

IDENTIFICATION OF QUANTITATIVE TRAIT LOCI AND MOLECULAR
MARKERS FOR DISEASE, INSECT AND AGRONOMIC
TRAITS IN SPRING WHEAT (*Triticum aestivum* L.)

By

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Abstract

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Wheat (*Triticum aestivum* L.) is a staple global crop, resulting in significant efforts to create sustainable cropping systems through improving agronomic traits and enhancing disease and insect pest resistance in commercial cultivars. Our first objective was to develop a genetic linkage map using 188 recombinant inbred lines from a 'Louise' by 'Penawawa' cross to identify quantitative trait loci (QTL) for high-temperature, adult-plant resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*). The genetic linkage map was constructed using 295 polymorphic simple sequence repeat and one single nucleotide polymorphism markers. F_{5,6} lines were evaluated for stripe rust reaction, and subsequent agronomic traits, under natural infection in replicated field trials at five locations in the U.S. Pacific Northwest in 2007 and 2008. One major QTL, designated *QYrlo.wpg-2BS*, associated with HTAP resistance in Louise, was detected on chromosome 2BS (LOD scores ranging from 5.5 to 62.3 across locations and years) and flanked by *Xwmc474* and *Xgwm148*. Our second objective was to identify DNA markers associated with the Hessian fly (*Mayetiola destructor* [Say]) resistance gene *H3*. Fifteen plants per line were evaluated under greenhouse conditions, scored as either resistant or susceptible and data were converted to percentage of plants susceptible. Two markers, *Xpsp2999* and *Xcfd15*

were significantly associated with *H3* at a LOD score of 65.2, and accounted for 83% of the phenotypic variation. Our third objective was to identify QTL associated with seedling growth habit, leaf color, plant height, heading date, maturity date, grain volume weight, grain protein concentration, and grain volume. The QTL *QFlt.wak-2D* and *QMat.wak-2D* were associated with the *Ppd-D1* gene for photoperiod insensitivity. Variation in plant height was associated with three QTL on chromosome 3B and one QTL on chromosome 2D. A QTL for leaf color was identified on chromosome 2B. Grain yield QTL were attributed to the effects other QTL had on pest resistance, plant growth and adaptability. Seedling growth habit mapped to chromosome 2D, and a significant QTL for grain volume weight was detected on chromosome 1D. Forward breeding strategies implementing selection using these markers will increase agronomic performance and the durability of pest resistance in developed cultivars.

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Dedication

This dissertation is dedicated to those who not only get satisfaction from knowing the answer, but from knowing why it is the answer.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Wheat (*Triticum* spp.) is the primary food grain directly consumed by humans worldwide, and more acreage is dedicated to the commercial production of wheat than any other crop in the world (Briggle and Curtis, 1987). In the United States (U.S.), 49 million metric tons of wheat are produced annually on over 23 million hectares, with nearly 20% of production occurring in California and the Pacific Northwest (PNW: Idaho, Oregon, and Washington) (NASS, 2007). Stripe rust, also known as yellow rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., is one of the most destructive diseases of wheat on a global scale (Line, 2002; Chen et al., 2002). Wheat stripe rust has been reported in more than 60 countries and on every continent except Antarctica, with the United States (U.S.) reporting its identification in 1915 (Line, 2002; Chen, 2005).

Stripe rust has been the most important disease of wheat in the western U.S. since the 1960's, and is becoming increasingly more prominent in the central and south-central U.S. (Line, 2002; Line and Qayoum, 1992). Due to high production of susceptible cultivars, along with constant green-bridge opportunities, severe disease epidemics have repeatedly occurred in the PNW over the past 45 years (Chen, 2005). Since 2000, nearly one million metric tons of wheat has been lost per year, on average, in the U.S. due to stripe rust (Cereal Disease Laboratory, 2007). Severe epidemics occurred in 2003 and 2005, when stripe rust related grain losses reached 2.7 and 2.2 million metric tons, respectively. Stripe rust epidemics are forecasted to continue to plague wheat crops in the region as long as large acres of susceptible cultivars are planted.

Economic Impact of Stripe Rust on Wheat

Historically, the most severe stripe rust epidemics in the U.S. have occurred in the PNW. In 2002, extremely favorable weather conditions for stripe rust infection occurred in Washington State, resulting in infection of 178,000 spring wheat hectares (70% of total hectares) and 18,200 winter wheat hectares (2.5% of total hectares) (Chen, 2005). Approximately 69,000 hectares were sprayed with fungicide at a cost of over \$2.5 million. The value of yield losses without fungicide use was estimated at \$26 to \$33 million (Chen, 2005). In California, the disease caused yield losses of 25% statewide in 2003, prompting the use of fungicides in the state for the first time (Chen, 2005). Since 2000, stripe rust has been detected at alarming rates in the south central states and the central Great Plains. In Arkansas, yield losses of 7%, 5%, and 3% resulted from stripe rust infection in 2000, 2002, and 2003, respectively, whereas prior to this the only yield losses greater than 2% occurred in 1987 (Cereal Disease Laboratory, 2007). Stripe rust seldom caused significant damage in Kansas before 2000; however, the state wheat crop suffered yield losses of 7.3 and 10.6% in 2001 and 2003, respectively (Chen, 2005). Due to the large acreage of wheat grown in the central Great Plains, record yield losses resulting from stripe rust occurred in 2003, with losses exceeding 2.4 million metric tons (Chen et al., 2004).

Importance of Wheat

In 2005, over 219 million hectares of wheat were harvested around the world, with 10% of that being grown in the U.S. (FAO, 2007). Wheat is grown in 42 of the 50 states, but is best adapted to the diverse climatic regions in the PNW, where yields can be 50% higher than those from other U.S. production regions (NASS, 2007). Two growth habit types, winter (fall-sown) and spring (spring-sown) are in commercial production in the PNW, and five market classes are grown, including soft white, soft white club, hard white, hard red winter, and hard red spring

(Washington State University, 2007). Market classes are distinguished by grain color (red or white), grain hardness (soft or hard), head type (club or lax), and in some cases growth habit type. All four traits are simply inherited. Red seed color is associated with dormancy, milling, and phenols (Flintham, 2000), whereas hardness impacts flour extraction rate and water absorption. Head type is determined by spike rachis internode length, where short internodes result in a compacted, or club, head type (Jones and Cadle, 1997).

Each wheat market class is associated with distinct end-use products (Table 1). Flour from hard wheat grain generally has strong gluten, which allows the dough to hold carbon dioxide produced by yeast and bacterial fermentation resulting in leavened bread. Flour from hard wheat also has higher damaged starch, which leads to higher water absorption by the flour (Hatcher et al., 2002). Under similar milling conditions, 1 to 3% more flour can be extracted from hard white compared to hard red grain, since more white wheat bran can be included in the flour without adversely affecting flour color and flavor (Boland and Dhuyvetter, 2002). Hard white wheat is superior to hard red wheat for making Asian noodle due to a more desirable end product color (Seib et al., 2000). Flour from soft wheat has weaker gluten and lower moisture absorption, making it suitable for the production of cakes, cookies, and crackers, for which strong gluten would impart a tough and chewy texture (Morris and Rose, 1996). Flour extraction rates are higher for club than common wheat (Jones and Cadle, 1997). The ability to produce an array of wheat market classes in the PNW allows producers to base planting decisions on market forecasting.

Based on its high grain yield potential, winter wheat production predominates in the PNW; however, spring wheat plays an important role as a rotation crop and is used for spring re-seeding when the fall sown crop is lost to winter injury (Papendick et al., 1983). Spring wheat

also is better adapted to reduced tillage systems, which are being adopted by many farmers in the PNW to control soil erosion (Papendick, 1998). Since soil erosion is the most severe crop management problem facing farmers in the PNW, more spring grains may be included in crop rotations in the future as the benefits of reduced tillage are demonstrated.

History and Biology of Stripe Rust

Stripe rust was first described in Europe by Gadd in 1777, although the disease probably occurred long before this time in both cultivated and native landscapes (Eriksson and Henning, 1896). The pathogen was originally referred to as *Uredo glumarum* by Schmidt (1827) and as *Puccinia glumarum* by Eriksson and Henning (1894) following closer evaluation of the pathogen's life cycle. In 1953, Hylander changed *P. glumarum* to *P. striiformis*, the currently accepted name, based on the stripes the pathogen causes on adult plant leaves.

Stripe rust was first recognized in North America in 1915 by F. Kolpin Ravn, a visiting scientist from Copenhagen, Denmark, while surveying wheat cultivars near Sacaton, AZ (Line, 2002; Carleton, 1915; Humphrey and Johnson, 1916; Humphrey, Hungerford, and Johnson, 1924). Once recognized, it also was observed at damaging levels in the PNW that same year (Carleton, 1915). Since its identification, stripe rust has been identified every year in the PNW at varying infection levels (Chen, 2005).

Puccinia striiformis is an obligate parasite, requiring a host plant to grow and reproduce, and only infects green tissue of cereal crops and grasses such as wheat, barley (*Hordeum spp.*), and jointed goatgrass (*Aegilops cylindrica*) (Chen, 2005). Infection can occur as long as the plant is green, and symptoms appear approximately one week after infection (Chen, 2005). The fungus forms tiny, yellow- to orange-colored rust pustules called uredia on plant leaves. Uredia, which contain thousands of urediniospores, are the fruiting structures of the fungus. These

pustules erupt, allowing the powdery yellow-orange urediniospores to spread to other plants through contact or wind dispersal. Late in the season, typically during grain fill, black telia form on leaves (Agrios, 1997). Germinated teliospores form haploid basidiospores, which require an alternate host to infect. The stripe rust pathogen has no known alternate host for the basidiospores to infect, and thus no sexual stage can occur (Chen, 2005). Urediniospores from late maturing spring wheat also can infect early planted winter wheat and volunteer wheat, overwinter, and infect plants in the spring. The presence of green plants between the harvest of one crop and planting of the next crop is referred to as a green-bridge (Cook and Vesseth, 1991). Typically, this is how the disease is transmitted to the following wheat crop in the PNW, although wind direction and intensity also plays a significant role (Line, 2002; Chen, 2005). The life cycle of *P. striiformis* consisting of the uredial and telial stages is shown in Figure 1.

The ability of stripe rust to infect a plant closely follows the gene-for-gene concept (Chen, 2005), which was described in detail by H.H. Flor (1971). Flor (1971) states that for each gene that confers virulence to the pathogen there is a corresponding gene in the host that confers resistance. Virulence refers to the pathogenicity, or relative ability, of a microbe to cause disease (Agrios, 1997). Avirulence refers to the inability of a pathogen to cause disease (Agrios, 1997). Virulence is essential for stripe rust to infect the host plant, grow, and reproduce (Chen, 2005). A pathogen with avirulence genes produce gene products known as elicitors (Agrios, 1997). If the plant has resistance genes (R), the R gene-coded host receptor recognizes the pathogen elicitor and triggers defense reactions. If the host lacks the receptor, the host is susceptible to the pathogen. Pathogens with virulence genes produce no specific elicitor. Thus, although the host may have a gene for resistance, infection results because the pathogen lacks the gene for avirulence that is recognized specifically by this particular gene for resistance (Agrios, 1997).

Table 2 identifies the gene combinations and disease reaction types in a host-pathogen system operating on the gene-for-gene concept. The more virulence genes a pathogen has, the more resistance genes it is able to overcome (Chen, 2005). If the host carries many resistance genes, the pathogen will need to accumulate more virulence genes before it can infect the host (Flor, 1971). This can be done through sexual recombination, mutations, or somatic fusion.

A pathogen race is a genetically distinct pathogen genotype that can infect a given set of plant varieties (Agrios, 1997). Races of stripe rust are differentiated by infection produced on a set of selected genotypes that are referred to as differentials. A list of the 20 differentials used to distinguish races is shown in Table 3. Races are distinguished based on which of the 20 differentials they are virulent on. For example, if one isolate of stripe rust is virulent on differentials 1, 6, 8 and 11, and another isolate is virulent on differentials 1, 4, 7, and 10, they are considered different races. Unique races are issued an identification number, preceded by the prefix PST (for example PST-12). A list of 109 races and differentials subject to virulence is provided by Chen (2005). To date, a total of 138 races have been identified (Chen, personal communication)

Since no sexual stage occurs in the life cycle of the stripe rust pathogen, new races with different virulence arise from mutations and/or somatic fusion. Mutations in the DNA of the pathogen can induce novel virulence genes so that the pathogen can infect plants which were previously resistant (Flor, 1971; Chen et al., 2002). New races also evolve through somatic recombination when mixtures of races are present in one production area. Somatic recombination occurs when the dikaryotic urediniospores from two different races form mycelia in the same plant (Flor, 1971; Chen et al., 2002). New urediniospores form when mycelia carrying unique nuclei fuse, resulting in the formation of urediniospores with two nuclei, each

from a different race (Taylor, 1976; Wright and Lennard, 1980). These recombinant spores can then infect wheat plants which were not infected by either of the two progenitor spores because they have combined virulence genes novel to each donor parent.

Infection Types

Uredia form on leaves of susceptible seedlings in irregular shaped patches. Stripes of uredia, which gives rise to the pathogen's common name of 'stripe rust', do not form on the leaves until after stem elongation (Chen, 2005). Line et al. (1970) developed a rating system based on the degree of infection on both seedlings and adult plants (Table 4). A scale of zero to nine is used, based upon the amount of chlorosis or necrosis produced as a hypersensitive response, which can occur in the presence or absence of fungal sporulation. Infection rating varies depending on resistance gene composition of the plant and the average daytime temperature. Wheat plants express ten distinct reaction types in response to the pathogen (Figure 2). Plants are typically considered resistant when infection type (IT) is between zero and three (Chen, personal communication).

Impact of Infection on Plants

Plants infected with stripe rust exhibit decreased vigor and growth because the pathogen robs the plant of water and energy (Agrios, 1997). Photosynthesis also is considerably reduced when infection type (IT) reaches 2, owing to the destruction of much of the photosynthetic tissue, especially on the flag leaf, resulting in a reduction in grain yield and grain quality (Agrios, 1997). Seed produced from wheat plants infected with stripe rust also have low vigor, which leads to poor emergence after germination (Chen, 2005). Chen (2005) estimates yield losses from stripe rust range from 10% to 70% depending on the susceptibility level of the plant, and highly susceptible cultivars exhibit 100% yield losses.

Environmental Factors Affecting Stripe Rust

Stripe rust is highly sensitive to environmental factors including moisture and temperature. *Puccinia striiformis* is considered to be a cool temperature pathogen compared to the other rusts (*P. triticina* and *P. graminis*) that infect wheat, which prefer higher temperatures for germination (Chen, 2005). The optimum temperature for spore germination is between 7 and 12 °C, and the minimum temperature is near 0 °C (Line, 2002). Rapilly (1979) reported that temperatures below -10 °C or above 33 °C inhibit germination and sporulation of the stripe rust pathogen. Night temperatures have a more critical effect on the development of stripe rust than daytime temperatures do (Stubbs, 1985). Hot nights (>12 °C) greatly limit spore infection and survival of the pathogen.

Moisture affects spore germination, infection and survival (Chen, 2005). According to Rapilly (1979), stripe rust urediniospores require at least 3 hours of continuous moisture on the plant surface, through dew formation, rain, or overhead irrigation, to germinate and infect the plant. Excessive moisture also can adversely affect spore survival. When temperatures are not ideal for spore germination, high moisture levels cause spores to lose viability more quickly than dry spores. Hence, dry weather in late summer allows urediniospores on late-harvested spring wheat to survive the summer and infect seedlings of winter wheat planted in the fall (Chen, 2005). Rain also impacts spore dispersal since raindrops release urediniospores, either by direct impact or splashing, which can then immediately germinate if the temperature range is optimal (Rapilly, 1979).

Wind serves multiple functions as far as stripe rust dispersal is concerned. Wind can disperse rust spores from plant to plant and field to field, as well as contribute to long-distance dispersal across continents and around the globe (Brown and Hovmøller, 2002). Hovmøller et

al. (2002) reported that rust spores can travel up to 1700 km in Europe. This long-distance aerial dispersal potential transports stripe rust into new wheat production areas, leading to shifts in race prevalence or increasing diversity in rust populations. Wind also can reduce dew formation on the plants, which limits germination and infection; however, this also can lead to longer spore viability due to low moisture levels.

In the U.S., stripe rust epidemics are most prevalent in California and the PNW where mild winters, followed by cool, wet springs and dry summers are typical (Chen, 2005). Warm days and cool nights, along with high dew formation, also make conditions favorable for stripe rust infection and germination. Chen et al. (2002) reports that stripe rust has become increasingly important in the South Central and Great Plains states when winters are mild and springs are cooler and wetter than normal, thus favoring the environmental conditions needed for stripe rust germination and infection. Due to the increasing severity of stripe rust across the nation, integrated management practices are being implemented to slow progression of the pathogen.

Chemical Control and Economic Thresholds

Stripe rust management strategies include cultural practices, fungicide applications, and growing resistant cultivars as a means of controlling the spread and development of the pathogen. Effective cultural practices include altering planting dates and removal of volunteer wheat plants to avoid the green bridge, along with changes in irrigation timing and application method to eliminate periods of leaf wetness (Brown et al., 2001). Stripe rust infection often is controlled by the application of systemic fungicides, both as seed treatments and foliar sprays. Generally, seed treatments are less expensive than foliar applications, yet only control stripe rust for 8 to 12 weeks after application (Yan, 2006). Thus, if stripe rust infects a crop after this

period, a foliar treatment might still be necessary to control the pathogen. Triadimefon (Bayleton) was the first effective commercial fungicide for stripe rust control in the U.S (Line, 2002). Foliar fungicides Tilt (propiconazole) (Syngenta Crop Protection, Greensboro, NC), Quadris (azoxystrobin) (Syngenta Crop Protection, Greensboro, NC), Quilt (azoxystrobin + propiconazole) (Syngenta Crop Protection, Greensboro, NC), and Headline (strobilurin) (BASF Corporation, Research Triangle Park, NC) have proven highly effective in controlling stripe rust on wheat (Cartwright, 2000; Chen and Wood, 2002; Davis and Jackson, 2007). Cost of chemical application varies from \$20 to \$50 per hectare depending on whether application is made by ground or air (McGregor Company, 2007). When profit margins are narrow, the economic risks associated with fungicide application are high. In contrast, when economic returns and profit potential are high, chemical control methods are warranted.

The pathogen density at which management intervention must be taken to prevent stripe rust from reaching the economic injury level is termed economic threshold (Paveley et al., 1997). Economic injury level is the amount of stripe rust per plant at which the cost of controlling the pathogen equals the value of the crop yield that would be lost if no control measures were taken (Paveley et al., 1997). Published work on the economic threshold of stripe rust infection on wheat is limited. In the PNW, spray recommendations for spring wheat are based on the equation:

$$\text{grain price (dollars/MT)} \times \text{metric tons saved/hectare} = \text{cost of application}$$

(dollars/hectare) (Chen, personal communication).

At an average application cost of \$35 per hectare (McGregor Company, 2007) and a current grain price of \$160 per metric ton (Chicago Board of Trade, 2007), 0.2 metric tons of grain per hectare would have to be gained after chemical application to justify treatment. In other words,

if inoculum of stripe rust in a crop was expected to cause yield losses of more than 0.2 metric tons, the economic return of spraying would be greater than that of not spraying.

Due to varying prices of grain and fungicide costs, economic thresholds to justify fungicide application change each year. Yield loss also is difficult to predict since it depends upon disease pressure, cultivar resistance/susceptibility level, and yield potential. In 2005, for example, yield losses of 7 and 10% occurred on spring wheat cultivars ‘Wawawai’ (PI 574538) and ‘Alpowa’ (PI 566596), respectively, under heavy inoculation pressure in Washington State (Chen, unpublished data). In contrast, cultivars ‘Scarlet’ (PI 601814) and ‘Penawawa’ (PI 495916) exhibited yield losses of 20 and 30%, respectively, in the same experiment. When the pathogen was controlled by fungicide, the yields of Scarlet and Penawawa increased by 25 and 43%, respectively, as compared to the control. Fungicide application to Wawawai and Alpowa did not result in significant yield increases when compared to unsprayed controls (Chen, unpublished data). In this example it would be cost effective to spray Scarlet and Penawawa but not Wawawai or Alpowa. Crop consultants typically recommend spraying when 5% of the crop is infected with stripe rust at an infection type of 4 or higher (McGregor Company, 2007; Duff et al., 2006). Dr. Xianming Chen, plant pathologist for the USDA in Pullman, WA, recommends controlling stripe rust when disease severity is 5 to 10% on 10% of the plants (Chen, personal communication).

Genetic Control of Stripe Rust

Although chemical control is an option, growing resistant cultivars is the most effective, environmentally friendly, and economical choice for wheat stripe rust control (Line and Chen, 1995). Infected seedlings and adult plants display various forms of hypersensitive response, resulting from host resistance mechanisms and temperature (Chen, 2005). There are two main

types of resistance. All-stage resistance is race specific and protects the plant during all stages of development (Chen, 2005). High-temperature adult-plant (HTAP) resistance, which is non-race specific and more durable, increases in effectiveness as the plant ages and the temperature increases (usually after stem elongation) (Chen, 2005).

All-stage resistance (also called seedling resistance) can be detected at the seedling stage, but is expressed at all stages of plant growth (Chen, 2005). All-stage resistance is race specific, only providing resistance to certain races of the fungus, according to the gene-for-gene concept. Due to the rapid evolution of new races with new virulence genes, cultivars with single, race-specific genes often become susceptible after release (Line and Qayoum, 1992; Line and Chen, 1996). For example, the cultivar ‘Zak’ was released from Washington State University in 2002 (Kidwell et al., 2002). This cultivar underwent 7 years of stripe rust testing and always displayed high levels of resistance to the pathogen. Unfortunately, a race shift occurred in its first year of commercial production and 40,000 hectares of Zak were highly susceptible to the new race, PST-78, that invaded the region (WASS, 2006). This one race shift cost farmers an estimated \$1.5 million in crop losses due to reduced grain yields, inferior grain quality, and/or fungicide application costs. To prevent situations like this from occurring, durable, non race-specific resistance must be identified and incorporated into new wheat cultivars.

High-temperature adult-plant (HTAP) resistance, present in many winter wheat cultivars and some spring cultivars, has proven to be durable and non-race specific (Qayoum and Line, 1985; Chen and Line, 1995a, 1995b; Line and Chen, 1995; Chen et al., 1998a). Cultivars with only HTAP resistance are susceptible to pathogen attack at the seedling stage; however, as temperatures increase and the plant ages, the plant becomes resistant and rust development slows and may even stop, regardless of race structure (Chen, 2005). Temperature plays an important

role both in host resistance as well as spore infection. Sharp (1962) observed susceptible symptoms on wheat plants at 15 °C, whereas a resistant reaction was detected at 25 °C, indicating that host plant resistance is affected by the temperature.

The level of HTAP resistance also varies among cultivars. Alpowa has HTAP resistance; however, in field trials under heavy infection pressure, an infection type of 2-3 over 30-50 percent of the leaf area is often observed (Chen, unpublished data). This can result in yield losses of 4 to 10% even with HTAP resistance. Under moderate disease pressure, no yield loss is detected. In contrast, the spring wheat cultivar 'Louise' also has HTAP resistance, and under both moderate and heavy disease pressure, infection is limited and no significant yield losses occur (Kidwell et al., 2006; Chen, unpublished data). It appears that these two cultivars either carry different HTAP resistance genes, or genetic background influences the expression of this HTAP gene.

Need for Durable Resistance

With infections occurring annually, genetic resistance is the most economical protection producers can use against the pathogen (Line, 2002). All-stage resistance is widely deployed, but often is quickly overcome by new race changes (Chen, 2005). HTAP resistance is deployed in only 16% of spring wheat cultivars in the PNW, leaving many planted fields vulnerable to infection (Chen, unpublished data). Susceptible spring wheat cultivars are easily infected by increased inoculum levels from earlier maturing susceptible winter wheat cultivars (Chen, 2005). Due to its durability, incorporation of HTAP resistance genes into locally adapted germplasm is essential, along with the ability to move this resistance into adapted germplasm in an efficient and cost effective manner (Börner et al., 2000; Boukhatem et al., 2002; Bariana et al., 2001; Singh et al., 2000).

Gene Mapping

Servin et al. (2004) states that “recently there have been advances in the mapping of genes involved in the variation of quantitative traits, through quantitative trait loci mapping experiments and analysis of genomic data.” Such studies lead to the identification of the genetic factors responsible for complex quantitative traits. These genetic factors are identified as either a gene or a quantitative trait loci (QTL). A gene is a sequence of DNA that occupies a specific location and determines a particular characteristic (Lynch and Walsh, 1998). A qualitative gene refers to a single genetic factor which results in phenotypes that can be distinctly categorized (Pierce, 2005). Quantitative genes are several genes which act in conjunction with the environment, resulting in a specific phenotype (Pierce, 2005). As such, each gene making up a quantitative trait can be responsible for varying levels of trait expression. A QTL is a locus segregating for alleles that have different, measurable effects on the expression of a quantitative trait (Hartl and Jones, 2006). The wide range of genes and possible permutations of genetic coding allows for a wide range of phenotypes. Genetic mapping identifies the chromosome that the gene/QTL is associated with as well as the location on that chromosome. Molecular markers are typically DNA segments that occupy a specific location on a specific chromosome and can be used to identify the presence of these genetic factors, although the presence of certain proteins and enzymes can also be used as markers (Lynch and Walsh, 1998).

Types of Markers

Markers can either be dominant or co-dominant. Co-dominant markers identify both the homozygotes as well as the heterozygotes (Gerber et al, 2000). This allows both alleles in the population to be visualized and tracked. Dominant markers only detect two patterns, presence or absence of a band (Jiang and Zeng, 1997). Jiang and Zeng (1997) report that there has been

concern about the use of dominant markers because the heterozygote can have the same band pattern as one of the homozygotes, which provide partial missing information. If mapping populations contain only partial marker data, the distance between the marker and the gene of interest is underestimated (Liu, 1998). Linkage groups and marker orders are not well determined as a consequence of this as well (Liu, 1998). As a result, co-dominant markers are favored for use in genetic studies over dominant markers when available (Masojc, 2002).

Determining Genetic Linkage

Molecular markers can only be used to indicate presence of genetic factors if they are linked to the gene/QTL of interest. Linkage occurs when particular alleles, in this case the marker and the genetic factor, are inherited together more than 50% of the time (Hartl and Jones, 2006). Linkage is determined by using a χ^2 analysis, which tests if the observed genotypic classes deviate from the expected genotypic frequencies with independent assortment (Pierce, 2005; Dubcovsky, 2007). The null hypothesis states the observed genotypic classes do not deviate from those expected with independent assortment. Thus, if the results of the χ^2 reveal significant differences between the observed class and the expected classes, the marker and genetic factor are considered linked (Allard, 1956; Dubcovsky, 2007). Given two linked markers, the next step is to calculate how far apart the two loci are. Distance between two markers are reported in centiMorgans (cM), or map units (mu), which is a unit of distance equal to 1% recombination (Hartl and Jones, 2006). Recombination is the sorting of alleles into new combinations (Pierce, 2005). Hence, if two markers are located 2 centimorgans apart, recombination would occur between them 2 out of 100 times. The closer the genetic factor is to the marker, the greater probability that they will be inherited together (Semagn et al., 2006). The

farther apart they are, the greater the chance of recombination and separation of the genetic factor and marker.

The simplest method of determining distance is to divide the total number of recombination events detected by the total number of meiotic events (Allard, 1956). This will identify the percentage of recombination between the two markers, which can be converted to map units. While this method allows for an approximation, more accurate methods for distance determinations are available which adjust the proportion of observed recombinant genotypes by counting single crossover events once and double crossover events twice. Double crossover events occur when two single crossover events take place at the same time between two loci (Singh, 2003). If double crossover events, which provide the same genotypic class as no crossover events, are not taken into consideration, estimations of distance between loci is shortened. The improved estimation is calculated as:

$$m = -(1/2)\ln(1-2p)$$

where p is the observed recombination fraction and m is the map distance between loci (Ridout et al., 1998; Martin and Hospital, 2006).

Calculations based on maximum likelihood give the best determination for genetic distances because maximum likelihood equations are quite general and may be applied to any genetic data providing information about linkage (Allard, 1956). For example, given marker M and disease resistant trait D , the recombination fraction between the gene and the marker is θ , and the proportion of escapes is ρ (Liu, 1998). Escapes are individuals that are susceptible to the disease but do not show the disease phenotype under experimental conditions. $1-\rho$ is the probability that an individual with the susceptible genotype has the disease phenotype (Liu, 1998). There are four different genotypes that can be observed: $+D$ (genotype with the marker

band and with the resistance allele, probability $0.5(1-\theta)$), +d (genotype with the marker band and without the resistance allele, probability 0.5 θ), -D (genotype without the marker band and with the resistance allele, probability 0.5 θ), and -d (genotype without the marker band and without the resistance allele, probability $0.5(1-\theta)$) (Liu, 1998). Thus, the probability that an individual with phenotype (P) +D is a recombinant (R) is:

$$p_1 (R|P) = 0.5\theta\rho / 0.5 (1 - \theta + \theta\rho)$$

where $0.5\theta\rho$ is the expected frequency of a recombinant individual with '+' marker and 'D' allele, and $0.5 (1 - \theta + \theta\rho)$ is the marginal probability of the same individual (Liu, 1998). If the observed count of the number of individuals is $f_1 = 173$, then the count for the number of recombinants from this observed category is:

$$f_1 p_1 (R|P) = 173 \times 0.5\theta\rho / 0.5 (1 - \theta + \theta\rho)$$

Maximum likelihood is determined through iteration, with an initial guess, θ' and ρ' . The number of recombinants (R) and number of escapes (E) are calculated as explained by Liu (1998). The maximization step computes new estimates for the parameters, which are:

$$\theta'' = R / \text{total sum of individuals}$$

$$\rho'' = E / \text{expected number of susceptible individuals}$$

Once this iteration is complete, θ' and ρ' are set equal to the new estimates θ'' and ρ'' for the next iteration (Liu, 1998). The iterations can be stopped once the likelihood reaches a maximum.

Once the maximum likelihood is reached, the value for θ' is the estimate of the recombination fraction between M and D. Fortunately, computer programs such as Mapmaker (Lander et al.,

1989) have been developed which use these formulas to calculate the distances given large data sets.

The strength of the association between the trait and marker is measured with the LOD score. The LOD score is calculated as: $LOD = \log (\text{probability of obtaining the observed data with linkage}) / \text{probability of obtaining the observed data with random assortment}$ (Ott, 1999). The higher the LOD score, the more probable a marker and gene are linked. For example, a LOD of 4 indicates that it is 10,000 times more likely to obtain the observed data from linkage and not independent assortment. For determining linkage, a LOD score of 3.0 is commonly used as it gives 1000:1 odds that linkage is present (Strachan and Read, 1999; Ghosh and Collins, 1996). Using the above techniques, markers can be placed in linkage groups and the genetic distance between each marker estimated, thus developing a genetic linkage map specific to the mapping population.

Identification of genetic factors controlling traits is typically executed by utilizing mapping populations. To be suitable for mapping, a population must be segregating for the trait of interest. Mapping populations are created by cross-hybridizing parent plants that are phenotypically distinct for the specific characteristic(s) of interest (Rousset et al., 2001). The progeny from this hybridization will be segregating for the trait(s) which are distinct in each parent. Population size varies among mapping populations. Generally the more individuals the more accurate the marker order and distance between markers are because more recombination events are being examined (Ferreira et al., 2006). Although Ferreira et al. (2006) state that populations over 500 individuals maximize accuracy, populations with individuals of 150 to 200 are typically recommended to minimize input costs for population development, marker and trait screening, while still maintaining 98% accuracy of linkage group identification.

Types of Mapping Populations

Different types of segregating populations are used to develop genetic linkage maps, and subsequently identify molecular markers linked to traits for which the parents differ. There are three commonly used mapping populations in self-pollinated crops such as wheat. These include F_2 populations, recombinant inbred line (RIL) populations, and doubled haploid (DH) populations. Each population has benefits and disadvantages when used to develop genetic linkage maps and identify QTL.

Molecular mapping can use F_2 populations, which require little time from initial cross to analysis of the population and have high levels of linkage disequilibrium (Peng et al., 1999; Sun et al., 1997; Börner et al., 2000; Ma et al., 2001; Masojc, 2002). Linkage disequilibrium is the tendency of certain combinations of alleles to be inherited together (FAO, 2003). They are inherited together due to the limited recombination that occurs between the two genes, an indication about the distance between the two genes. An association between a given allele from a marker locus and the target gene exists only when linkage disequilibrium is imposed (Masojc, 2002). Linkage disequilibrium between a marker and a QTL implies that there is an association between the marker locus and the QTL across all genetic populations, not just within the specific mapping population (Hayes et al., 2006). Linkage disequilibrium is consistently decreased in consecutive generations of self-pollination. Thus, due to a lower number of recombination events, F_2 populations have shorter (100 cM per chromosome instead of 110 cM) genetic maps than RIL populations, which undergo 6 generations or more of inbreeding. As a result, genetic maps built on F_2 mapping populations underestimate linkage distances (Ferreira et al., 2006; Masojc, 2002).

Genetic distance also can vary depending on whether the alleles are in coupling (A_iB_j gametes are overrepresented) or repulsion (A_iB_j gametes are underrepresented) phase linkage (Lynch and Walsh, 1998). Coupling phase exists when the recombinant gametes provide heterozygous allele combinations, whereas the parental types are homozygous (Lynch and Walsh, 1998). Repulsion phase exists when the parental gametes provide heterozygous allele combinations and recombinant gametes are in the homozygous state (Lynch and Walsh, 1998). F_2 populations do not permit efficient mapping of dominant marker loci with alleles linked in repulsion due to fewer recombination events and under-representation of recombinant gametes (Allard, 1956; Haley et al., 1994). Conversely, Haley et al. (1994), using an F_2 population, report that RAPD (random amplification of polymorphic DNA) markers linked in repulsion to an allele provide dramatically improved efficiency compared to RAPD markers linked in coupling with the same allele, 81.8 versus 26.3%, respectively, in identification of homozygous resistant genotypes among pea cultivars. The authors do acknowledge that the only prerequisite for using repulsion-phase linkages as indirect selection criteria in breeding programs is the ability to identify such markers using mapping populations other than F_2 populations (Haley et al., 1994).

Although production of F_2 mapping populations in wheat is quick and efficient, a limited amount of seed from each F_2 line is obtained. Thus, if testing needs to be performed over multiple locations and/or years, seed is a limiting factor. At times F_3 populations derived from the F_2 generation are used to mimic the F_2 population, and provide the ability to replicate (Börner et al., 2000). Since most quantitative traits require testing over years and locations, populations such as RIL and DH are better suited given that they produce homozygous lines which can be replicated in the field several times and over several cycles.

RIL populations composed of highly inbred individuals are commonly used in molecular mapping of self-pollinated crops such as wheat (Boukhatem et al., 2002; Santra et al., 2008; Anderson et al., 2001). A wheat RIL population is developed by cross-hybridizing two plants to obtain an F₁ hybrid (Carter et al., 2005). The two original plants are selected as parents based on detectable variation for a trait, or traits, of interest. For example, if disease resistance is the trait of interest, one parent must carry the alleles for resistance, whereas the second parent must carry susceptible alleles in order for the trait to be mapped in this cross. One seed is selected from the F₁ progeny and self-pollinated to generate the F₂. If a large population size is desired, or the plant produces small amounts of seed from the F₁, multiple seeds can be selected from the F₁ for advancement (Prioul et al., 2004). Each population created from a different F₁ seed must first be tested for homogeneity before data can be pooled across them. The F₁ progeny are heterozygous for the trait, whereas the F₂ population segregates 1:2:1 (homozygous dominant: heterozygous: homozygous recessive) for the trait (considering a simple gene). Each F₂ seed is planted individually with each plant self-pollinated multiple generations (6-8) using single seed decent (Masojc, 2002). Single seed decent requires one random seed to be chosen from the previous generation which undergoes self-pollination to produce the subsequent generation (Masojc, 2002). Each generation the heterozygous trait continues to segregate in a 1:2:1 ratio, whereas the homozygous traits remain fixed. The amount of herterozygosity left in the population after each generation is calculated as:

$$0.5^{n-1} \times 100$$

where n is the number of self-pollinated generations (Carr and Dudash, 2003). A minimum of six generations, or 97% homozygosity, is typically needed to create a linkage map (Masojc, 2002). Multiple generations of self-pollination are often desired to increase the number of

recombination events, which breaks up the association of large linkage groups and creates genetic disequilibrium (Masojc, 2002). Thus, marker associations from these populations are more reliable over generations and less likely to have recombination occur between them. This is in contrast to F_2 and DH populations which undergo one meiotic event and have large linkage blocks. As more meiotic events occur, the probability of recombination increases and the marker and gene can dissociate (Masojc, 2002).

Doubled haploid populations also are frequently used in wheat to map agronomic traits (Suenaga et al., 2003; Eriksen et al., 2004; Bariana et al., 2001; Castro et al., 2003a). DH populations are created by cross-hybridization of two distinct genotypes, although the resulting F_1 progeny are not self-pollinated. Instead, microspore culture (Hansen and Andersen, 1998), anther culture (Zhou and Konzak, 1989), or interspecific/intergeneric pollination (Suenga, 1994), is employed to produce haploid embryos. The haploid embryos undergo a chromosome doubling event, usually with exposure to colchicine, which fixes genes in a homozygous state. This is the quickest method for producing a homozygous mapping population. Homozygous mapping populations (be it RIL or DH) are desired since they have the ability to undergo multiple self-pollination events, thus allowing seed of each line to be increased, without changing the genetic structure. Even though instant inbred lines are created, similar to those produced from RIL populations, only one meiotic event occurred, producing large linkage blocks similar to F_2 populations (Masojc, 2002). Another concern with DH populations is that certain techniques and genotypes may cause skewed segregation due to genotypic effects, which would not allow for genetic mapping (Baenziger et al., 1984). Based on the success of haploid embryo production and chromosome doubling, time required to develop different populations will vary.

After the mapping population is created, DNA is extracted from each individual within the population and analyzed with molecular markers (Suenaga et al., 2003). The markers used for analysis on the mapping population must first be tested on the two parental lines to confirm that they are polymorphic (Santra et al., 2008). This analysis allows researchers to determine if each individual line is genetically similar to parent A or parent B for a specific marker, allowing the segregation to be determined. As explained previously, programs like Mapmaker can assist in converting the marker segregation data to genetic linkage maps.

QTL Analysis

Once a genetic linkage map has been constructed, the chromosomal location of the genetic factor(s) controlling the trait of interest can be located on the map. This is done by utilizing phenotypic trait data collected from the same individuals used to generate the genetic linkage map. Marker-trait associations can be assessed using one-, two-, or multiple-locus marker genotypes. Using single-marker analysis, the distribution of trait values is examined separately for each marker locus (Lynch and Walsh, 1998). Lynch and Walsh (1998) report that this analysis is desirable when the goal is simple detection of a QTL linked to a marker rather than an estimation of its chromosomal location.

To detect and identify chromosomal location of a QTL, interval mapping (two-marker system) can be utilized. Interval mapping uses two observable flanking markers to construct an interval within which to search for QTL (Zeng, 1994). Lander and Bostein (1989) developed the interval mapping approach in which one marker interval at a time is analyzed to construct a putative QTL by performing a likelihood ratio test at every position in the interval. Interval mapping provides a systematic way to scan the entire genome for evidence of QTL.

Unfortunately, both single-marker and interval mapping approaches are biased because the test

QTL is subject to other linked QTL on the same chromosome (Zeng, 1994). Ideally, when testing a marker interval for presence of a QTL, the test statistic should be independent of other QTL at other regions of the chromosome (Lynch and Walsh, 1998).

Composite interval mapping (multiple-marker system) allows the testing position (interval) to be constructed with a pair of markers, and at the same time the genetic background is examined through multiple regression analysis using randomly selected markers (Zeng, 1993). Zeng (1993) states that by utilizing this approach any bias is removed because the test statistic for the QTL of interest is independent of the effects of alternate QTL. Using interval and composite interval mapping, a LOD score is calculated at each increment in the interval and a LOD score profile is calculated for the whole genome. When a peak has exceeded the threshold value, a QTL is declared at that location (Zeng, 1994). QTL Cartographer is a typical computer program that utilizes composite interval mapping to determine QTL location on genetic linkage maps (Wang et al., 2006).

Identification of Linked Molecular Markers

Mapping populations have been very useful for the identification of disease resistance genes, especially for stripe rust resistance. Santra et al. (2008) used a RIL population consisting of 114 individuals to map HTAP resistance from the winter wheat cultivar 'Stephens'. The population was screened at six locations, with disease reaction scored on multiple dates, to calculate area under the disease progress curve (AUDPC) (Jeger and Viljanen-Rollinson, 2001). The AUDPC values were used in the data analysis. 496 DNA markers were used to screen the RIL population and construct the map. QTL analysis identified two QTL associated with HTAP resistance on chromosome 6BS. *QYrst.wgp.6BS.1* was located within a 3.9 cM interval flanked by *Xbarc136* and *Xbarc101*. *QYrst.wpg.6BS.2* was located within a 17.5 cM region spanned by

the SSR markers *Xgwm132*, *Xgwm705*, *Xgwm508* and *Xgdm113*. Boukhatem et al. (2002) also used RIL to identify molecular markers associated with stripe rust resistance, although the population only consisted of 98 plants, and relied on already published linkage maps to identify markers linked to disease resistance. Two loci (QYR1 and QYR3) on chromosome 2BL and 2BS, respectively, were associated with resistance to stripe rust. QYR1 was located between SSR markers *gwm501* and *gwm47* (distance between these markers was 3.4 cM), whereas QYR3 was between the RFLP (restriction fragment length polymorphism) loci *Xcdo405* and *Xbcd152* (distance between these markers was 6.7 cM).

Suenga et al. (2003) used a DH population of 107 individuals from a cross between a Japanese line 'Fukuho-komugi' and an Israeli genotype 'Oligoculm' to map the HTAP resistance gene *Yr18*. The genetic linkage map was composed of 47 RFLP and 400 SSR, and the QTL analysis located *Yr18* to chromosome 7DS. Lagudah et al. (2006) did further molecular genetic characterization on *Yr18*, and has developed a co-dominant sequence tagged site for use as a molecular marker. The genetic linkage between *csLV34* and *Yr18* was estimated at 0.4 cM, and has proven diagnostic for use in marker-assisted selection programs (Lagudah et al. 2006).

Sun et al. (1997) used RFLP and RAPD markers in an F₂ population of 123 individuals created from the cross between the resistant *Yr15* donor line, *T. dicoccoides* G-25, and a susceptible line *T. durum* cultivar D447. One RAPD marker, OPB13₁₄₂₀ was present in all 123 individuals and segregated 3:1, indicating normal segregation for a dominant allele. Linkage analysis using marker and trait data found that the distal marker OPB13₁₄₂₀ is 27.1 cM away from the resistance gene. Linkage analysis using the RFLP probes identified *Nor1* as being 11.0 cM proximal from the gene. More recent mapping efforts by Murphy et al. (2009) have identified two SSR markers, *Xbarc8* and *Xgwm413*, which appear to be completely linked in their mapping

population with *Yr15*. These markers have proven useful in marker-assisted selection programs and will facilitate the incorporation of *Yr15* into regionally adapted cultivars.

Stripe Rust Resistance Genes

Due to the high percentage of acreage dedicated to wheat production in the U.S. and other countries, wheat producers have been increasing their demand for durable stripe rust resistance. Currently, molecular markers have been associated with several genes and QTL for HTAP resistance (Chen, 2005; Lin and Chen, 2007, 2008; Guo et al., 2008). The gene *Yr18* is located on chromosome 7DS (Suenaga et al., 2003), *Yr36* on chromosome 6BS (Uauy et al., 2005), *QYrst.wgp-6BS.1* and *QYrst.wgp-6BS.2* also on chromosome 6BS (Santra et al., 2008), three QTL (*QYrex.wgp-6AS*, *QYrex.wgp-3BL* and *QYrex.wgp-1BL*) on chromosomes 6AS, 3BL, and 1BL, respectively (Lin and Chen, 2008), and *Yr39* on chromosome 7BL (Lin and Chen, 2007). Other genes or QTL, reported to confer adult-plant and presumably HTAP resistance, have been located on many chromosomes. These genes or QTL include *Yr16* on 2D (Worland and Law, 1986); *Yr29* on 1BL (McIntosh et al., 2001); *Yr30* on 3BS (McIntosh et al., 2001); *Yrns-B1* on 3BS (Börner et al., 2000); *YrCK* on 2DS (Navabi et al., 2005); *QPst.jic-1B*, *QPst.jic-2D* and *QPst.jic-4B* on 1BL, 2D and 4B, respectively (Melichar et al., 2008); *QYrtm.pau-2A* and *QYrtb.pau-5A* on 2A and 5A, respectively (Chhuneja et al., 2008); and *QYr.inra-2AL* on 2AL, *QYr.inra-2DS* on 2DS, and *QYr.inra-5BL.1* and *QYr.inra-5BL.2* on 5BL (Mallard et al., 2005).

Several genes for stripe rust resistance (*Yr5*, *Yr7*, *Yr27*, *Yr31*, and *Yr41*) have been reported on chromosome 2BS, all of which confer all-stage resistance (Macer, 1966; McDonald et al., 2004; McIntosh et al., 2006; Lou et al., 2008). *YrSp* (McIntosh et al., 1995), *YrSte* (Chen et al., 1998) and *YrV23* (Chen et al., 1998) have been reported on chromosome 2B, but their locations need to be identified to particular arms, and all of which confer race-specific all-stage

resistance. Mallard et al. (2005) identified two QTL, in a French winter wheat cultivar ‘Camp Remy’ (PI 452119), on both long and short arms of chromosome 2B. *QYr.inra-2BL* confers all-stage resistance and *QYr.inra-2BS* confers adult-plant resistance. Rosewarne et al. (2008) detected a QTL on 2BS for slow rusting resistance to stripe rust in ‘Attila’ (PI 35159) spring wheat. The Attila 2BS QTL was not consistently detected in their study and was considered a minor QTL to the *Lr46/Yr29* complex on chromosome 1BL (Rosewarne et al., 2006, 2008). Guo et al. (2008) identified two QTL for HTAP resistance on chromosome 2BS from the winter wheat ‘Luke’ (CItr 14586).

Marker-Assisted Selection (MAS)

Marker-assisted selection (MAS) is the process whereby the presence of a molecular marker is used for indirect selection for the presence of a gene (FAO, 2003). The greater the distance the marker is from the gene, the lower the probability that they will be inherited jointly in the next generation (Semagn et al., 2006). Populations can be screened at any generation of development, but typically F₂ to F₄ generation screening is performed (Dekkers and Hospital, 2002). The F₂ progeny are grown to the seedling stage and DNA extracted from the plant. Using the identified marker(s) for the gene, the DNA is assayed, and using resulting marker profiles, inferences are made as to the presence of the gene (FAO, 2003; Helguera et al., 2005). Plants without the gene are discarded, whereas plants with the gene are advanced in the breeding program

Marker-assisted selection is valuable for breeding programs interested in transferring specific genes to recipient germplasm (Dekkers and Hospital, 2002). One method typically used by breeding programs is marker-assisted backcrossing (Semagn et al., 2006). The recurrent parent is cross-hybridized to the donor parent to produce F₁ progeny. The F₁ is then cross-

hybridized back to the recurrent parent to create a BC₁F₁ population (Babu et al., 2005). The BC₁F₁ population is screened with the marker(s) for the gene and plants which are heterozygous and/or homozygous for the marker(s) are selected. These selected BC₁F₁ individuals are then cross-hybridized with the recurrent parent to create the BC₂F₁ population (Babu et al., 2005). Multiple rounds of backcrossing can be repeated following this strategy. Once backcrossing is complete, the BC_nF₁ plants are self-pollinated to produce BC_nF₂ individuals. Phenotypic selection is then conducted in the field allowing variation and new gene integrations in the non-target region to be evaluated by the breeder (Hospital and Charcosset, 1997; Semagn et al., 2006). Dreher et al. (2003) estimate that a 22% cost savings can occur when using MAS over traditional phenotypic screening.

Although the concept of MAS is quite simple, the reality is very complex. Populations must be developed and managed, molecular markers do not always work as reported, and distances between marker and gene of interest can be larger than desired. MAS also has been limited by the lack of markers and difficulties in application (FAO, 2003; Masojc, 2002). The first application of MAS in practical breeding was reported for transferring strawbreaker foot rot (*Pseudocercospora herpotrichoides*) resistance from *Aegilops ventricosa* into wheat (McMillin et al., 1984). The authors discovered an association of a distinct endopeptidase allele from *A. ventricosa* to the trait. Thus, if the wheat plant exhibited this distinct endopeptidase, it also carried the resistance allele. Further MAS work involved introgressing disease resistance genes into common bean (*Phaseolus vulgaris* L.) (Kelly and Miklas, 1998). Hanson et al. (1997) used MAS to identify plants of *Brassica napus* with restored male-fertility. More recently, Helguera et al. (2005) developed molecular markers to transfer the leaf rust resistance gene *Lr51*

from *Triticum speltoides* to *T. aestivum*. These markers were then used to develop isogenic hard red spring wheat lines carrying *Lr51*

Gene Pyramiding

Gene pyramiding is extremely useful for traits where multiple genes have been identified, such as in stripe rust. Pyramiding multiple resistance genes into a single cultivar is an effective way to increase the durability of resistance (Servin et al., 2004). The probability that a race will become homozygous for two or more new virulence genes is much less than becoming homozygous for one gene (Flor, 1971). Therefore, cultivars with two or more genes for rust resistance should be less apt to succumb to new races than cultivars possessing a single resistance gene (Flor, 1971). The presence of multiple resistance genes will continue to provide resistance even if one gene is overcome by the pathogen.

For disease resistance, the most effective way, and sometimes the only way, to introgress multiple resistance genes into one cultivar is through marker-assisted gene pyramiding (Servin et al., 2004), because the presence of more than one gene conferring resistance in different plants may produce identical phenotypes. Markers linked to the resistance genes can be used to screen DNA from populations segregating for multiple genes and to determine how many genes are present in each plant. Due to the rapid origin of new pathotypes of powdery mildew (*Blumeria graminis* DC. f. *tritici*), the only effective method for control is pyramiding two or more *Pm* genes into wheat. Liu et al. (2000) pyramided different genes for powdery mildew resistance in wheat, facilitated by markers. Of the 30 known loci for mildew resistance, 10 had markers linked to them, of which three, *Pm2*, *Pm4a*, and *Pm21*, were pyramided into susceptible germplasm.

Similar gene pyramiding in barley (*Hordeum vulgare*) has enhanced resistance to barley stripe rust (*P. striiformis* Westend. f. sp. *hordei*). Like wheat, barley has both qualitative and quantitative resistances to stripe rust. Castro et al. (2003a) used a DH mapping population to map a qualitative resistance gene (*Rpsx*) in barley to the long arm of chromosome 1(7H). Through a series of crosses and marker-assisted selection, the *Rpsx* gene was combined with three mapped and validated barley stripe rust resistance QTL alleles located on chromosomes 4(4H), 5(1H), and 7(5H) (Chen et al., 1994; Toojinda et al., 2000). Quantitative resistance effects were mainly additive, although certain gene combinations provided higher levels of resistance than others. The results from Castro et al. (2003a) indicated that combining qualitative and quantitative resistance in the same genotype is feasible, although the durability of this resistance remains in question.

The barley stripe rust QTL underwent further investigation by evaluating resistance at the seedling and adult plant stage (Castro et al., 2003b; Richardson et al., 2006). Castro et al. (2003b) identified the QTL on chromosome 4(4H) and 5(1H) were the two most important QTL out of four for providing resistance to barley stripe rust. While a single resistance allele at either QTL showed a resistant phenotype, combining both QTL in the same genotype increased the resistance by an average of 62% (Castro et al., 2003b). Richardson et al. (2006) evaluated disease resistance at the adult plant stage, examining latent period, infection efficiency, lesion size, and pustule density. The 4H QTL had the largest effect, followed by the QTL on 1H and 5H. Pyramiding multiple QTL for stripe rust resistance led to higher levels of resistance in terms of all components measured except for latent period (Richardson et al., 2006). This work verifies that MAS is effective for introgressing qualitative and quantitative disease resistance genes into a common genetic background. It also proves that, although resistance levels may

vary between genes, pyramiding resistance QTL can lead to high expression of disease resistance.

Since HTAP resistance has been durable, but does not provide stripe rust resistance during the seedling stage, it would be ideal to develop cultivars with both all-stage and HTAP resistance. Pyramiding differing resistance mechanisms in wheat is essential for controlling stripe rust since multiple resistance alleles would provide various forms of resistance. Multiple resistance genes would allow for non-race specific resistance (HTAP resistance) to provide resistance if all-stage resistance were to be overcome by race shifts. Pyramiding resistance genes is nearly impossible to do phenotypically, since all-stage resistance masks the expression of HTAP resistance (Chen, personal communication). The most efficient and effective way to pyramid both types of resistance into the same plant is through the use of marker detection systems.

Molecular marker analysis allows researchers to identify germplasm carrying multiple resistance genes, even though this may not be seen phenotypically. MAS can be used to identify genotypes carrying the gene before advancement to the next generation of evaluation. By using MAS to introgress and pyramid resistance genes into new spring wheat germplasm, costs can potentially be reduced and release of resistant germplasm can be expedited (Hospital and Charcosset, 1997; Knapp, 1998; Dreher et al., 2003; Kuckel et al., 2005; Semagn et al., 2006). Optimization and identification of molecular markers linked to stripe rust resistance, as well as the ability to pyramid these resistance genes into adapted germplasm, will advance the progression of sustainable agriculture by maintaining high yielding, high quality lines while limiting annual applications of fungicides.

Goals of the Project

Objectives:

1. Identify DNA markers closely associated with the HTAP resistance from the spring wheat cultivar 'Louise' through genetic linkage analysis.
2. Identify chromosomal location and DNA markers associated with Hessian fly resistance gene *H3*.
3. Identify DNA markers closely associated with important agronomic traits from the Louise by Penawawa mapping population.

References

- AACC. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. The Association, St. Paul, MN, USA.
- Agrios, G.N. 1997. *Plant Pathology 4th Edition*. Pp. 371-375. San Diego, CA: Academic Press.
- Allard, R.W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. *Hilgardia* 24: 235-278.
- Anderson, J.A., Stack, R.W., Liu, S., Waldron, B.L., Fjeld, A.D., Coyne, C., Moreno-Sevilla, B., Mitchell Fetch, J., Song, Q.J., Cregan, P.B., and Frohberg, R.C. 2001. DNA markers for Fusarium head blight resistance QTLs in two wheat populations. *Theoretical and Applied Genetics* 102:1164-1168.
- Babu, R., Nair, S.K., Kumar, A., Venkatesh, S., Sekhar, J.C., Singh, N.N., Srinivasan, G., and Gupta, H.S. 2005. Two-generation marker-aided backcrossing for rapid conversion of normal maize lines to quality protein maize (QPM). *Theoretical*

- and Applied Genetics 111:888-897.
- Baenziger, P.S., Kudirka, D.T., Schaeffer, G.W., and Lazar, M.D. 1984. The significance of doubled haploid variation. P. 385-414. *In* J.P. Gustafson (ed.) Gene manipulation in plant improvement. Stadler Genetics Symposium, 16th, University of Missouri, Columbia, MO. May 7th, 1984. Plenum Press, New York.
- Bariana, H.S., Hayden, M.J., Ahmed, N.U., Bell, J.A., Sharp, P.J., and McIntosh, R.A. 2001. Mapping of durable adult plant and seedling resistances to strip rust and stem rust diseases in wheat. *Australian Journal of Agricultural Research* 52:1247-1255.
- Boland, M., and Dhuyvetter, K.C. 2002. Economic Issues with Milling Hard White Wheat. Kansas State University Cooperative Extension Service Bulletin MF-2499, May, 2002.
- Börner, A., Röder, M.S., Unger, O., and Meinel, A. 2000. The detection and molecular mapping of a major gene for non-specific adult-plant resistance against stripe (*Puccinia striiformis*) in wheat. *Theoretical and Applied Genetics* 100:1095-1099.
- Boukhatem, N., Baret, P.V., Mingeot, D., and Jacquemin, J.M. 2002. Quantitative trait loci for resistance against yellow rust in two wheat-derived recombinant inbred line populations. *Theoretical and Applied Genetics* 104:111-118.
- Briggle, L.W. and Curtis, B.C. 1987. Worldwide wheat. pp. 1-31. *In* Heyne, E.G. (ed), Wheat and Wheat Improvement. Agronomy Monograph 13. ASA, CSSA, SSSA, Madison, WI.
- Brown, W.M.Jr., Hill, J.P., and Velasco, V.R. 2001. Barley yellow rust in North

- America. *Annual Review of Phytopathology* 39:367-384.
- Brown, J.K.M., and Hovmöller, M.S. 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* 297:537-541.
- Carleton, M.A. 1915. A serious new wheat rust in this country. *Science* 42:58-59.
- Carr, D.E., and Dudash, M.R. 2003. Recent approaches into the genetic basis of inbreeding depression in plants. *Philosophical Transactions of the Royal Society B* 358:1071-1084.
- Carter, A., Hansen, J., Koehler, T., Chen, X., and Zemetra, R. 2005. Development of a recombinant inbred line (RIL) population in soft white winter wheat [online]. *In* 2005 ASA Annual Meeting, Nov. 6-10, 2005, Salt Lake City, UT. pp.161 [abstract].
- Cartwright, R.D., Parsons, C.E., Welch, J., and Dodgen, B.J. 2000. Evaluation of foliar fungicides for control of stripe rust of wheat. *Fungicide and Nematode Tests* 56, CF14.
- Castro, A.J., Capettini, F., Cory, A.E., Filichkina, T., Hayes, P.M., Kleinhofs, A., Kudrna, D., Richardson, K., Sandoval-Islas, S., Rossi, C., and Vivar, H. 2003a. Mapping and pyramiding of qualitative and quantitative resistance to stripe rust in barley. *Theoretical and Applied Genetics* 107:922-930.
- Castro, A.J., Chen, X., Hayes, P.M., and Johnston, M. 2003b. Pyramiding quantitative trait loci (QTL) alleles determining resistance to barley stripe rust: effects on resistance at the seedling stage. *Crop Science* 43:651-659.
- Cereal Disease Laboratory. 2007. United States Department of Agriculture, Agricultural Research Service Cereal Disease Laboratory. Retrieved February 19, 2007. (http://www.ars.usda.gov/main/site_main.htm?modecode=36-40-05-00).

- Chen F.Q., Prehn, D., Hayes, P.M., Mulroney, D., Corey, A., and Vivar, H.E. 1994. Mapping genes for resistance to barley stripe rust (*Puccinia striiformis* f. sp. *hordei*). *Theoretical and Applied Genetics* 88:215-219.
- Chen, X.M. 2005. Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. *Canadian Journal of Plant Pathology* 27:314-337.
- Chen, X.M. and Line, R.F. 1995a. Gene action in wheat cultivars for durable high-temperature adult-plant resistance and interactions with race-specific, seedling resistance to stripe rust caused by *Puccinia striiformis*. *Phytopathology* 85:567-572.
- Chen, X.M. and Line, R.F., 1995b. Gene number and heritability of wheat cultivars with durable, high-temperature, adult-plant resistance and race-specific resistance to *Puccinia striiformis*. *Phytopathology* 85:573-578.
- Chen, X.M., Line, R.F., Shi, Z.X., and Leung, H. 1998. Genetics of wheat resistance to stripe rust. *In Proceedings of the 9th International Wheat Genetics Symposium*. 2-7 August, 1998, University of Saskatchewan, Saskatoon, Sask. *Edited by* A.E. Slinkard. University Extension Press, University of Saskatchewan, Saskatoon, Sask. Vol. 3. pp. 237-239.
- Chen, X.M., Milus, E.A., Long, D.L., and Jackson, L.F. 2004. Impact of wheat stripe rust and races of *Puccinia striiformis* f. sp. *tritici* in the United States. *In Proceedings of the 11th International Cereal Rusts and Powdery Mildews Conferences*. 22-27 August, 2004, John Innes Centre, Norwich, UK. European and Mediterranean Cereal Rust Foundation, Wageningen, Netherlands. *Cereal Rusts and Powdery Mildews Bulletin*, Abstract A2.11. Available from <http://www.crpmb.org/icrpsc11/abstracts.htm>.

- Chen, X.M. and Moore, M. 2002. Epidemics and races of *Puccinia striiformis* in North America in 2001. *Phytopathology* 92:S14.
- Chen, X.M., Moore, M., Milus, E.A., Long, D.L., Line, R.G., Marshall, D., and Jackson, L. 2002. Wheat stripe rust epidemics and races of *Puccinia striiformis* f. sp. *tritici* in the United States in 2000. *Plant Disease* 86:39-46.
- Chen, X.M., Soria, M.A., Yan, G.P., Sun, J., and Dubcovsky, J. 2003. Development of sequence tagged site and cleaved amplified polymorphic sequence markers for wheat stripe rust resistance gene *Yr5*. *Crop Science* 43:2058-2064.
- Chen, X.M., and Wood, D.A. 2002. Control of stripe rust of spring wheat with foliar fungicides, 2001. *Fungicide and Nematicide Test* [serial online], Report 57:CF03. The American Phytopathological Society, St. Paul, Min. doi: 10.1094/FN57.
- Chen, X.M., and Wood, D.A. 2003. Control of stripe rust of spring wheat with foliar fungicides, 2002. *Fungicide and Nematicide Test* [serial online], Report 58:CF004. The American Phytopathological Society, St. Paul, Min. doi: 10.1094/FN58.
- Chen, X.M., and Wood, D.A. 2004. Control of stripe rust of spring wheat with foliar fungicides, 2003. *Fungicide and Nematicide Test* [serial online], Report 59:CF022. The American Phytopathological Society, St. Paul, Min. doi: 10.1094/FN59.
- Chhuneja, P., Kaur, S., Garg, T., Ghai, M., Kaur, S., Prashar, M., Bains, N.S., Goel, R.K., Keller, B., Dhaliwal, H.S., and Singh, K. 2008. Mapping of adult plant stripe rust resistance genes in diploid A genome wheat species and their transfer to bread wheat. *Theoretical and Applied Genetics* 116:313-324.

- Chicago Board of Trade. 2007. "Wheat Futures." Retrieved online April 17, 2007.
(<http://www.cbot.com/>).
- Cook, R.J. and Veseth, R.J. 1991. Wheat health management before planting. *In* Wheat Health Management. St. Paul, MN: American Phytopathological Society. pp.105–120.
- Davis, R.M., and Jackson, L.F. 2007. UC IPM pest management guidelines: small grains. UC ANR Publication 3466, University of California, Davis, CA.
- de la Peña, R.C., Murray, T.D., and Jones, S.S. 1996. Linkage relations among eyespot resistance gene *Pch2*, endopeptidase *Ep-A1b*, and RFLP marker *Xpsr121* on chromosome 7A of wheat. *Plant Breeding* 115:273-275.
- Dekkers, J.C.M., and Hospital, F. 2002. The use of molecular genetics in the improvement of agricultural populations. *Nature Reviews: Genetics* 3:22-32.
- Dreher, K., Khairallah, M., Ribaut, J., Morris, M. 2003. Money matters (I): costs of field and laboratory procedures associated with conventional and marker-assisted maize breeding at CIMMYT. *Molecular Breeding* 11:221-234.
- Dubcovsky, J. 2007. QTL Mapping Workshop, Davis, CA. 18-20 January, 2007.
- Duff, C., Hamblin, P., and Poole, N. 2006. "Stripe Rust Management in Wheat" Grains Research and Development Corporation, 40 Blackall Street, Barton, ACT 2600.
- Eriksen, L., Afshari, F., Christiansen, M.J., McIntosh, R.A., Jahoor, A., and Wellings, C.R. 2004. *Yr32* for resistance to stripe (yellow) rust present in the wheat cultivar Carstens V. *Theoretical and Applied Genetics* 108:567-575.
- Eriksson, J. and Henning, E. 1894. Die Hauptresultate einer neuen Untersuchung über die Getreiderostpilze. *A. Pflanzenkr.* 4:197-203.

- Eriksson, J. and Henning, E. 1896. *Die Getreideroste*. Stockholm: Norsteadt and Söner.
- FAO. 2003. Food and Agriculture Organization of the United Nations Electronic Forum on Biotechnology in Food and Agriculture. “Molecular marker assisted selection as a potential tool for genetic improvement of crops, forest trees, livestock, and fish in developing countries” Conference 10: November 17 to December 14, 2003. Retrieved April 18th, 2007 (<http://www.fao.org/biotech/C10doc.htm>).
- FAO. 2007. Food and Agriculture Organization of the United Nations. Retrieved February 8, 2007. (<http://www.fao.org/>).
- Ferreira, A., da Silva, M.F., da Costa e Silva, L., and Cruz, C.D. 2006. Estimating the effects of population size and type on the accuracy of genetic maps. *Genetics and Molecular Biology* 29:187-192.
- Flintham, J.E. 2000. Different genetic components control coat-imposed and embryo-imposed dormancy in wheat. *Seed Science Research* 10:43-50.
- Flor, H.H. 1971. Current status of the gene-for-gene concept. *Annual Review of Phytopathology* 9:275-296.
- Gerber, S., Mariette, S., Streiff, R., Bodénès, C., and Kremer, A. 2000. Comparison of microsatellites and amplified fragment length polymorphism markers for parentage analysis. *Molecular Ecology* 9:1037-1048.
- Ghosh, S., and Collins, F.S. 1996. The geneticist’s approach to complex disease. *Annual Review of Medicine* 47:333-335.
- Guo, Q., Zhang, Z.J., Xu, Y.B., Li, G.H., Feng, J., and Zhou, Y. 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.

- Hansen, N.P.J., and Andersen, S.B. 1998. *In vitro* chromosome doubling with colchicine during microspore culture in wheat (*Triticum aestivum* L.). *Euphytica* 102:101-108.
- Hartl, D.L., and Jones, E.W. 2006. *Essential Genetics: A Genomics Perspective*. Sudbury, MA: Jones and Bartlett Publishers.
- Hatcher, D.W., Anderson, M.J., Desjardins, R.G., Edwards, N.M., and Dexter, J.E. 2002. Effects of flour particle size and starch damage on processing and quality of white salted noodles. *Cereal Chemistry* 79: 64-71.
- Hayes, B.J., Gjuvslund, A., and Omholt, S. 2006. Power of QTL mapping experiments in commercial Atlantic salmon populations, exploiting linkage and linkage disequilibrium and effect of limited recombination in males. *Heredity* 97:19-26.
- Hayley, S.D., Afanador, L., and Kelly, J.D. 1994. Selection for monogenic pest resistance traits with coupling- and repulsion-phase RAPD markers. *Crop Science* 34:1061-1066.
- Helguera, M., Vanzetti, L., Soria, M., Khan, I.A., Kolmer, J., and Dubcovsky, J. 2005. PCR markers for *Triticum speltoides* leaf rust resistance gene *Lr51* and their use to develop isogenic hard red spring wheat lines. *Crop Science* 45:728-734.
- Holland, J.B., Nyquist, W.E., and Cervantes-Martinez, C.T. 2003. "Estimating and Interpreting Heritability for Plant Breeding: An Update" *Plant Breeding Reviews*, Vol. 22, pp. 9-112. Edited by Jules Janick. Hoboken, NJ: John Wiley & Sons, Inc.
- Hospital, F., and Charcosset, A. 1997. Marker assisted introgression of quantitative trait loci. *Genetics* 147:1469-1485.

- Hovmøller, M.S., Justesen, A.F., and Brown, J.K.M. 2002. Clonality and long-distance migration of *Puccinia striiformis* f. sp. *tritici* in northwest Europe. *Plant Pathology* 51:24-32.
- Humphrey, H.B., Hungerford, C.W., and Johnson, A.G. 1924. Stripe rust (*Puccinia glumarum*) of cereals and grasses in the United States. *Journal of Agricultural Research* 29:209-227.
- Humphrey, H.B. and Johnson, A.G. 1916. Observations on the occurrence of *Puccinia glumarum* in the United States. *Phytopathology* 6:96-97.
- Hylander, N., Jorstad, I., and Nanfeldt, J.A. 1953. Enumeratio uredinearum Scandinavicarum. *Opera Botanica* 1:1-102.
- Jeger, M.J., and Viljanen-Rollinson, S.L.H. 2001. The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. *Theoretical and Applied Genetics* 102:32-40.
- Jiang, C., and Zeng, Z. 1997. Mapping quantitative trait loci with dominant and missing markers in various crosses from two inbred lines. *Genetica* 101:47-58.
- Jones, S.S., and Cadle, M.M. 1997. Effect of variation at *Glu-D1* on club wheat end-use quality. *Plant Breeding* 116:69-72.
- Kelly, J.D., and Miklas, P.N. 1998. The role of RAPD markers in breeding for disease resistance in common bean. *Molecular Breeding* 4:1-11.
- Kidwell, K.K., Shelton, G.B., Demacon, V.L., Burns, J.W., Carter, B.P., Chen, X.M., Morris, C.F., and Bosque-Pérez, N.A. 2006. Registration of 'Louise' wheat. *Crop Science* 46:1384-1386.
- Kidwell, K.K., Shelton, G.B., Demacon, V.L., Morris, C.F., Engle, D.A., Burns, J.W.,

- Line, R.F., Konzak, C.F., and Hatchett, J.H. 2002. Registration of 'Zak' wheat. *Crop Science* 42:661-662.
- Knapp, S.J. 1998. Marker-assisted selection as a strategy for increasing the probability of selection superior genotypes. *Crop Science* 38:1164-1174.
- Kuchel, H., Ye, G., Fox, R., and Jefferies, S. 2005. Genetic and economic analysis of a targeted marker-assisted wheat breeding strategy. *Molecular Breeding* 16:67-78.
- Lagudah, E.S., McFadden, H., Singh, R.P., Huerta-Espino, J., Bariana, H.S., and Spielmeyer, W. 2006. Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. *Theoretical and Applied Genetics* 114:21-30.
- Lander, E., and Bostein, D. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185-199.
- Lander, E., Green, P., Abrahamson, J., Barlow, A., Baly, M.J., Lincoln, S.E., and Newburg, L. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174-181.
- Lin, F., and Chen, X.M. 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. *Theoretical and Applied Genetics* 114:1277-1287.
- Lin, F., and Chen, X.M. 2008. Quantitative trait loci for non-race-specific, high-temperature adult-plant resistance to stripe rust in wheat cultivar Express. *Theoretical and Applied Genetics* 118:631-642.
- Line, R.F. 2002. Stripe rust of wheat and barley in North America: A retrospective

- historical review. *Annual Review of Phytopathology* 40:75-118.
- Line, R.F. and Chen, X.M. 1995. Successes in breeding for and managing durable resistance to wheat rusts. *Plant Disease* 79:1254-1255.
- Line, R.F. and Chen, X.M. 1996. Wheat and barley stripe rust in North America. *In* Proceedings of the 9th European and Mediterranean Cereal Rusts and Powdery Mildews Conference. 2-6 September, 1996, Lunteren, Netherlands. *Edited by* G.H.J. Kema, R.E. Nike, and R.A. Damen. European and Mediterranean Cereal Rust Foundation, Wageningen, Netherland. *Cereal Rusts and Powdery Mildews Bulletin*, 24(Suppl.): 101-104.
- Line, R.F. and Qayoum, A. 1992. Virulence, aggressiveness, evolution, and distribution of races of *Puccinia striiformis* (the cause of stripe rust in wheat) in North America, 1968-1987. United States Department of Agriculture, Agricultural Research Service Technical Bulletin 1788.
- Line, R.F., Sharp, E.L., and Powelson, R.L. 1970. A system for differentiating races of *Puccinia striiformis* in the United States. *Plant Disease Reporter* 54:992-994.
- Liu, B.H. 1998. *Statistical Genomics: Linkage, Mapping, and QTL Analysis*. Boca Raton, NY: CRC Press LLC.
- Liu, J., Liu, D., Tao, W., Li, W., Wang, S., Chen, P., Cheng, S., and Gao, D. 2000. Molecular marker-facilitated pyramiding of different genes for powdery mildew resistance in wheat. *Plant Breeding* 119:21-24.
- Lou, P.G., Hu, X.Y., Ren, Z.L., Zhang, H.Y., Shu, K., and Yang, Z.J. 2008. Allelic analysis of stripe rust resistance genes on wheat chromosome 2BS. *Genome* 51:922-927.
- Lynch, M., and Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sunderland,

- MA: Sinauer Associates, Inc.
- Ma, J., Zhou, R., Dong, Y., Wang, L., Wang, Z., and Jia, J. 2001. Molecular mapping and detection of the yellow rust resistance gene *Yr26* in wheat transferred from *Triticum turgidum* L. using microsatellite markers. *Euphytica* 120:219-226.
- Macer, R.C.F. 1966. The formal and monosomic genetic analysis of stripe rust (*Puccinia striiformis*) resistance in wheat. In Proceedings of the 2nd International Wheat Genetics Symposium. 19-24 August 1963, Lund, Sweden. Edited by J. MacKey. *Hereditas* 2(Suppl.):127-142.
- Mallard, S., Gaudet, D., Aldeia, A., Abelard, C., Besnard, A.L., Sourdille, P., and Dedryver, F. 2005. Genetic analysis of durable resistance to yellow rust in bread wheat. *Theoretical and Applied Genetics* 110:1401-1409.
- Martin, O.C., and Hospital, F. 2006. Two- and three-locus tests for linkage analysis using recombinant inbred lines. *Genetics* 173:451-459.
- Masojc, P. 2002. The application of molecular markers in the process of selection. *Cellular and Molecular Biology Letters* 7:499-509.
- McDonald, D.B., McIntosh, R.A., Wellings, C.R., Singh, R.P., and Nelson, J.C. 2004. Cytogenetical studies in wheat. XIX. Location and linkage studies on gene *Yr27* for resistance to stripe (yellow) rust. *Euphytica* 136: 239–248.
- McGregor Company. 2007. “Cost of fungicide application.” Colfax, WA 99111.
- McIntosh, R.A., Wellings, C.R., and Park, R.F. 1995. Wheat rusts: an atlas of resistance genes. Commonwealth Scientific and Industrial Research Organization, Australia, and Kluwer Academic Publishers, Dordrecht, Netherlands.

- McIntosh, R.A., Devos, K.M., Dubcovsky, J., and Rogers, W.J. 2001. Catalogue of gene symbols for wheat: 2001 supplement [online]. Available from <http://grain.jouy.inra.fr/ggpages/wgc/2001upd.html> [accessed 15 January 2009].
- McIntosh, R.A., Devos, K.M., Dubcovsky, J., Rogers, W.J., Morris, C.F., Appels, R., and Anderson, O.A. 2006. Catalogue of gene symbols for wheat: 2006 supplement [online]. Available from <http://www.shigen.nig.ac.jp/wheat/komugi/genes/macgene//supplement2006.pdf> [accessed 05 December 2008].
- McMillin, D.E., Allan, R.E. and Roberts, D.E. 1984. Association of an isozyme locus and strawbreaker foot rot resistance derived from *Aegilops ventricosa* in wheat. *Theoretical and Applied Genetics* 72:743-747.
- Melichar, J.P.E., Berry, S., Newell, C., MacCormack, R., and Bord, L.A. 2008. QTL identification and microphenotype characterisation of the developmentally regulated yellow rust resistance in the UK wheat cultivar Guardian. *Theoretical and Applied Genetics* 117:391-399.
- Morris, C.F., and Rose, S.P. 1996. Wheat, p. 3-54, *In Cereal Grain Quality*, R.J. Henry and P.S. Kettlewell, eds. Chapman and Hall, London.
- Murphy, L.R., Santra, D., Kidwell, K., Yan, G.P., Chen, X.M., and Garland-Campbell, L. 2009. Linkage maps of wheat stripe rust resistance genes *Yr5* and *Yr15* for use in marker assisted selection. *Crop Science* (In Press).
- NASS. 2007. United States Department of Agricultural National Agricultural Statistics Service. Retrieved February 10, 2007. (<http://www.nass.usda.gov/>).

- Navabi, A., Tewari, J.P., Singh, R.P., McCallum, B., Laroche, A., and Briggs, K.G. 2005. Inheritance and QTL analysis of durable resistance to stripe and leaf rusts in an Australian cultivar, *Triticum aestivum* 'Cook'. *Genome* 48:97-106.
- Ott, J. 1999. *Analysis of Human Genetic Linkage*. Pp. 37-83. Baltimore, MD: Johns Hopkins University Press.
- Papendick, R.I. 1998. Farming with the wind: Best management practices for controlling wind erosion and air quality on Columbia Plateau croplands. Misc Publ MISC0208. Washington State University, College of Agriculture and Home Economics, Pullman, WA.
- Papendick, R.I., McCool, D.K., and Krauss, H.A. 1983. Soil Conservation: Pacific Northwest. P. 273-290. In H.E. Dregne and W.O. Willis (eds) *Dryland Agriculture*. Agronomy Monograph No. 23. ASA-CSSA-SSSA, Madison, WI.
- Paterson, A.H. 1998. *Molecular Dissection of Complex Traits*. Boca Raton, FL: CRC Press LLC.
- Patterson, H.D., and Williams, E.R. 1976. A new class of resolvable incomplete block designs. *Biometrika* 63:83-92.
- Paveley, N.D., Lockly, K.D., Sylvester-Bradley, R. and Thomas, J. 1997. Determinants of fungicide spray decisions for wheat. *Pesticide Science* 49:379-388.
- Peng, J.H., Fahima, T., Röder, M.S., Huang, Q.Y., Dahan, A., Li, R.C., Grama, A., and Nevo, E. 2000. High-density molecular map of chromosome region harboring stripe-rust resistance genes *YrH52* and *Yr15* derived from wild emmer wheat, *Triticum dicoccoides*. *Genetica* (The Hague) 109:199-210.
- Pierce, B.A. 2005. *Genetics: A Conceptual Approach, Second Edition*. New York, NY: W.H.

Freeman and Company.

- Prioul, S., Frankewitz, A., Deniot, G., Morin, G., and Baranger, A. 2004. Mapping of quantitative trait loci for partial resistance to *Mycosphaerella pinodes* in pea (*Pisium sativum* L), at the seedling and adult plant stages. Theoretical and Applied Genetics 108:1322-1334.
- Pumphrey, M.O., Bernardo, R., and Anderson, J.A. 2007. Validating the *Fhb1* QTL for Fusarium head blight resistance in near-isogenic wheat lines developed from breeding populations. Crop Science 47:200-207.
- Qayoum, A., and Line, R.F. 1985. High-temperature adult-plant resistance to stripe rust of wheat. Phytopathology, 75:1121-1125.
- Rapilly, F. 1979. Yellow rust epidemiology. Annual Review of Phytopathology 17:59-73.
- Richardson, K.L., Vales, M.I., Kling, J.G., Mundt, C.C., and Hayes, P.M. 2006. Pyramiding and dissecting disease resistance QTL to barley stripe rust. Theoretical and Applied Genetics 113:485-495.
- Ridout, M.S., Tong, S., Vowden, C.J., and Tobutt, K.R. 1998. Three-point linkage analysis in crosses of allogamous plant species. Genetical Research 72:111-121.
- Roelfs, A.P., Singh, R.P., and Saari, E.E. 1992. Rust Diseases of Wheat: Concepts and methods of disease management. Mexico, D.F.: CIMMYT.
- Rogers, S.O., and Bendich, A.J. 1985. *In* Plant molecular Biology Manual, edited by S.B. Gelvin and R.A. Schilperoort. Kluwer Academic Publishers, Dordrecht A6:pp 1-10.
- Rosewarne, G.M., Singh, R.P., Huerta-Espino, J., William, H.M., Bouchet, S., Cloutier, S., McFadden, H., and Lagudah, E.S. 2006. Leaf tip necrosis, molecular markers and β 1-

- proteasome subunits associated with the slow rusting resistance genes *Lr34/Yr29*.
Theoretical and Applied Genetics 112:500-508.
- Rosewarne, G.M., Singh, R.P., Huerta-Espino, J., and Rebetzke, G.J. 2008. Quantitative trait loci for slow-rusting resistance in wheat to leaf rust and stripe rust identified with multi-environment analysis. Theoretical and Applied Genetics 116:1027-1034.
- Rousset, M., Brabant, P., Kota, R.S. Dubcovsky, J., and Dvorak, J. 2001. Use of recombinant substitution lines for gene mapping and QTL analysis of bread making quality in wheat. Euphytica 119:81-87.
- Santra, D.K., Chen, X.M., Santra, M., Campbell, K.G., and Kidwell, K.K. 2008. Identification and mapping QTL for high-temperature adult-plant resistance to stripe rust in winter wheat (*Triticum aestivum* L.) cultivar 'Stephens'. Theoretical and Applied Genetics 117:793-802.
- SAS (Statistical Analysis Systems). 2002. SAS/STAT users guide, version 9.1. SAS Institute Inc., Cary, NC.
- Schmidt, J.K. 1827. Allgemeine ökonomisch-technische Flora oder Abbildungen und Beschreibungen aller in bezug auf Ökonomie und Technologie, merkwürdigen Gewächse. Jena, Germany. Vol. I. pp. 27.
- Seah, S., Bariana, H., Jahier, J., Sivasithamparam, K., and Lagudah., E.S. 2001. The introgressed segment carrying rust resistance genes *Yr17*, *Lr37*, and *Sr38* in wheat can be assayed by a cloned disease resistance gene-like sequence. Theoretical and Applied Genetics 102:600-605.
- Seib, P.A., Liang, X., Guan, Y.T., Liang, Y.T., and Yang, H.C. 2000. Comparison of Asian noodles from some hard white and hard red wheat flours. Cereal Chemistry 80:91-98.

- Semagn, K., Bjørnstad, A., and Ndjioudjop, M.N. 2006. Progress and prospects of marker assisted backcrossing as a tool in crop breeding programs. *African Journal of Biotechnology* 5:2588-2603.
- Servin, B., Martin, O.C., Mézard, M., and Hospital, F. 2004. Toward a theory of marker-assisted gene pyramiding. *Genetics* 168:513-523.
- Sharp, E.L. 1962. Effect of pre-inoculation host temperature on infection of cereal seedlings by *Puccinia striiformis*. *Nature* 194:593-594.
- Singh, R.J. 2003. *Plant Cytogenetics, Second Edition*. Boca Raton, FL: CRC Press LLC.
- Singh, R.P., Nelson, J.C., and Sorrells, M.E. 2000. Mapping *Yr28* and other genes for resistance to stripe rust in wheat. *Crop Science* 40:1148-1155.
- Smith, P.H., Koebner, R.M.D., and Boyd, L.A. 2002. The development of a STS marker linked to a yellow rust resistance derived from the wheat cultivar Moro. *Theoretical and Applied Genetics* 104:1278-1282.
- Strachan, T., and Read, A.P. 1999. *Human Molecular Genetics 2*. New York, NY: John Wiley and Sons Inc.
- Stubbs, R.W. 1985. Stripe rust. Pages 61-101. *In: The Cereal Rusts*. Vol. 2. Disease, distribution, epidemiology, and control. A.P. Roelfs and W.R. Bushnell, eds. Academic Press, Orlando, FL.
- Suenaga, K. 1994. Doubled haploid system using the intergeneric crosses between wheat (*Triticum aestivum*) and maize (*Zea mays*). *Bulletin of the National Institute of Agrobiological Resources* 9:83-139.
- Suenaga, K., Singh, R.P., Huerta-Espino, J., and William, H.M. 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, G.L., Fahima, T., Korol, A.B., Turpeinen, T., Grama, A., Ronin, Y.I., Nevo, E. 1997. Identification of molecular markers linked to the *Yr15* stripe rust resistance gene of wheat originated from wild emmer wheat, *Triticum dicoccoides*. *Theoretical and Applied Genetics* 95:622-628.
- Taylor, E.C. 1976. The production and behavior of somatic recombinants in *Puccinia striiformis*. *Proceedings of the European and Mediterranean Cereal Rusts Conference* 4:36-38.
- Toojinda, T., Broers, L.H., Chen, X.M., Hayes, P.M., Kleinhofs, A., Korte, J., Kudrna, D., Leung, H., Line, R.F., Powell, W., Ramsay, L., Vivar, H.E., and Waugh, R. 2000. Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley (*Hordeum vulgare*). *Theoretical and Applied Genetics* 101:580-589.
- Uauy, C., Brevis, J.C., Chen, X., Khan, I.A., Jackson, L., Chicaiza, O., Distelfeld, A., Fahima, T., and Dubcovsky, J. 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*. *Theoretical and Applied Genetics* 112:97-105.
- Wang, S., Basten, C.J., and Zeng, Z.-B. 2006. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC. (<http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>).
- Washington State University. 2007. "WSU Extension Uniform Cereal Variety Testing Program." Pullman, WA: Washington State University, Retrieved February 12, 2007. (<http://variety.wsu.edu/>).

- WASS. 2002-2007. Washington Agricultural Statistics Service. Olympia, Washington, USA.
Retrieved March 9th, 2007. (http://www.nass.usda.gov/Statistics_by_State/Washington/index.asp).
- Worland, A.J., and Law, C.N. 1986. Genetic analysis of chromosome 2D of wheat. I. The location of genes affecting height, day-length insensitivity, hybrid dwarfism and yellow rust resistance. *Z Pflanzenzücht* 96:331-345.
- Wright, R.G., and Lennard, J.H. 1980. Origin of a new race of *Puccinia striiformis*.
Transactions of the British Mycological Society 74:283-287.
- Yan, G.P. 2006. "Molecular mapping of barley genes for resistance to stripe rust." Ph.D. dissertation, Department of Plant Pathology, Washington State University, Pullman, WA.
- Zadoks, J.C., Chang, T.T., and Konzak, C.F. 1974. A decimal code for the growth stages of cereals. *Weed Research* 14:415-421.
- Zeng, Z. 1993. Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proceedings of the National Academy of Sciences* 90:10972-10976.
- Zeng, Z. 1994. Precision mapping of quantitative trait loci. *Genetics* 136:1457-1468.
- Zhou, H., and Konzak, C.F. 1989. Improvement of anther culture methods for haploid production in wheat. *Crop Science* 29:817-821.

Table 1. Characteristics of each market class of wheat grown in the Pacific Northwest.

Market Class	Endosperm type	Grain color	Growth habit	Head type	Protein content target	Gluten strength	End products
Soft White	Soft	White	Spring or Winter	Lax	< 12%	Weak	Cookies, cakes, pastries, crackers
Club	Soft	White	Spring or Winter	Club	<11%	Very weak	Flaky pastries
Hard White	Hard	White	Spring or Winter	Lax	10-14%	Intermediate	Bread, Asian noodles
Hard Red Winter	Hard	Red	Winter	Lax	11-12%	Strong	Bread
Hard Red Spring	Hard	Red	Spring	Lax	13-14%	Strong	Bread

Table 2. Gene combinations and disease reaction types in a host-pathogen system operating under one gene in the gene-for-gene concept.

Virulence or avirulence genes in the pathogen	Resistance or susceptibility genes in the host plant	
	R (resistant)	r (susceptible)
A (avirulent)	AR (resistant)	Ar (susceptible)
a (virulent)	aR (susceptible)	ar (susceptible)

Adapted from Agrios, 1997.

Table 3. Wheat genotypes used to differentiate races of *Puccinia striiformis* f. sp. *tritici* in the United States.

Differential number	Cultivar or line	Identification number	Growth habit type	<i>Yr</i> gene ^{ab}	Year of incorporation
1	‘Lemhi’	CI 011415	Spring	<i>Yr21</i>	1968
2	‘Chinese 166’	CI 011765	Winter	<i>Yr1</i>	1968
3	‘Heines VII’	PI 201195	Winter	<i>Yr2, YrHVII</i>	1968
4	‘Moro’	CI 013740	Winter	<i>Yr10, YrMor</i>	1968
5	‘Paha’	CI 014485	Winter	<i>YrPa1, YrPa2, YrPa3</i>	1974
6	‘Druchamp’	CI 013723	Winter	<i>Yr3a, YrD, YrDru</i>	1969
7	‘Riebesel 47-51’ or Yr5	Yr 00004	Spring	<i>Yr5</i>	2004
8	‘Produra’	CI 017460	Spring	<i>YrPr1, YrPr2</i>	1974
9	‘Yamhill’	CI 014563	Winter	<i>Yr2, Yr4a, YrYam</i>	1974
10	‘Stephens’	CI 017596	Winter	<i>Yr3a, YrS, YrSte</i>	1976
11	‘Lee’	CI 012488	Spring	<i>Yr7, Yr22, Yr23</i>	1977
12	‘Fielder’	CI 017268	Spring	<i>Yr6, Yr20</i>	1980
13	‘Tyee’	CI 017773	Winter	<i>YrTye</i>	1983
14	‘Tres’	CI 017917	Winter	<i>YrTr1, YrTr2</i>	1989
15	‘Hyak’	PI 511674	Winter	<i>Yr17</i>	1990
16	‘Express’	DA 984034	Spring	<i>YrExp1, YrExp2</i>	1998
17	Yr8	YR 000008	Spring	<i>Yr8</i>	2000
18	Yr9	YR 000009	Spring	<i>Yr9</i>	2000
19	‘Clement’	PI 518799	Winter	<i>Yr9, YrCle</i>	2000
20	‘Compare’	PI 325842	Spring	<i>Yr8, Yr19</i>	2000

^a Yr = yellow rust

^b Official (*Yr* followed by a number) and provisional (*Yr* followed by letters) symbols are reported (Chen, 2005)

Adapted from Chen, 2005.

Table 4. Rating scale for recording stripe rust (*Puccinia striiformis*) infection types.

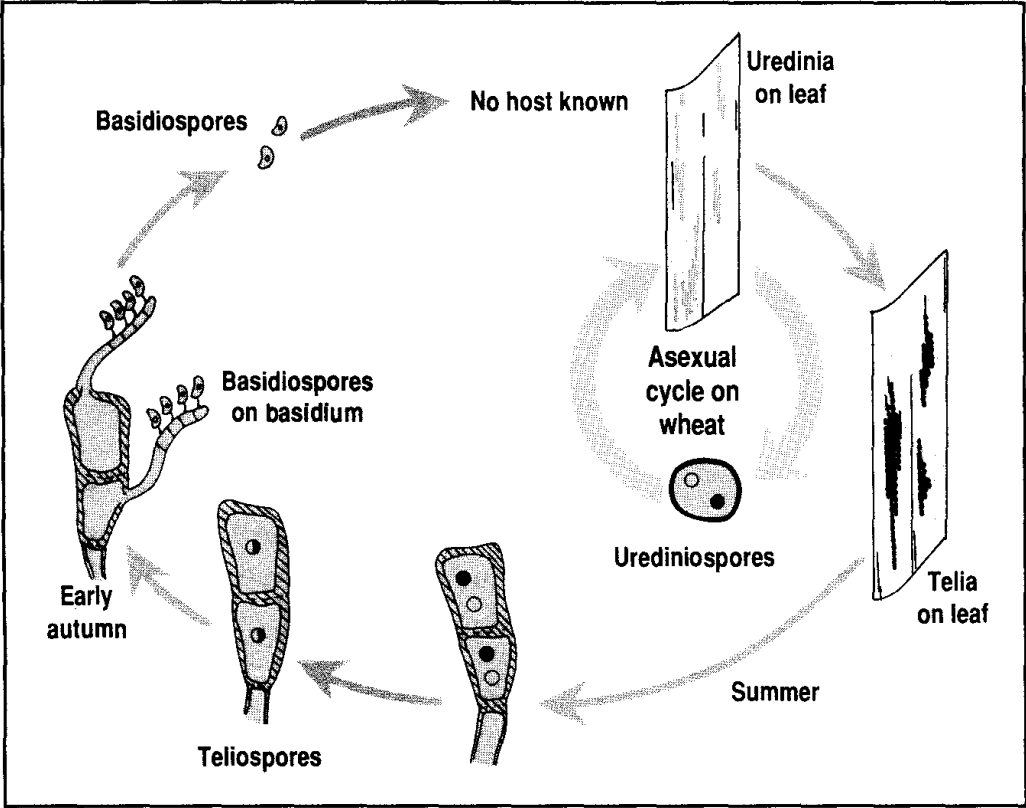
IT ^a	Signs and symptoms for infection types ^b
0	No visible signs or symptoms
1	Necrotic and/or chlorotic flecks; no sporulation
2	Necrotic and/or chlorotic blotches or stripes; no sporulation
3	Necrotic and/or chlorotic blotches or stripes; trace sporulation
4	Necrotic and/or chlorotic blotches or stripes; light sporulation
5	Necrotic and/or chlorotic blotches or stripes; intermediate sporulation
6	Necrotic and/or chlorotic blotches or stripes; moderate sporulation
7	Necrotic and/or chlorotic blotches or stripes; abundant sporulation
8	Chlorosis behind sporulating area; abundant sporulation
9	No necrosis or chlorosis; abundant sporulation

^a IT = infection type

^b Blotches are formed on leaves of susceptible seedlings, whereas stripes are formed on leaves of susceptible adult plants.

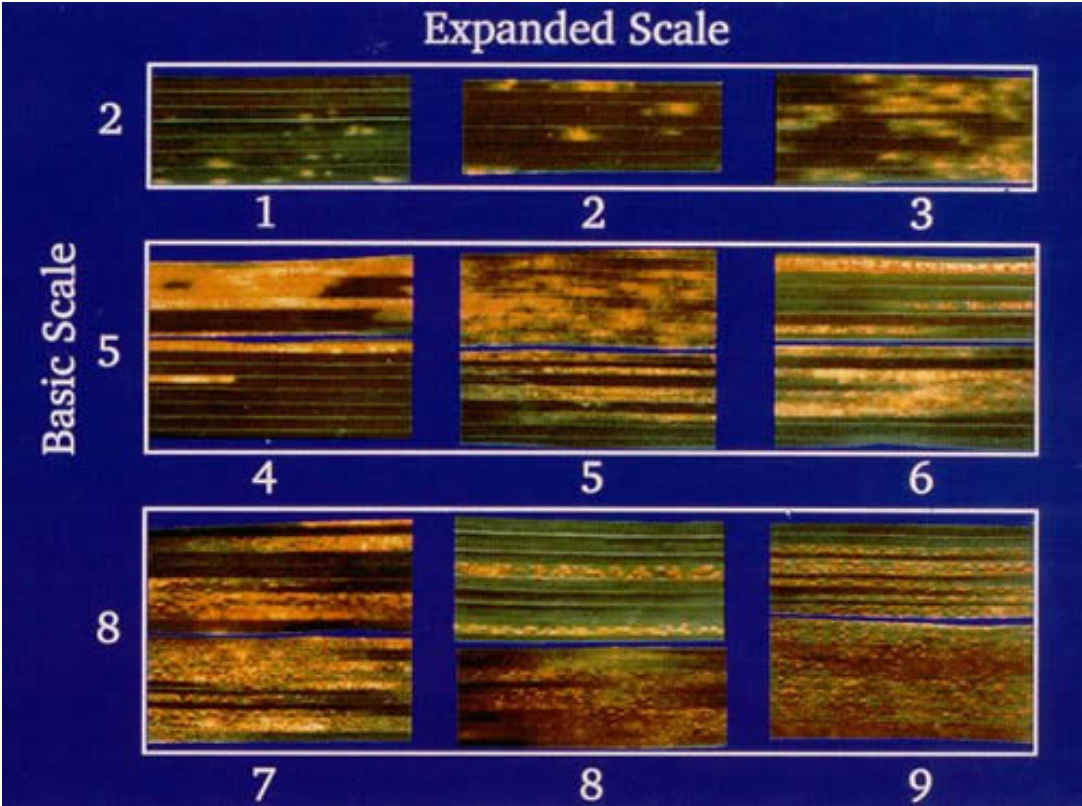
Adapted from Line et al., 1970.

Figure 1. Life cycle of *Puccinia striiformis*.



From Rust Diseases of Wheat, 1992 (Roelfs et al., 1992)

Figure 2. Visual scale for rating stripe rust infection types.



From http://variety.wsu.edu/Updates/StripeRust_5_20_05.htm

CHAPTER 2

**Identifying QTL for high-temperature adult-plant resistance to stripe
rust (*Puccinia striiformis* f. sp. *tritici*) in the spring wheat
(*Triticum aestivum* L.) cultivar ‘Louise’**

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Abstract

Over time, many single, all-stage resistance genes to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in wheat (*Triticum aestivum* L.) are circumvented by race changes in the pathogen. In contrast, high-temperature, adult-plant resistance (HTAP), which only is expressed during the adult-plant stage and when air temperatures are warm, provides durable protection against stripe rust. Our objective was to identify major quantitative trait loci (QTL) for HTAP resistance to stripe rust in the spring wheat cultivar ‘Louise’. The mapping population consisted of 188 recombinant inbred lines (RIL) from a Louise (resistant) by ‘Penawawa’ (susceptible) cross. F_{5,6} lines were evaluated for stripe rust reaction under natural infection in replicated field trials at five locations in the U.S. Pacific Northwest in 2007 and 2008. Infection type (IT) and disease severity (DS)

were recorded for each RIL two to four times per location. In all environments, Penawawa, the susceptible parent, was rated with an IT ranging from 6 to 8 at all growth stages evaluated. In contrast, Louise, the resistant parent, was rated with an IT of 2 or 3 across growth stages. Distribution of IT values was bimodal, indicating a single major gene was affecting the trait. The parents and RIL population were evaluated with 295 polymorphic simple sequence repeat (SSR) and one single nucleotide polymorphism (SNP) markers. One major QTL, designated *QYrlo.wpg-2BS*, associated with HTAP resistance in Louise, was detected on chromosome 2BS (LOD scores ranging from 5.5 to 62.3 across locations and years) within a 16.9 cM region flanked by *Xwmc474* and *Xgwm148*. SSR markers associated with *QYrlo.wpg-2BS* are currently being used in marker-based forward breeding strategies to transfer the target region into adapted germplasm to improve the durability of resistance in resulting cultivars.

Keywords: wheat; stripe rust; high-temperature adult-plant resistance; molecular markers; marker-assisted selection

Introduction

Wheat (*Triticum* spp.) is the primary food grain directly consumed by humans worldwide, and more acreage is dedicated to the commercial production of wheat than any other crop in the world (Briggle and Curtis 1987). As a result of its broad adaptation, numerous pathogens plague the crop. Stripe rust (also known as yellow rust), caused by the obligate biotroph fungus *Puccinia striiformis* Westend. f. sp. *tritici* Eriks. (*Pst*), is among the most destructive of these pathogens, causing substantial losses to wheat production annually on a global scale (Stubbs 1985; Chen et al. 2002; Chen 2005). Genetic resistance is the most economical and

environmentally friendly way to control stripe rust; however, adequate levels of resistance are not currently available in adapted germplasm in all wheat producing regions of the world.

Genetic resistance to stripe rust is either race-specific or non-race-specific. All-stage resistance is typically race-specific and qualitatively inherited, closely adhering to the gene-for-gene interaction model of Flor (1971). This type of resistance can be detected during the seedling stage of the plant, is frequently expressed at high levels, and continues throughout the life cycle of the plant. Due to their specific nature and the strong selection pressure placed on the pathogen, all-stage resistance is frequently overcome by race changes in the pathogen population (Chen and Line 1995). In contrast, high-temperature, adult-plant (HTAP) resistance is non-race-specific, often durable, and generally quantitatively inherited (Qayoum and Line 1985; Line and Chen 1995; Line 2002; Chen 2005). This type of resistance is manifested during later stages of plant development, when temperatures are typically above 21 °C (Chen and Line 1995a, 1995b).

More than 30 different genes for all-stage resistance have been identified and mapped to date, allowing wide deployment into germplasm through marker-assisted selection (reviewed in Chen 2005). Several reports identifying and mapping the more durable HTAP resistance to stripe rust also have been published in wheat (Chen and Line 1995a, 1995b; Chen 2005; Uauy et al. 2005; Chicaiza et al. 2006; Lin and Chen 2007, 2008; Santra et al. 2008). Multiple quantitative trait loci (QTL) are typically associated with HTAP resistance; however, in all previous reports, a few major QTL accounted for a significant portion of the variation (Uauy et al. 2005; Lin and Chen 2007).

In the U.S., stripe rust epidemics are most prevalent in California and the Pacific Northwest (PNW) where mild winters, followed by cool, wet springs and dry summers are

typical (Chen 2005). Many adapted winter wheat cultivars have combinations of HTAP and all-stage resistances to stripe rust. Relatively few spring wheat cultivars carry HTAP resistance, leaving the crop vulnerable to the disease. Louise (Kidwell et al. 2006), a soft white spring wheat cultivar released in 2006 has high levels of HTAP resistance. The objectives of this research were to: 1) identify major QTL for HTAP resistance in Louise through genetic linkage analysis; and 2) identify DNA markers linked to the resistance genes for use in marker-assisted selection.

Materials and methods

Plant materials

One hundred and eighty eight F_{5:6} recombinant inbred lines (RIL) were developed through single seed descent from F₂ seed harvested from a single F₁ plant of Louise (resistant parent; PI 634865) x Penawawa (susceptible parent; PI 495916). Louise, a soft white spring wheat, has moderate grain volume, low grain protein concentration, and high grain yield potential in non-irrigated production scenarios (Kidwell et al. 2006). Penawawa, also a soft white spring wheat, has moderate grain volume, moderate grain protein concentration, and low grain yield potential without irrigation (unpublished data). Both cultivars are adapted to growing conditions in the PNW.

Field experiments

The 188 RIL along with parental controls were evaluated for stripe rust resistance in the field in four locations in 2007. These locations included Pullman, WA (at Spillman Agronomy Farm and Whitlow Agronomy Farm); Mt. Vernon, WA (Northwestern Washington Research and Extension Center); and Genesee, ID (Kambitsch Farm). In 2008, trials were planted at similar

locations except the Genesee, ID site was changed to Moscow, ID (Parker Farm). Five grams of seed were planted in rows of 0.5 meter length spaced 30 cm apart in a randomized complete block design, with three replications per location. All trials in all locations were evaluated under natural infections of stripe rust. Spreader rows of the susceptible cultivar ‘Lemhi’ (CI 011415) were planted every 30 rows to increase the uniformity of stripe rust inoculum across the trial. Lemhi contains the all-stage resistance gene *Yr21*, which is ineffective against almost all races identified in the U.S. In 2007, planting dates were April 19th, 24th, 26th, and 24th for Mt. Vernon, Spillman Farm, Genesee, and Whitlow Farm, respectively. In 2008, planting dates were April 22nd, 21st, May 2nd, and April 17th for Mt. Vernon, Spillman Farm, Moscow, and Whitlow Farm, respectively. Before planting, the field was cultivated and fertilized with nitrogen (formulated as urea) at a rate of 101 kg ha⁻¹. Stripe rust symptoms were rated as infection type (IT) based on a 0-9 scale (Line and Qayoum 1992) and disease severity (DS) as a percentage of plants (0-100) in the row that were infected. Disease ratings were first taken when all plots of the susceptible parent Penawawa had a disease severity of 20% and continued until initiation of senescence (Feekes 11.2; Feekes 1941). Data were collected every three to seven days depending on disease progression and location.

Greenhouse experiment

Parental lines, as well as the 188 RIL, were planted in a randomized complete block design with three replicates. To determine HTAP resistance levels, three seeds of each line were planted in three liter pots using #1 Sunshine Mix (Sun Gro Horticulture, Bellevue, WA) and fertilized with 250 mg nitrogen (formulated as ammonium nitrate) per pot over a one month period. Plants were grown under a 16 h photoperiod, with daytime temperatures ranging from 21 to 24 °C and nighttime temperatures ranging from 15 to 18 °C until heading (Feekes 10.2). Light intensity

was 400 μmol at bench level. At initiation of heading (Feekes 10.2), plants were placed in a dew chamber (Percival Scientific, Inc, Perry, IA), inoculated with fresh urediniospores of *P. striiformis* f. sp. *tritici* race PST-100, the most predominant race throughout the U.S. since 2003, in a mixture of one part spores and twenty parts talc, and incubated for 24 h at 100% humidity in the dark at 10 °C. After 24 h incubation in the dew chamber, plants were returned to the greenhouse chamber at a diurnal temperature cycle of 15 °C at night and 28 °C at day with a daily 16 h photoperiod. The IT data was collected 18 to 20 days after inoculation as described previously. To determine seedling resistance levels, seeds were planted in 96 well trays, replicated three times. Seedlings were inoculated at the three to five leaf stage as described above. After 24 h incubation in the dew chamber, trays were placed in a growth room set at a diurnal temperature cycle of 4 °C at night and 20 °C at day with a daily 16 h photoperiod (Chen and Line 1992). Disease ratings for seedlings were evaluated 18 to 20 days after inoculation as previously described.

Statistical analysis for disease ratings

Area under the disease progress curve (AUDPC) was calculated for each RIL and the parental lines using the IT and DS data collected according to the formula: $\text{AUDPC} = \sum_i [(x_i + x_{i+1})/2]t_i$, where x_i is the severity value on date i , t_i the time in days between dates i and $i+1$ (Chen and Line 1995a). Relative AUDPC (rAUDPC) was calculated for each line as a percentage of the most susceptible AUDPC value in each of the experiments. The statistical analysis of rAUDPC was performed within each environment using the statistical package SAS v. 9.1 (SAS Institute, Raleigh, NC). Genotype and replication*genotype effects were tested using the Proc GLM procedure. Broad-sense heritability (h^2) was calculated for all locations using the formula: $h^2 = \text{Var}(G)/\text{Var}(P)$ (where $\text{Var}(G)$ is the variance of the genotypic effect and $\text{Var}(P)$ is the variance

of the phenotypic effect) using SAS code provided by Holland et al. (2003). The mean rAUDPC values for each line within each environment were used in QTL mapping.

Molecular marker analysis

Fresh leaf tissue of three individuals from each F_{5:6} RIL or parent was collected at the five leaf stage, and used to extract genomic DNA using the CTAB method as described by Anderson et al. (1992). Sequences of available SSR markers along with their previously determined chromosomal locations were obtained from Graingenes (<http://wheat.pw.usda.gov/>). SSR marker analyses were conducted using the PCR conditions described by Röder et al. (1998) except that primers were synthesized to include the M13-tail (Oetting et al. 1995). The 10 µL reaction mixture consisted of 50 ng of template DNA, 1.0 µL Mg-free 10X PCR buffer, 0.5 units of Taq DNA polymerase, 1.5 mM of MgCl₂ (Promega, Madison, WI, USA), 200 µM each of dCTP, dGTP, dTTP, and dATP (Fermentas, Glen Burnie, MD) and 0.25 µM of each primer pair synthesized by MWG-Biotech (High Point, NC, USA). Appropriate flourophores for either the Global IR² analysis system (LiCor Biosciences, Lincoln, NE, USA) or the ABI 3130xl (Applied Biosystems, Foster City, CA) fragment detection systems were included in the PCR mix. Amplification conditions were an initial 5 min denaturation at 94 °C, followed by 41 cycles of 1 min of denaturation at 94 °C, 1 min of annealing at 50 to 65 °C (depending on primers), and a 1 min extension at 72 °C. The final extension step was 10 min at 72 °C.

Identification and sequences of SNP markers were obtained from Dr. Shiaoman Chao at the USDA-ARS Biosciences Research Laboratory, Fargo, ND. SNP marker analyses were conducted according to PCR conditions provided by Dr. Shiaoman Chao (personal communication). Pre-amplification of DNA was conducted with the forward and reverse primer corresponding to each SNP using the PCR conditions described above. After pre-amplification,

SNP detection was completed following protocols outlined using the AcycloPrime II Combination SNP Detection Kit (PerkinElmer, Boston, MA, USA).

Linkage map construction and QTL analysis

Segregation of marker loci was tested for goodness-of-fit to the expected 1:1 ratio using the χ^2 test. Linkage maps were constructed using Mapmaker V3.0 (Lander et al. 1987). Linkage groups were established using the “group” command with a recombination value of 0.5 and a constant LOD score of 3.0. Three-point linkage analyses were performed using the “compare” command to determine the most likely order of markers with the shortest genetic distance within each group. For large linkage groups, a framework order was established using the above procedure and additional markers were mapped using the “try” command and verified using the “ripple” command. The Kosambi map function was applied to calculate the genetic distances in centiMorgans (cM) between the ordered markers (Kosambi 1944). Each linkage group was assigned to a wheat chromosome based on previously published wheat genome maps available at Graingenes.

Single marker analysis using one-way ANOVA with a comparison-wise probability level of $P < 0.01$ was used to identify markers with significant effects on HTAP resistance. The QTL detection was performed using composite interval mapping (CIM) (Zeng 1993, 1994) with the software WinQTLCart V2.5 (Basten et al. 1997) and was targeted toward linkage groups previously identified through the one-way ANOVAs. In order to detect significant QTL, a critical LOD threshold value of 3.0 was used. Percentage of phenotypic variation (R^2) explained by the whole model was determined using multiple interval mapping (MIM).

QTL validation

One F₅ breeding population was used to validate the expression of the Louise HTAP QTL (Pumphrey et al. 2007). The F₅ population was a cross between Louise and Nick, a cultivar developed by Westbred, LLC, that is rated as susceptible to prevalent PNW races of stripe rust. Six separate F₄ headrows from this cross were growing in non-replicated F₅ yield plots at the Spillman Agronomy Farm in Pullman, WA. Tissue was harvested from twelve plants selected at random from each plot for marker analysis. DNA was extracted from each plant as described above. IT and DS values were recorded for each plant from each plot as described earlier.

Five F₂ breeding populations were used to identify the utility of markers flanking the HTAP resistance QTL for marker-assisted selection. The five populations consisted of crosses between Louise and various other cultivars and experimental breeding lines, all of which were susceptible to stripe rust. The pedigrees of the five populations were: 1) Alpowa (PI 566596)/Citr14689//Louise; 2) Louise/Citr14734//Louise; 3) Louise/Citr14689//Louise; 4) Louise//Blanca Grande (PI 631481)//Otis (PI 634866)*2/P985RE1-16 (Purdue University); and 5) Louise//WA7919 (Washington State University)/WA7921 (Washington State University)//IDO000586 (PI 632713). Sixteen plants from each population were selected at random and allowed to self-pollinate in the field, and resulting F₃ seed was collected. The 16 plants per population were genotyped for the presence or absence of *Xwmc474* and *Xgwm148*. Plants were then phenotyped in the greenhouse using the methods previously described for HTAP resistance screening. Race PST-100 was used to inoculate the flag leaf of plants at Feekes 10.2, and IT and DS readings were collected as described previously.

To identify polymorphisms between the HTAP flanking markers in other germplasm, a panel of 45 lines was evaluated including currently grown spring (30) and winter (15) wheat

cultivars, stripe rust differentials, and advanced breeding lines. Level of polymorphism in current germplasm determines uniqueness and current deployment of the QTL, as well as usefulness of markers for marker-assisted selection (MAS). DNA was extracted as previously described when plants reached the five leaf stage. Allele band size was determined using a 50 to 350 bp ladder (LiCor Biosciences, Lincoln, NE, USA)

Results

Stripe rust evaluation

Significant genotype effects ($P < 0.0001$) for each environment were observed for both IT and DS values (Supplementary Table 1). In 2007, race PST-114 was the most predominant race in all locations. In 2008, race PST-54 was the most predominant race in Mt. Vernon, whereas race PST-114 was still the most predominant races in the other locations. In all environments, Penawawa, the susceptible parent, was rated with an IT ranging from 6 to 8 at all growth stages evaluated. In contrast, Louise, the resistant parent, was rated with an IT of 2 or 3 across growth stages. The rAUDPC values for IT ranged from 1 to 100 for the entire population across locations. The frequency distribution of mean rAUDPC values for IT of the 188 RIL obtained from each environment was bimodal (Fig. 1), indicating a single major gene confers HTAP resistance in this population. Under greenhouse conditions, Louise and Penawawa were both susceptible to race PST-100 of *P. striiformis* f. sp. *tritici* at the seedling stage indicating the absence of effective all-stage resistance genes to this race.

Louise, the resistant parent, was rated with a DS of 1 to 15%, whereas Penawawa, the susceptible parent, was rated with a DS ranging from 20 to 70%, at all growth stages evaluated. The rAUDPC values for DS for the entire RIL population ranged from 1 to 100 across locations.

The frequency distribution of rAUDPC values for DS was continuous and skewed toward resistance (Fig. 1). Although the IT and DS values were correlated ($R^2=0.48$, $P<0.0001$), the bimodal nature of the IT data was not evident in the DS data suggesting that DS is impacted by more genes than IT.

Linkage map construction

Over 1000 SSR and 250 SNP markers were tested for polymorphism between Louise and Penawawa. Of the markers tested, 322 SSR and 40 SNP markers were polymorphic between the parental lines. Of the 322 SSR markers, 295 produced distinguishable polymorphism among RIL and were used for genetic linkage map construction. Due to difficulties with the SNP protocol and lack of distinguishable polymorphism, only one of the 40 SNP markers was used for linkage map construction. Linkage analysis of the 296 segregating markers at LOD 3.0 established 29 linkage groups consisting of 1 SNP and 242 SSR markers and the remaining 53 SSR markers were unlinked. The LOD value was lowered to 1.8, which reduced the number of linkage groups to 25. These 25 linkage groups were assigned to 21 wheat chromosomes, which covered a total genetic distance of 2,181.8 cM. Chromosomes 1A, 3A, 4A, and 5B each were comprised of two linkage groups. The shortest chromosome was 4D (27.6 cM) and the longest chromosome was 5D (237.1 cM). The seven A-genome chromosomes ranged from 47.9 cM (4A) to 118.0 cM (5A) and covered a total of 587.7 cM. The seven B-genome chromosomes ranged from 73.4 cM (7B) to 137.8 cM (2B) and covered a total of 662.4 cM. The seven D-genome chromosomes ranged from 27.6 cM (4D) to 237.1 cM (5D) and covered a total of 931.7 cM. The average distances between markers on the A-, B-, and D-genome were 8.6 cM, 7.8 cM and 10.4 cM, respectively. The genome-wide average distance between markers was 9.0 cM.

HTAP resistance QTL identification

Based on single marker analysis, six markers were significantly associated with the rAUDPC values derived from the IT and DS data in each of the eight environments. One major QTL was identified on chromosome 2B based on composite interval mapping and was significantly associated with the HTAP resistance in Louise for both IT and DS readings. This QTL was consistently detected using rAUDPC values from each of the eight environments (Fig. 2) and was designated *QYrlo.wpg-2BS* (Louise designated as lo). This QTL mapped to the short arm of chromosome 2B and explained 19 to 68% of the phenotypic variation for IT and 11 to 57% of the phenotypic variation for DS, depending upon year and location (Table 1). *QYrlo.wpg-2BS* is located within a 27 cM region spanned by the six markers identified using single marker analysis. The most likely location of *QYrlo.wpg-2BS* is within a 16.9 cM region flanked by SSR markers *Xwmc474* and *Xgwm148* (Fig. 2). Resistance was always associated with the allele derived from Louise; however, variation in significance levels associated with *QYrlo.wpg-2BS* and stripe rust resistance was detected across years and locations. LOD values ranged from 7.5 to 62.3 for IT and 5.5 to 30.0 using DS depending on environment (Table 1). Heritability values ranged from 0.50 to 0.89 for IT and 0.22 to 0.84 for DS across environments.

Flanking markers for HTAP resistance QTL

Molecular markers *Xwmc474* and *Xgwm148* flank *QYrlo.wpg-2BS*. The peak of the QTL mapped between these two markers, 2.4 cM from *Xwmc474* and 14.5 cM from *Xgwm148*. *Xwmc474* is a co-dominant marker (annealing temperature 51 °C) represented by a 154 bp band in Louise and a 150 bp band in Penawawa. *Xgwm148* also is a co-dominant marker (annealing temperature 60 °C), represented by a 178 bp band in Louise and a 160 bp band in Penawawa.

Both marker loci were verified to be on chromosome 2B based on analysis using Chinese Spring nulli-tetrasomic lines (data not shown).

QTL validation

The selected F₅ populations from the Louise by Nick cross were used to validate the expression of *QYrlo.wpg-2BS* in advanced breeding lines. Nick is susceptible to stripe rust and does not contain the Louise alleles for either *Xwmc474* or *Xgwm148*. Marker analysis confirmed the presence of both the Louise alleles for *Xwmc474* and *Xgwm148* in all selected plants of all six F₅ populations evaluated. Under field conditions, the 12 selected lines from five of the six populations had IT values of 2 to 3 and DS values of 5 to 15%. Selected plants from the other population had IT values ranging from 2 to 5 and DS values from 20 to 30%. Even though the IT and DS values are slightly higher in this population, a resistant reaction was present on the plants selected for marker analysis.

Evaluation of F₂ breeding populations were used to validate the usefulness of *Xwmc474* and *Xgwm148* in MAS. In all five breeding populations tested, lines homozygous for the presence of the Louise allele for both markers resulted in a resistant phenotype, whereas lines homozygous for the absence of the Louise alleles demonstrated a susceptible phenotype. In addition, one population contained lines that were homozygous for one but not both markers. The presence of the Louise allele for *Xwmc474* and the absence of *Xgwm148* resulted in a resistant phenotype. The absence of the Louise allele for *Xwmc474* and the presence of *Xgwm148* demonstrated a susceptible phenotype. Based on the map distances between these markers and the peak of the QTL, *Xwmc474* appears to be more diagnostic in determining the presence of this QTL.

A total of 45 wheat cultivars were tested for polymorphism in comparison to the flanking markers. Based on marker phenotypes, *QYrlo.wpg-2BS* has not been widely deployed in current cultivars (Table 2). *Xwmc474*, the closest flanking marker for *QYrlo.wpg-2BS*, detected differences from Louise in 87% of the genotypes tested, whereas *Xgwm148* detected differences in only 69% of the tested genotypes. Both the Louise alleles for *Xwmc474* and *Xgwm148* were present in three of the 20 spring lines but none of the winter lines tested (Table 2). The presence of these alleles in Wakanz, WA8089, and WA8090 were expected since Wakanz is a parent of Louise and WA8089, and WA8090 is progeny from a Louise cross. These three lines are confirmed to have HTAP (using the rating system previously described) based on multiple year and field testing locations under natural inoculation (Supplementary Table 2). Two other cultivars, Kelse (Kidwell et al. 2009) and Lee (CItr 12488), were positive for the Louise allele for *Xwmc474* but not for *Xgwm148*. Kelse is a hard red spring line confirmed to have HTAP based on four years of field testing and one year of greenhouse testing, although it is unknown what the sources of this resistance is (Kidwell et al., 2009). Lee, another spring line, carries three all-stage resistance genes (*Yr7*, *Yr22*, and *Yr23*) and no HTAP resistance genes (Chen 2005). Excluding the three lines mentioned above, nine spring and one winter line contained the Louise allele for *Xgwm148*.

Discussion

Of the 32 all-stage resistance genes officially identified to date, only two (*Yr5* and *Yr15*) are still resistant to all known races in the U.S. It is predicted that, with time, these two genes also will be circumvented by new races. High-temperature, adult-plant resistance genes have proven durable over many years (Chen 2005). The concern with HTAP resistance is that the mechanism

is only active during the adult-plant growth stage, leaving seedlings susceptible to infection unless adequate all-stage resistance genes also are present (Chen et al. 1998; 2002). Cool summer temperatures also can extend the length of susceptible reactions on wheat leaves exacerbating the risk. The ability to deploy both all-stage and HTAP resistance genes into a single cultivar would provide a more effective and durable source of stripe rust resistance. Since all-stage resistance masks the phenotypic expression of HTAP resistance, molecular markers provide the only efficient way of pyramiding all-stage resistance with HTAP (Lin and Chen 2007).

Currently, molecular markers have been associated with several genes and QTL for HTAP resistance (Chen 2005; Lin and Chen 2007, 2008; Guo et al. 2008). The gene *Yr18* is located on chromosome 7DS (Suenaga et al. 2003), *Yr36* on chromosome 6BS (Uauy et al. 2005), *QYrst.wgp-6BS.1* and *QYrst.wgp-6BS.2* also on chromosome 6BS (Santra et al. 2008), three QTL (*QYrex.wgp-6AS*, *QYrex.wgp-3BL* and *QYrex.wgp-1BL*) on chromosomes 6AS, 3BL, and 1BL, respectively (Lin and Chen 2008), and *Yr39* on chromosome 7BL (Lin and Chen 2007). Other genes or QTL, reported to confer adult-plant and presumably HTAP resistance, have been located on chromosomes other than 2B. These genes or QTL include *Yr16* on 2D (Worland and Law 1986); *Yr29* on 1BL (McIntosh et al. 2001); *Yr30* on 3BS (McIntosh et al. 2001); *Yrns-B1* on 3BS (Börner et al. 2000); *YrCK* on 2DS (Navabi et al. 2005); *QPst.jic-1B*, *QPst.jic-2D* and *QPst.jic-4B* on 1BL, 2D and 4B, respectively (Melichar et al. 2008); *QYrtm.pau-2A* and *QYrtb.pau-5A* on 2A and 5A, respectively (Chhuneja et al. 2008); and *QYr.inra-2AL* on 2AL, *QYr.inra-2DS* on 2DS, and *QYr.inra-5BL.1* and *QYr.inra-5BL.2* on 5BL (Mallard et al. 2005).

Several genes for stripe rust resistance (*Yr5*, *Yr7*, *Yr27*, *Yr31*, and *Yr41*) have been reported on chromosome 2BS, all of which confer all-stage resistance (Macer 1966; McDonald et al. 2004; McIntosh et al. 2006; Lou et al. 2008). *YrSp* (McIntosh et al. 1995), *YrSte* (Chen et al. 1998) and *YrV23* (Chen et al. 1998) have been reported on chromosome 2B, but their locations need to be identified to particular arms, and all of which confer race-specific all-stage resistance. Mallard et al. (2005) identified two QTL, in a French winter wheat cultivar ‘Camp Remy’ (PI 452119), on both long and short arms of chromosome 2B. *QYr.inra-2BL* confers all-stage resistance and *QYr.inra-2BS* confers adult-plant resistance. Interestingly, *QYr.inra-2BL* is linked to *Xgwm148* and *QYr.inra-2BS* is 45 cM away from *QYr.inra-2BL* and further away from the marker. Because of the significant difference in genetic distance between *Xgwm148* and *QYr.inra-2BS* and between *QYrlo.wgp-2BS* and the markers identified in the present study, these two QTL are unlikely to represent the same locus.

Rosewarne et al. (2008) detected a QTL on 2BS for slow rusting resistance to stripe rust in ‘Attila’ (PI 35159) spring wheat. The relationship between *QYrlo.wgp-2BS* and the Attila 2BS slow rusting QTL could not be determined because different types of markers were used in these studies. However, *QYrlo.wgp-2BS* was consistently detected across locations and years in the present study, whereas the Attila 2BS QTL was not consistently detected in their study and was considered a minor QTL to the *Lr46/Yr29* complex on chromosome 1BL (Rosewarne et al. 2006, 2008). Guo et al. (2008) identified two QTL for HTAP resistance on chromosome 2BS from the winter wheat ‘Luke’ (Citr 14586). Although both *QYrlu.cau-2BS2* and *QYrlo.wpg-2BS* are linked to *Xgwm148*, linkage map comparisons localized *QYrlu.cau-2BS2* more distal to the centromere than *QYrlo.wpg-2BS*. Thus, it appears that the Louise QTL for HTAP resistance found on chromosome 2BS is likely novel to currently known HTAP resistance genes.

Many QTL identified for HTAP resistance are not consistently detected in all years and locations and are identified as multiple QTL (Santra et al. 2008; Lin and Chen, 2007, 2008; Rosewarne et al. 2008). The ability to detect *QYrlo.wpg-2BS* consistently over locations and years will have significant impact on breeding programs focused on stripe rust resistance. *QYrlo.wpg-2BS* has consistently demonstrated high level of expression in all germplasm it has been incorporated into (Supplementary Table 2), and it accounts for large portion of the phenotypic variation, indicating that environmental variation will have minimal effects on expression levels. Most interesting is the observation that *QYrlo.wpg-2BS* acts as a single gene, similar to the adult plant resistance gene *Yr18* and the slow-rusting gene *Yr36* (Spielmeyer et al. 2008; Fu et al. 2009). This simplifies the process of incorporating *QYrlo.wpg-2BS* into other germplasm through MAS. Although it is not known why this form of HTAP resistance manifests as a single gene, it has continued to demonstrate non-race specific resistance to stripe rust.

Since circumvention of all stage resistance is a significant problem in wheat, along with the lack of diversity for HTAP resistance in current cultivars, combining different sources of resistance within a single genotype will enhance the durability of resistance in cultivars, and will prolong the life (i.e. utility) of individual resistance genes (Smith et al. 2002). Of the germplasm tested, 64% had neither Louise allele and 94% had only one Louise allele, suggesting that these markers will be useful in MAS strategies designed to pyramid *QYrlo.wpg-2BS* with other stripe rust resistance genes, due to the availability of useful polymorphism in targeted cultivars. Based on validation studies it appears *QYrlo.wpg-2BS* will continue to provide high levels of expression when incorporated into other genetic backgrounds, thus broadening deployment into new adapted cultivars. The uniqueness of *QYrlo.wpg-2BS* compared to other HTAP resistance genes,

and the opportunity to combine *QYrlo.wpg-2BS* with all-stage resistance genes, creates the opportunity for wheat breeders worldwide to enhance the genetic diversity and durability of stripe rust resistance in adapted cultivars.

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References

- Anderson JA, Ogihara Y, Sorrells ME, Tanksley SD (1992) Development of chromosomal arm map for wheat based on RFLP markers. *Theor Appl Genet* 83:1035-1043
- Basten JC, Weir BS, Zeng ZB (1997) QTL CARTOGRAPHER. A reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh, N.C.
- Börner A, Röder MS, Unger O, Meinel A (2000) The detection and molecular mapping of a major gene for non specific adult plant disease resistance against stripe rust (*Puccinia striiformis*) in wheat. *Theor Appl Genet* 100:1095-1099

- Briggle LW, Curtis BC (1987) Worldwide wheat. pp. 1-31. *In* Heyne, E.G. (ed), Wheat and Wheat Improvement. Agronomy Monograph 13. ASA, CSSA, SSSA, Madison, WI.
- Chen XM (2005) Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. *Can J Plant Pathol* 27:314-337
- Chen XM, Line RF (1992) Inheritance of stripe rust resistance in wheat cultivars used to differentiate races of *Puccinia striiformis* in North America. *Phytopathol* 82:633-637
- Chen XM, Line RF (1995a) Gene action in wheat cultivars for durable high-temperature adult-plant resistance and interactions with race-specific, seedling resistance to stripe rust caused by *Puccinia striiformis*. *Phytopathol* 85:567-572
- Chen XM, Line RF (1995b) Gene number and heritability of wheat cultivars with durable, high-temperature, adult-plant resistance and race-specific resistance to *Puccinia striiformis*. *Phytopathol* 85:573-578
- Chen XM, Line RF, Shi ZX, Leung H (1998) Genetics of wheat resistance to stripe rust. *In* Proceedings of the 9th International Wheat Genetics Symposium. 2–7 August 1998, University of Saskatchewan, Saskatoon, Sask. *Edited by* A.E. Slinkard. University Extension Press, University of Saskatchewan, Saskatoon, Sask. Vol. 3. pp. 237–239
- Chen XM, Moore M, Milus EA, Long DL, Line RG, Marshall D, Jackson L (2002) Wheat stripe rust epidemics and races of *Puccinia striiformis* f. sp. *tritici* in the United States in 2000. *Plant Dis* 86:39-46
- Chhuneja P, Kaur S, Garg T, Ghai M, Kaur S, Prashar M, Bains NS, Goel RK, Keller B, Dhaliwal HS, Singh K (2008) Mapping of adult plant stripe rust resistance genes in diploid A genome wheat species and their transfer to bread wheat. *Theor Appl Genet* 116:313-324

- Chicaiza O, Chan IA, Zhang X, Brevis CJ, Jackson L, Chen X, Dubcovsky J (2006)
Registration of five wheat isogenic lines for leaf rust and stripe rust resistance
genes. *Crop Sci* 46:485-487
- Feekes W (1941) De tarwe en haar milieu. p. 560–561. *In* Vers. XVII Tech. Tarwe Comm,
Groningen
- Flor HH (1971) Current status of the gene-for-gene concept. *Annual Review of*
Phytopathol 9:275-296
- Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen X, Sela H, Fahima T, Dubcovsky J
(2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe
rust. *Science* 323:1357-1360
- Guo Q, Zhang ZJ, Xu YB, Li GH, Feng J, Zhou Y (2008) Quantitative trait loci for high-
temperature adult-plant and slow-rusting resistance to *Puccinia striiformis* f. sp. *tritici* in
wheat cultivars. *Phytopathol* 98:803-809
- Helguera M, Vanzetti L, Soria M, Khan IA, Kolmer J, Dubcovsky J (2005) PCR markers for
Triticum speltoides leaf rust resistance gene *Lr51* and their use to develop isogenic hard
red spring wheat lines. *Crop Sci* 45:728-734
- Holland JB, Nyquist WE, Cervantes-Martinez CT (2003) Estimating and interpreting
heritability for plant breeding: an update. *Plant Breeding Reviews*, Vol. 22, pp.
9-112. Edited by Jules Janick. John Wiley & Sons, Inc.
- Kidwell KK, Shelton GB, Demacon VL, Burns JW, Carter BP, Chen XM, Morris CF, Bosque-
Pérez NA (2006) Registration of ‘Louise’ wheat. *Crop Sci* 46:1384-1386
- Kidwell KK, Shelton GB, DeMacon VL, Chen X, Kuehner J, Baik B, Engle DA,
Bosque-Pérez NA (2009) Registration of ‘Kelse’ wheat. *J Plant Regist* (accepted)

- Kosambi DD (1944) The estimation of map distances from recombination values. *Ann Eugen* 12:172-175
- Lander ES, Green P, Abrahamson J, Barlow A, Daly JM, Lincoln SE, Newberg L (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174-181
- Lin F, Chen XM (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
- Lin F, Chen XM (2008) Quantitative trait loci for non-race-specific, high-temperature adult-plant resistance to stripe rust in wheat cultivar Express. *Theor Appl Genet* 118:631-642
- Line RF (2002) Stripe rust of wheat and barley in North America: A retrospective historical review. *Ann Rev Phytopathol* 40:75-118
- Line RF, Chen XM (1995) Successes in breeding for and managing durable resistance to wheat rusts. *Plant Dis* 79:1254-1255
- Lou PG, Hu XY, Ren ZL, Zhang HY, Shu K, Yang ZJ (2008) Allelic analysis of stripe rust resistance genes on wheat chromosome 2BS. *Genome* 51:922-927
- Macer RCF (1966) The formal and monosomic genetic analysis of stripe rust (*Puccinia striiformis*) resistance in wheat. *In Proceedings of the 2nd International Wheat Genetics Symposium*. 19-24 August 1963, Lund, Sweden. *Edited by J. MacKey*. *Hereditas* 2(Suppl.):127-142
- Mallard S, Gaudet D, Aldeia A, Abelard C, Besnard AL, Sourdille P, Dedryver F (2005) Genetic analysis of durable resistance to yellow rust in bread wheat. *Theor Appl Genet* 110:1401-1409

- McDonald DB, McIntosh RA, Wellings CR, Singh RP, Nelson JC (2004) Cytogenetical studies in wheat. XIX. Location and linkage studies on gene *Yr27* for resistance to stripe (yellow) rust. *Euphytica* 136: 239–248
- McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. Commonwealth Scientific and Industrial Research Organization, Australia, and Kluwer Academic Publishers, Dordrecht, Netherlands
- McIntosh RA, Devos KM, Dubcovsky J, Rogers WJ (2001) Catalogue of gene symbols for wheat: 20001 supplement [online]. Available from <http://grain.jouy.inra.fr/ggpages/wgc/2001upd.html> [accessed 15 January 2009]
- McIntosh RA, Devos KM, Dubcovsky J, Rogers WJ, Morris CF, Appels R, Anderson OA (2006) Catalogue of gene symbols for wheat: 2006 supplement [online]. Available from <http://www.shigen.nig.ac.jp/wheat/komugi/genes/macgene//supplement2006.pdf> [accessed 05 December 2008]
- Melichar JPE, Berry S, Newell C, MacCormack R, Bord LA (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
- Navabi A, Tewari JP, Singh RP, McCallum B, Laroche A, Briggs KG (2005) Inheritance and QTL analysis of durable resistance to stripe and leaf rusts in an Australian cultivar, *Triticum aestivum* ‘Cook’. *Genome* 48:97-106
- Oetting WS, Lee HK, Flanders DJ, Wiesner GL, Sellers, TA, King RA (1995) Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. *Genomics* 30:450-458

- Pumphrey MO, Bernardo R, Anderson JA (2007) Validating the *Fhb1* QTL for fusarium head blight resistance in near-isogenic wheat lines developed from breeding populations. *Crop Sci* 47:200-206
- Qayoum A, Line RF (1985) High-temperature adult-plant resistance to stripe rust of wheat. *Phytopathol* 75:1121-1125
- Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal, MW (1998) A microsatellite map of wheat. *Genetics* 149:2007-2023
- Rosewarne GM, Singh RP, Huerta-Espino J, William HM, Bouchet S, Cloutier S, McFadden H, Lagudah ES (2006) Leaf tip necrosis, molecular markers and β 1-proteasome subunits associated with the slow rusting resistance genes *Lr34/Yr29*. *Theor Appl Genet* 112:500-508
- Rosewarne GM, Singh RP, Huerta-Espino J, Rebetzke GJ (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
- Santra DK, Chen XM, Santra M, Campbell KG, Kidwell KK (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
- Smith PH, Koebner RMD, Boyd LA. (2002) The development of a STS marker linked to a yellow rust resistance derived from the wheat cultivar Moro. *Theor Appl Genet* 104:1278-1282
- Stubbs RW (1985) Stripe rust. Pages 61-101. *In: The Cereal Rusts. Vol. 2. Disease, distribution, epidemiology, and control.* A.P. Roelfs and W.R. Bushnell, eds. Academic Press, Orlando, FL.

- Suenaga K, Singh RP, Huerta-Espino J, Williams HM (2003) Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. *Phytopathol* 93:881-890
- Uauy C, Brevis JC, Chen XM, Khan IA, Jackson L, Chicaiza O, Distelfeld A, Fahima T, Dubcovsky J (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
- Worland AJ, Law CN (1986) Genetic analysis of chromosome 2D of wheat. I. The location of genes affecting height, day-length insensitivity, hybrid dwarfism and yellow rust resistance. *Z Pflanzenzücht* 96:331-345
- Zeng ZB (1993) Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proc Natl Acad Sci USA* 90:10972-10976
- Zeng ZB (1994) Precision mapping of quantitative trait loci. *Genetics* 136:1457-1468

Table 1 Quantitative trait locus and heritability (h^2) analysis for relative area under the disease progress curve (rAUDPC) values of both infection type (IT) and disease severity (DS) for five locations across two years. LOD values are those given for chromosome 2B.

Location	Year	IT rAUDPC values				DS rAUDPC values			
		LOD	R^2	h^2	Confidence interval (h^2)	LOD	R^2	h^2	Confidence interval (h^2)
Spillman Farm ^a	2007	34.2	0.62	0.50	0.46 - 0.54	25.0	0.39	0.22	0.17 - 0.27
	2008	61.5	0.67	0.76	0.73 - 0.79	9.4	0.18	0.31	0.25 - 0.37
Whitlow Farm	2007	59.9	0.68	0.89	0.88 - 0.90	30.0	0.47	0.84	0.82 - 0.86
	2008	59.3	0.58	0.85	0.83 - 0.87	29.6	0.57	0.74	0.71 - 0.77
Genesee, ID	2007	62.3	0.68	0.92	0.91 - 0.93	16.4	0.35	0.67	0.64 - 0.70
Moscow, ID	2008	15.0	0.24	0.58	0.54 - 0.62	7.2	0.14	-- ^b	--
Mount Vernon, WA	2007	7.5	0.19	0.82	0.80 - 0.84	7.6	0.16	0.81	0.79 - 0.83
	2008	19.0	0.38	0.65	0.62 - 0.68	5.5	0.11	0.69	0.66 - 0.72
Range		7.5 - 62.3	0.19 - 0.68	0.50 - 0.92		5.5 - 30.0	0.11 - 0.57	0.22 - 0.84	

^a Spillman Farm and Whitlow Farm are located in Pullman, WA

^b Could not be calculated due to missing values

Table 2 Polymorphism in selected 30 spring and 15 winter wheat genotypes for *Xwmc474* and *Xgwm148*, the flanking markers for the major quantitative trait locus for high-temperature adult-plant resistance to stripe rust in Louise.

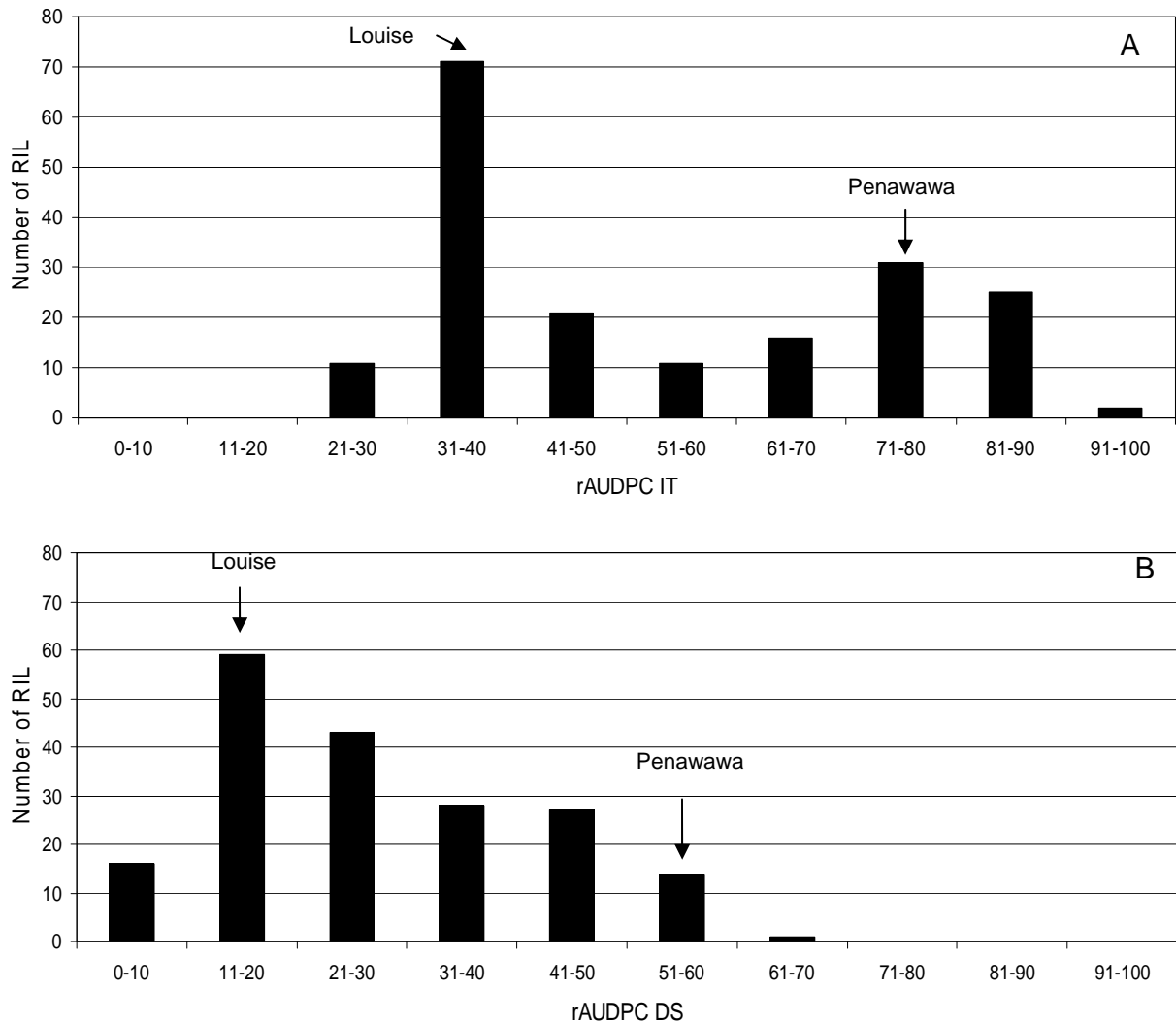
Genotype	Growth habit	Presence of Louise HTAP resistance	<i>Xwmc474</i>	<i>Xgwm148</i>
Wawawai (PI 574598)	Spring	-	150	178
Wakanz (PI 506352)	Spring	+	154	178
Louise (PI 634865)	Spring	+	154	178
Penawawa (PI 495916)	Spring	-	150	156
WA8089 (WSU ^a)	Spring	+	154	178
WA8090 (WSU)	Spring	+	154	178
Alpowa (PI 566596)	Spring	-	150	178
WA8039 (WSU)	Spring	-	150	178
WA7985 (WSU)	Spring	-	150	156
WA8058 (WSU)	Spring	-	152	176
WA8043 (WSU)	Spring	-	150	178
WA8045 (WSU)	Spring	-	152	161
Otis (PI 634866)	Spring	-	150	175
Hollis (PI 632857)	Spring	-	150	178
Kelse (PI 653842)	Spring	-	154	175
Tara2002 (PI 617073)	Spring	-	150	178
Hank (PI 613581)	Spring	-	150	178
Macon (PI 617072)	Spring	-	150	156
Whit (PI 653841)	Spring	-	150	156
Zak (PI 607839)	Spring	-	150	175
Farnum (PI 638535)	Winter	-	150	175
Stephens (GSTR 11901)	Winter	-	150	178
Bauermeister (PI 634717)	Winter	-	150	175
Centurk (CI 015075)	Winter	-	152	141
Norstar (CI 017735)	Winter	-	152	174
Karl (PI 527480)	Winter	-	152	148
Fielder (CI 017268)	Spring	-	146	141
Moro (CI 013740)	Winter	-	156	170
Chinese166 (CI 011765)	Winter	-	152	170
Yr9 (YR 000009)	Spring	-	150	147
Hyak (PI 511674)	Winter	-	150	170
Druchamp (CI 013723)	Winter	-	152	147
Produra (CI 017460)	Spring	-	150	143
Express (PI 573003)	Spring	-	152	178
Lemhi (CI 011415)	Spring	-	159	178
Clement (PI 518799)	Winter	-	152	173
Compair (PI 325842)	Spring	-	144	152
Yr8 (YR 000008)	Spring	-	150	171
Tres (CI 017917)	Winter	-	156	170

Table 2 cont.

Genotype	Growth habit	Presence of Louise HTAP resistance	<i>Xwmc474</i>	<i>Xgwm148</i>
Paha (CI 014485)	Winter	-	156	170
Tyee (CI 017773)	Winter	-	150	170
Riebesel (YR 000004)	Spring	-	152	170
Lee (CI 012488)	Spring	-	154	147
HeinesVII (PI 201195)	Winter	-	152	162
Avocet (PI 464644)	Spring	-	151	174
Polymorphism			87%	69%

^a Washington State University Breeding Line

Figure 1 Distribution of relative area under the disease progress curve (rAUDPC) values from the Louise by Penawawa mapping population averaged across locations and years for A) infection type^a (IT) values and B) disease severity^b (DS) values.

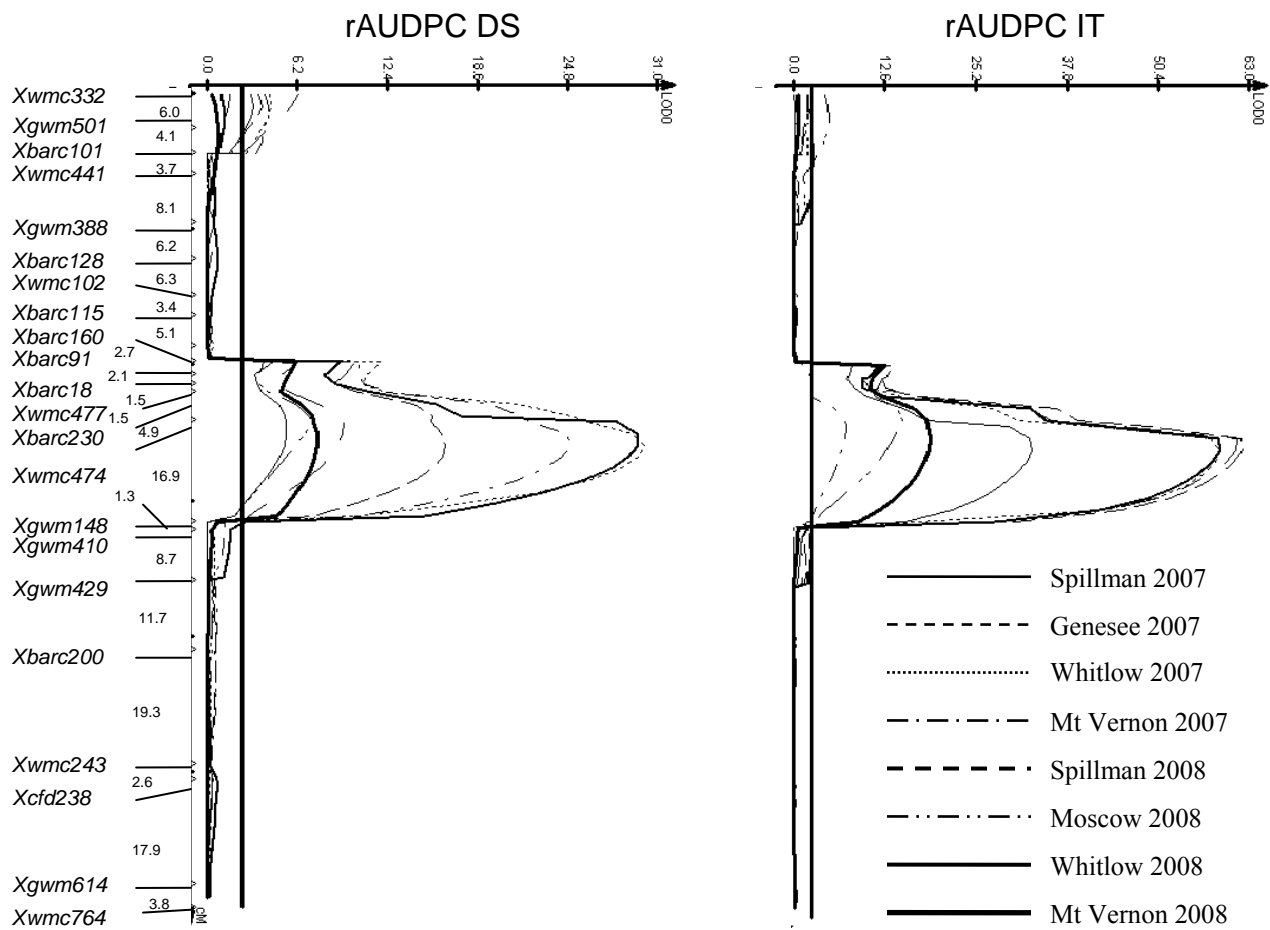


^a Average IT rAUDPC value for Louise and Penawawa are 33 and 85, respectively with an LSD value of 4.0

^b Average DS rAUDPC value for Louise and Penawawa are 13 and 52, respectively with an LSD value of 3.5

Figure 2 High-temperature adult-plant resistance quantitative trait locus on chromosome 2B identified by composite interval mapping. Positions (cM) of the molecular markers along the chromosome are on the vertical axis.

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Supplementary Table 1a Analysis of variance for replicate and genotype effect for relative area under the disease progress curve for infection type values analyzed for each year-location.

Year, location Effect	df	MS	F-value	P-value	R ²	Coefficient of variance
Mount Vernon, 2007						
Replicate effect	2	1039.58	7.28	0.0008	0.88	17.42
Genotype effect	187	2047.13	14.35	<.0001		
Error	373	142.72				
Mount Vernon, 2008						
Replicate effect	2	515.08	4.85	0.0083	0.77	14.73
Genotype effect	187	687.21	6.47	<.0001		
Error	374	106.22				
Genesee, 2007						
Replicate effect	2	10246.23	201.03	<.0001	0.95	12.60
Genotype effect	187	1717.10	33.69	<.0001		
Error	374	50.97				
Moscow, 2008						
Replicate effect	2	1704.47	3.16	0.0437	0.72	79.09
Genotype effect	187	2739.69	5.01	<.0001		
Error	374	539.90				
Spillman, 2007						
Replicate effect	2	6603.85	22.65	<.0001	0.68	38.29
Genotype effect	187	1165.03	4.00	<.0001		
Error	373	291.52				
Spillman, 2008						
Replicate effect	2	13294.39	60.89	<.0001	0.85	28.21
Genotype effect	187	2306.05	10.56	<.0001		
Error	374	218.35				
Whitlow, 2007						
Replicate effect	2	29.96	0.32	0.7230	0.94	18.96
Genotype effect	187	2725.64	29.54	<.0001		
Error	363	92.27				
Whitlow, 2008						
Replicate effect	2	4.12	3.60	0.0283	0.90	22.87
Genotype effect	187	20.17	17.61	<.0001		
Error	374	1.15				

Supplementary Table 1b Analysis of variance for replicate and genotype effect for relative area under the disease progress curve for disease severity values analyzed for each year-location.

Year, location Effect	df	MS	F-value	P-value	R ²	Coefficient of variance
Mount Vernon, 2007						
Replicate effect	2	1213.63	11.97	<.0001	0.87	21.7
Genotype effect	187	1369.80	13.51	<.0001		
Error	374	101.38				
Mount Vernon, 2008						
Replicate effect	2	649.64	5.44	0.0047	0.79	34.70
Genotype effect	187	899.24	7.52	<.0001		
Error	374	119.51				
Genesee, 2007						
Replicate effect	2	3077.75	32.22	<.0001	0.78	34.51
Genotype effect	187	665.01	6.96	<.0001		
Error	374	95.51				
Moscow, 2008						
Replicate effect	2	880.62	5.98	0.0032	0.56	135.89
Genotype effect	134	202.31	1.37	0.0297		
Error	149	147.30				
Spillman, 2007						
Replicate effect	2	4906.53	37.62	<.0001	0.53	56.54
Genotype effect	187	241.24	1.85	<.0001		
Error	374	130.42				
Spillman, 2008						
Replicate effect	2	13097.02	116.85	<.0001	0.68	65.24
Genotype effect	185	285.15	2.54	<.0001		
Error	316	112.08				
Whitlow, 2007						
Replicate effect	2	383.30	2.86	0.0588	0.83	43.33
Genotype effect	187	2298.70	17.12	<.0001		
Error	374	134.24				
Whitlow, 2008						
Replicate effect	2	1301.36	7.99	0.0004	0.90	26.81
Genotype effect	187	1617.22	9.92	<.0001		
Error	373	162.95				

Supplementary Table 2 High-temperature adult-plant resistance infection type (IT) and disease severity (DS) ratings for four spring wheat lines containing the QTL *QYrlo.wpg.2B* identified from the cultivar Louise. Data was collected at multiple locations over four years.

Year	Location	Feekes Stage	Louise		WA8089		WA8090		Wakanz		Lemhi ^a	
			IT ^b	DS	IT	DS	IT	DS	IT	DS	IT	DS
2008	Walla Walla, WA	11.1	3	20	3	5	1	5	3	20	8	5
	Lind, WA	11.1	0	0	0	0	0	0	2	2	0	0
	Mount Vernon, WA	5	1	1	1	5	8	20	1	5	8	50
	Mount Vernon, WA	10.54	3	20	3	5	3	20	2	10	9	100
	Whitlow Farm ^c	11.1	3	5	3	5	1	1	2	5	8	90
	Plant Pathology Farm	11.1	2	5	2	5	2	1	2	1	8	100
	Spillman Farm	10.5	0	0	0	0	0	0	1	0	8	70
	Spillman Farm	10.54	2	2	2	2	2	2	2	2	8	90
2007	Mount Vernon, WA	5	5	10	2	20	2	10	- ^d	-	8	30
	Mount Vernon, WA	11.1	3	30	3	30	3	30	-	-	8	80
	Whitlow Farm	10.54	3	5	5	40	5	20	-	-	8	80
	Spillman Farm	10.5	0	0	0	0	0	0	-	-	8	50
	Spillman Farm	10.54	3	5	3	5	1	1	-	-	8	80
2006	Spillman Farm	10.5	2	5	2	2	0	0	-	-	-	-
	Spillman Farm	10.54	2	5	2	2	2	1	-	-	-	-
2005	Spillman Farm	10.5	2	15	3	15	2	10	-	-	-	-
	Spillman Farm	10.54	2	20	2	20	2	15	-	-	-	-

^a Susceptible spring wheat check cultivar

^b IT scored on a scale of 0-9; DS scored on a scale of 0-100%

^c Whitlow Farm, Plant Pathology Farm, and Spillman Farm are all located in Pullman, WA

^d Cultivars not included in these trials

CHAPTER 3

Genetic linkage analysis indicates that Hessian fly resistance gene *H3* is located on chromosome 1AS of wheat

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Abbreviations: AFLP, amplified fragment length polymorphism; cM, centiMorgans; MAS, marker-assisted selection; PNW, Pacific Northwest; RAPD, random amplified polymorphic DNA; RIL, recombinant inbred line; RF, recombination fraction; STS, sequence-tagged site; SSR, simple-sequence-repeat.

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Abstract

The Hessian fly (*Mayetiola destructor* [Say]) is a major yield limiting factor in high residue management wheat (*Triticum aestivum* L.) production systems throughout the world. The objectives of this study were to: 1) identify the chromosomal location of *H3*, a widely deployed Hessian fly resistance gene; 2) identify DNA markers associated with *H3* for use in marker-assisted selection; and 3) evaluate these markers in resistant cultivars suspected of containing *H3*. One hundred eighty-eight F_{5:6} recombinant inbred lines (RIL) from a genetic mapping population derived from a 'Louise' (resistant) by 'Penawawa' (susceptible) cross were evaluated under greenhouse conditions for resistance to Hessian fly biotype C. Fifteen plants per line were scored for resistance, and data were converted to reflect the percentage of susceptible plants within each line. Louise and Penawawa had susceptibility values of 9% and 100%, respectively, whereas

the individuals within the RIL population ranged from 6 to 100% susceptible. Distribution of susceptibility values for the RIL population was bimodal, and skewed toward highly susceptible, indicating that a single major gene affected this trait. A genetic linkage map consisting of one single nucleotide polymorphism (SNP) and 296 simple sequence repeat (SSR) markers was used for QTL analysis. Two SSR markers were significantly associated with *H3* at a LOD score of 65.2, which accounted for 83% of the phenotypic variation. *H3* was localized to a 20.1 cM region on chromosome 1AS, flanked by the markers *Xpsp2999* and *Xcfd15*. SSR markers associated with *H3* are currently being used in marker-based forward breeding strategies to transfer and pyramid this gene with other Hessian fly genes in regionally adapted germplasm to improve the durability of resistance in resulting wheat cultivars.

Introduction

The World Health Organization estimates that two-thirds of the world population relies on wheat (*Triticum aestivum* L.) as a staple food (World Health Organization, 2009), which makes protecting the crop from yield limiting pests a high priority in wheat improvement programs (Quarrie et al., 1999; Anderson et al., 2001; Kong et al., 2005). The Hessian fly (*Mayetiola destructor* [Say]) is a devastating pest of both winter and spring sown wheat, resulting in average annual yield losses of 5 to 10% worldwide (Buntin, 1999). The adult Hessian fly lives for approximately four days, during which time reproduction occurs (Veseth, 1988). Hessian fly females only mate once and deposit 100 to 400 eggs on the adaxial surface of wheat leaves. Larvae hatch from these eggs within three to four days and migrate to the base of the nearest node to feed (Stuart

et al., 2008). After feeding for about two weeks, the larvae develop to the pupa stage (Veseth, 1988). The pupa stage, which ranges from seven to 35 days, occurs within a dark brown puparium, after which the adult fly emerges to repeat the life cycle. (Harris et al., 2001).

Hessian fly biotypes are distinguished with respect to their ability to survive on plants carrying unique *H* genes (Stuart et al., 2008). Current biotypes of Hessian fly identified throughout the United States (US) include the series ‘A’ through ‘O’ and biotype ‘GP’ (Great Plains; Ratcliffe et al., 2000). In the Pacific Northwest (PNW) region of the US, prevalent biotypes include GP, D, E, F, G, H, J, L, M, N, and O (Ratcliffe et al., 2000). Biotype GP, avirulent to *H3*, represented 47 to 75% of the population at sites sampled in Washington (Ratcliffe et al., 2000). Shifts in the dominant biotype can occur, necessitating deployment of other effective resistance genes in commercial wheat cultivars (Smiley et al., 2004).

Infestations of Hessian fly typically occur in high-residue and annual cropping systems in years with cool, wet springs (Pike and Antonelli, 1981; Pike et al., 1993). Although crop losses in the PNW associated with Hessian fly damage are inconsistent due to environmental fluctuations (Pike and Antonelli, 1981), researchers speculate that population sizes will increase as more wheat producers incorporate reduced tillage into their crop management systems, since surface residue provides excellent over-wintering habitats for insects (Pike et al., 1993; Smiley et al., 2004). Insecticides can be used to control the insect, but are not desirable due to high costs and environmental contamination concerns. The most effective, economical and environmentally friendly

method of controlling the Hessian fly is sowing cultivars with genetic resistance to this pest (Liu et al., 2005a).

Genetic resistance to Hessian fly operates on a gene-for-gene basis (Hatchett and Gallun, 1970; Williams et al., 2002). The resistant/susceptible phenotype of the plant and the virulent/avirulent phenotype of the insect dictates resistance, which Ratcliffe et al. (2000) stated is expressed as larval antibiosis. During compatible interactions, larvae feed, grow, and mature near the crown of infected seedlings (Williams et al., 2002). Larval feeding causes abnormal stem and leaf growth, stunting, and can eventually lead to seedling death (Anderson and Harris, 2006). During incompatible interactions, first-instar Hessian fly fail to increase in length and die within three to five days (Painter, 1930; Williams et al., 2002).

Thirty-two Hessian fly resistance genes, designated *H1* to *H32*, have been identified to date in wheat (Kong et al., 2005; McIntosh et al., 2008). Many of these resistance genes have been assigned to specific wheat chromosomes by other researchers. Gallun and Patterson (1977) assigned *H6* to chromosome 5A through monosomic analysis. Genes *H3* and *H9* also were determined to be linked in a block to *H6* on chromosome 5A, and thus were designated as *H3-H6-H9* (Stebbins et al., 1982). *H10* also was reported to be located on chromosome 5A based on its linkage to *H9* (Carlson et al., 1978). *H12*, *H14*, *H15*, *H16*, *H17*, *H19*, *H28* and *H29* also were reported to be located on chromosome 5A (Stebbins et al., 1982; Obanni et al., 1988; Ohm et al., 1995; Cebert et al., 1996; McIntosh et al., 2008). Using monosomic analysis, *H5*, which is linked to *H11*, was localized to chromosome 1AS (Roberts and Gallun, 1984). Results of recent genetic linkage mapping efforts indicated that a cluster of genes, *H9*, *H10*, and

H11, were located on the distal region of chromosome 1AS instead of on chromosome 5A as was previously reported (Kong et al., 2005; Liu et al., 2005a). Based on the discrepancy between recent results and previous reports on the chromosomal location of *H9* and *H10*, other genes previously reported as being linked to these genes also may be located on wheat chromosome 1AS within a cluster of Hessian fly resistance genes.

In the US, Hessian fly resistance genes are frequently circumvented by the insect as new mutations arise in existing populations (Stuart et al., 2008). Gould (1986) predicted that pyramiding multiple Hessian fly resistance genes into a single wheat cultivar offers the highest relative durability of resistance, and this durability could last up to 400 fly generations. Pyramiding resistance genes into a single cultivar can reduce the impact of the Hessian fly on wheat produced in reduced tillage systems, as well as slow the circumvention of deployed resistance genes. However, the phenotypes of plants with multiple resistance genes to the Hessian fly are indistinguishable from plants with only one resistance gene, since the expression of one gene masks the expression of others. Pyramiding can only be efficiently achieved by utilizing unique molecular markers that co-segregated with the respective Hessian fly resistance genes, thereby allowing confirmation of the presence of multiple genes in a single genotype (Williams et al., 2003).

Dweikat et al. (1994, 1997, 2002) identified random amplified polymorphic DNA (RAPD) and sequence-tagged site (STS) markers associated with *H3*, *H5*, *H6*, *H9-H14*, *H16*, and *H17*. However, marker-assisted selection (MAS) using RAPD markers is often unreliable due to lack of repeatability (Kong et al., 2005). Williams et al. (2003) used amplified fragment length polymorphism (AFLP) and STS markers to map *H31* to

chromosome 5BS. More recently, Liu et al. (2005c, 2005b) identified simple-sequence-repeat (SSR) markers associated with Hessian fly resistance genes on wheat chromosome 1AS (tentatively named *Hdic*) and chromosome 6DS (*H13*). Identifying linkage between the more reliable SSR markers and previously identified genes will allow efficient deployment of resistance in commercial cultivars.

In the PNW, *H3* has been widely deployed since it confers resistance to predominant Hessian fly biotypes in the region (Smiley et al., 2004). It would be beneficial to pyramid *H3* with other Hessian fly genes to enhance the durability of resistance in commercial wheat cultivars. The objectives of this study were to: 1) determine the chromosomal location of *H3*; 2) identify SSR markers linked to Hessian fly resistance gene *H3* for use in MAS; and 3) confirm these markers in other PNW germplasm suspected of carrying *H3*.

Materials and Methods

Plant materials

One hundred and eighty-eight F_{5:6} recombinant inbred lines (RIL) were developed from a single F₁ plant, derived from the hybridization of ‘Louise’ (resistant parent; Kidwell et al., 2006) x ‘Penawawa’ (susceptible parent; PI 495916), using single-seed descent. A complete description of the parental lines and mapping population is available in Carter et al. (2009). Based on pedigree analysis, Louise carries *H3*, since both of its parents have ‘W38’ (PI 410582) in their pedigrees, which is the germplasm in which *H3* was initially identified (Caldwell et al., 1946). Penawawa is susceptible to the Hessian fly.

Greenhouse experiment

The 188 RIL and parental checks were evaluated for phenotypic response to Hessian fly infestation. Approximately 15 seeds of each line were planted in uniformly spaced rows in flats containing a 1:1 mixture of soil (#1 Sunshine Mix; Sun Gro Horticulture, Bellevue, WA) and vermiculite. Trays were placed in the greenhouse at 18 °C with 14:10 h (L: D) photoperiod. Light intensity was 400 μmol at bench level. Seedlings in each flat were infested at the one-leaf stage with biotype C, which was selected because it demonstrates excellent avirulence to *H3* compared to PNW biotypes, which demonstrate varying degrees of virulence to this gene. Infested plant material with emerging adults was placed under cheesecloth netting in close proximity to the seedlings. The plant materials were removed when approximately 10-15 eggs were visible on each seedling. Three weeks after infestation, seedlings were visually examined to determine resistance response. Susceptible seedlings were stunted with abnormally dark green leaves, whereas resistant plants were not stunted with typical green leaves (Maas et al., 1987). The number of susceptible and resistant seedlings among the 15 plants tested per RIL and parental line were recorded. Data were converted to percentage of susceptible plants for QTL analysis.

DNA isolation and marker analysis

Fresh leaf tissue of three individuals from each $F_{5,6}$ RIL or parent was collected at the five leaf stage, and used to extract genomic DNA using the CTAB method described by Anderson et al. (1992). The genetic linkage map described by Carter et al. (2009) was used for QTL analysis. Twenty additional markers located on chromosome 5A and 1A were screened for polymorphism between the parents as this was the suspected location

of the gene. Sequences of available SSR markers along with their previously determined chromosomal locations were obtained from Graingenes (<http://wheat.pw.usda.gov/>). SSR marker analysis was conducted using the PCR conditions described by Röder et al. (1998) except that primers were synthesized to include the M13-tail (Oetting et al. 1995). The 10 μ L reaction mixture consisted of 50 ng of template DNA, 1.0 μ L Mg-free 10X PCR buffer, 0.5 units of Taq DNA polymerase, 1.5 mM of MgCl₂ (Promega, Madison, WI, USA), 200 μ M each of dCTP, dGTP, dTTP, and dATP (Fermentas, Glen Burnie, MD) and 0.25 μ M of each primer pair synthesized by MWG-Biotech (High Point, NC, USA). Appropriate fluorophores for the Global IR² analysis system (LiCor Biosciences, Lincoln, NE, USA) were included in the PCR mix. Amplification conditions were an initial 5 min denaturation at 94 °C, followed by 41 cycles of 1 min of denaturation at 94 °C, 1 min of annealing at 50 to 65 °C (depending on primers), and a 1 min extension at 72 °C. The final extension step was 10 min at 72 °C.

Statistical analysis

One-way analysis of variance was performed with genotype as the main effect using the statistical package SAS V9.1 (SAS Institute, Raleigh, NC). Linkage maps were constructed using Mapmaker V3.0 (Lander et al., 1987). Additional markers were added to the established genetic linkage map using the “try” command and verified using the “ripple” command. The Kosambi map function was applied to calculate genetic distances in centiMorgans (cM) between the ordered markers (Kosambi, 1944). The software WinQTLCart V2.5 (Basten et al., 1997) was used for QTL analysis. Single marker analysis using one-way ANOVA with a probability level of $P < 0.01$ was used to identify markers with significant effects on Hessian fly resistance. QTL analysis was

targeted toward linkage groups previously identified using single marker analysis, and detection was performed using composite interval mapping (Zeng 1993, 1994). In order to detect significant associations between a marker and *H3*, a critical LOD threshold value of 3.0 and a maximum distance of 50 cM were used.

***H3* germplasm validation**

The wheat cultivars used to validate the presence of *H3* are listed in Table 1 along with information regarding susceptibility to the Hessian fly. Hessian fly genes predicted to be present are based on previous greenhouse testing results, as well as molecular characterization. Advanced breeding lines WA8039, WA8089, and WA8090 are from the Washington State University Spring Wheat Breeding and Genetics Program.

WA8039 has a pedigree of ‘Alpowa’ (PI 566596)/3/‘Centennial’ (PI 537303)/‘Wawawai’ (PI 574598)//Alpowa; WA8089 has a pedigree of ‘Wakanz’ (PI 506352)/‘Wadual’ (PI506354)//Centennial/3/ Louise; and WA8090 has a pedigree of ‘Treasure’ (PI 468962)/Wawawai//Louise. Identified markers flanking *H3* were evaluated using previously described leaf tissue collection, DNA extraction, PCR conditions and fragment analysis methods. Sequences of the flanking SSR markers were obtained from Graingenes. Seed of resistant cultivars and experimental breeding lines were provided by the Washington State University Spring Wheat Breeding and Genetics Program.

Results and Discussion

Hessian fly response

Significant differences ($P < 0.0001$) were observed between the susceptibility levels of Louise (9%) and Penawawa (100%) to the Hessian fly biotype C. A significant (P

<0.0001) range (6% to 100%) of susceptibility levels also was detected within the RIL population. The data were bimodally distributed, and skewed toward highly susceptible (100%; Figure 1). It is interesting to note that although many plants demonstrated 100% susceptibility, no RIL exhibited 100% resistance (Figure 1). Possible explanations include: 1) the Hessian fly population used for screening was heterogeneous; or 2) the expression levels of this gene do not confer 100% resistance to Hessian fly biotype C. The parents of Louise, Wakanz (PI 506352) and Wawawai (PI 574538) were determined to be 60% and 71% susceptible to biotype C, respectively. Based on field and controlled environment screening analysis, the parental lines are moderately resistant to Hessian fly biotypes that predominate in the PNW (Dr. Nilsa Bosque-Pérez, personal communication).

Genetic mapping

The genetic map developed by Carter et al. (2009) was used to identify molecular markers associated with *H3*. Of the additional 20 markers evaluated, only one marker on chromosome 1A was polymorphic in the population. Based on single marker analysis, three markers were significantly associated with Hessian fly susceptibility. Composite interval mapping localized *H3* to the distal end of chromosome 1AS in the same region as *H9* and *H11* (Kong et al., 2005; Liu et al., 2005a), within a 20.1 cM region flanked by *Xpsp2999* and *Xcfd15* (Figure 2). The LOD value of this association was 65.2, and accounted for 83% of the phenotypic variation. The peak of the gene mapped 4.0 cM proximal to *Xpsp2999* and 16.1 cM distal to *Xcfd15*. *Xpsp2999* is a co-dominant marker (annealing temperature 55 °C) represented by three bands in Penawawa (147, 154, 162 bp) and three bands in Louise (154, 162, 164 bp). *Xcfd15* is a dominant marker

(annealing temperature 60 °C) represented by two bands in Penawawa (177 and 194 bp) and one band in Louise (177 bp). Resistance is associated with the allele derived from Louise. Both marker loci were verified to be on chromosome 1A based on analysis of the Chinese Spring nulli-tetrasomic lines.

Due to the close proximity in which *H3* mapped to *H9*, the markers *Xcfa2153*, *Xbarc263*, and *Xwmc329*, which co-segregate with *H9*, were tested in this population (Liu et al., 2005a). The susceptible marker alleles were fixed in this population, indicating that *H9* is not present. Based on molecular mapping and comparison to other genetic linkage maps, *H3* appears to be distal to the *H9* complex.

Marker analysis of other cultivars

Many spring wheat cultivars in the PNW are resistant to Hessian fly (Kidwell et al., 2002, 2003, 2006; Westbred, LLC., personal communication), which, according to Smiley et al. (2004), may result from excessive deployment of *H3* in regional germplasm. As a result, many of these known resistant cultivars, including their suspected donor parent, were screened for the presence of *XpSP2999* and *Xcfd15*. Of the 17 resistant spring cultivars/breeding lines tested from the PNW, four had the same marker allele profiles as Louise (Table 1). Two of the cultivars were Wawawai and Wakanz, the parental lines of Louise, although Wakanz was identified as being heterogeneous for this banding profile (Table 1). The pedigrees of Wawawai and Wakanz are very diverse, although both have the winter wheat line ‘Arthur’ (CItr 14425) in their lineage, which is confirmed to have *H3* (Stebbins et al., 1980). One of the parents of Arthur, the winter wheat line W38 (PI 410582), was the original line in which *H3* was identified (Caldwell et al. 1946), and therefore, may be the source of the resistance in Louise. Since Wakanz is a

heterogeneous population, and the original line used to make the hybridization is unavailable, it is difficult to determine from which parent Louise inherited its resistance from. In greenhouse tests conducted at Purdue University and field tests in the PNW (Dr. Nilsa Bosque-Pérez, personal communication), Wawawai and Wakanz had mixed resistance reactions, which have resulted from heterogeneity within the populations.

The cultivar ‘Zak’ (PI 607839; Kidwell et al., 2002), another soft white spring cultivar adapted to the PNW with resistance to Hessian fly, had the same marker allele profile as Louise and was expected to carry *H3* (Table 1). However, Zak was 100% susceptible to Hessian fly biotype C, indicating that it does not carry *H3* (data not shown). The suspected resistant donor parent of this line, Treasure (Sunderman and O’Connell, 1988), had a similar marker allele profile as that of Louise for *Xp_{sp}2999*, but did not have the null allele for *Xcfd15*. *Xp_{sp}2999* also was reported to be linked to the *Pm3* resistance allele to powdery mildew (*Blumeria graminis* (DC) E.O. Speer f. sp. *tritici*) in wheat (Bougot et al., 2002). Pedigree analysis of Louise, Zak, and Treasure indicated that all three cultivars may carry the *Pm3a* allele from ‘Asosan’ (PI 155256). If this is the case, the presence of the Louise allele for *Xp_{sp}2999* in Zak and Treasure may result from linkage to *Pm3a* and not *H3*. Since many of the resistant spring wheat cultivars tested had unique marker allele profiles compared to Louise, they may contain different forms of resistance to Hessian fly.

One advanced experimental spring wheat breeding line from Washington State University, WA8090 (derived from a Wawawai by Louise cross), was confirmed phenotypically to be resistance to the Hessian fly (Dr. Nilsa Bosque-Pérez, personal communication). A subsequent evaluation of the flanking markers indicated that *H3* is

present this line (Table 1). Another breeding line, WA8089 (derived from a Wakanz by Louise cross), also confirmed to be resistant to Hessian fly, had a similar allele profile as Louise using *Xpsp2999* but not *Xcfd15*, and probably carries *H3*. Our results indicate that the resistance from *H3* is heritable, and that the flanking markers *Xpsp2999* and *Xcfd15* can be useful for identifying the presence of this gene.

Previous discussions by Liu et al. (2005a) and Kong et al. (2005) have disputed the location of many Hessian fly genes, including *H6* and *H9*. These authors suspect that *H6* may have been erroneously mapped by Gallun and Patterson (1977) to chromosome 5A due to misinterpretation of the monosomic mapping data. Thus, all genes linked to *H6* are incorrectly positioned on chromosome 5A as well. Previously, *H3* was assigned to chromosome 5A because of its linkage to *H6* (Patterson and Gallun, 1977; Stebbins et al., 1982). *H9* also was originally placed on chromosome 5A due to its linkage to *H6* (Stebbins et al., 1980; 1982). Recently, three distinct F₂ populations ('Iris' (PI 562615) x 'Newton' (CItr 17715); Iris x 'Len' (CItr 17790); and 'Ella' (CItr 17938) x Len) have provided evidence that *H9* resides on chromosome 1AS (Liu et al., 2005a; Kong et al., 2005). With the new information that *H9-H10-H11* form a cluster of Hessian fly resistance genes on chromosome 1AS (Liu et al., 2005a), along with the identification of *H5* and *Hdic* on this chromosome as well (Roberts and Gallun, 1984; Liu et al., 2005c), it is probable that many other Hessian fly genes also reside in this region. Our results indicate that *H3* resides in the same region as *H5*, *H9*, *H10*, *H11*, and *Hdic* on chromosome 1AS. This information continues to clarify the linkage relationships among Hessian fly genes, which may define their utility in breeding for resistance.

Conclusion

Until recently, breeding for Hessian fly resistance relied on phenotypic selection through bioassays or selection based on morphological or agronomic traits (Stuart et al., 2008).

These methods are labor-intensive, costly, and often inconclusive. In contrast, MAS more accurately detects the presence of a gene by using DNA markers linked to the specific resistance gene, employing a more efficient method of selecting desirable resistant genotypes (Bernardo, 2008). Since MAS is unaffected by environmental variables or plant developmental stages, it can facilitate the selection of resistant genotypes in the early stages of the breeding process (Bernardo, 2008). The flanking markers identified in this study will assist in deploying *H3* into regionally adapted germplasm, as well as in combination with other Hessian fly resistance genes.

Selection using DNA markers identifies resistant genotypes with a certain level of accuracy. Primer set PSP2999 was originally developed to identify the presence of the low-molecular-weight glutenin gene *Glu-3* on wheat chromosome 1AS (Pitts et al., 1988; Devos et al., 1995) and was found to be 4.0 cM distal to *H3*. Thus, the recombination fraction (RF) between this marker and *H3* is 3.9% based on Kosambi index (Kosambi, 1944). The RF between *Xcfd15* and *H3* is 13.8%. These RFs translate into selection accuracies for *Xpsp2999* and *Xcd15* of 96.1% and 86.2%, respectively (Liu, 1998).

Using the product rule of the probability, the selection accuracy will increase to 99% when both markers are used for selection (Liu et al., 2005a). Understanding that recombination will occur between the gene and the flanking markers at an elevated frequency due to the large distance between them, larger breeding populations will need to be evaluated to ensure the gene is carried in advanced lines (Witcombe and Virk,

2001). Even though the flanking markers cover a 20.1 cM distance, these markers will still be very useful in breeding applications and MAS, especially when pyramiding *H3* with other Hessian fly genes, until closer markers are identified to improve selection efficiency.

Many growers in the PNW are converting to reduced tillage systems, raising the concern that Hessian fly damage will increase in these fields if susceptible cultivars are grown (Veseth, 1988; Smiley et al., 2004). To reduce this risk, public and private breeding programs in the region are focused on developing cultivars with resistance to the Hessian fly. Identification of SSR markers *Xpsp2999* and *Xcfd15* flanking *H3* will improve the reliability of MAS compared to previously reported RAPD markers (Kong et al., 2005), and will help facilitate the deployment of *H3* in breeding programs. These markers also will facilitate pyramiding multiple Hessian fly genes into a single cultivar to enhance the durability of Hessian fly resistance in future wheat cultivars.

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References

- Anderson, J.A., R.W. Stack, S. Liu, B.L. Waldron, A.D. Fjeld, C. Coyne, B. Moreno-Sevilla, J. M. Fetch, Q.J. Song, P.B. Cregan, and R.C. Froberg. 2001. DNA markers for Fusarium head blight resistance QTLs in two wheat populations. *Theor. Appl. Genet.* 102:1164-1168.
- Anderson, J.A., Y. Ogihara, M.E. Sorrells, and S.D. Tanksley. 1992. Development of chromosomal arm map for wheat based on RFLP markers. *Theor. Appl. Genet.* 83:1035-1043.
- Anderson, K.G., and M.O. Morris. 2006. Does R gene resistance to Hessian fly allow wheat to prevent plant growth effects associated with Hessian fly attack? *J. Econ. Entomol.* 99:1842-1853.
- Basten, J.C., B.S. Weir, and Z.B. Zeng. 1997. QTL CARTOGRAPHER. A reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh, N.C.
- Bernardo, R. 2008. Molecular markers and selection for complex traits in plants: learning from the last 20 years. *Crop Sci.* 48:1649-1664.
- Bougot, Y., J. Lemoine, M.T. Pavoine, D. Barloy, and G. Doussinault. 2002. Identification of a microsatellite marker associated with *Pm3* resistance alleles to powdery mildew in wheat. *Plant Breed.* 121:325-329.
- Buntin, G.D. 1999. Hessian fly (Diptera: Cecidomyiidae) injury and loss of winter wheat grain yield and quality. *J. Econ. Entomol.* 92:1190-1197.
- Caldwell, R.M., W.B. Cartwright, and L.E. Compton. 1946. Inheritance of Hessian fly resistance derived from W38 and Durum P.I. 94587. *Agron. J.* 38:398-409.

- Carlson, S.K., F.L. Patterson, and R.L. Gallun. 1978. Inheritance of resistance to Hessian fly derived from *Triticum turgidum* L. *Crop Sci.* 18:1011-1014.
- Carter, A.H., X.M. Chen, K.G. Campbell, and K.K. Kidwell. 2009. Identification and genetic mapping of QTL for high-temperature adult-plant resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in the spring wheat (*Triticum aestivum* L.) cultivar 'Louise'. *Theor. Appl. Genet.* (in review).
- Cebert, E, H.W. Ohm, F.L. Paterson, R.H. Ratcliffe, and S. Cambron. 1996. Genetic analysis of Hessian fly resistance (*H28*) in Durum wheat. In: *Agronomy abstracts. 1996 Annual Meetings of ASA, CSSA, and SSSA. Indianapolis, IN, USA, Nov. 3-8, 1996, pp 88.*
- Devos K.M., G.J. Bryan, A.J. Collins, P. Stephenson, and M.D. Gale. 1995. Application of two microsatellite sequences in wheat storage proteins as molecular markers. *Theor. Appl. Genet.* 90:247-252.
- Dweikat, I., H. Ohm, F. Patterson, and S. Cambron. 1997. Identification of RAPD markers for 11 Hessian fly resistance genes in wheat. *Theor. Appl. Genet.* 94:419-423.
- Dweikat, I., H. Ohm, S. MacKenzie, F. Patterson, S. Cambron, and R. Ratcliffe. 1994. Association of a DNA marker with Hessian fly resistance gene *H9* in wheat. *Theor. Appl. Genet.* 89:964-968.
- Dweikat, I., W. Zhang, and H. Ohm. 2002. Development of STS markers linked to Hessian fly resistance gene *H6*. *Theor. Appl. Genet.* 105:766-770.
- Gallun, R.L., and F.L. Patterson. 1977. Monosomic analysis of wheat for resistance to Hessian fly. *J. Hered.* 68:223-226.

- Gould, F. 1986. Simulation models for predicting durability of insect-resistant germplasm: Hessian fly (Diptera: Cecidomyiidae) resistant winter wheat. *Environ. Entomol.* 15:11-23.
- Harris, M.O., M. Sandanayaka, and W. Griffin. 2001. Oviposition preferences of the Hessian fly and their consequences for the survival and reproductive potential of offspring. *Ecol. Entomol.* 26:473-486.
- Hatchett, J.H., and R.L. Gallun. 1970. genetics of the ability of the Hessian fly, *Mayetiola destructor*, to survive on wheats having different genes for resistance. *Ann. Entomol. Soc. Am.* 63:1400-1407.
- Kidwell, K.K., G.B. Shelton, V.L. DeMacon, C.F. Morris, D.A. Engle, J.W. Burns, R.F. Line, C.F. Konzak, and J.H. Hatchett. 2002. Registration of Zak wheat. *Crop Sci.* 42:661.
- Kidwell, K.K., G.B. Shelton, V.L. DeMacon, J.W. Burns, B.P. Carter, X.M. Chen, C.F. Morris, and N.A. Bosque-Pérez. 2006. Registration of 'Louise' wheat. *Crop Sci.* 46:1384-1386.
- Kidwell, K.K., V.L. DeMacon, G.B. Shelton, J.W. Burns, B.P. Carter, C.F. Morris, X. Chen and N.A. Bosque-Perez. 2003. Registration of 'Macon' Wheat. *Crop Sci.* 43:1561-1563.
- Kong, L., H.W. Ohm, S.E. Cambron, and C.E. Williams. 2005. Molecular mapping determines that Hessian fly resistance gene *H9* is located on chromosome 1A of wheat. *Plant Breed.* 124:525-531.
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Ann. Eugen.* 12:172-175.

- Lander, E.S., P. Green, J. Abrahamson, A. Barlow, J.M. Daly, S.E. Lincoln, and L. Newberg. 1987. Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174-181.
- Liu, B.H. 1998. *Statistical Genomics: Linkage, Mapping, and QTL Analysis*. Boca Raton, NY: CRC Press LLC.
- Liu, X.M., A.K. Fritz, J.C. Reese, G.E. Wilde, B.S. Gill, and M.-S. Chen. 2005a. *H9*, *H10*, and *H11* compose a cluster of Hessian fly-resistance genes in the distal gene-rich region of wheat chromosome 1AS. *Theor. Appl. Genet.* 110:1473-1480.
- Liu, X.M., B.S. Gill, and M.S. Chen. 2005b. Hessian fly resistance gene *H13* is mapped to a distal cluster of R genes in chromosome 6DS of wheat. *Theor. Appl. Genet.* 111:243-249.
- Liu, X.M., G.L. Brown-Guedira, J.H. Hatchett, J.O. Owuoché, and M.S. Chen. 2005c. Genetic characterization and molecular mapping of a Hessian fly-resistance gene transferred from *T. turgidum* ssp. *dicoccum* to common wheat. *Theor. Appl. Genet.* 111:1308-1315.
- Maas, III, F.B., F.L. Patterson, J.E. Foster, and J.H. Hatchett. 1987. Expression and inheritance of resistance of 'Marquillo' wheat to Hessian fly biotype D. *Crop Sci.* 27:49-52.
- McIntosh, R.A., K.M. Devos, J. Dubcovsky, W.J. Rogers, C.F. Morris, R. Appels, and O.A. Anderson. 2008. Catalogue of gene symbols for wheat [online]. Available from <http://wheat.pw.usda.gov/GG2/Triticum/wgc/2008/> [accessed 02 February 2009].

- Obanni, M., F.L. Patterson, J.E. Foster, and H.W. Ohm. 1988. Genetic analyses of resistance of durum wheat PI 428435 to the Hessian fly. *Crop Sci.* 28:223-226.
- Oetting, W.S., H.K. Lee, D.J. Flanders, G.L. Wiesner, T.A. Sellers, and R.A. King. 1995. Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. *Genomics* 30:450-458.
- Ohm, H.W., H.C. Sharma, F.L. Patterson, R.H. Ratcliffe, and M. Obanni. 1995. Linkage relationships among genes on wheat chromosome 5A that condition resistance to Hessian fly. *Crop Sci.* 35:1603-1607.
- Painter, R.H. 1930. The biological strains of Hessian fly. *J. Econ. Entomol.* 23:322-326.
- Patterson, F.L. and R.L. Gallun. 1977. Linkage in wheat of the *H3* and *H6* genetic factors for resistance to Hessian fly. *J. Hered.* 68:293-296.
- Pike, K.S. and A.L. Antonelli. 1981. The Hessian fly in Washington. Washington State University Agriculture Bulletin No. XB0909. pp. 12.
- Pike, K., R. Veseth, B. Miller, R. Schirman, L. Smith, and H. Homan. 1993. Hessian fly management in conservation tillage systems for the inland Pacific Northwest. PNW Conservation Tillage Handbook Series, Ch. 8, No. 15. Pullman, WA.
- Pitts E.G., J.A. Rafalski, and C. Hedgeoth. 1988. Nucleotide sequence and encoded amino-acid sequence of a genomic gene region for a low-molecular-weight glutenin. *Nucleic Acids Res.* 16:11376.
- Quarrie, S.A., J. Stojanović, and S. Pekić. 1999. Improving drought resistance in small-grained cereals: A case study, progress and prospects. *J. Plant Growth Regul.* 29:1-21.
- Ratcliffe, R.H., S.E. Cambron, K.L. Flanders, N.A. Bosque-Perez, S.L. Clement, and

- H.W. Ohm. 2000. Biotype composition of Hessian fly populations from the southeastern, midwestern, and northwestern United States and virulence to resistance genes in wheat. *J. Econ. Entomol.* 93:1319-1328.
- Roberts, J.J., and Gallun, R.L. 1984. Chromosome location of the *H5* gene for resistance to the Hessian fly in wheat. *J. Hered.* 75:147-148.
- Smiley, R.W., J.A. Gourlie, R.G. Whittaker, S.A. Easley, and K.K. Kidwell. 2004. Economic impact of Hessian fly (Diptera: Cecidomyiidae) on spring wheat in Oregon and additive yield losses with *Fusarium* crown rot and lesion nematode. *J. Econ. Entomol.* 97:397-408.
- Stebbins, N.B., F.L. Patterson, and R.L. Gallun. 1980. Interrelationships among wheat genes for resistance to Hessian fly. *Crop Sci.* 20:177-180.
- Stebbins, N.B., F.L. Patterson, and R.L. Gallun. 1982. Interrelationships among genes *H3*, *H6*, *H9* and *H10* for Hessian fly resistance. *Crop Sci.* 22:1029-1032.
- Stuart, J.J, M.-S. Chen, and M.O. Harris. 2008. Hessian Fly. p. 93-102. *In* W. Hunter and C. Kole (ed.) *Genome Mapping and Genomics in Animals, Volume 1: Genome Mapping and Genomics in Arthropods*. Springer-Verlag, Berlin.
- Sunderman, D.W., and B. O'Connell. 1988. Registration of Treasure wheat. *Crop Sci.* 28:576.
- 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.
- Veseth, R. 1988. Effective Hessian fly control: plant resistance. *Pacific Northwest Conservation Tillage Handbook, Chapter 8: Crops and Varieties, No. 9, Fall 1988*. Available from <http://pnwsteep.wsu.edu/tillagehandbook/index.htm> [Accessed 06

- March, 2009].
- Witcombe, J.R., and D.S. Virk. 2001. Number of crosses and population size for participatory and classical plant breeding. *Euphytica* 122:451-462.
- Williams, C.E., C.C. Collier, J.A. Nemacheck, C. Liang, and S.E. Cambron. 2002. A lectin-like wheat gene responds systemically to attempted feeding by avirulent first-instar Hessian fly larvae. *J. Chem. Ecol.* 28:1411-1428.
- Williams, C.E., C.C. Collier, N. Sardesai, H.W. Ohm, and S.E. Cambron. 2003. Phenotypic assessment and mapped markers for *H31*, a new wheat gene conferring resistance of Hessian fly (Diptera: Cecidomyiidae). *Theor. Appl. Genet.* 107:1516-1523.
- World Health Organization. 2009. World Health Organization: Data and statistics. <http://www.who.int/research/en/> Accessed January 19, 2009.
- Zeng, Z.B. 1993. Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proc. Natl. Acad. Sci. USA* 90:10972-10976.
- Zeng, Z.B. 1994. Precision mapping of quantitative trait loci. *Genetics* 136:1457-1468.

Table 1 Polymorphism among 26 wheat genotypes detected by *Xpsp2999* and *Xcfd15*, two molecular markers flanking the Hessian fly (HF) resistance gene *H3*.

Genotype	HF response	HF gene predicted to be present	Banding profile for <i>Xpsp2999</i>											Banding profile for <i>Xcfd15</i>	
			147	151	154	158	159	160	162	164	165	167	177	194	
Louise (PI 634865)	Resistant	H3			154					162	164			177	-
Penawawa (PI 495916)	Susceptible	None	147		154					162				177	194
Wakanz (a) ^a (PI 506352)	Resistant	H3			154					162	164			177	-
Wakanz (b) (PI 506352)	Susceptible	None	147		154					162				177	194
Wawawai (PI 574598)	Resistant	H3			154					162	164			177	194
Arthur ^b (CI 014425)	Resistant	H3			154					162	164			177	-
W38 (PI 410582)	Resistant	H3			154					162	164			177	194
WA8089 ^c	Resistant	H3			154					162	164			177	194
WA8090	Resistant	H3			154					162	164			177	-
Treasure (PI 468962)	Resistant	unknown			154					162	164			177	194
Zak (PI 607839)	Resistant	unknown			154					162	164			177	-
Whit (PI 653841)	Resistant	unknown					158						165	177	194
Macon (PI 617072)	Resistant	unknown					158						165	177	194
Otis (PI 634866)	Resistant	unknown					158						165	177	194
Kelse (PI 653842)	Resistant	unknown					158						165	177	194
Jefferson (PI 603040)	Resistant	unknown		151				159						177	-
Hollis (PI 632857)	Resistant	unknown		151				159						177	-
Tara 2002 (PI 617073)	Resistant	unknown		151				159						177	194

Table 1 cont.

Genotype	HF response	HF gene predicted to be present	Banding profile for <i>XpSP2999</i>										Banding profile for <i>Xcfd15</i>		
			147	151	154	158	159	160	162	164	165	167	177	194	
Westbred 906R (PI 483455)	Resistant	unknown		151				159						177	-
Westbred 926 (Westbred, LLC)	Resistant	unknown		151				159						177	-
Hank (PI 613581)	Resistant	unknown		151				159						177	194
Jerome (PI 632712)	Resistant	unknown	147		154						162			177	194
Challis (PI 630935)	Susceptible	None	147		154						162			177	-
Alpowa (PI 566596)	Susceptible	None	147		154						162			177	194
WA8039	Susceptible	None	147		154						162			177	194
IDO586 (PI 632713)	Resistant	H25	147		154						162			177	194
P985RE1-16 (Purdue University)	Resistant	H9							160				167	177	194

^a Wakanz is heterogeneous for Hessian fly resistance

^b Arthur and W38 are winter wheat cultivars, whereas remaining lines are spring type

^c WA8089, WA8090, and WA8039 are advanced experimental breeding lines from the Washington State University Spring Wheat Breeding Program

Figure 1 Distribution of the percentage of susceptible plants to Hessian fly infection in a recombinant inbred line mapping population derived from a Louise (resistant) by Penawawa (susceptible) cross, averaged over 15 plants per line.

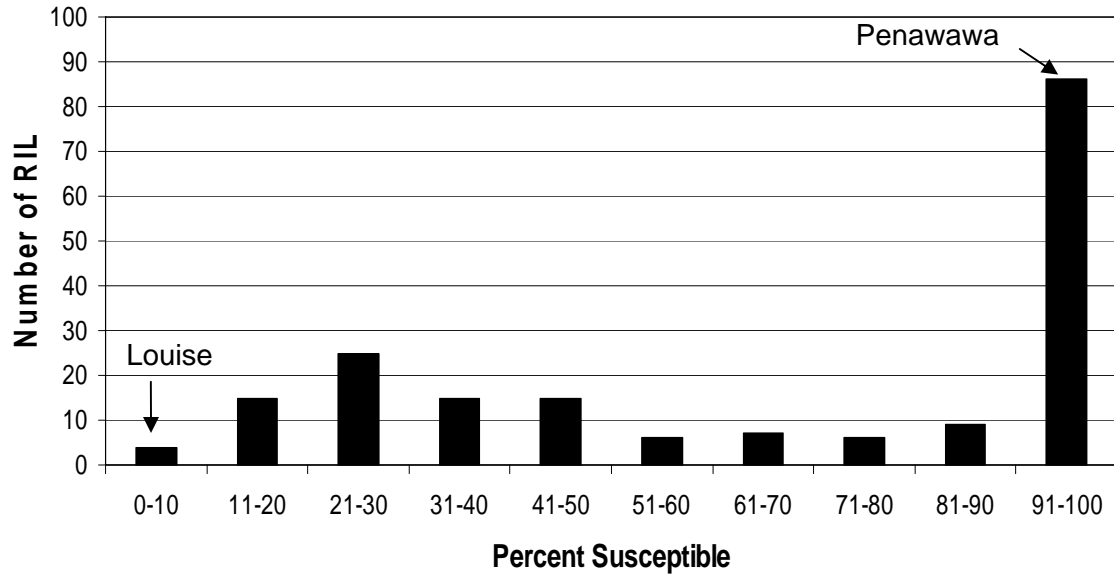
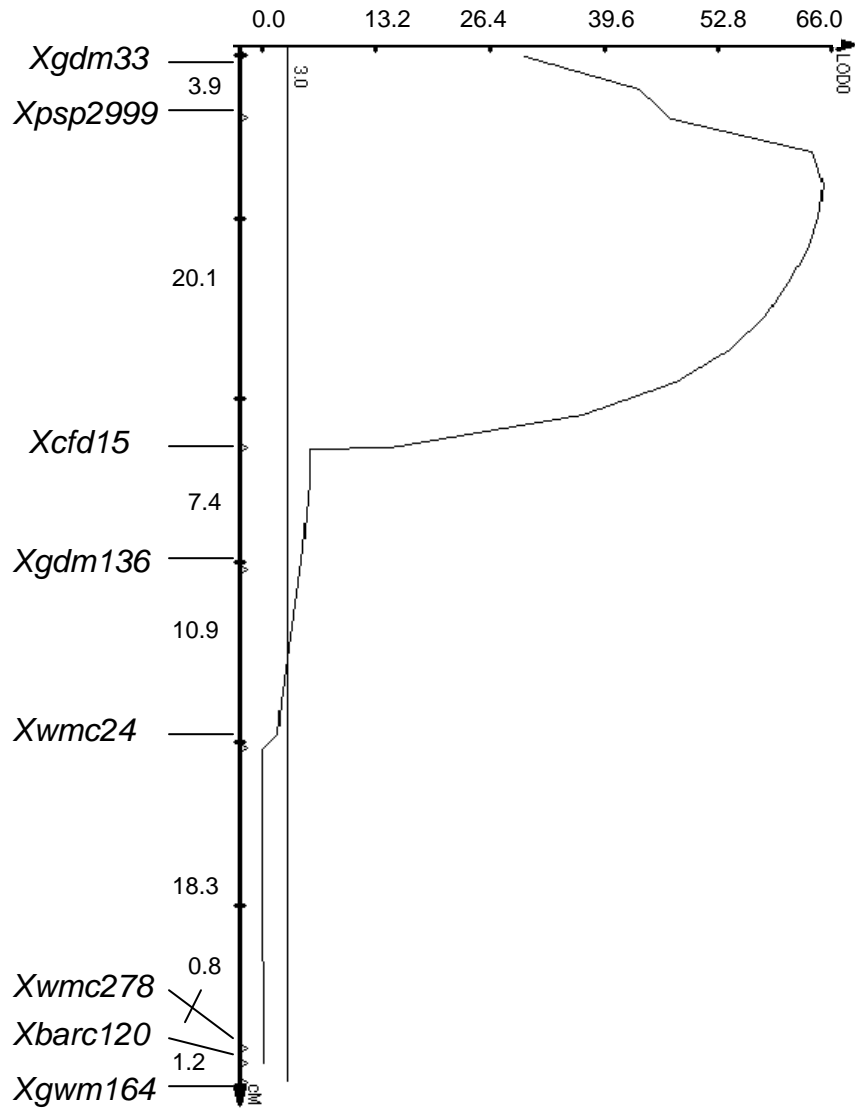


Figure 2 Mapping analysis of Hessian fly resistance gene *H3* identified on chromosome 1A by composite interval mapping. Positions (cM) of the molecular markers along the chromosome are on the vertical axis.



CHAPTER 4

**Genetic mapping of quantitative trait loci associated with important
agronomic traits in the spring wheat (*Triticum aestivum* L.)
cross ‘Louise’ by ‘Penawawa’**

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Abbreviations: cM, centiMorgans; CIM, composite interval mapping; ITMI, International Triticeae Mapping Initiative; MAS, marker-assisted selection; PNW, Pacific Northwest; QTL, quantitative trait loci; RIL, recombinant inbred line

(To be submitted as a research paper to Crop Science)

Abstract

Little is known about the genetic factors underlying many important agronomic traits in common wheat (*Triticum aestivum* L.). A set of 188 recombinant inbred lines from the ‘Louise’ by ‘Penawawa’ mapping population was grown in two crop years at two locations in the Pacific Northwest region of the United States to identify quantitative trait loci (QTL) associated with: seedling growth habit, leaf color, plant height, flowering date, maturity date, grain volume weight, grain protein content, and grain yield. Using an established genetic linkage map, composite interval mapping was used to identify QTL associated with agronomic traits. QTL for flowering date and maturity date were associated with the *Ppd-D1* gene for photoperiod insensitivity. Variation in the QTL for plant height was dependent on location and year, and localized to DNA regions on

chromosome 2D and 3B. A QTL for leaf color (ranging from yellow-green to blue) was identified on chromosome 2B. Seedling growth habit mapped to chromosome 2D, and a significant QTL for grain volume weight was detected on chromosome 1D. QTL were identified for grain yield, but were associated with other known QTL or photoperiod insensitivity. Yield increases were associated with the effects other QTL had on pest resistance, seedling growth habit and adaptability. The identification of QTL for agronomic traits is the first step to dissecting their complex genetic nature.

Introduction

Due to the importance of wheat globally (Briggle and Curtis, 1987), many efforts have been undertaken to enhance the efficiency of genetic improvement in wheat through breeding. Efforts have been undertaken to improve traits such as flowering rate, grain yield, grain volume weight, and grain protein concentration through field-based breeding methods; however, with the advent of molecular markers, plant breeders also have used quantitative trait loci (QTL) analysis and marker-assisted selection (MAS) to select for desired traits (Gupta et al., 1999; Bernardo, 2008). The identification of QTL defines the chromosomal location and number of loci underlying the genetic control of quantitatively inherited traits. This knowledge facilitates incorporating these traits into regionally adapted cultivars.

Many genetic mapping populations have been created to evaluate of one or two traits (Lin and Chen, 2008; Liang et al., 2006), such as specific resistance to a specific disease. In contrast, the analysis of mapping populations segregating for multiple important plant traits (Nelson et al., 1995) allows the discernment of both genetic

associations and genetic correlations with other traits. In wheat, the population used for the International Triticeae Mapping Initiative (ITMI) has been used for numerous QTL studies of this sort (Nelson et al., 1998; Singh et al., 2000; Somers et al., 2004). One disadvantage of the ITMI mapping population is its lack of relevance and adaptation to most commercial wheat production areas. Thus, it would be beneficial to have access to mapping populations that segregate for essential traits required for successful commercial wheat production in specific geographic regions.

QTL analysis has been undertaken for many traits in wheat including those associated with agronomic adaptation and grain production. Kato et al. (2000) identified multiple QTL on chromosome 5A of wheat associated with grain yield and yield components. Traits such as grain yield, tiller number, and spikelet number were associated with multiple chromosomal regions, but were largely explained by allelic variation at the *Vrn-A1* locus (Kato et al., 2000). In an analysis of chromosome 3A, Campbell et al. (2003) identified 14 QTL for plant height, kernel weight, grain yield, and yield components. All grain yield QTL were associated with QTL identified for 1000-kernel weight, kernels per spike, and spikes per square meter.

McCartney et al. (2005) identified 35 major QTL located on 16 of the 21 wheat chromosomes in a spring wheat doubled haploid mapping population. Six major agronomic traits (plant height, maturity, lodging, grain yield, grain volume weight, and 1000-kernel weight) were evaluated and at least three QTL were identified for each trait, many having pleiotropic effects with other QTL. Börner et al. (2002) used the ITMI mapping population to evaluate 20 morphological, agronomical, and disease resistance traits. Over 60 major QTL were discovered using this population, which covered 20 of

the 21 wheat chromosomes. Huang et al. (2004) created a backcross population from a cross between a German elite winter wheat and a synthetic wheat, which was evaluated for seven agronomic traits. A total of 57 QTL covering 18 of the 21 wheat chromosomes were identified, most of which were associated with plant height, 1000-kernel weight, and grain weight per ear. While some of these QTL are useful in marker-assisted breeding schemes, many of them were specific to the environments and populations in which they were identified, which limit their utility (Bernardo, 2008).

Two soft white spring wheat cultivars, ‘Louise’ (Kidwell et al. 2006; PI 634865) and ‘Penawawa’ (PI 495916), which are adapted and widely grown in the Pacific Northwest (PNW) region of the United States, differ for many important agronomic and disease resistance traits. The objectives of this study were to: a) collect phenotypic data on a Louise by Penawawa recombinant inbred line (RIL) population; and b) identify DNA markers associated with QTL for important agronomic traits in this population. By doing so, a better understanding of the genetic control of multiple agronomic traits will be available to wheat researchers and plant breeders. The mapping population and genetic linkage map also are available to other researchers interested in studying QTL and their effects in wheat.

Materials and Methods

Field experiment

A RIL population of 188 individuals from a cross between Louise and Penawawa was selected for phenotypic and genotypic analyses. Louise, a soft white spring wheat released in 2005, has moderate grain volume weight, low grain protein concentration, and

high yield potential under non-irrigated production. Penawawa, a soft white spring wheat released in 1985, has moderate grain volume weight, moderate grain protein concentration, and lower yield potential in non-irrigated production. Field trials were conducted in Genesee, ID, and Pullman, WA, in 2007, and in Moscow, ID, and Pullman, WA, in 2008. Plots were planted using an alpha lattice design with three replicates at each location (Mason et al., 2003). Each plot was 0.8 meters wide by 5 meters long. The plots were planted using an 8-row planter with double disc openers spaced 15 cm apart (Wintersteiger planter model TRM 2200, Wintersteiger Co., Salt Lake City, UT). In 2007, plots were planted on April 5th and April 6th in Pullman and Genesee, respectively. In 2008, plots were planted on April 21st and May 2nd in Pullman and Moscow, respectively. Before planting, the field was cultivated and fertilized with nitrogen (formulated as urea) at a rate of 101 kg ha⁻¹. Due to differences in resistance to foliar fungal diseases, primarily stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) to which Louise is resistant and Penawawa is susceptible, plots were sprayed with Tilt (propiconazole; Syngenta, Basel, Switzerland) at the rate of 4 oz per acre at stem extension (Feekes 10; Feekes, 1941) to prevent confounding results with disease susceptibility.

Agronomic trait data collection

Seedling growth habit at early tillering (Feekes 4) was identified as either erect (score of one, similar to Louise) or prostrate (score of two, similar to Penawawa). Flowering date was recorded as the day (Julian) when 50% of the heads had emerged from the flag leaf (Feekes 10.3). Leaf color was recorded as either being yellow-green (score of one), green (score of two, similar to Louise), green-blue (score of three, similar

to Penawawa), or blue (score of four) at late flowering (Feekes 10.54). Plant maturity was recorded as the day (Julian) when 50% of the peduncles had turned from green to yellow (Feekes 11.2). Plant height was recorded as the height (cm) from the ground to the middle of the head at full plant maturity (Feekes 11.4). A mechanical small plot combine (Nurserymaster Classic, Wintersteiger Co., Salt Lake City, UT) was used to harvest the trials. Grain yield was measured from seed collected from the combine as grams per plot and reported as kg ha^{-1} . Grain protein concentration was analyzed on an Infratec 1229 Whole Grain Analyzer (Foss, Eden Prairie, MN) and reported as g kg^{-1} . Grain volume weight was measured using a Seedburo filling hopper and stand (Seedburo Equipment Co., Chicago, IL). Grain volume weight was measured in lb bu^{-1} and reported as kg m^{-3} . This population also was screened for resistance to Hessian fly (*Mayetiola destructor* [Say]) and stripe rust following the methods reported in Carter et al. (2009a; 2009b).

Statistical and QTL analysis

Statistical analysis of the agronomic trait data was performed using the statistical package SAS V9.1 (SAS Institute, Raleigh, NC). Levene's test for heterogeneity was used to test for variation across environments. A log transformation was used on plant height data to eliminate variation between environments. ANOVA was performed using Proc GLM and means for each trait within each environment were determined using LSMEANS. The statistical model for the ANOVA was: $Y = \text{Environment} + \text{Replication}(\text{Environment}) + \text{Genotype} + \text{Genotype} * \text{Environment} + \text{Error}$. Genotype was considered random whereas environment was fixed. Phenotypic correlations were determined using the Pearson product-moment correlation (SAS Institute, Raleigh, NC).

Broad-sense heritability (h^2) was calculated across all locations using the formula: $h^2 = \text{Var}(G)/\text{Var}(P)$ (where $\text{Var}(G)$ is the variance of the genotypic effect and $\text{Var}(P)$ is the variance of the phenotypic effect) using SAS code provided by Holland et al. (2003).

The genetic linkage map consisting of one single nucleotide polymorphism (SNP) and 296 simple sequence repeat (SSR) markers developed by Carter et al. (2009b) was used for QTL analyses. The marker Ppd-D1 was used to detect the presence of the photoperiod insensitivity gene *Ppd-D1* (Hanocq et al., 2004; Beales et al., 2007). Segregation of marker loci was tested for goodness-of-fit to the expected 1:1 ratio using the χ^2 test. Linkage maps were constructed using Mapmaker V3.0 (Lander et al., 1987). Linkage groups were established using the “group” command with a recombination value of 0.5 and a constant LOD score of 3.0. Three-point linkage analyses were performed using the “compare” command to determine the most likely order of markers with the shortest genetic distance within each group. For large linkage groups, a framework order was established using the above procedure and additional markers were mapped using the “try” command and verified using the “ripple” command. The Kosambi map function was applied to calculate the genetic distances in centiMorgans (cM) between the ordered markers (Kosambi, 1944). Each linkage group was assigned to a wheat chromosome based on previously published wheat genome maps available at Graingenes (<http://wheat.pw.usda.gov/GG2/index.shtml>).

WinQTLCart V2.5 (Basten et al., 1997) was used for single marker and QTL analysis. Single marker analysis using one-way ANOVA with a probability level of $P < 0.01$ was used for preliminary identification of markers with significant effects on agronomic traits. The statistical model for this analysis was: $Y = B + M$, where Y equals

the trait, B equals the block, and M equals the marker. QTL detection was then performed within each environment using composite interval mapping (CIM) (Zeng, 1993; 1994) and targeted toward linkage groups identified in the single marker analysis. A critical LOD threshold value of 3.0 was used to detect significant QTL. Percentage of phenotypic variation (R^2) explained by a single QTL in each environment was determined using multiple interval mapping (MIM).

Results and Discussion

Phenotypic data

Heterogeneity of variance was significant ($P < 0.0001$) only for plant height. Plant height data were log transformed to correct for heterogeneity. Analysis of variance indicated that Louise and Penawawa were significantly different from each other ($P < 0.0001$) for all traits analyzed (Table 1). The RIL population also differed significantly for the environment and genotype effect for all traits analyzed ($P < 0.0001$; Table 1), although the environment effect was not significant for grain yield. Transgressive segregation was observed for all traits, except for seedling growth habit and leaf color (Figure 1). This population also segregated for resistances to the Hessian fly and to stripe rust (Carter et al., 2009a; 2009b). Flowering date spanned a 22 day period, whereas maturity date spanned a 24 day period. Although both parents carry *Rht-B1a* and *Rht-D1b*, identical alleles at the major dwarfing genes, a range in plant height of 43.5 cm was detected among the RIL indicating that other genetic or environmental factors may be contributing to plant height. The average grain volume weights among RIL ranged from 680 to 770 kg m⁻³. Grain yield average ranged from 4000 to 5700 kg

ha⁻¹ among RIL. As expected, means of the RIL population for all traits were nearly identical to the mean of trait values of the two parental lines (Table 2).

Flowering date, seedling growth habit, and leaf color had high heritability estimates, whereas those for maturity date, plant height, and grain volume weight were moderate (Table 2). The heritability estimates for grain protein concentration and grain yield were very low. Similar results on the heritability of agronomic traits have been identified by McCartney et al. (2005), Talbert et al. (2001), and Huang et al. (2006). The concept of heritability is defined by Hanson (1963) as the fraction of the selection differential expected to be gained when selection is practiced on a defined reference unit. By selecting the QTL for a trait such as flowering date with high heritability estimates, there is a high probability that selections carrying this QTL will have the resulting phenotype of early flowering (Holland et al., 2003). In contrast, if the QTL alleles associated with high grain yield were selected, there is a lower probability that selected lines will actually have higher grain yield due to the low heritability estimate.

Trait correlations

Phenotypic correlations were calculated among RIL for the agronomic traits measured in the population (Table 3), and significance was determined at the $P < 0.0001$ value for all traits. Although many of the correlations were significant, weak correlations of a magnitude $r \leq 0.3$ will not be discussed (Moore, 2007). Seedling growth habit was significantly correlated to flowering and maturity date (Table 3). RIL with the Penawawa allele for prostrate seedling growth exhibited delays in flowering and maturity date of four days. Flowering date and maturity date also were highly correlated (Table 3). RIL which flowered earlier also matured earlier. Leaf color and maturity date were

correlated; the blue leaf color resulting in an earlier maturity date (Table 3). It is not fully understood why the correlation between leaf color and maturity date exist.

Maturity date and grain protein concentration were significantly correlated, with earlier maturing lines having higher grain protein concentration (Table 3). During grain fill, proteins are accumulated before the full accumulation of starch (Altenbach et al., 2003; Dupont and Altenbach, 2003). Plants which mature earlier have higher protein to starch ratios, which translates to higher grain protein concentration. Grain volume weight and protein concentration were negatively correlated (Table 3), which agreed with previous reports (Bhatt and Derera, 1974; Ohm et al., 1998; Blanco et al., 2002). Plant height was correlated to grain yield, with the taller plants having a higher grain yield potential (Table 3). Allan (1989) compared the effects of *Rht-B1* and *Rht-D1*, and found genotypes with *Rht-B1*, which resulted in a taller phenotype, had higher grain yield potential. McCartney et al. (2005) associated the *Rht-D1b* allele to decreases in grain yield, grain volume weight, and 1000-kernel weight. Richards (1992) identified that shorter populations produced more grain than taller populations even though they all contained *Rht-B1b*. In contrast, Flintham et al. (1997) identified that within a population containing a given *Rht* gene, the taller plants had higher grain yield potential, supporting the results presented here.

Seedling growth habit QTL

One QTL for seedling growth habit, designated *QSgh.wak-2D*, was detected on chromosome 2D using composite interval mapping (Figure 2). This QTL was not detected in the Moscow, 2008 location. *QSgh.wak-2D* was localized on chromosome 2D in a 6.7 cM interval associated with five SSR markers. *Xgwm608* is 2.8 cM distal to the

peak, and markers *Xcfd62*, *Xbarc228*, *Xcfd168*, and *Xwmc41* are 0.1, 0.4, 0.7, and 3.9 cM proximal to the peak, respectively. *QSgh.wak-2D* had a LOD score ranging from 2.5 to 4.1, and explained only 5 to 8% of the phenotypic variation across environments. The prostrate seedling growth habit was associated with the allele from Penawawa.

Li et al. (2002) identified three QTL for seedling growth habit using the ITMI mapping population. The QTL were located on wheat chromosome 1DS, 2DS, and 6AS, although only the 2DS QTL was identified at both locations. Li et al. (2002) noted that seedling growth habit was affected by number of tillers per plant, as well as days to flowering (controlled by the presence of the *Ppd-D1* gene for photoperiod insensitivity). In contrast, Kulwal et al. (2003), also using the ITMI population, identified four QTL for seedling growth habit, one on chromosome 2DL, one on chromosome 4D, and two on chromosome 5DL. Differences in the method of data collection or environmental variation may account for the discrepancy in results across experiments. No associations between days to flowering and seedling growth habit were detected by Kulwal et al. (2003). Based on comparison of molecular maps and interactions between seedling growth habit and days to flowering, the 2DL QTL identified by Kulwal et al. (2003) appears to be in the same location as *QSgh.wak-2D*.

Time to flowering and maturity QTL

A QTL for flowering date and a QTL for maturity date both mapped to a 42.1 cM region on chromosome 2D. The peak of both QTL coincided with the dominant *Ppd-D1a* allele for photoperiod insensitivity, which is associated with Penawawa. The QTL, designated *QFlt.wak-2D* and *QMat.wak-2D*, had LOD scores ranging from 8.8 to 19.7 and 8.7 to 17.6, respectively. Both *QFlt.wak-2D* and *QMat.wak-2D* were flanked by

Xbarc168 and *Xcfd36* at distances of 12.1 and 30.0 cM, respectively (Figure 2). These QTL explained 24 to 41% and 27 to 40% of the phenotypic variation for flowering date and maturity date, respectively. RIL with the dominant allele flowered four days earlier and matured three days earlier than lines with the recessive allele.

Ppd-D1 has been characterized in many genetic studies, although its effect in different environments varies. Foulkes et al. (2004) noted that *Ppd-D1a* advances flowering in the winter cultivars ‘Mercia’ and ‘Cappelle-Desprez’ by 12 and 9 days, respectively. Dyck et al. (2004) stated that hard red spring wheat lines which are photoperiod insensitive flower three days earlier than those which are photoperiod sensitive. Maturity date also was accelerated by two days. Snape et al. (2001) reported that the *Ppd-D1a* allele can accelerate flowering date anywhere from 6 to 14 days. Using the ITMI mapping population, Li et al. (2002) stated that *Ppd-D1a* reduces days to flowering by five days. Thus, the results from our study align with previous reports of acceleration in flowering date resulting from the *Ppd-D1a* allele, although it is evident that genetic background and environment impact response.

Leaf color QTL

A single QTL was detected for leaf color and mapped to a 17.9 cM region on chromosome 2BL. *QCol.wak-2B* had LOD scores ranging from 13.0 to 25.6 across locations and explained 33 to 50% of the phenotypic variation. *QCol.wak-2B* was flanked by *Xcfd238* and *Xgwm614* at distances of 14.1 and 3.8 cM, respectively (Figure 2). The Louise allele contributed to a lighter leaf color ranging from yellow-green to green, whereas the Penawawa allele contributed to a darker leaf color ranging from blue-

green to blue. Leaf color varied significantly across years and locations, indicating that environmental influences affect the expression of this trait.

Leaf color is mainly attributed to the accumulation of wax on the epidermis, also known as glaucousness, which imparts a bluish-green cast commonly referred to as bloom (Johnson et al., 1983). Although this trait is thought to be associated with photosynthetic efficiency and drought tolerance, few examples of QTL identification are available (Kulwal et al., 2003). Kulwal et al. (2003) identified 14 molecular markers with significant association with leaf color using tests of independence. These markers were present on wheat chromosomes 1A, 1D, 2B, 2D, 6A, 7A, and 7D. Markers of interest are those on chromosome 2B, as this corresponds to *QCol.wak-2B*. One marker was associated with the short arm, and one with the long arm, although based on map comparisons it was difficult to determine if these locations were similar to *QCol.wak-2B*.

Plant height QTL

Seven QTL for plant height were detected on chromosome 3B, and all mapped within a 55.2 cM region (Figure 2). Three QTL were detected at Pullman in 2008, two at Pullman in 2007, and one at Genesee in 2007 and Moscow in 2008. These seven QTL correspond to three distinct adjoining regions on chromosome 3B, none of which have been identified to carry genes controlling height in previous reports (McIntosh et al., 2008). *QHt.wak-3B.1* had a LOD score of 5.1 and was only identified at Pullman in 2008. This QTL spanned a 21.5 cM region flanked by *Xbarc101* and *Xwmc777*. *QHt.wak-3B.2* had LOD scores of 5.2, 7.6, and 10.5 at Pullman, 2008; Pullman, 2007; and Genesee, 2007, respectively. This QTL spanned an 18.1 cM region flanked by *Xwmc777* and *Xbarc164*. *QHt.wak-3B.3* had LOD scores of 3.1, 8.1, and 9.6 at Pullman,

2008; Pullman, 2007; and Moscow, 2008, respectively. This QTL spanned a 15.6 cM region flanked by *Xbarc164* and *Xbarc344*. Penawawa was, on average, 13 cm shorter than Louise, even though both cultivars carry the *Rht-D1b* allele. RIL with the Penawawa allele at any of the three QTL were 4 cm shorter than RIL with the Louise allele.

Variation in height within the *Rht* genes appears to be dependent on environmental conditions and genetic backgrounds. Richards (1992) identified plant height variations among isogenic line sets containing either the *Rht-B1* or the *Rht-D1* gene. Depending on the environment, lines within each set varied in height by 30 cm. Rebetzke et al. (1999) also identified significant differences between lines containing the same *Rht* alleles, and attributed differences to time of sowing as well as environmental factors. Trethowan et al. (2001) identified a significant interaction between *Rht-B1* and genotype. Depending on the genetic background, removal of the gene increased plant height by 22 cm to 45 cm. Thus, incorporation of the *Rht* gene is not enough to determine short stature; it also requires consideration of the genetic background and environmental conditions.

One additional QTL, designated *QHt.wak-2D*, was detected on chromosome 2D and coincided with the QTL for flower and maturity date (Figure 2). *QHt.wak-2D* was not detected in Genesee, 2007, and had LOD scores ranging from 4.4 to 6.3 at the other locations. Presence of *QHt.wak-2D* is associated with a height reduction of 3 cm. In a study of the role of *Ppd-D1* on flowering time, Snape et al. (2001) stated that pleiotropic effects were consistent with producing shorter plants; therefore, the gene also behaves as a height reducing factor. Dyck et al. (2004) demonstrated that photoperiod insensitive

lines with the *Ppd-D1* gene were 5.5 cm shorter than lines without the gene, and similar reports were published by Marshall et al. (1989) and Worland (1996). Albeit small, the consistent reduction in plant height associated with the *Ppd-D1a* allele will be useful for most cultivars grown in high rainfall or irrigated production conditions where earliness and short plant stature are desirable.

Grain volume weight QTL

One QTL was detected for grain volume weight in three of the four locations tested. It was not present in Pullman, 2007, and had a LOD score ranging from 3.4 to 4.8 at the other locations. *QGvwt.wak-1B* spanned a 17.7 cM region of chromosome 1B and was flanked by markers *Xgwm247* and *Xcfd48* (Figure 2). The presence of the Penawawa allele at this locus resulted in an increase in grain volume weight of 2.6 kg m⁻³. Other studies have attributed increases in grain volume weight to earliness traits (Busch et al., 1984; Marshall et al., 1989), whereas others have not (Knott, 1986; Dyck et al., 2004), and earliness may be in part responsible for the increases detected in this study. This QTL, although significant, would have little value in a breeding program as it minimally increases grain volume weight.

Grain yield QTL

Two significant QTL for grain yield (*QYld.wak-2D.1* and *QYld.wak-2D.2*) were identified at the Genesee, 2007 location and one QTL (*QYld.wak-1A*) at the Moscow, 2008 location. All three of the QTL identified mapped to regions where other QTL had been identified or known genes reside (Figure 2). The QTL identified at Genesee, 2007 coincides with the QTL identified for seedling growth habit and the *Ppd-D1* gene. The presence of the Louise allele for erect seedling growth habit resulted in a yield advantage

of 144 kg ha⁻¹. Presence of the *Ppd-D1a* allele resulted in a grain yield advantage of 81 kg ha⁻¹. *QYld.wak-1A* identified at the Moscow, 2008 location mapped to chromosome 1A, and coincided with the location of the Hessian fly resistance gene *H3* (Carter et al., 2009a). Although Hessian fly infection was not recorded in this experiment, a cool, wet spring resulted in an 11 day delay in spring planting, which created an ideal environment for Hessian fly infection (Smiley et al., 2004). RIL containing the *H3* gene had an average grain yield advantage of 336 kg ha⁻¹ in this location.

Yield QTL are typically associated with yield components or environmental adaptation traits. Li et al. (2002) found genetic evidence that *Ppd-D1* reduces both tiller number per plant as well as spikelet number per spike, indicating a possible reduction in grain yield. Worland et al. (1998) confirmed reductions in tiller and spikelet number, but detected an increase in spikelet fertility, compensating for these reductions. In Southern and Central Europe, winter wheat cultivars carrying the *Ppd-D1a* allele have a 30% and 15% yield advantage over photoperiod sensitive cultivars, respectively (Worland et al., 1994; Worland, 1996). Environmental conditions in Southern Europe favor cultivars with early heading dates, since they set and fill their grain before the hot, desiccating summer conditions arrive. Studies by Dyck et al. (2004) suggest that photoperiod sensitive lines are generally higher yielding than insensitive lines when grown in Canada. In contrast, Busch et al. (1984) and Marshall et al. (1989) reported that photoperiod insensitivity in the spring wheat growing regions of North Dakota and Minnesota was not associated with grain yield penalties. Thus, grain yield potential due to photoperiod insensitivity is highly dependent on the environment and should be examined closely in the target production region before being used as a selection criterion.

Increases in yield are associated with other important genes such as the *Rht* and *Vrn* genes (Quarrie et al., 2005, Rebetzke et al., 1999). Groos et al. (2003) identified a major QTL for grain yield on chromosome 7D near the *Pch1* locus, which confers resistance to *Pseudocercospora* foot rot. It was postulated that the yield increase did not come as a direct result of *Pch1*, since no disease symptoms were present and the trials were treated with fungicide. Groos et al. (2003) suggest that other important genes influencing grain yield may have been introgressed with the *Pch1* locus. Many QTL identified for grain yield result from pleiotropic effects of other genes (pest resistance and photoperiod sensitivity/insensitivity) that cause plants to be better adapted to their growing environment. Hence, it is important to understand the relationship these yield QTL have with other known traits, otherwise incorporation of these QTL into new cultivars will not result in yield increases.

Conclusion

QTL analysis on quantitatively inherited agronomic traits unlocks their genetic complexity. It also helps determine the interaction among traits and environmental conditions. Photoperiod insensitivity has a pleiotropic effect on many agronomic traits such as plant height, flowering date, maturity date, and grain yield; however, the value of the *Ppd-D1a* allele is environmentally dependent. Variations in plant height are readily apparent aside from those conferred by major known genes for reduced height and also are environmentally dependent. Leaf color and seedling growth habit can be effective selection criteria when breeding cultivars for specific environments. Identified QTL for higher grain yield potential were pleiotropic to *Ppd-D1* and QTL identified for seedling

growth habit and insect resistance. Understanding these agronomic QTL and their relationships will help facilitate the breeding of complex traits into regionally adapted wheat cultivars.

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References

- Allan, R.E. 1989. Agronomic comparisons between *Rht1* and *Rht2* semidwarf genes in winter wheat. *Crop. Sci.* 29:1103-1108.
- Altenbach, S.B., F.M. DuPont, K.M. Kothari, R. Chan, E.L. Johnson, and D. Lieu. 2003. Temperature, water and fertilizer influence the timing of key events during grain development in a US spring wheat. *J. Cereal Sci.* 37:9-20.
- Basten, J.C., B.S. Weir, and Z.B. Zeng. 1997. QTL CARTOGRAPHER. A reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh, N.C.
- Beales, J., A. Turner, S. Griffiths, J.W. Snape, D.A. Laurie 2007. A pseudo-response regulator is misexpressed in the photoperiod insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 115:721-733.

- Bernardo, R. 2008. Molecular markers and selection for complex traits in plants: learning from the last 20 years. *Crop Sci.* 48:1649-1664.
- Bhatt, G.M., and Derera, N.F. 1974. Genotype x environment interactions for, heritabilities of, and correlations among quality traits in wheat. *Euphytica* 24:597-604.
- Blanco, A., A. Pasqualone, A. Troccoli, N. Di Ronzo, and R. Simeone. 2002. Detection of grain protein content QTLs across environments in tetraploid wheats. *Plant Mol. Biol.* 48:615-623.
- Börner, A., E. Schumann, A. Fürste, H. Cöster, B. Leithold, M.S. Röder, and W.E. Weber. 2002. Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 105:921-936.
- Briggle, L.W., and B.C. Curtis. 1987. Worldwide wheat. pp. 1-31. *In* Heyne, E.G. (ed), *Wheat and Wheat Improvement*. Agronomy Monograph 13. ASA, CSSA, SSSA, Madison, WI.
- Busch, R.H., F.A. Elsayed, and R.E. Heiner. 1984. Effect of day length insensitivity on agronomic traits and grain protein in hard red spring wheat. *Crop Sci.* 24:1106-1109.
- Carter, A.H., S. Cambron, H.W. Ohm, and K.K. Kidwell. 2009a. Molecular mapping of Hessian fly gene *H3* indicates its location is on chromosome 1AS. *Crop Sci.* (in review).
- Carter, A.H., X.M. Chen, K.G. Campbell, and K.K. Kidwell. 2009b. Identification and genetic mapping of QTL for high-temperature adult-plant resistance to stripe rust

- (*Puccinia striiformis* f. sp. *tritici*) in the spring wheat (*Triticum aestivum* L.) cultivar 'Louise'. Theor. Appl. Genet. (in review).
- Campbell, B.T., P.S. Baenziger, K.S. Gill, K.M. Eskridge, H. Budak, M. Erayman, I. Dweikat, and Y. Yen. 2003. Identification of QTLs and environmental interactions associated with agronomic traits on chromosome 3A of wheat. *Crop Sci.* 43:1493-1505.
- DuPont, F.M., and Altenbach, S.B. 2003. Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. *J. Cereal Sci.* 38:133-146.
- Dyck, J.A., M.A. Matus-Cádiz, P. Hucl, L. Talbert, T. Hunt, J.P. Dubuc, H. Nass, G. Clayton, J. Dobb, and J. Quick. 2004. Agronomic performance of hard red spring wheat isolines sensitive and insensitive to photoperiod. *Crop Sci.* 44:1967-1981.
- Feekes, W. 1941. De tarwe en haar milieu. p. 560–561. *In* Vers. XVII Tech. Tarwe Comm, Groningen.
- Flintham, J.E., A. Börner, A.J. Worland, and M.D. Gale. 1997. Optimizing wheat grain yield: effects of *Rht* (gibberellin-insensitive) dwarfing genes. *J. Agricul. Sci.* 128:11-25.
- Foulkes, M.J., R. Sylvester-Bradley, A.J. Worland, and J.W. Snape. 2004. Effects of a photoperiod-response gene *Ppd-D1* on yield potential and drought resistance in UK winter wheat. *Euphytica* 135:63–73.
- Groos, C., N. Robert, E. Bervas, and G. Charmet. 2003. Genetic analysis of grain protein-content, grain yield, and thousand-kernel weight in bread wheat. *Theor. Appl. Genet.* 106:1032-1040.

- Gupta, P.K., R.K. Varshney, P.C. Sharma, and B. Ramesh. 1999. Molecular markers and their applications in wheat breeding. *Plant Breed.* 118:369-390.
- Hanocq, E., M. Niarquin, E. Heumez, M. Rousset, J. Le Gouis 2004. Detection and mapping of QTL for earliness components in a bread wheat recombinant inbred lines population. *Theor. Appl. Genet.* 110:106-115.
- Hanson, W.D. 1963. Statistical genetics and plant breeding. In: W.D. Hanson and H.F. Robinson (eds.) p. 125-139. Publ. 982. Natl. Acad. Sci.-Natl. Res. Counc., Washington DC.
- Holland, J.B., W.E. Nyquist, and C.T. Cervantes-Martinez. 2003. Estimating and interpreting heritability for plant breeding: an update. *Plant Breeding Reviews*, Vol. 22, pp. 9-112. Edited by Jules Janick. John Wiley & Sons, Inc.
- Huang, X.Q., H. Kempf, M.W. Ganal, and M.S. Röder. 2004. Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and a synthetic wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 109:933-943.
- Huang, X.Q., S. Cloutier, L. Lycar, N. Radovanovic, D.G. Humphreys, J.S. Noll, D.J. Somers, and P.D. Brown. 2006. Molecular detection of QTLs for agronomic and quality traits in a doubled haploid population derived from two Canadian wheats (*Triticum aestivum* L.). *Theor. Appl. Genet.* 113:753-766.
- Johnson, D.A., R.A. Richards, and N.C. Turner. 1983. Yield, water relations, gas exchange, and surface reflectances of near-isogenic wheat lines differing in glaucousness. *Crop Sci.* 23:318-325.
- Kato, K., H. Miura, and S. Sawada. 2000. Mapping QTLs controlling grain yield and its

- components on chromosome 5A of wheat. *Theor. Appl. Genet.* 101:1114-1121.
- Kidwell, K.K., G.B. Shelton, V.L. DeMacon, J.W. Burns, B.P. Carter, X.M. Chen, C.F. Morris, N.A. Bosque-Pérez. 2006. Registration of ‘Louise’ wheat. *Crop Sci.* 46:1384-1386.
- Knott, D.R. 1986. Effects of genes for photoperiodism, semi-dwarfism, and awns on agronomic characters in a wheat cross. *Crop Sci.* 26:1158–1162.
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Ann Eugen.* 12:172-175.
- Kulwal, P.L., J.K. Roy, H.S. Balyan, and P.K. Gupta. 2003. QTL mapping for growth and leaf characters in bread wheat. *Plant Sci.* 164:267-277.
- Lander, E.S., P. Green, J. Abrahamson, A. Barlow, J.M. Daly, S.E. Lincoln, and L. Newberg. 1987. Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174-181.
- Li, W.L., J.C. Nelson, C.Y. Chu, L.H. Shi, S.H. Suang, and D.J. Liu. 2002. Chromosomal locations and genetic relationships of tiller and spike characters in wheat. *Euphytica* 125:357-366.
- Liang, S.S., K. Suenga, Z.H. He, Z.L. Wang, H.Y. Liu, D.S. Wang, R.P. Singh, P. Sourdille, and X.C. Xia. 2006. Quantitative trait loci mapping for adult-plant resistance to powdery mildew in bread wheat. *Phytopathol.* 96:784-789.
- Lin, F., and X.M. Chen. 2008. Quantitative trait loci for non-race-specific, high-temperature adult-plant resistance to stripe rust in wheat cultivar Express. *Theor. Appl. Genet.* Online First.

- Marshall, L., R. Busch, F. Cholick, I. Edwards, and R. Froberg. 1989. Agronomic performance of spring wheat isolines differing for day length response. *Crop Sci.* 29:752-757.
- Mason, R.L., R.F. Gunst, and J.L. Hess. 2003. *Statistical Design and Analysis of Experiments (Second Edition)*. John Wiley & Sons, Inc. Hoboken, New Jersey.
- McCartney, C.A., D.J. Somers, D.G. Humphreys, O. Lukow, H. Ames, J. Noll, S. Cloutier, and B.D. McCallum. 2005. Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross RL4452 x 'AC Domain'. *Genome* 48:870-883.
- McIntosh, R.A., K.M. Devos, J. Dubcovsky, W.J. Rogers, C.F. Morris, R. Appels, and O.A. Anderson. 2008. Catalogue of gene symbols for wheat [online]. Available from <http://wheat.pw.usda.gov/GG2/Triticum/wgc/2008/> [accessed 02 February 2009].
- Moore, D.S. 2007. *The Basic Practice of Statistics (Fourth Edition)*. W.H. Freeman and Company, New York, New York.
- Nelson, J.C., J.E. Autrique, G. Fuentes-Dávila, and M.E. Sorrells. 1998. Chromosomal location of genes for resistance to karnal bunt in wheat. *Crop Sci.* 38:231-236.
- Nelson, J.C., M.E. Sorrells, A.E. Van Deynze, Y.-H. Lu, M. Atkinson, M. Bernard, P. Leroy, J.D. Faris, and J.A. Anderson. 1995. Molecular mapping of wheat: major genes and rearrangements in homoeologous groups 4, 5, and 7. *Genetics* 141:721-731.
- Ohm, J.B., O.K. Chung, and C.W. Deyoe. 1998. Single-kernel characteristics of hard winter wheats in relation to milling and baking quality. *Cereal Chem.* 75:156-161.

- Quarrie, S.A., A. Steed, C. Calestani, A. Semikhodskii, C. Lebreton, C. Chinoy, N. Steele, D. Pljevljakusić, E. Waterman, J. Weyen, J. Schondelmaier, D.Z. Habash, P. Farmer, L. Saker, D.T. Clarkson, A. Abugalieva, M. Yessimbekova, Y. Turupekov, S. Abugalieva, R. Tuberosa, M.-C. Sanguineti, P.A. Hollington, R. Aragués, A. Royo, and D. Dodig. 2005. A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring X SQ1 and its use to compare QTLs for grain yield across a range of environments. *Theor. Appl. Genet.* 110:865-880.
- Rebetzke, G.J., R.A. Richards, V.M. Fisher, and B.J. Mickelson. 1999. Breeding long coleoptile, reduced height wheat. *Euphytica* 106:159-168.
- Richards, R.A. 1992. The effect of dwarfing genes in spring wheat in dry environments. I. Agronomic characteristics. *Aust. J. Agric. Res.* 43:517-527.
- 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.
- Singh, R.P., J.C. Nelson, and M.E. Sorrells. 2000. Mapping *Yr28* and other genes for resistance to stripe rust in wheat. *Crop Sci.* 40:1148-1155.
- Smiley, R.W., J.A. Gourlie, R.G. Whittaker, S.A. Easley, and K.K. Kidwell. 2004. Economic impact of Hessian fly (Diptera: Cecidomyiidae) on spring wheat in Oregon and additive yield losses with *Fusarium* crown rot and lesion nematode. *J. Econ. Entomol.* 97:397-408.
- Snape, J.W., K. Butterworth, E. Whitechurch, and A.J. Worland. 2001. Waiting for fine times: genetics of flowering times in wheat. *Euphytica* 119:185-190.
- Somers, D.J., P. Isaac, and K. Edwards. 2004. A high-density microsatellite consensus

- map for bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 109:1105-1114.
- Talbert, L.E., S.P. Lanning, R.L. Murphy, and J.M. Martin. 2001. Grain fill duration in twelve hard red spring wheat crosses: genetic variation and association with other agronomic traits. *Crop Sci.* 41:1390-1395.
- Trethowan, R.M., R.P. Singh, J. Huerta-Espino, J. Crossa, and M. van Ginkel. 2001. Coleoptile length variation of near-isogenic *Rht* lines of modern CIMMYT bread and durum wheat. *Field Crop Res.* 70:167-176.
- Worland, A.J. 1996. The influence of flowering time genes on environmental adaptability in European wheats. *Euphytica* 89:49-57.
- Worland, A.J., A. Börner, V. Korzun, W.M. Li, S. Petrovic, and E.J. Sayers. 1998. The influence of photoperiod genes on the adaptability of European winter wheats. *Euphytica* 100:385-394.
- Worland, A.J., M.L. Appendino, and E.J. Sayers. 1994. The distribution, in European winter wheats, of genes that influence ecoclimatic adaptability whilst determining photoperiodic insensitivity and plant height. *Euphytica* 80:219-228.
- Zeng, Z.B. 1993. Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proc. Natl. Acad. Sci. USA* 90:10972-10976.
- Zeng, Z.B. 1994. Precision mapping of quantitative trait loci. *Genetics* 136:1457-1468.

Table 1 Mean square values for agronomic traits in the Louise by Penawawa mapping population for both the parental lines as well as the recombinant inbred line (RIL) population.

Trait Variable	Growth Habit		Leaf Color	
	Parents [†]	RIL	Parents	RIL
Environment	0.033	0.409*	7.622***	166.526***
Genotype	28.033***	2.092***	90.133***	4.596***
Genotype*Environment	0.033	0.143***	0.378*	0.215***
<i>R</i> ² value	0.94	0.89	0.92	0.87
	Flowering Date		Maturity Date	
	Parents	RIL	Parents	RIL
Environment	236.364***	277.376***	636.811***	11295.855***
Genotype	14.008***	34.743***	108.900***	70.753***
Genotype*Environment	9.763***	3.744***	15.700**	7.927***
<i>R</i> ² value	0.90	0.97	0.86	0.90
	Plant Height		Grain Protein Content	
	Parents	RIL	Parents	RIL
Environment	72.633***	1336.055***	15.359***	328.762***
Genotype	672.133***	33.014***	6.394***	1.483***
Genotype*Environment	2.333	2.584***	1.345*	0.586***
<i>R</i> ² value	0.84	0.83	0.58	0.72
	Grain Volume Weight		Grain Yield	
	Parents	RIL	Parents	RIL
Environment	20.211***	2300.010***	309.983**	1511.087
Genotype	90.480***	23.931***	1239.399***	201.722***
Genotype*Environment	3.966***	2.084***	109.211	3.372***
<i>R</i> ² value	0.87	0.92	0.41	0.55

[†] Parents and RIL were estimated using different error terms

*, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001

Table 2 Means, ranges, and heritabilities of eight agronomic traits in the Louise by Penawawa recombinant inbred line (RIL) population.

Traits	Parental Means			RIL Population			Heritability (h^2)	Confidence interval (h^2)
	Louise	Penawawa	Mean	Minimum	Maximum	Mean		
Seedling Growth Habit [†]	1.0	2.0	1.5	1.0	2.0	1.4	0.70	0.67-0.73
Flowering (Julian)	182.4	183.1	182.8	172	194	182.8	0.85	0.84-0.86
Maturity (Julian)	217.6	215.4	216.5	207.1	231.0	216.6	0.58	0.55-0.61
Leaf Color [‡]	1.5	3.3	2.4	1.0	4.0	2.3	0.69	0.67-0.71
Height (cm)	81.4	69.4	75.4	51.2	94.7	76.3	0.57	0.54-0.60
Grain Volume Weight (kg m ⁻³)	765.8	743.9	754.9	622	819	749	0.61	0.58-0.64
Grain Protein Content (g kg ⁻¹)	10.7	11.2	11.0	7.2	15.1	11.1	0.13	0.11-0.15
Grain Yield (kg ha ⁻¹)	5127	4697	4912	2838	7149	4923	0.14	0.12-0.16

[†] 1=erect; 2=prostrate

[‡] 1=yellow-green; 2=green; 3=blue-green; 4=blue

Table 3 Phenotypic correlations among eight agronomic traits from data collected from the Louise by Penawawa recombinant inbred line (RIL) population.

Traits	Seedling Growth Habit	Flowering	Maturity	Leaf Color	Plant Height	Grain Volume Weight	Grain Protein Content	Grain Yield
Flowering	0.496***	1						
Maturity	0.341***	0.850***	1					
Leaf Color	-0.010	-0.189***	-0.309***	1				
Plant Height	0.028	0.096***	0.001	0.249***	1			
Grain Volume Weight	-0.022	-0.057***	0.165***	-0.118***	-0.048*	1		
Grain Protein Content	0.025	-0.084***	-0.379***	0.224***	0.131***	-0.455***	1	
Grain Yield	0.026	-0.059**	0.144***	0.045*	0.376***	0.139***	-0.131***	1

*, p < 0.05; **, p < 0.01; ***, p < 0.001

Figure 1 Frequency distributions of eight agronomic traits using data collected from the Louise by Penawawa recombinant inbred line (RIL) population. The L and P represent the means of Louise and Penawawa, respectively.

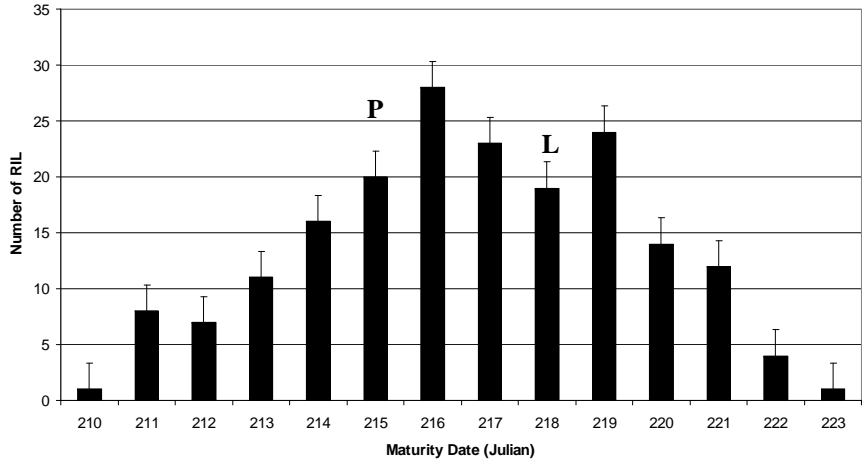
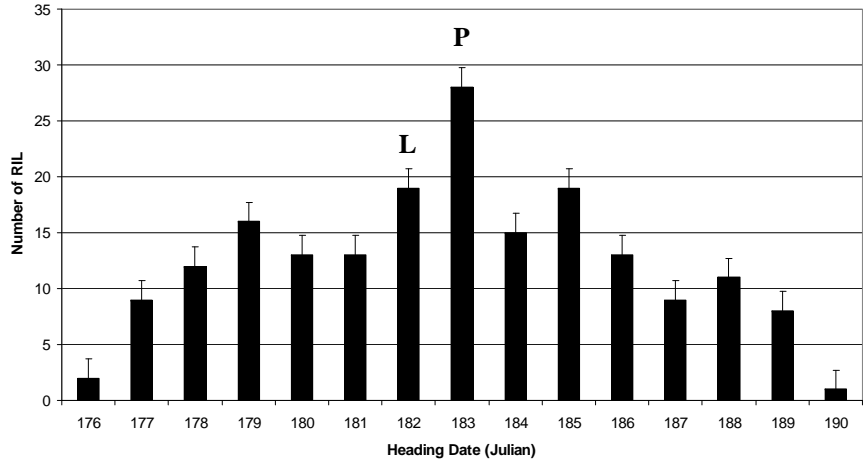
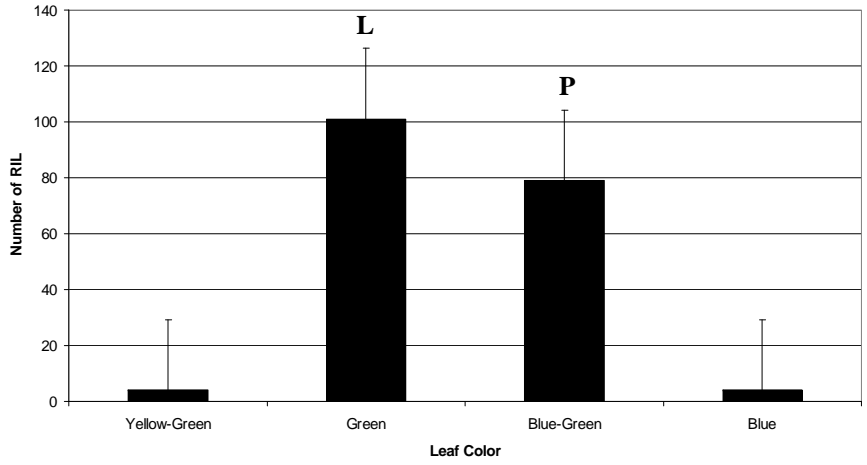
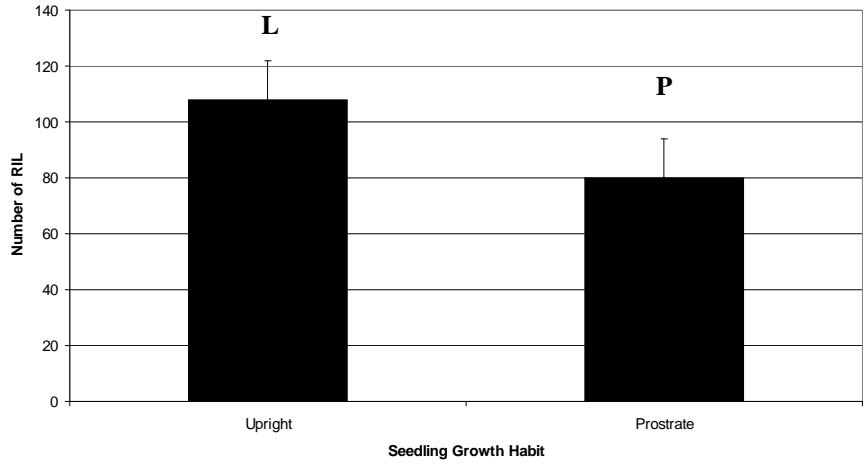


Figure 1 cont.

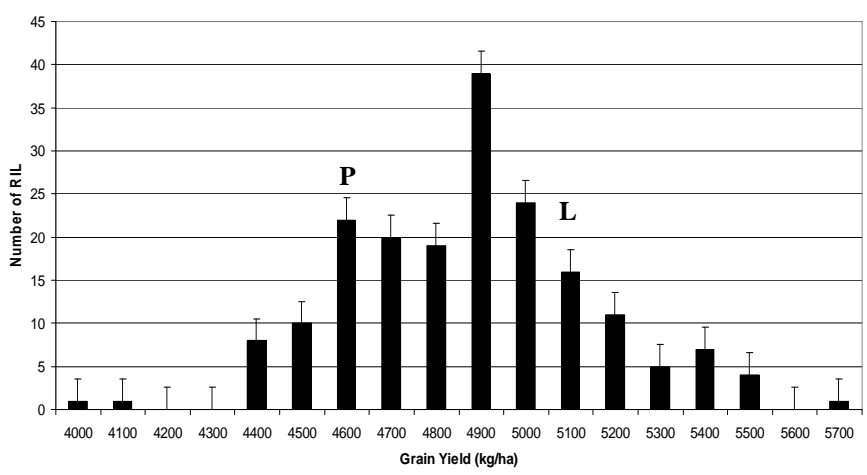
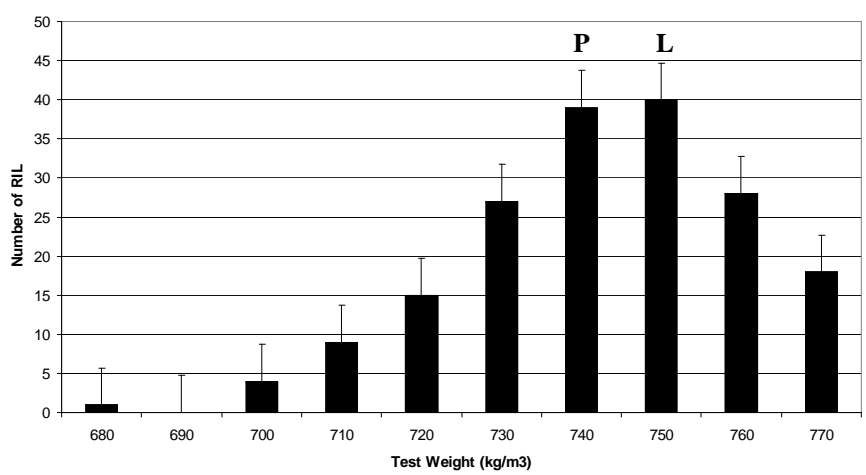
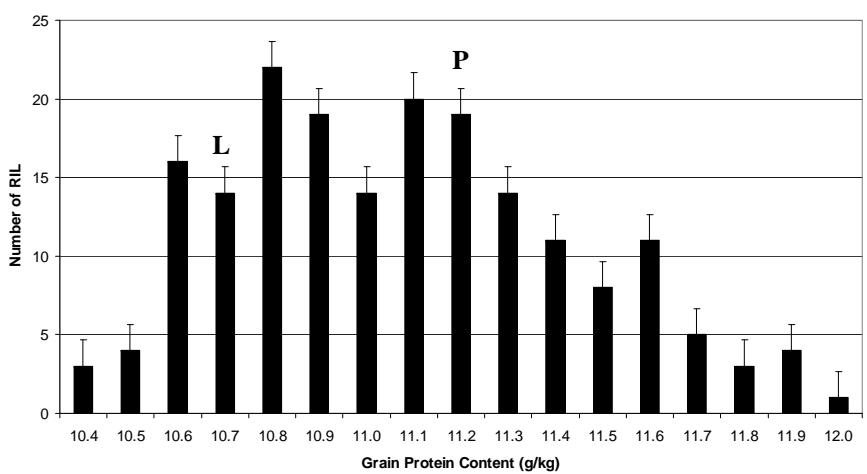
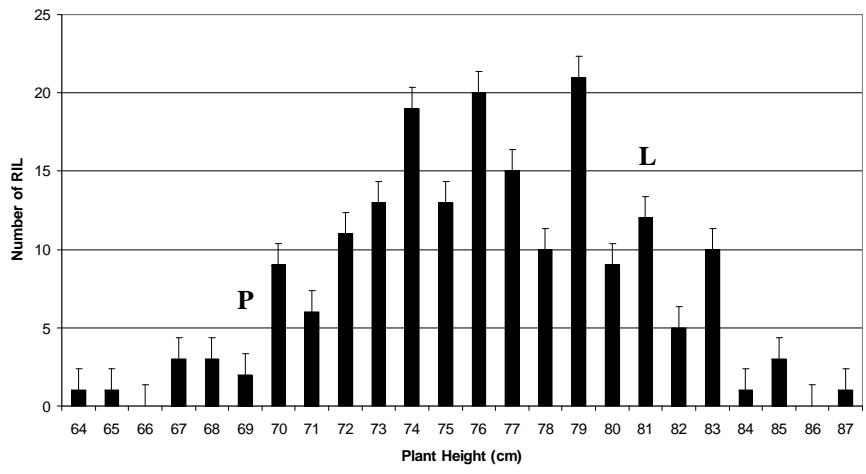
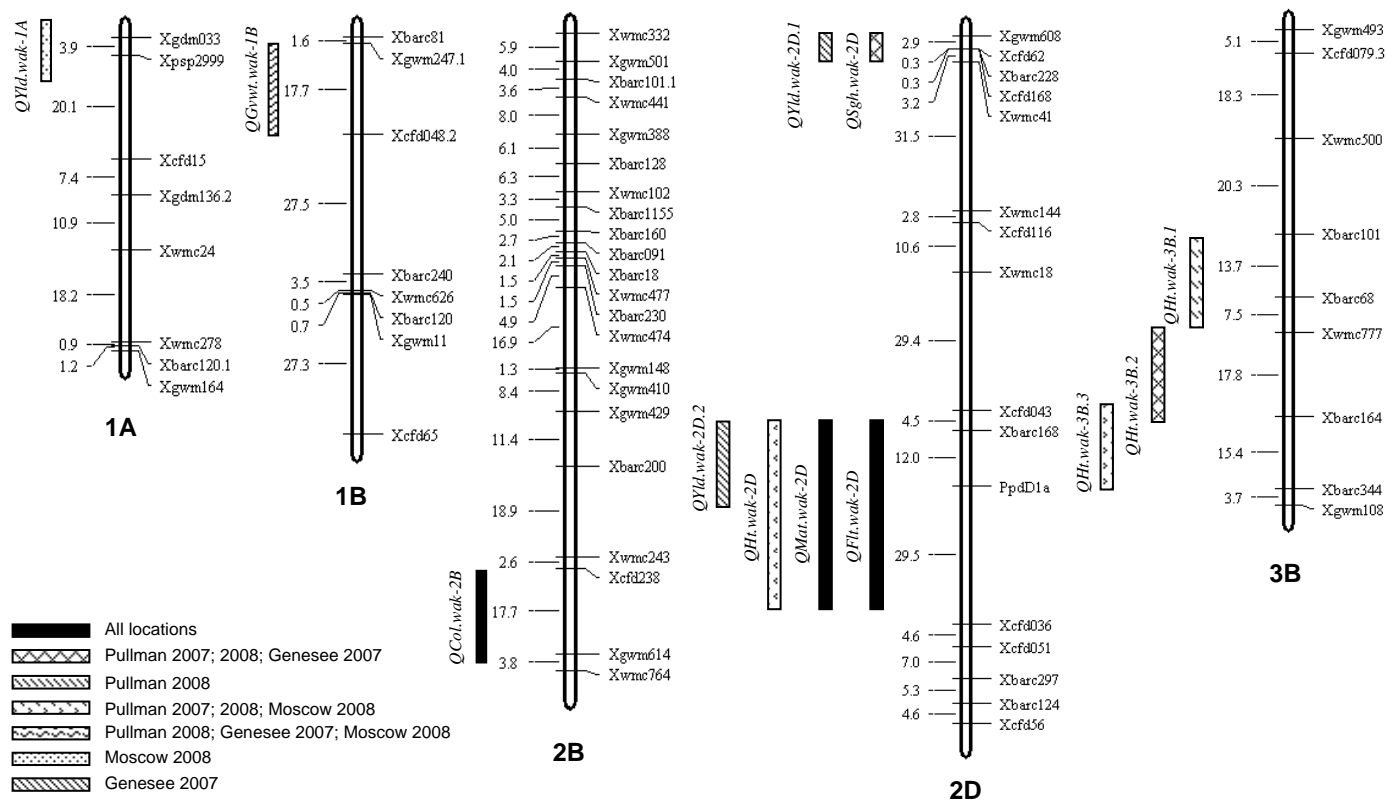


Figure 2 Partial genetic linkage map from the cross Louise by Penawawa reporting 12 agronomic trait QTL over five chromosomes. The vertical bar indicates the QTL confidence interval defined by a one LOD dropoff. Genetic map positions are indicated on the left of each chromosome in Kosambi centiMorgans.



CHAPTER 5

INTERPRETIVE SUMMARY

Utilization of molecular markers in plant breeding can enhance and expedite the incorporation of essential traits into regionally adapted cultivars. Identifying molecular markers associated with QTL requires the development of genetic linkage maps, precise phenotypic data collection, and data analysis. Although many resources are devoted to the detection of QTL and their associated markers, few of these markers are actually used in marker-assisted selection (MAS) programs. This is primarily due to the identification of many QTL associated with a single trait, or the low amount of phenotypic variation the QTL accounts for. Other problems arise with the difficulty of markers to work across laboratories and inconsistency in QTL detection. The genetic background in which the QTL is introgressed also influences the expression of the QTL. Although many difficulties arise when identifying and utilizing molecular markers associated with QTL, efforts continue to proceed to find them, with the hopes that they will be useful for developing new cultivars in the future.

Developing wheat cultivars with durable resistance to disease and insects has been difficult over the years. Costly and time consuming field screening methods with the uncertainty of infection plague mid-generation selection procedures in breeding programs. Race and biotype shifts quickly circumvent race-specific single genes, halting production of once effective cultivars. The ability to pyramid resistance genes together improves the durability of the resistance genes deployed in wheat breeding programs. Since one resistance gene usually masks the presence of another resistance gene, the only way to efficiently and effectively pyramid multiple genes together is with the use of molecular makers.

Many sources of resistance to stripe rust are controlled by single genes, and have been circumvented by new races (Chen, 2005). High-temperature adult-plant resistance genes are typically more durable, although they only provide resistance during the adult growth stages, leaving the seedlings vulnerable (Chen, 2005). The QTL *QYr.wpg.2B* and its associated markers identified from the cultivar Louise provides high levels of durable non-race specific resistance to stripe rust. This QTL, flanked by the markers *Xpsp2999* and *Xgwm148*, has been consistently detected in five locations over a two year period. *QYr.wpg.2B* provides the same level of resistance when validated using other genetic backgrounds. The markers associated with *QYr.wpg-2B* are highly polymorphic in wheat germplasm and will prove useful in marker-assisted selection programs. Pyramiding durable resistance with single-gene race specific resistance will improve the durability of wheat cultivars and limit the dependence on fungicide use.

Hessian fly, another globally important pest, also is controlled using single gene resistance. Like the stripe rust genes, many Hessian fly genes are beginning to become ineffective as new biotypes of the fly emerge. Many genes for Hessian fly resistance have been identified, but the markers associated with them are difficult to use and often inconsistent (Kong et al., 2005; Liu et al., 2005). *H3*, an important Hessian fly gene in the Pacific Northwest, was mapped using SSR markers to develop reliable markers to pyramid this resistance with others. *H3* mapped to chromosome 1AS and flanked by markers *Xpsp2999* and *Xcfd15*. These markers will be useful in deploying *H3* and pyramiding it with other Hessian fly genes. Genotypic data from other resistant cultivars in the PNW indicate that other genes besides *H3* may be present, contrary to previous thought.

Identification of molecular markers useful in breeding for agronomic traits is often difficult due to the multiple QTL that are often associated with each trait. More importantly may be the correlations that are identified between agronomic traits in a growing region. Twelve QTL for agronomic traits were identified on five chromosomes in the Louise by Penawawa mapping population and corresponded to seedling growth habit, leaf color, plant height, flowering date, maturity date, grain volume weight, and yield. Seedling growth habit and leaf color corresponded to a single QTL associated on chromosome 2D and 2B, respectively. Although identified, these traits can easily be score phenotypically and would not be useful in MAS programs. Correlation analysis did identify significant correlations between seedling growth habit and flowering date.

Eight QTL were identified for plant height, seven of them corresponding to a 55.2 cM region on chromosome 3B and the other to the *Ppd-D1* gene on chromosome 2D. Since both parents carry the same dwarfing gene, this variation in height must be a result of another unknown genetic factor. Flowering date and maturity date both corresponded to the *Ppd-D1* gene on chromosome 2D. This gene increases flowering date by three to four days and would be useful in environments requiring early flowering. The QTL for grain volume weight was only significant in three of the four locations and was localized on chromosome 1B. Three yield QTL were identified, but were only significant at certain locations and were associated with other known QTL and genes. In Genesee, 2007, drought stress was present and yield QTL were associated with the *Ppd-D1* gene and the QTL for seedling growth habit. Those lines which flowered earlier had higher yield potential, possibly because they escaped the moisture limiting conditions during grain fill. Another yield QTL was identified in Moscow, 2008, and corresponded to the

H3 gene. Cool wet spring conditions resulted in late planting and infection by Hessian fly. Lines containing resistance to Hessian fly as manifested by the presence of *H3* had higher yields.

The sustainability of wheat production around the globe relies on the dissection of complex pest, agronomic and end-use quality traits. Efforts to identify QTL associated with these traits defines the chromosomal location and number of loci underlying the genetic control of quantitatively inherited traits. If one or two loci are identified conferring the majority of the phenotypic variation, these loci could then be introgressed into new cultivars. By effectively Mendelizing these traits, they can easily be incorporated into breeding programs via marker-assisted selection. Since each QTL in distinct genetic backgrounds will behave differently across environments, this process is not a cure-all. It is, however, a process to begin to understand complex traits and manipulate them in wheat breeding programs.

Future work

The deployment of these QTL via forward breeding marker-assisted selection procedures will help create durable cultivars for resistance to stripe rust and Hessian fly. Breeding lines previously identified as having stripe rust resistance (either all-stage resistance or HTAP), had been crossed to Louise and were in the F₂ generation in the Washington State University Spring Wheat Breeding and Genetics Program. These populations were sampled, and 100 seeds of each population were planted and genotyped with the flanking markers for the desired genes. The gene combinations were: *Yr5*, *Yr15*, HTAP-L (HTAP resistance from Louise), and *H3*; two populations containing *Yr15*,

HTAP-L, and *H3*; HTAP-L and HTAP-A (HTAP resistance from Alpowa); and HTAP-L alone. After genotyping, lines homozygous for the desired gene combinations were increased in the greenhouse. Mature heads were snapped, and headrows were prepared for field screening in the spring of 2009. These headrows will undergo normal selection procedures, and will be advanced based on their agronomic performance for further evaluation.

Crosses were also initiated to transfer and pyramid *H3* and HTAP-L with other forms of resistance. Breeding lines verified to have *H9* and *H25* were topcrossed with Louise in the effort to incorporate multiple *H* genes into the same cultivar. Crosses were also made in an effort to pyramid HTAP sources. Lines containing the HTAP genes from Louise, Whit, Alpowa, and Stephens were crossed and the F₁ seed planted. Once F₂ seed is available, markers will be used to identify lines homozygous for the different HTAP and Hessian fly resistance sources

The variability in PNW cultivars for the markers flanking *H3* raises questions as to the source of Hessian fly resistance in these cultivars. It has long been proposed that these cultivars receive their resistance from *H3* (Smiley et al., 2004). Thus, either there are different allelic forms of *H3* present in the germplasm, or a different gene all together. A genetic linkage map is currently being developed for the RIL population of ‘Macon’ (resistant, Kidwell et al., 2003) by ‘Sunco’ (susceptible, Australian wheat). Macon was previously thought to carry *H3*, but genetic analysis using flanking markers indicates the absence of the resistant allele for this gene. The population will be tested for Hessian fly resistance following similar methods as proposed in Chapter 3. Analysis of the data will identify QTL and markers corresponding to the resistance in Macon. If needed, allelism

tests will be conducted to determine if the resistance from Louise is different from that of Macon. This future research will further the understanding of Hessian fly resistance genes in the PNW and their association to each other.

The Louise by Penawawa mapping population has the potential to identify many more QTL than what the present study evaluated. One potential is the end-use quality of harvested grain. Preliminary data indicate the two parents are significantly different from each other with regards to end-use quality. Data from a single location in 2007 identified eight putative QTL for quality. Grain samples from two locations in 2008 were submitted to the Western Regional Wheat Quality Laboratory in Pullman, WA, to evaluate the grain for end-use quality. Once data is collected, QTL analysis similar to those done in this study will be performed, and QTL for end-use quality identified. This will begin to dissect the complex nature of many end-use quality traits, with the hopes of finding one or two QTL which can be introgressed into new cultivars to improve grain end-use quality.

The genetic linkage map created from this mapping population is made publically available through Graingenes (<http://wheat.pw.usda.gov/GG2/index.shtml>). Seed from this population are also available from the Small Grains Seed Repository in Aberdeen, ID. The variability of this population has not been fully exploited, and may be useful in future studies. If a certain trait needs to be genetically dissected, the parents from this population should first be tested for differences. If different, this population can be used to do QTL analysis, thus eliminating the need to develop a new mapping population and expediting the dissection of the trait of interest.

References

- Chen, X.M. 2005. Epidemiology and control of stripe rust [*Puccinia striiformis* f. sp. *tritici*] on wheat. *Can. J. Plant Pathol.* 27:314-337.
- Kidwell, K.K., V.L. DeMacon, G.B. Shelton, J.W. Burns, B.P. Carter, C.F. Morris, X. Chen and N.A. Bosque-Perez. 2003. Registration of 'Macon' Wheat. *Crop Sci.* 43:1561-1563.
- Kong, L., H.W. Ohm, S.E. Cambron, and C.E. Williams. 2005. Molecular mapping determines that Hessian fly resistance gene *H9* is located on chromosome 1A of wheat. *Plant Breed.* 124:525-531.
- Liu, X.M., A.K. Fritz, J.C. Reese, G.E. Wilde, B.S. Gill, and M.-S. Chen. 2005. *H9*, *H10*, and *H11* compose a cluster of Hessian fly-resistance genes in the distal gene-rich region of wheat chromosome 1AS. *Theor. Appl. Genet.* 110:1473-1480.
- Smiley, R.W., J.A. Gourlie, R.G. Whittaker, S.A. Easley, and K.K. Kidwell. 2004. Economic impact of Hessian fly (Diptera: Cecidomyiidae) on spring wheat in Oregon and additive yield losses with *Fusarium* crown rot and lesion nematode. *J. Econ. Entomol.* 97:397-408.