications (Holland et al., 2003; SAS 9.1.3, 2005). For all tests, a probability level of $P<0.05$ was used.

Contrast sites analysis was done using PROC MIXED procedure in SAS software. RILs, environment, replication*environment, RILs*environment were considered to be random. Due to the importance given to GxE interaction within each location and its effect to disease response, SREG biplot analysis was used as an approach to visualize such interactions by clustering groups of similar response together in same quadrants using PROC MIXED procedure in SAS software with fixed effects (SAS 9.1.3. 2005). Biplot analysis was based on genotype; genotype x environment interactions effects (GGE) model, also called sites regression (SREG) model, a special case of general lineal-bilineal model (GLBM) that test for additive and multiplicative effects(Yan and Kang 2003; Yang et al., 2009).The model is expressed:

$$Y_{ij} = \mu + E_j + (\sum \lambda_k \alpha_{ik} \gamma_{jk}) + e_{ij}$$

where Yij is the mean of the ith genotype and jth environment; μ is the overall mean, E is the effect of the jth environment; λ is a constant for α, γ as multiplicative terms for genotypes and environments and eij is the residual error (Yang et al., 2009).

Due results obtained from genotype x environment interaction, two sets of data were used to perform separate QTL analyses. One dataset was compounded of Mt. Vernon 2008 and 2009. A second dataset was produced from the remaining six locations/years. QTL analysis was performed using composite interval mapping (CIM) in WinQTL Cartographer v. 2.5 software (Wang et al., 2007); window size was set at 10 cM with five cofactors identified for each dataset section; forward and backward stepwise. Likelihood-odds (LOD) thresholds for declaring statistical significance were calculated by 1000 permutations (Churchill and Doerge, 1994).Permutation threshold at a type I error rate with a probability of 0.05, were 2.9 on each individual

environment and 3.0 for combined environments. The additive effects (a) and phenotypic coefficients of determination (R^2) for individual QTL were estimated by CIM (Tang et al., 2006).

2.4 RESULTS

2.4.1 Molecular Analysis and Map Construction

The map with 161 markers, 7 SSR and 154 DArT, was comprised of 32 linkage groups, representing areas from the 21 chromosomes of common wheat and covering 1786 cM. Final linkage groups were assigned to each chromosome with data provided by Triticarte wheat map alignment (Triticarte, 2009) and maps available on the database GrainGenes 2.0 (2009). The largest chromosome mapped was 2B with 224.5 cM and the shortest was 3D with 3.6 cM length (APPENDIX B).

2.4.2 Phenotypic Values and Statistical Analysis

For each location, disease scores of parents; least square mean for the RILs; population maxima and minima; coefficient variation (CV); heritability and genotype term p-values are presented in Table 2.1.Significant disease pressure was obtained in each location, with ratings for the susceptible parent ‘Platte’ ranging from 37 to 100%. The resistant parent, ‘Stephens’, scored consistently low for stripe rust severity, ranging from 0 to a maximum of 10%. Heritabilites (H^2) were moderate to high depending on the environment, ranging from 0.41 in Toluca 2009 to 0.82 in Mt. Vernon 2009. Coefficients of variation (CVs) ranged 23 to 81%, considered to be due to variation in disease severity magnitude and for hence the wide dispersion of the data; p-values for the genotype term suggest significant differences among genotype performance for each location.

Disease severity on the RILs at each location/year suggests the response is of a quantitative, rather than qualitative trait (Fig 2.1). Responses were normally distributed in some environments, or slightly skewed to lower infection ratings in others. There is little indication of transgressive segregation, as severity ratings of RIL’s generally fell within parental values. Contrast analysis shows that resistance response differed among locations (Table 2.2)

Table 2.1 Means (± standard error); highest and lowest disease severity score; coefficient variation; narrow-sense heritability on plot basis (± standard error) for parents and the 156 recombinant inbred lines in each location/year. Disease severity score is based on % severity (leaf area covered by stripe rust on a plot basis)

Year	Location	'Platte' (%)	'Stephens' (%)	Mean (%)	Minimun (%)	Maximun (%)	H^2 Plot Basis	Coefficient variation	p-value genotype
2008	Mt. Vernon	100	2.0	45.0(±9.8)	1.0	100	0.78(±0.03)	31.28	<.0001
	Whitlow	37	6.0	27.9(±7.5)	0.5	70	0.51(±0.05)	37.4	<.0001
	Toluca	83	2.0	22.9(±7.0)	1.0	85	0.69(±0.04)	46.6	<.0001
	Corvallis	100	0.0	29.5(±9.5)	0.5	100	0.79(±0.02)	47.0	<.0001
	Pendleton	86	0.0	15.9(±8.6)	0.5	87	0.57(±0.05)	81.0	<.0001
2009	Mt. Vernon	81	10.0	36.2(±7.5)	5.0	97	0.82(±0.02)	23.0	<.0001
	Toluca	50	9.0	21.1(±6.1)	5.0	50	0.41(±0.06)	41.2	<.0001
	Corvallis	100	2.0	50.0(±8.0)	0.5	100	0.64(±0.04)	29.0	<.0001

Figure 2.1. Frequency distributions of stripe rust ratings for 156 RILs derived from a cross between ‘Stephens’ and ‘Platte’ in eight environments. Susceptible and resistant parents are noted on each frequency distribution. The environments are: a) Mt. Vernon 2008; b) Mt. Vernon 2009; c) Whitlow 2008; d) Pendleton 2008; e) Corvallis 2008; f) Corvallis 2009; g) Toluca 2008; and h) Toluca 2009

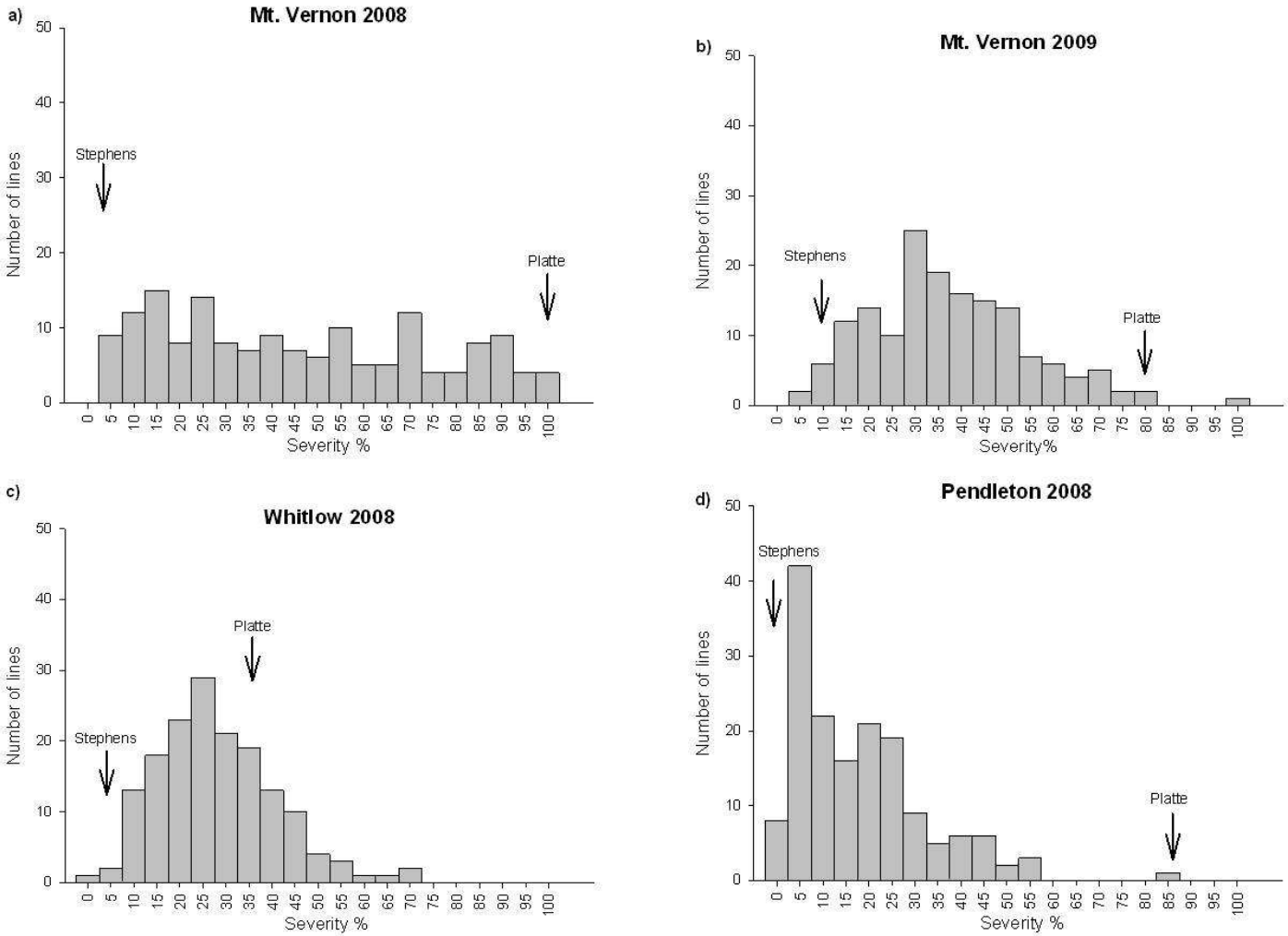
a)
Mt. Vernon 2008
Number of lines
Stephens
Platte
Severity %
b)
Mt. Vernon 2009
Number of lines
Stephens
Platte
Severity%
c)
Whitlow 2008
Number of lines
Stephens
Platte
Severity %
d)
Pendleton 2008
Number of lines
Stephens
Platte
Severity %

Figure 2.1 (Continue)

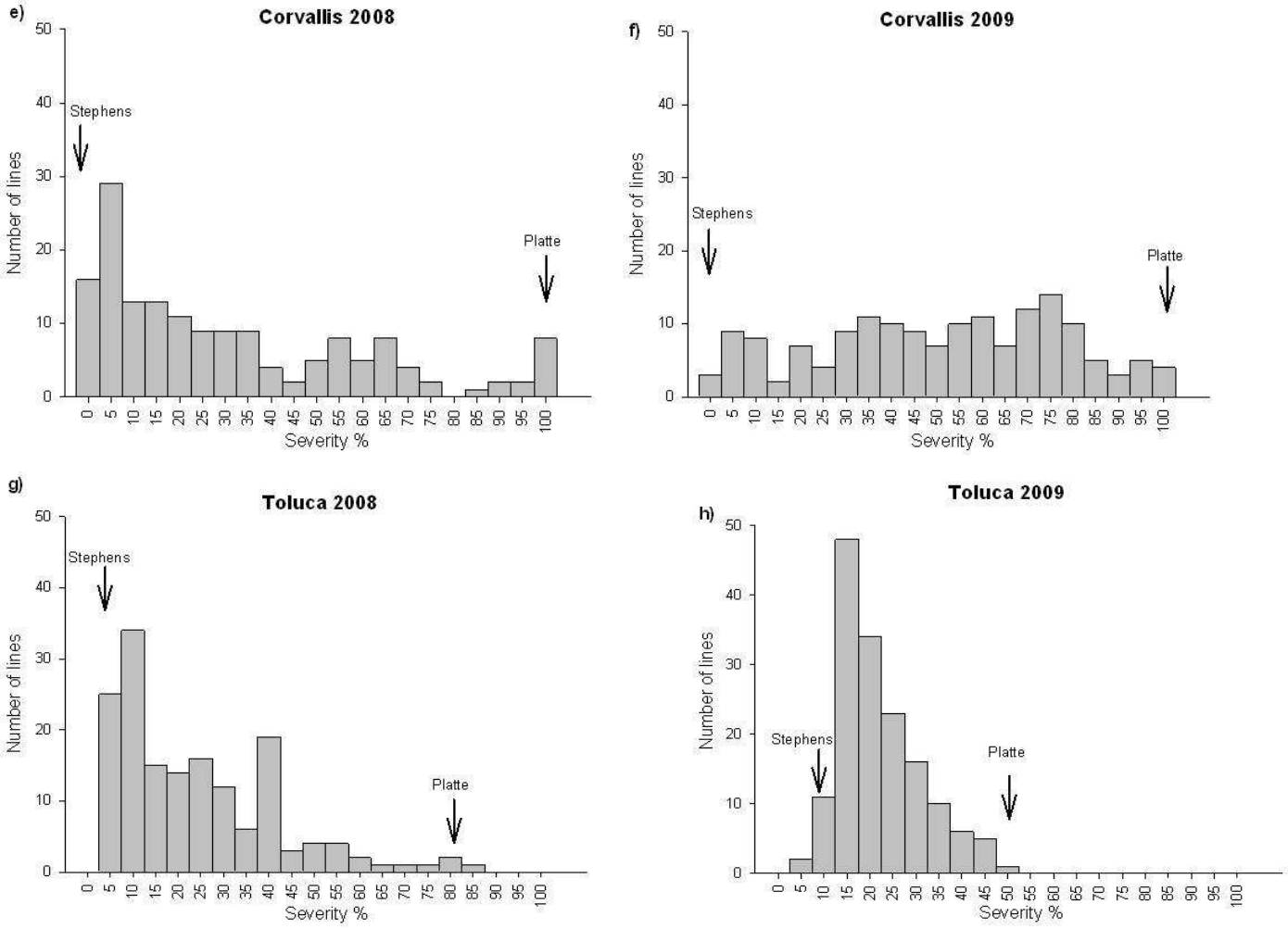

Table 2.2. Contrast analysis results for disease severity based on random model.

Source of variation	Num DF	Den DF	Chi-Square	F Value	Pr>ChiSq	Pr>F
Mt. Vernon vs rest of locations	1	1270	31.88	31.88	<.0001	<.0001
Mt. Vernon and Whitlow vs rest of the locations	1	1270	17.15	17.75	<.0001	<0.0001
Covallis and Pendleton vs Toluca	1	1270	14.38	14.38	0.0001	0.0002

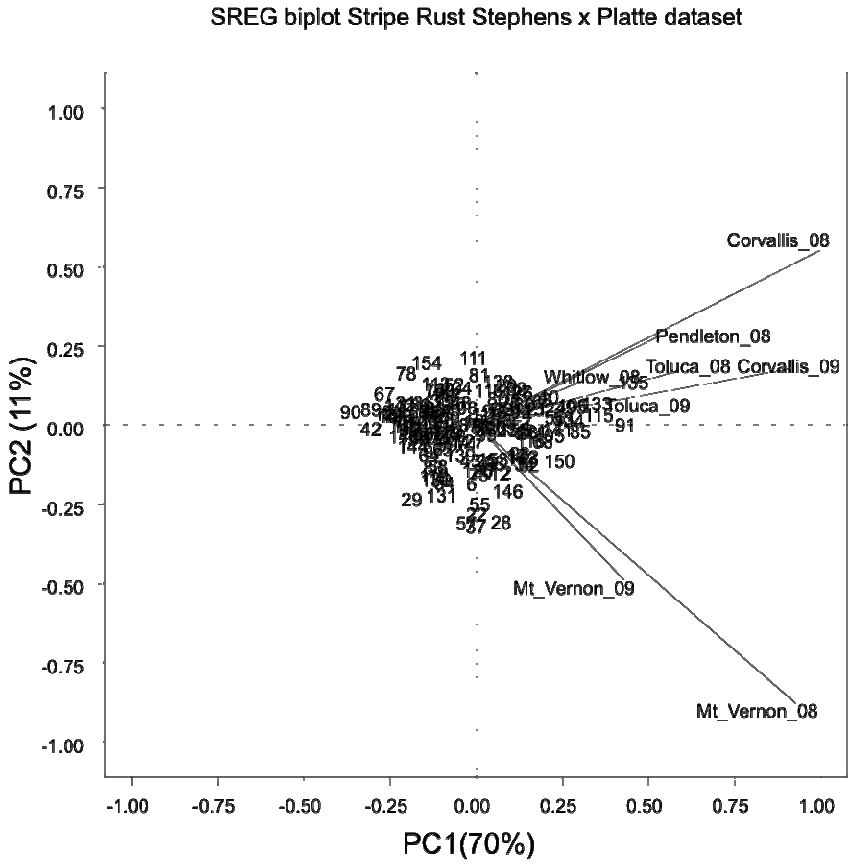

Figure 2.2.Genotype and genotype x environment biplot based on SREG model.

The length of an environment vector indicates the magnitude of differences among genotypes in that specific environment. The biplot for disease response explained 81% of the variance (Fig. 2.2) in RILs and environments. Two groups of

environments were readily apparent: Mt. Vernon in 2008 and 2009 vs all remaining environments. Due to the presence of two mega-environments showing different disease-patterns in the biplot graph, data were then analyzed in two separate sets. Figure 2.2 also suggests the genotypes responded somewhat differently in each environment, but did not clearly differentiate into separate clusters.

2.4.3 QTL Analysis - Mt. Vernon 2008 and 2009Dataset

The QTL that contributed to disease resistance at Mt. Vernon were located on chromosomes 1AL, 1DS, 3AL, 4AL, 4BS, 6AL and 7BS (Table 2.3 and Figure 2.3). Three of the QTL were significant in both years. The QTL in chromosome 1AL was linked to wPt4399; 4BS linked to marker wPt5265; and 6AL linked to marker 378849. These QTL accounted for 12, 11 and 6% of the phenotypic variance, respectively, as compared with 37% of phenotypic variance that was explained by all QTL together from this dataset (Table 2.4). QTL also were identified on 1DS, 3AL, 4AL and 7BS based on significance in one of the two years of testing. Phenotypic variance accounted for by these QTL ranged from 6 to 11% (Table 2.3). With one exception, all resistance QTL were donated by 'Stephens'. The susceptible parent 'Platte' donated a QTL for resistance on 6ALsignificant only in 2009.

2.4.4 QTL Analysis for All Locations/Years Except Mt. Vernon

When data from Mt. Vernon were excluded from analyses, QTL contributing to disease resistance were identified on 2AS, 2BS.1, 2BS.2, 4BL, 5AS and 7AS (Figure 2.4). QTL that were significant over multiple locations were 2AS, 2BS.2, 4BL and 7AS, each explaining 19, 11, 9 and 15% of the phenotypic variance respectively. Total phenotypic variance explained by these four QTL is approximately 50% (Table 2.4). Two QTL were significant in each of the five locations/years: 2BS.2 linked to marker wPt0408 and 7AS linked to marker 376425. The 2AS QTL, linked to marker wPt0003, was significant in four locations/years. The QTL on 4BL, linked to marker 312980, was significant in three locations. The QTL identified on 2BS.1, linked to marker wPt5738, was significant in two locations. A QTL on 5AS, linked to marker tPt4184, was significant in analysis in just one of the locations. The locations that share similar significant QTL are Toluca, Corvallis and Pendleton (Table 2.5). With

one exception, all resistance QTL were donated by ‘Stephens’. The resistance QTL identified on 4BLcomes from susceptible parent ‘Platte’.(Table 2.5).

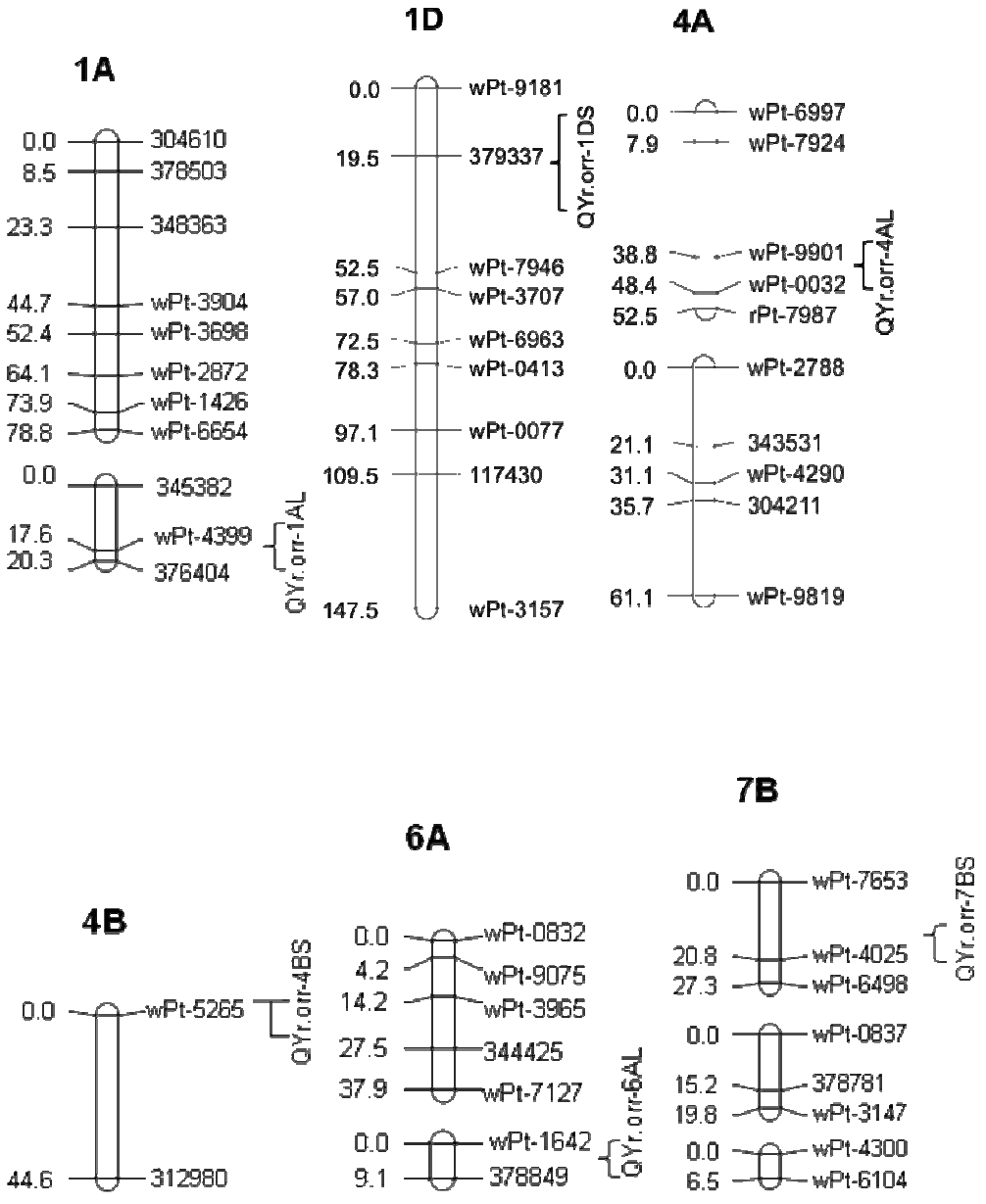

Figure 2.3 Linkage map with reduced markers showing chromosomes with significant QTL for Mt. Vernon 2008, 2009 dataset analysis.

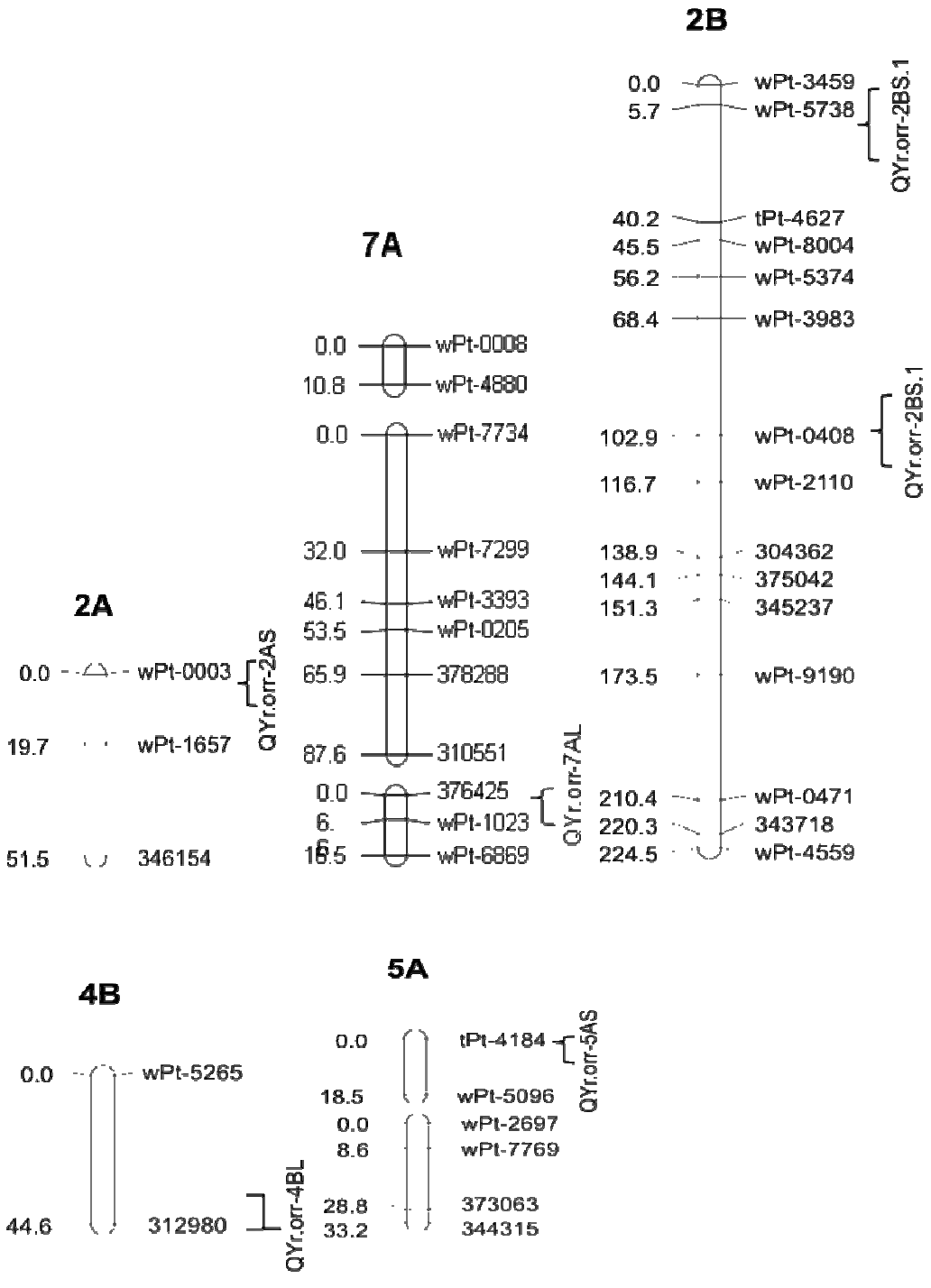

Figure 2.4. Linkage map with reduced number of markers showing chromosomes with significant QTL for all locations except Mt. Vernon.

Table 2.3. QTL associated with disease response for Mt. Vernon in 2008 and 2009, including position and peak on the linkage map; closest linked markers; likelihood odds (LOD) scores; phenotypic coefficients (R^2) and estimated additive effects (a). Negative additive effect values indicate that the resistance allele is derived from 'Stephens'

QTL name	QTL peak cM	Closest marker	Mt. Vernon 2008			Mt. Vernon 2009		
			LOD	a	R^2	LOD	a	R^2
QYr.orr-1AL	17.8	wPt4399	6.8	-10.8	14	.	.	.
QYr.orr-3AL	29.6	wPt1652	3.1	-9.1	10	.	.	.
QYr.orr-4AL	44.8	wPt9901	3.0	-7.6	7	.	.	.
QYr.orr-7BS	0.0	wPt7653	3.1	-7.3	6	.	.	.
QYr.orr-1DS	23.0	379337	.	.	.	3.3	-5.6	11
QYr.orr-6AL	0.0	378849	.	.	.	3.0	4.2	6
QYr.orr-4BS	44.0	wPt5265	2.8	-7.1	6	5.2	-5.9	12

Table 2.4 Combined QTL analysis associated with disease response for Mt. Vernon and the rest of the locations, including position and peak on the linkage map; closest linked markers; likelihood odds (LOD) scores; phenotypic coefficients (R^2); total phenotypic coefficients (TR^2).and estimated additive effects (a). Negative additive effect values indicate that the resistance allele is derived from 'Stephens'

QTL name	QTL peak cM	Closest marker	a) Mt. Vernon				b) All locations except Mt. Vernon			
			LOD	a	R^2	TR^2	LOD	a	R^2	TR^2
QYr.orr-1AL	16.0	wPt4399	5.2	-7.2	12	38	.	.	.	.
QYr.orr-4BS	44.0	wPt5265	5.4	-7.2	11	37	.	.	.	.
QYr.orr-6AL	0.0	378849	3.1	5.2	6	36	.	.	.	.
QYr.orr-2AS	6.0	wPt0003	.	.	.	.	7.8	-7.8	19	55
QYr.orr-2BS.2	94.0	wPt0408	.	.	.	.	4.1	-6.1	11	53
QYr.orr-4BL	0.0	312980	.	.	.	.	4.4	5.4	9	46
QYr.orr-7AS	2.0	376425	.	.	.	.	8.4	-7.1	15	49

Table 2.5. QTL associated with disease response for all locations except Mt. Vernon, including position and peak on the linkage map; closest linked markers; likelihood odds (LOD) scores; phenotypic coefficients (R^2) and estimated additive effects (a). Negative additive effect values indicate that the resistance allele is derived from 'Stephens'.

QTL name	QYr.orr-2BS.2	QYr.orr-7AS	QYr.orr-2AS	QYr.orr-4BL	QYr.orr-2BS.1	QYr.orr-5AS
QTL peak cM	96.0	2.0	6.0	0.0	9.7	2.0
Closest marker	wPt0408	376425	wPt0003	312980	wPt5738	tpt4184
Toluca 2008						
LOD	3.2	5.9	3.8	4.50	.	.
a	-5.3	-6.4	-5.5	5.40	.	.
R^2	9.0	13.0	9.0	9.00	.	.
Toluca 2009						
LOD	2.7	5.7	.	.	.	.
a	-3.0	-3.0	.	.	.	.
R^2	10.0	13.0	.	.	.	.
Corvallis 2008						
LOD	2.8	6.1	5.2	5.0	.	.
a	-8.2	-10.1	-11.5	9.4	.	.
R^2	8.0	12.0	15.0	10.0	.	.
Corvallis 2009						
LOD	4.3	10.0	6.7	.	.	.
a	-7.9	-12.1	-9.7	.	.	.
R^2	8.0	20.0	13.0	.	.	.
Pendleton 2008						
LOD	3.1	5.2	5.9	.	3.0	.
a	-6.2	-6.1	-7.9	.	-5.5	.
R^2	13.0	12.0	20.0	.	10.0	.
Whitlow 2008						
LOD	.	.	.	4.90	3.6	3.80
a	.	.	.	4.50	-4.4	-4.30
R^2	.	.	.	11.00	10.0	10.00

2.5 Discussion

2.5.1 Disease Resistance Response at Two Mega-Environments

In the present study, many QTL were detected that contribute to the disease resistance of 'Stephens'. QTL detected in Mt. Vernon were clearly different from those detected in the remaining locations. There are two main groups of locations (mega-environments) that correspond to two different environmental-disease response interactions, each independent of the other. This phenomenon has been reported before by Vales et al. (2005) for *P. striformis* f. sp. *hordei*. The identification of two mega-environments suggests a different resistance response to the disease that we hypothesize is due to presence of seedling epidemics, different races prevalent in the region, and/or different environmental conditions.

Mt. Vernon is a wheat-producing region known to produce its own stripe rust inoculum and epidemics at the seedling stage are not rare (Chen, 2005). Stripe rust races present at this location could be more compared to the other locations. Various races could target different resistance genes, resulting different QTL being identified at this site. Seedling epidemics could also trigger a different response at adult plant stage in 'Stephens'.

Santra et al., (2008), reported that QTL on the 6BS chromosome was mainly responsible for the resistance in 'Stephens'. They also suggested that this QTL was of the high temperature, adult plant (HTAP) type, i.e. resistance that is expressed only after the reproductive state of the plant is reached and after temperatures rise above a threshold. In this study we did not detect a significant QTL on 6B. Prevalent races present at the seedling stage in Mt. Vernon might have activated and/or alleles contributed by the susceptible parent could be interfering in the expression of the QTL on 6B. Also, 'Stephens' is a heterogeneous cultivar, and the RILs in our population could be different than those of Santra et al. (2008) with respect to QTL on 6B.

Based on this study, resistance in the cultivar 'Stephens' is controlled by the combined effects of several resistance loci with large effects, along with others with small effects. Some of them are stable across locations that share similar environment conditions. From the 11 resistance loci found to be significant for 'Stephens', three (on

2AS, 2BS.2 and 7AS), are considered to confer to adult plant resistance genes but no seedling test was performed in the locations where those QTL are significant. These loci showed stable effects across five locations that were grouped together in a mega-environment due to a similar environment-disease response pattern. QTL on 2BS.2 showed an increase in additive effect as the plant stage advanced and disease pressure increased, but its effect never was higher than the effects shown by 7AS and 2AS, the latter having the highest effect.

The QTL in 1DS and 5AS are considered to be seedling resistance genes or all stage genes, based in their appearance as significant QTL at a seedling field test from Mt. Vernon (APPENDIX C). The QTL on chromosomes 1A, 2BS.1, 3AL, 4AL, 4BS, 7B are considered to be partial resistance genes, defining as partial genes those present during the entire wheat life but the expression increase in adult plants during the critical grain-filling stage offering a broader spectrum of resistance than seedling or also known as “all stage” genes (Fu et al., 2009; Krattinger et al., 2009; Line, 2002; Mallard et al., 2008). The QTL considered controlled by partial genes were expressed at the seedling stage in Mt. Vernon, but their effect increased at the adult plant stage. We do not know if this resistance behavior corresponds to the nature of the genes or to environmental conditions.

QTL 4BL from the susceptible parent ‘Platte’ is considered to be an adult plant resistance gene since it was stable over locations and its effect was seen only in adult plant stages, as in the case of 2AS, 2BS.1, 2BS.2 and 7AS. QTL on 6A, also from ‘Platte’, corresponds to a seedling gene, also based on Mt. Vernon field seedling analysis. None of the QTL identified in this study had an additive effect larger than 10 or a phenotypic variance (R^2) larger than 20%. The percentage of phenotypic variance explained by a QTL seems to have been subject to disease pressure.

2.5.2 Relationship With Published Disease Resistance QTL

QYr.orr-2A, QYr.orr-2BS.2, and QYr.orr-7AL were notably stable across Toluca, Corvallis and Pendleton; probably similar *P.striiformis* f.sp *tritici* races seem to be present in those locations, since Pendleton and Corvallis share similar races and MX 94-11 used in Toluca is considered a mixture of races (Chen personal

communication, 2009). In these three locations, the disease resistance response corresponds to adult plant resistance; no seedling stage analysis was performed. It is difficult to infer is those genes correspond to minor or major genes, but we interpret those QTL seem to be effective to the *P. striiformis* recent races across locations. Also, those QTL seems to have relatively high, stable effects.

The QTL QYr.orr-2A in our study had a strong and stable effect among the QTL. Several QTL on 2A have been reported as seedling and adult plant resistances. Yr17 (Bariana and McIntosh, 1993; Dedryver et al., 2009; Varshney et al., 2006) and Yr32, (Eriksen et al., 2004), both are located in the short arm of 2A (Crossa et al., 2007). QTL located by Chhuneja et al. (2007) was reported in a diploid wheat linked to adult plant resistance and QTL in 2AL reported by Boukhatem et al. (2002) and Mallard et al. (2005) in "Camp Remy.

In chromosome 2BS were detected two QTL. The QTL QYr.orr-2BS.2 remained stable over several locations, having a stronger effect as disease severity increased over time when analyses on individual day-notes were done (unpublished data). Boukhatem et al. (2002) and Mallard et al. (2005) reported an adult plant resistance QTL on 2B in "Camp Remy". Börner et al. (2002) found a QTL on 2BS. In this study, QTL in QYr.orr-2BS.1 and QYr.orr-2BS.2 could be part of a resistance clustering against rusts that has been related to race and non-race specific resistance genes (Boukhatem et al., 2002; McDonald et al., 2004). Zhang et al. (2009) concluded than Yr5, allelic to Yr7 and located on 2BL, confer resistance to almost all isolates in the world. After comparing published maps, in this study, QTL on 2B are in a similar region as those found by several others (Bariana et al., 2001; Crossa et al., 2007; Guo et al., 2008; Rosewarne et al., 2008).

QYr.orr-7AS was stable over the locations tested. Börner et al., (2002) and Crossa et al., (2007) reported a QTL on 7AS.QYr.orr-5AS was detected only in Whitlow. Boukhatem et al., (2002), reported a QTL in 5A in cultivar "Camp Remy". Calonnec and Johnson, (1998) reported a QTL in 5AS related to durable resistance. Bariana et al. (2006), reported also a QTL in 5AL related to Yr34.

QYr.orr-4BL was significant in three locations. Crossa et al. (2007), Suenaga

et al. (2003) and Lu et al. (2009) also reported a QTL in 4BL, which seems to be positioned in the same location asQYr.orr-4BL in this study. Börner et al. (2002) and Lillemo et al. (2008), reported powdery mildew resistance genes in 4B; the last author reported a QTL linked to marker wPt6209, one of the markers linked to QYr.orr-4BL in this study. It is known that resistance to powdery mildew and rust pathogens might be under some common genetic control (Collins et al., 2007).QYr.orr-4BS, significant in Mt. Vernon, shares similar positions with QTL on 4BS reported by Crossa et al. (2007). 'Platte' the susceptible parent, contributed resistance alleles to QYr.orr-4BL and QYr.orr-6AL. The contribution of resistance alleles by susceptible parents has been reported before (Dedryver et al., 2009; Lillemo et al., 2008; Toojinda et al., 2000).

Chen et al. (1995) reported QTL on 1A, 1D, 3A and 4A. Later, Crossa et al. (2007) reported QTL for stripe rust resistance on these same chromosomes. It is reported that Yr25 is located on 1D. One QTL in 4A was located in "Nord Desprez", a parental line of 'Stephens' (Chen, 2005). Lillermo et al. (2008) reported a QTL on 6AL. Marais et al. (2006) reported a QTL on 6A designated as Yr38 but on an unknown arm. Crossa et al. (2007) also reported a QTL on an unknown arm of 6A. Suenaga et al. (2003) reported a QTL in chromosome 7BS; Crossa et al. (2007) make reference to a QTL Yr2 located on 7BS.

2.5.3 Implications to a Breeding Program

In this study have been detected many different QTL that provide resistance against stripe rust. This could mean for 'Stephens' that unique configuration of multiple alleles working with additive effect are behind its high and durable level of resistance. Chen and Line (1995 a, b) reported before the presence of two to three HTAP resistance genes behind the durable resistance in 'Stephens'. Overall three QTL were related to adult plant resistance but also data suggest other genes not related to APR are behind 'Stephens' durable resistance. Combinations of seedling and adult plant resistant genes have been in place to keep this cultivar resistant over the past 30 years.

This study is reporting QTL that are located in similar positions where other studies around the world have reported QTL linked to durable resistance. This represents an advantage for a breeding program where pyramiding QTL in 2A, 2BS, 7AS as APR along with other seedling resistance could provide good level of durable resistance. For purpose of Oregon State University Wheat Breeding program as seedling resistance genes could be picked those seen in Pendleton and Whitlow. This approach agrees to Singh et al., (2009), that three to five additive genes (mainly slow rusting genes) could provide durable resistance to cultivars.

In this regard population size is an important limitation factor in this study. The resistance behind ‘Stephens’ seems complex, with a special combination of APR and seedling genes. In this study it was not possible to get one line in the progeny with such combination as ‘Stephens’ look near-immunity as is seen in the tested regions.

Table 2.6. Summary of QTL identified, closest marker and relationship to previous studies of wheat stripe rust resistance

Chromosome	DArT marker associated	QTL name	References
1A	wPt4399	QYr.orr-1AL	Chen et al.1995 (YrDa1); Crossa et al. 2007.
1D	379331/wPt7946	QYr.orr-1DS	Calonnec and Jonhson, 1998(Yr25); Crossa et al. 2007.
2A	wPt0003/wPt1657	QYr.orr-2A	Bariana and McIntosh, 1993 (Yr17); Boukhatem et al. 2002; Chhuneja et al. 2007; Crossa et al. 2007; Eriksen et al. 2004 (Yr32); Mallard et al. 2005.
2B	wPt5738	QYr.orr-2BS.1	Bariana et al. 2001; Börner et al. 2002; Boukhatem et al. 2002; Crossa et al. 2007 ;
	wPt0408	QYr.orr-2BS.2	Dredyver et al. 2008; Guo et al. 2008; Mallard et al. 2005; McDonald et al. 2004 (Yr27, Yr31); Rosewarne et al. 2008 (Yr5, Yr7, Yr27); Luo et al.
3A	wPt1652	QYr.orr-3AL	Chen et al. 1995 (YrTr2); Crossa et al. 2007.
4A	wPt0032	QYr.orr-4AL	Chen et al. 1995 (YrHVII, YrMin); Crossa et al. 2007.
4B	wPt5265	QYr.orr-4BS	Chen et al. 1995 (YrCle, YrMor and YrYam); Crossa et al. 2007; Lu et al. 2009;
	312980	QYr.orr-4BL	Suenaga et al. 2003.
5A	tPt-4184	QYr.orr-5AS	Bariana et al. 2006 (Yr34); Boukhatem et al. 2002; Crossa et al. 2007; Calonnec and Jonhson, 1998.
6A	378849	QYr.orr-6AL	Lillemo et al. (2008); Lin and Chen (2008) ; Marais et al. 2006 (Yr38); Crossa et al. 2007.
7A	376425	QYr.orr-7AS	Börner et al. 2002; Crossa et al. 2007.
7B	wPt7653	QYr.orr-7BS	Crossa et al. 2007 (Yr2, Yr6, Yr39); Rosewarne et al. 2008 ; Suenaga et al. 2003.

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GENERAL CONCLUSIONS

CHAPTER 3

This research highlights the importance of the presence of seedling and adult plant resistance genes for the expression of durable resistance to stripe rust. All QTL found in this study are in chromosomes where previous studies reported regions for resistance genes to stripe rust. Many of the studies were conducted with cultivars related to ‘Stephens’, suggesting the presence of those regions in the genetic background of this cultivar. Due the nature of this study and resolution of the linkage map used, conclusions about the nature of QTL found and gene action are not possible but suggestions are made based on the results obtained.

Two main groups of locations (mega-environments) corresponded to two different environmental-disease response interactions, each independent of the other. Different resistant response to the disease could be due presence of seedling epidemics; different structure of the races prevalent in the region and/or different environmental conditions. It is possible that seedling epidemics could trigger a different response at adult plant stage in ‘Stephens’ over temperature variation.

Although a high number of QTL were found in this study, just 65% of the resistance is explained, meaning that a remaining 35% is unaccounted for. Population size is an important limitation factor in this study. The resistance behind ‘Stephens’ seems complex, with a special combination of major and minor genes. In this study, it was not possible to identify one line with the level of resistance ‘Stephens’ has in the field. However, lines were recovered similar to ‘Stephens’ with resistant allele combinations of the 11 QTL found. In this study is difficult to be precise relating QTL to known Yr genes due map resolution.

For a breeding program, the establishment of marker assisted selection for resistance to wheat stripe rust is challenging, many genetic regions seems to play a role in the resistance response. Different approaches can be used to incorporate those regions into a cultivar. Screening progeny to confirm presence of desirable allele to be

later tested in the field. Incorporation of many alleles increases difficulty and complications. High-throughput genotyping, superior phenotyping and complex statistical resources are all need it in the development of new varieties with durable disease resistance with combinations of other desirable traits.

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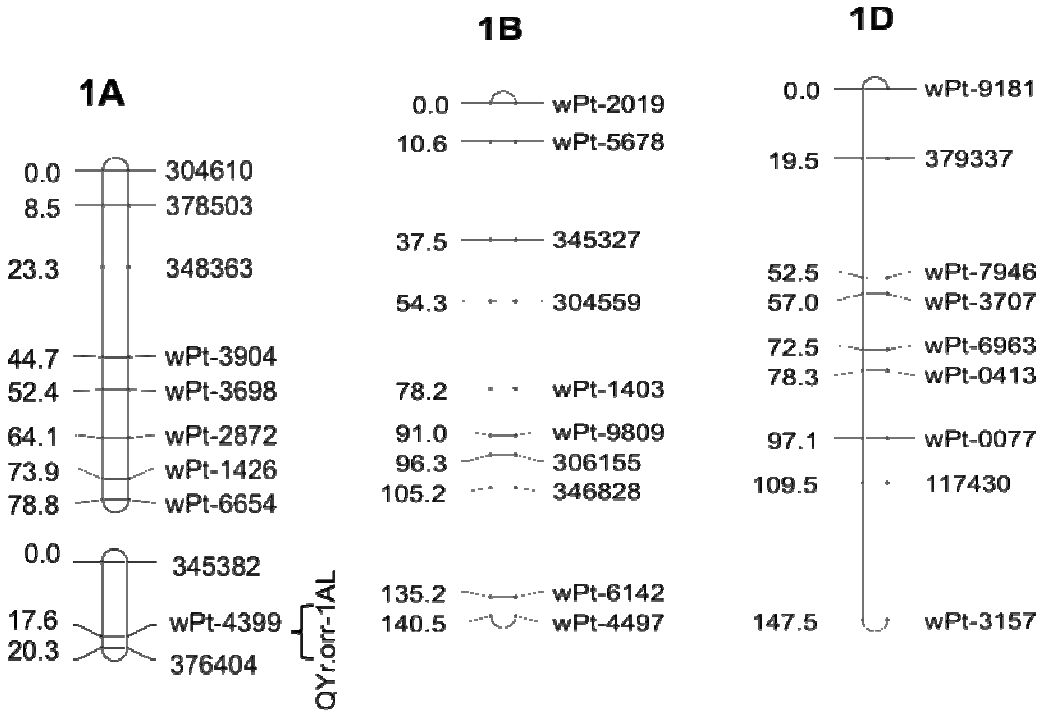
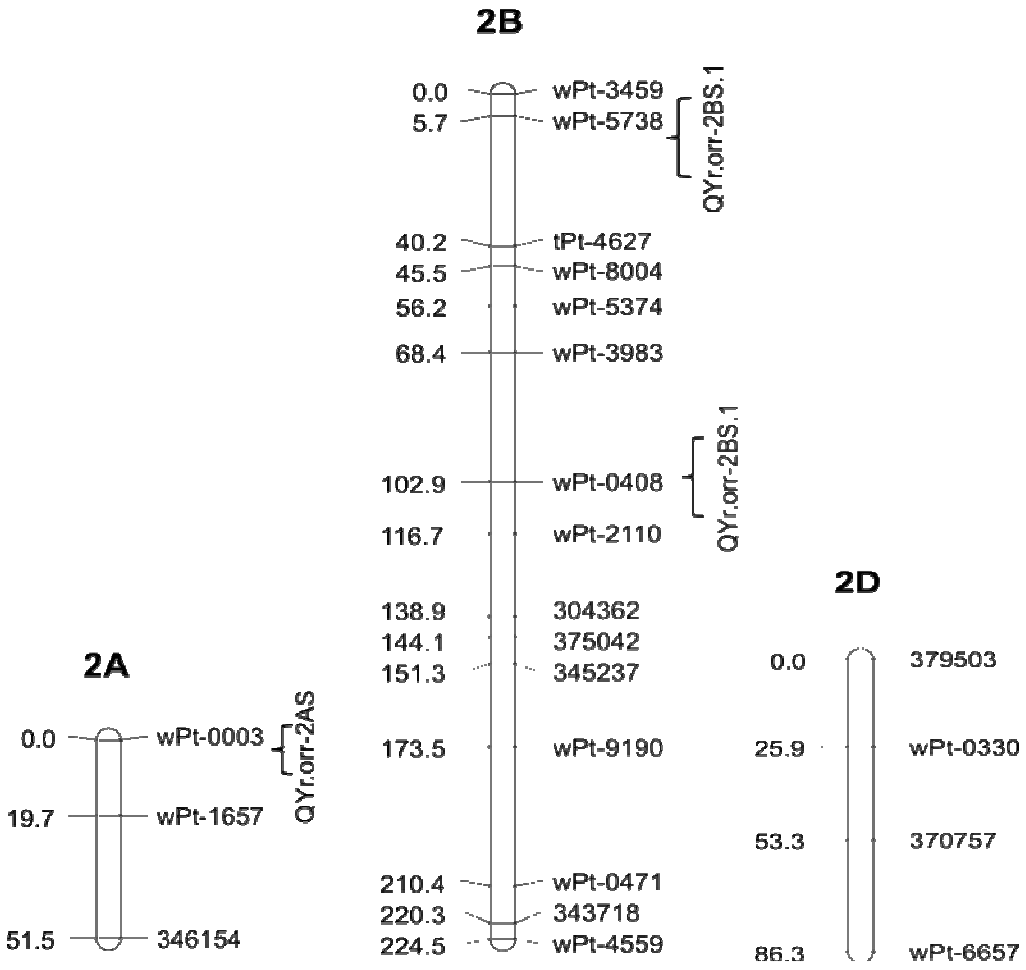
APPENDICES

APPENDIX A Dates and plant growth stage for each location/year

Year	Location	Date note	Growth stage	Zadok scale
2008	Corvallis	June 27	Early milk	Z73
	Mt. Vernon	June 05	Heading	Z55
	Pendleton	June 26	Early milk	Z73
	Toluca	Middle June	Late milk	Z77
	Whitlow	July 11	Soft dough	Z85
2009	Corvallis	June 02	Anthesis	Z65
	Mt. Vernon	June 06	Heading	Z55
	Toluca	June 10	Late milk	Z77

APPENDIX B A linkage map of wheat based on a mapping population from a cross between ‘Stephens’ x ‘Platte’

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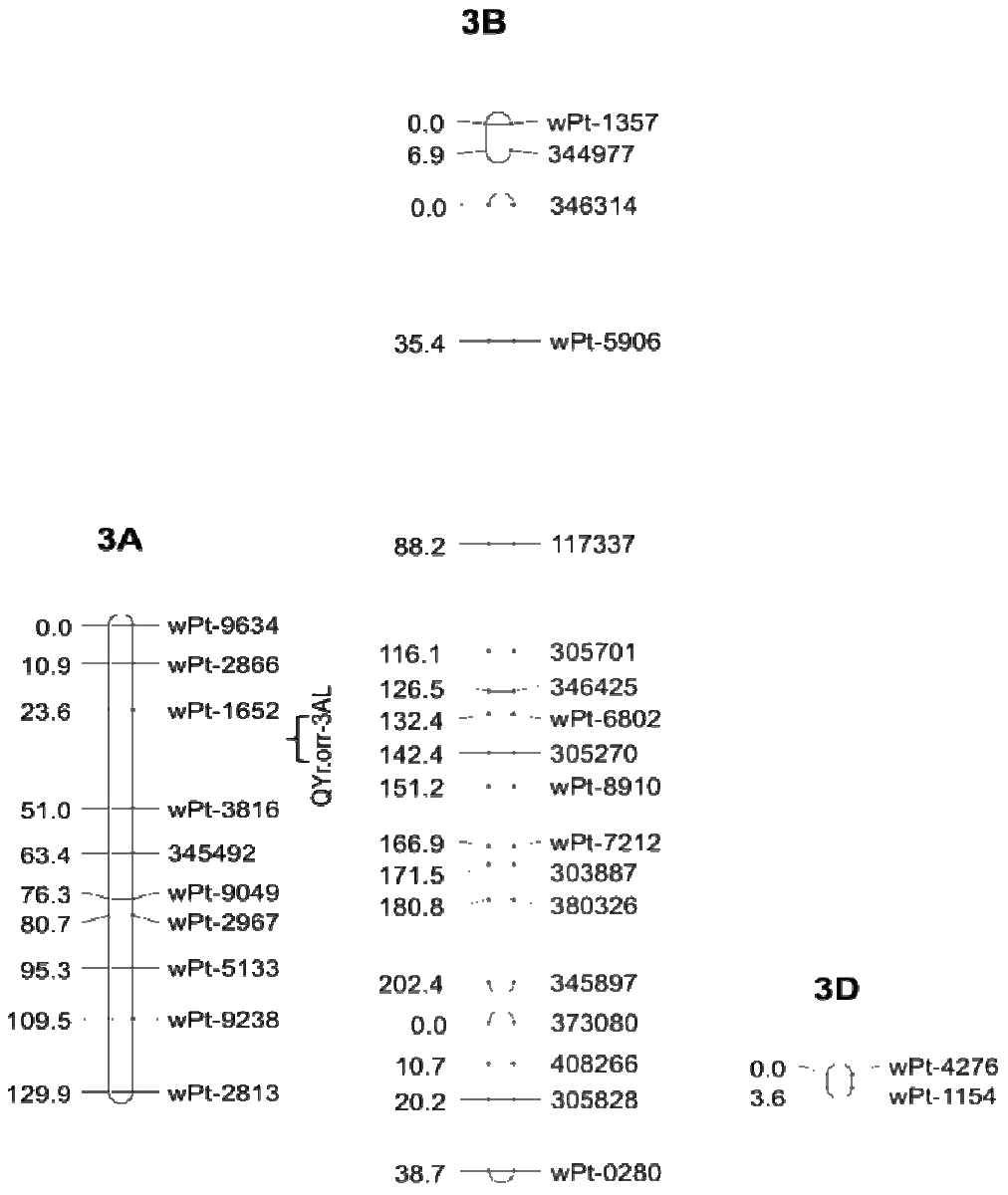
5.7
40.2
45.5
56.2
68.4
102.9
116.7
138.9
144.1
151.3
173.5
210.4
220.3
224.5
wPt-3459
wPt-5738
tPt-4627
wPt-8004
wPt-5374
wPt-3983
wPt-0408
wPt-2110
304362
375042
345237
wPt-9190
wPt-0471
343718
wPt-4559
QYr.orr-2BS.1
QYr.orr-2BS.1
2A
0.0
19.7
51.5
wPt-0003
wPt-1657
346154
QYr.orr-2AS
2D
0.0
25.9
53.3
86.3
379503
wPt-0330
370757
wPt-6657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0.0 346314
35.4 wPt-5906
3A
88.2 117337
0.0 wPt-9634
10.9 wPt-2866
23.6 wPt-1652
QYr.orr-3AL
51.0 wPt-3816
63.4 345492
76.3 wPt-9049
80.7 wPt-2967
95.3 wPt-5133
109.5 wPt-9238
129.9 wPt-2813
116.1 305701
126.5 346425
132.4 wPt-6802
142.4 305270
151.2 wPt-8910
166.9 wPt-7212
171.5 303887
180.8 380326
202.4 345897
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10.7 408266
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3D
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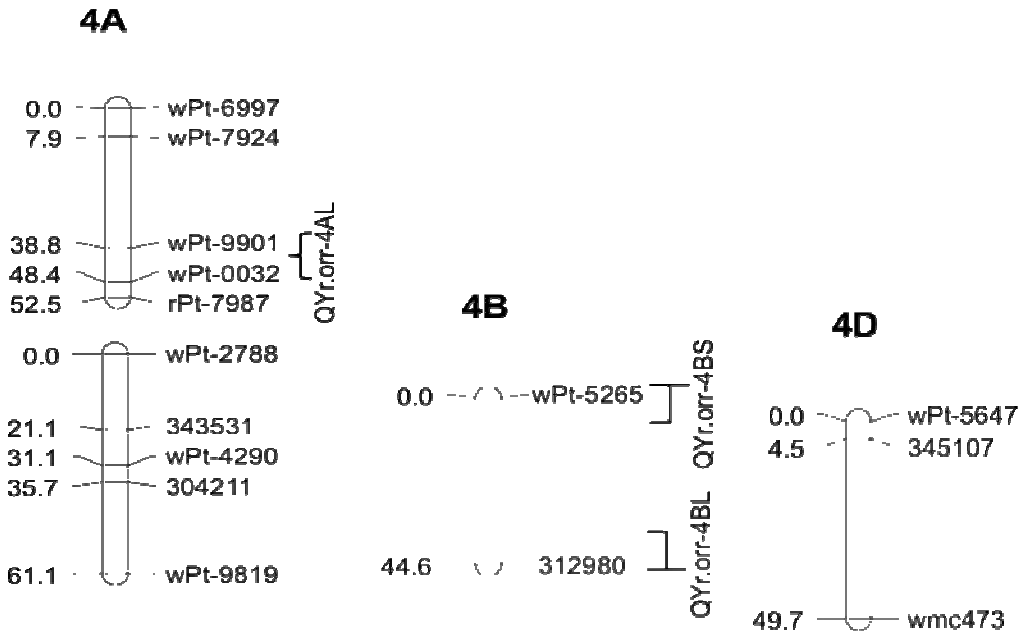
3.6 wPt-1154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7.9
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48.4
52.5
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wPt-9901
wPt-0032
rPt-7987
QYr.orr-4AL
0.0
21.1
31.1
35.7
61.1
wPt-2788
343531
wPt-4290
304211
wPt-9819
4B
0.0
44.6
wPt-5265
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4.5
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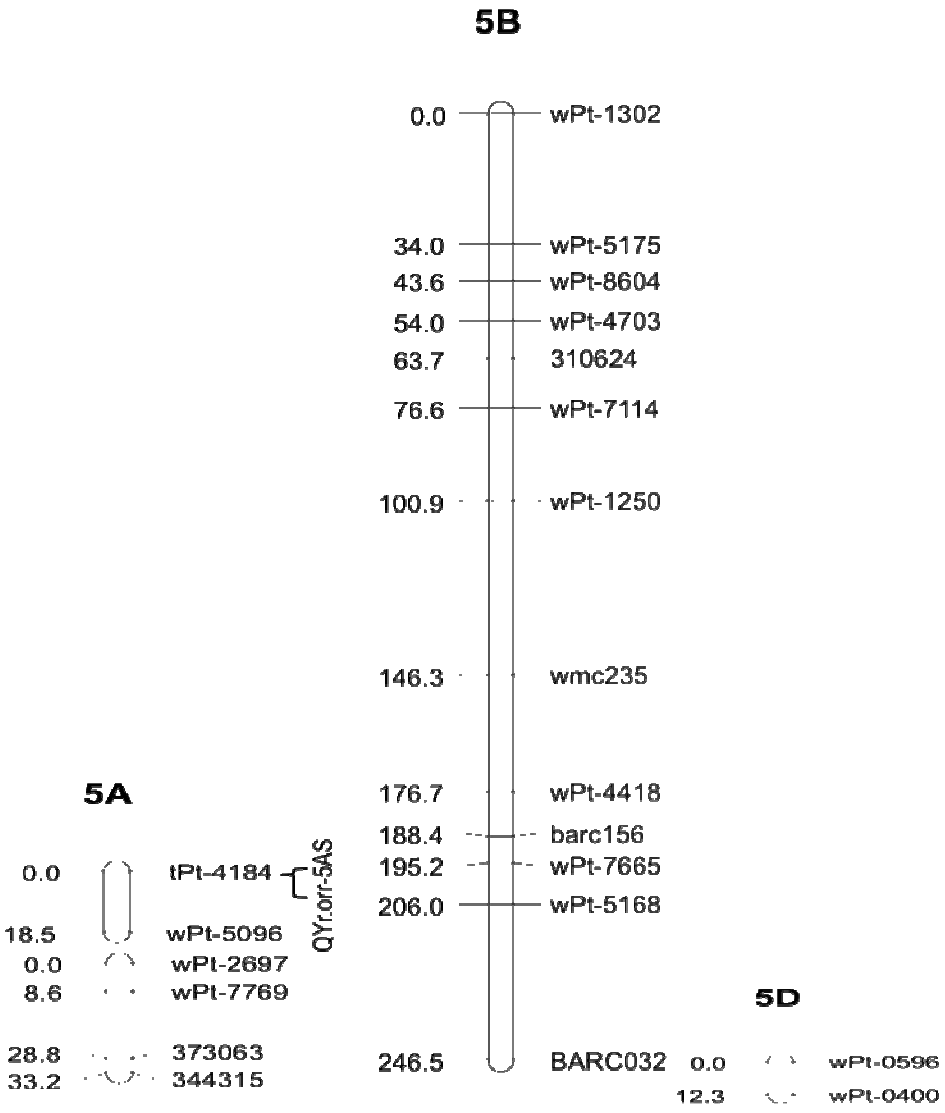
wmc473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wPt-4703
63.7
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76.6
wPt-7114
100.9
wPt-1250
146.3
wmc235
176.7
wPt-4418
188.4
barc156
195.2
wPt-7665
206.0
wPt-5168
246.5
BARC032
5A
0.0
tPt-4184
QYr.orr-5AS
18.5
wPt-5096
0.0
wPt-2697
8.6
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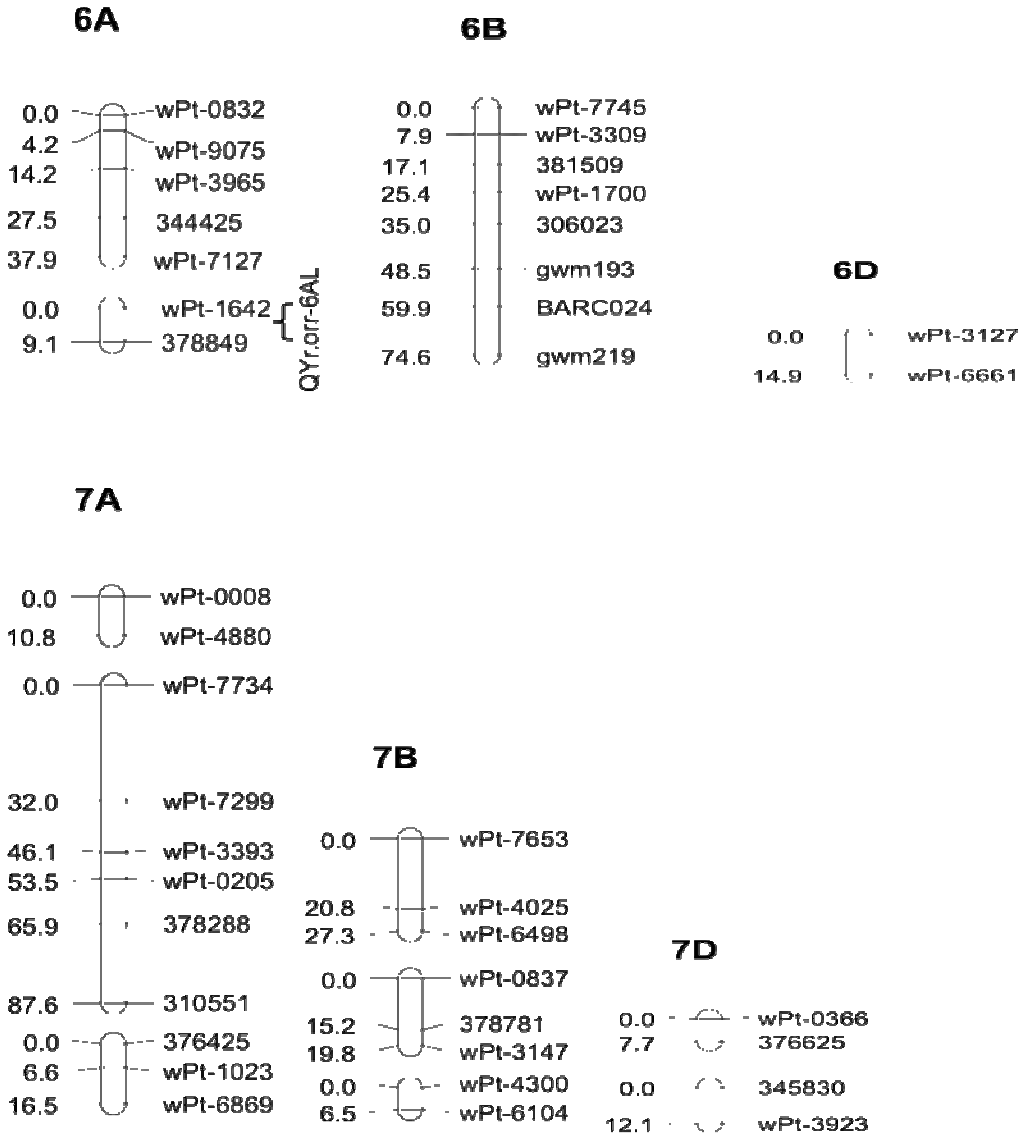
33.2
344315
5D
0.0
wPt-0596
12.3
wPt-0400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4.2 wPt-9075
14.2 wPt-3965
27.5 344425
37.9 wPt-7127
0.0 wPt-1642
9.1 378849
QYr.orr-6AL
6B
0.0 wPt-7745
7.9 wPt-3309
17.1 381509
25.4 wPt-1700
35.0 306023
48.5 gwm193
59.9 BARC024
74.6 gwm219
6D
0.0 wPt-3127
14.9 wPt-6661
7A
0.0 wPt-0008
10.8 wPt-4880
0.0 wPt-7734
32.0 wPt-7299
46.1 wPt-3393
53.5 wPt-0205
65.9 378288
87.6 310551
0.0 376425
6.6 wPt-1023
16.5 wPt-6869
7B
0.0 wPt-7653
20.8 wPt-4025
27.3 wPt-6498
0.0 wPt-0837
15.2 378781
19.8 wPt-3147
0.0 wPt-4300
6.5 wPt-6104
7D
0.0 wPt-0366
7.7 376625
0.0 345830
12.1 wPt-3923

APPENDIX C QTL associated with disease seedling response in Mt. Vernon 2008, 2009 and combined 2008-2009 analysis, including position and peak on the linkage map; closest linked markers; likelihood odds (LOD) scores; phenotypic coefficients (R^2) and estimated additive effects (a). Negative additive effect values indicate that the resistance allele is derived from 'Stephens'

			Mt. Vernon 2008			Mt. Vernon 2009			Combined 2008-2009		
QTLname	QTL peak cM	Closest marker	LOD	a	R^2	LOD	a	R^2	LOD	a	R^2
QYr.orr-2D	2.0	wPt0330	.	.	.	4.0	-3.0	9	.	.	.	.
QYr.orr-5AS	2.0	tPt4184	6.0	-8.2	13	.	.	.	6.1	-5.4	13
QYr.orr-6AL	2.0	378849	.	.	.	4.1	3.0	9	2.87	3.5	5
QYr.orr-1DS	27.0	379337	4.6	-10.8	23	2.9	-2.5	6	5.5	-6.8	21