ABSTRACT

Title of Document: MAPPING DISEASE RESISTANCE QTL FOR FUSARIUM HEAD BLIGHT AND LEAF RUST IN A WHEAT DOUBLED HAPLOID POPULATION

Benjamin Conway, M.S., 2014

Directed By: Professor Emeritus Dr. José Costa, Department of Plant Science and Landscape Architecture

Fusarium graminearum and *Puccinia triticina* are common wheat pathogens in the Mid-Atlantic region, causing *Fusarium* head blight (FHB) and leaf rust, respectively. Both diseases can cause serious yield losses in epidemic conditions and can be controlled by breeding resistant cultivars. MD01W233-06-1 is an adapted soft red winter wheat (SRWW) breeding line with previously uncharacterized "native" FHB resistance. SS8641 is an FHB-susceptible SRWW cultivar that has the leaf rust resistance gene *Lr37* and an additional unidentified source of resistance. These parents were used to generate a doubled haploid mapping population to map their resistance to these diseases. Four FHB resistance quantitative trait loci (QTL) were mapped to chromosomes 3B (3 QTL) and 1A (1 QTL). Several QTL in SRWW have been mapped to these regions. Two leaf rust resistance QTL were mapped to chromosomes 2A, the same location as *Lr37*, and *5B*, known to contain *Lr18*, previously unreported in either parent.

MAPPING DISEASE RESISTANCE QTL FOR FUSARIUM HEAD BLIGHT AND LEAF RUST IN A WHEAT DOUBLED HAPLOID POPULATION

By

Benjamin J. Conway

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Advisory Committee:
Professor Emeritus José Costa, Chair
Assistant Professor Jianhau Zhu
Associate Professor Shunyuan Xiao

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Table of Contents

List of Tables

List of Figures

Chapter 1: Literature Review

Introduction

Since its domestication 10000 years ago, wheat (*Triticum aestivum* L. ssp. *aestivum*) has become a major human staple. Today, it is the most widely cultivated crop, providing the main source of dietary calories to 35 percent of the global population (McCorriston, 2012). As with any plant, wheat can be affected by a variety of pathogens, pests, and abiotic stresses. Mitigating the harmful effects of these threats is a main goal of plant breeding, allowing crops to reach their full yield potential. Wheat is regularly attacked by fungal pathogens. In the Mid-Atlantic region of the United States, two of the most destructive are *Fusarium graminearum* Schwabe and *Puccinia triticina* Eriks., causal agents of wheat *Fusarium* head blight (FHB) and leaf rust, respectively. FHB is a destructive pathogen with a wide host range, including common wheat, durum wheat (*T. turgidum* L. ssp. *durum*), barley (*Hordeum vulgare* L.), and maize (*Zea mays* L.). FHB affects developing grains, causing reduced grain fill and the formation of shriveled white- to pink-colored "tombstones." FHB reduces test weight, and is capable of causing yield losses as high as 70% (Pirgozliev et al., 2003). *F. graminearum* is also associated with the production of mycotoxins. The most significant mycotoxin produced by *F. graminearum* in the United States is deoxynivalenol (DON), or vomitoxin (Goswami & Kistler, 2004). DON accumulates in seeds on infected wheat spikes, rendering them unsuitable for human consumption and livestock feed. The Food and Drug Administration has issued guidelines for the concentration of DON allowable for

human and livestock consumption, with 1ppm DON allowable on wheat products for human consumption, 10ppm for cattle and chickens, and 5ppm for swine and other animals (FDA, 2010). Together, yield losses and DON accumulation can have major economic impacts. In the 1990s alone, FHB epidemics caused losses estimated at $3 billion for wheat and barley farmers (Windels, 2000). In the Mid-Atlantic region, there have been destructive epidemics since 2000, with the 2003 epidemic causing $13.6 million in losses in Maryland, Virginia and North Carolina (Cowger & Sutton, 2005).

Leaf rust is another major fungal pathogen affecting wheat worldwide. This fungus can infect leaves of wheat at any growth stage, producing orange-brown-colored pustules, asexual spore-producing structures called uredinia, leading to the common name for this pathogen: brown rust. Leaf rust is capable of causing yield losses up to 50%, though this is largely dependent on host growth stage at infection (Huerta-Espino et al., 2011). Leaf rust is a persistent problem for wheat agriculture in all major wheat growing regions of the United States and around the world.

Fusarium Head Blight of Wheat

Causal Agent Taxonomy

Until recently, the causal agent *F. graminearum* was widely accepted to be a single species. With the advent of whole genome sequencing, phylogenetic studies have revealed numerous species of FHB causal agents based in large part on geographic distribution. There are over a dozen species comprising what is now referred to as the *F. graminearum* species complex (or sensu lato) (O'Donnell, et al., 2000; O'Donnell, et al., 2004; Aoki, et al., 2012). There have been reports of several

native FHB causal agents in the United States including, *F. graminearum* sensu stricto, *F. louisianense,* and *F. gerlachii,* the latter two being found in isolated regions (Starkey et al., 2007; Sarver et al., 2011). Additionally, the Asian-centered species *F. asiaticum* has recently been isolated from wheat in several parishes of Louisiana (Gale et al., 2011). *F. graminearum* sensu stricto (which will hereafter be referred to as *F. graminearum*) represents nearly 100% of FHB causal agents in the United States and can be found in major wheat producing regions around the world (Aoki et al., 2012).

Life Cycle

Fusarium graminearum (teleomorph *Gibberella zeae* [Schwein.] Petch) is an ascomycete with a complex life cycle, living as a facultative saprophyte with both sexual and asexual reproductive strategies. FHB is a polycyclic disease, it is homothallic in nature with the compatible mating type genes in the same genome (Kim et al., 2012), allowing sexual reproduction to occur without outcrossing (Cavinder et al., 2012). Successful sexual reproduction on crop residue produces black, pear-shaped perithecia, which contain ascospores, the primary source of inoculum (Dill-Macky & Jones, 2000). *F. graminearum* overwinters on residue until the spring (Fernando, 1997). Perithecia can form at temperatures above 3°C and produce ascospores above 10°C, with an optimum temperature range for ascospore production between 15-20°C (Xu & Nicholson, 2009). Ascospores are released during rain events or humid conditions and are carried by wind to exposed wheat spikes, where they germinate and begin the parasitic stage of the lifecycle.

In addition to sexually-produced ascospores, *F. graminearum* established on host tissues can sporulate asexually, producing macroconidia as a secondary source of inoculum. Macroconidia are produced in large orange- or pink-colored masses called sporodochia, a tell-tale sign of FHB infection. Macroconidia are primarily splash-dispersed by rain, contributing to local spread of the disease, although there have been reports of long-range wind distribution (Fernando et al., 1997; Doohan et al., 2003; Gilbert & Fernando, 2004).

Infection Biology

After landing on the floral tissues of the wheat spike, the hyphae of germinating ascospores or macroconidia proceed to invade the host. Infection can occur through a passive route via dehiscent anthers and stomata (Pritsch et al., 2000; Bushnell, 2001) or by direct penetration of vulnerable tissues within the floret (Rittenour & Harris, 2010). It has been reported that *F. graminearum* is a hemibiotroph, with both biotrophic and necrotrophic stages of pathogenesis. After invading dehiscent anthers or stomata of glumes or florets, hyphae proliferate within the apoplast of the floral tissues (Brown et al., 2010). Hyphal growth progresses from the initial site of infection, spreading through the spikelet and entering the rachis, moving up and down the spike. Behind this infection front, hyphae penetrate host cells, creating visible symptoms several days after initial infection (Brown et al., 2010). *F. graminearum* colonizes vascular tissues and grows laterally to the epidermal cells which are then ruptured, allowing the development of aerial mycelia and sporodochia. The necrosis of tissue gives infected spikes a prematurely bleached appearance.

DON plays an important role in the infection biology of *F. graminearum*. Biosynthesis of DON and other trichothecenes is controlled by the TRI gene pathway which consists of 15 genes (Proctor et al., 2009). DON functions as a virulence factor (Proctor et al., 1995; Desjardins et al., 1996), with greatest TRI gene expression at the biotrophic infection front (Brown et al., 2011). DON binds to the 60S subunit of eukaryotic ribosomes, inhibiting protein synthesis and causing ribotoxic stress response which induces apoptosis (Pestka, 2007; Boenisch & Schäfer, 2011). This protein synthesis mechanism is responsible for the mycotoxic effects in animals as well (Pestka, 2007; Sobrova et al., 2010; Arunachalam & Doohan, 2013). While DON production has been found to be important in *F. graminearum* pathogenicity in wheat, it is not the only factor that contributes to virulence. Baldwin et al. (2010) demonstrated topoisomerase I (*Top1*) mutant strains of *F. graminearum* produced limited visible symptoms after inoculation, suggesting there are other virulence genes involved in pathogenicity. Brown et al. (2012) evaluated the secretome of *F. graminearum* during infection of wheat spikes, identifying 171 secreted proteins that are believed to be involved in the degradation of wheat cuticle, cellulose, hemicelluloses, lignin, callose, pectin, lipids, starches, proteins, and choline, all of which may be implicated in cell penetration after biotrophic colonization of head tissues, although specific mechanisms have yet to be elucidated.

Management Strategies

With a host range that includes maize (*Zea mays* L.) and the capability of living as a saprophyte on crop residues, *F. graminearum* has become an emerging problem in US wheat production in recent decades. A large-scale shift in the US to

no-till cultural practices (Horowitz et al., 2010) and the prevalence of wheat-after-maize crop rotations has been responsible for the increased frequency of FHB epidemics (Dill-Macky & Jones, 2000; Windels, 2000; Cowger & Sutton, 2005). Control of this pathogen is difficult, with no one solution fully solving the problem. Management strategies include cultural practice selection, fungicide application, biological control, and selection of resistant cultivars.

One strategy to control FHB is through cultural practices, namely tilling of the soil prior to planting. This incorporates the saprophytic *F. graminearum* inoculum growing on crop residues into the soil, where it is a poor competitor against other soil microbes (Leplat et al., 2013). Studies examining the efficacy of tilling in lowering DON accumulation in wheat grain have reported reductions in the range of 65-70% compared to worst case scenarios (no-till planting a susceptible cultivar following maize without fungicide applications) (Beyer et al., 2006; Blandino et al., 2012). While this can be an effective means of suppressing FHB, many US farmers have shifted to no till. Maryland in particular has been eager to encourage no-till practices in order to protect the ecology of the Chesapeake Bay, while small grains acreage has increased due to state subsidies for planting cover crops to mitigate nitrification of the Bay. These factors preclude tilling as a management tool in this context and promote a cropping system that is more vulnerable to FHB epidemics.

Fungicide application is another management strategy for control of FHB in wheat. Triazole fungicides applied at anthesis are recommended for control of FHB. First developed in 1976, triazoles are locally systemic fungicides that act as ergosterol biosynthesis inhibitors, producing aberrant intermediate products, which then

accumulate around fungal hyphae, inhibiting further growth (Fera et al., 2009). While initially considered ineffective in controlling FHB symptoms and DON accumulation (Milus & Parsons, 1994), improved fungicide chemistry and application techniques have produced several effective options. As of 2013 there are 5 triazole fungicides labeled for use to control FHB. These are Prosaro (prothioconazole + tebuconazole), Caramba (metconazole), Tilt (propiconazole), Proline (prothioconazole), and Folicur (tebuconazole) (North Central Regional Committee on Management of Small Grain Diseases, 2013). Triazole fungicides applied at the proper time can generally achieve 50-60% reductions in FHB severity and DON accumulation (Beyer et al., 2006; US Wheat & Barley Scab Initiative, 2009; Blandino et al., 2012). Timing of fungicide application is critical for effective FHB control. Application at anthesis is most effective for preventing the spread of FHB infection, while later applications (approximately 20 days after anthesis) has been shown to reduce mycotoxin accumulation without improving visible symptoms (Yoshida et al., 2012).

With the difficulty in timing application of fungicides, the best way to control FHB is to plant wheat cultivars with genetic resistance. Selection of resistant cultivars has long been recognized as a major management strategy for FHB (Dickson, 1942). Resistance to FHB is inherited in a quantitative fashion and is characterized as either passive or active resistance. Passive resistance is largely controlled by agronomic and phenological traits. For example, tall plant height and loose spikelet density spike morphology can hinder access to the FHB inoculum and flowering in conditions unfavorable to ascospore discharge (disease escape)

(Mesterhazy, 1995). Active resistance mechanisms depend on host physiological resistance to *F. graminearum*, with 5 types of resistance being identified. These types are defined based on different traits and stages of infection. Resistance to the initial establishment of an infection is referred to as Type I resistance, while Type II resistance is defined as the resistance to spread of FHB after initial infection (Schroeder & Christensen, 1963). Type III refers to mycotoxin resistance (Miller & Wang, 1988). Types IV and V refer to resistance to infection of the kernel and tolerance, respectively (Mesterházy et al., 1999). Operating with this framework, numerous sources of genetic resistance have been characterized.

Many sources of FHB resistance have been found, with quantitative trait loci (QTL) mapped to every chromosome of the wheat genome (Buerstmayr et al., 2009). The most prominent sources of genetic resistance have been derived from Chinese cultivars with Sumai 3 and its derivative Ning 7840 providing Fhb1, a major quantitative trait locus (QTL) on the short arm of chromosome 3B, which explained over 40% of the FHB resistance in the population in which it was mapped (Anderson et al., 2001). Additionally, FHB resistance has also been identified in the Brazilian cultivar Frontana, which has been used by the International Center for Maize and Wheat Breeding (CIMMYT) (Magliano et al., 2013). Breeding exotic FHB-resistant cultivars with US winter wheat cultivars generally introduces undesirable characteristics for agronomic and end-use quality traits (Anderson, 2007; McCartney et al., 2007). This problem has spurred the search for “native” resistance to FHB within US wheat classes that will be adapted to local environments and present less of a barrier in development of new cultivars.

The soft red winter wheat (SRWW) class has several sources of characterized FHB resistance, such as Ernie (McKendry et al., 1995; Liu et al., 2005; Liu et al., 2007; Liu et al., 2013) Other moderately resistant cultivars reported include McCormick (Griffey, 2005), Truman (McKendry et al., 2005), Bess (McKendry et al., 2007), Roane (Griffey et al., 2001), Tribute (Griffey et al., 2005), and Jamestown (Griffey et al., 2010). FHB resistance from McCormick has not yet been characterized. The breeding line MD01W233-06-1, derived from a cross between McCormick and Choptank, has also been reported as FHB resistant in FHB-inoculated field nurseries (Costa et al., 2010). The objective of this study was to characterize and map the US native resistance of MD01W233-06-1. Mapping this source of resistance may provide wheat breeders with molecular tools to incorporate this source of FHB resistance into their breeding programs.

Leaf Rust of Wheat

Causal Agent: Taxonomy and Life Cycle

Leaf rust, or brown rust, is an important, global threat to wheat production. In contrast to FHB, leaf rust has but a single causal agent, the basidiomycete *Puccinia triticina* Eriks., and derives its nutrition from its host in a biotrophic manner. Within *P. triticina*, there are strains with very specific host ranges, termed *formae speciales*. *P. triticina* f. sp. *tritici* affects common wheat, durum wheat (*T. turgidum* L. ssp. *durum*), emmer wheat (*T. turgidum* L. ssp. *dicoccum*), triticale (x *Triticosecale*), and several *Aegilops* L. species and other wild wheat relatives (Bolton et al., 2008). Leaf rust is further classified within *P. triticina* f. sp. *tritici* based on physiologic

specialization with the host range. These specializations are termed races and are defined by pathogenicity (virulence versus avirulence) to hosts with known sources of genetic resistance (Huerta-Espino et al., 2011). *P. triticina* affects wheat in all regions where it is produced. Although it not as devastating a disease as related species like stem rust (*P. graminis* Pers.) as it normally causes yield losses under 10%, leaf rust epidemics can be severe when conditions are favorable, causing yield losses as high as 30-50% (Pretorius et al., 1988; Roelfs et al., 1992; Huerta-Espino et al., 2011).

The cereal rusts have heteroaecious lifecycles, involving both sexual and asexual reproductive stages on separate host species. The life cycle begins with telia on the primary cereal host tissue. Telia form near the end of the cereal host lifecycle and produce teliospores. Teliospores can survive the summer and begin producing basidiospores by meiosis in the autumn. Basidiospores are wind-dispersed and establish infection on the alternate host, in the case of leaf rust the alternate hosts include common meadow-rue (*Thalictrum speciosissimum* L.) and *Isopyrum fumarioides* L. (Roelfs et al., 1992; Bolton et al., 2008).

After infection of the alternate host, sexual reproduction can occur. *P. triticina* mycelia form specialized structures called pycnia on the upper side of the alternate host leaves, which produce haploid sexual spores called pycniospores. pycniospores are splashed or transported by insects to another pycnium, where they encounter receptive hyphae. After successful fertilization with a receptive hypha of a compatible mating type, dikaryotic hyphae will proliferate and form aecia on the underside of the leaf. Aecia produce aeciospores, which are then dispersed by the

wind and infect the cereal primary host. After infection of the cereal host, uredinia, or pustules, bearing urediniospores form on the upper surface of the leaves. When the cereal host approaches physiological maturity, *P. triticina* produces telia structures which begin the cycle again (Roelfs et al., 1992; Bolton et al., 2008).

Epidemiology and Infection Biology

While the life cycles of *Puccinia* sp. are complex involving 5 different spore stages, alternate hosts are not found in many wheat producing regions of the world, preventing sexual reproduction in wild populations. Instead, reproduction is clonal, with the disease cycle consisting of successive generations of uredinia producing urediniospores exclusively on the primary cereal host (Goyeau et al., 2007). The absence of the alternate host precludes not only sexual reproduction, but also overwintering in wheat-producing regions with cold winters, such as the Mid-Atlantic United States. In North America, rust epidemics normally begin in the southern US or Mexico. Urediniospores from the south are wind dispersed further north in successive stages as the wheat matures and temperatures rise with the advance of summer (Roelfs, 1989). In some instances, leaf rust can over winter as mycelium on volunteer wheat (Eversmeyer & Kramer, 2000). In most years, leaf rust can be found in the Gulf of Mexico coastal states in February, spreading to other regions of the southeastern US. By mid-May leaf rust can be found throughout the eastern US and southern Great Plains regions, with subsequent spread throughout the northern Great Plains completed by the end of July (Kolmer et al., 2007).

When a urediniospore lands on a wheat leaf, germination is induced by environmental conditions. The spores require high humidity, free water on the leaf

surface, and temperatures around 20°C to initiate germination. In suitable conditions infection can occur within 8 hours (Kolmer et al., 2009). Upon germination, a germ tube hypha will grow laterally across the leaf surface until it encounters a stoma. When over a stoma, the germ tube will produce appressorium that begins the infection process. The appressorium produces a penetration peg, which is forced between the closed guard cells. Once inside, hyphae grow toward the mesophyll cells. Upon contacting a mesophyll cell, another penetration peg is produced, which will invade the host cell and produce a haustorium, the primary feeding structure of *P. triticina*. With a source of nutrition, the hyphae proliferate and invade other mesophyll cells, producing a mycelial network. Uredinia are produced within 7-10 days, releasing more urediniospores that can begin new infections (Bolton et al., 2008). Spore production is continuous, with the rate of sporulation highly dependent on host growth stage and immune response and environmental conditions. Each uredinium is capable of producing hundreds to thousands of urediniospores per day (Eversmeyer & Kramer, 2000).

Host Resistance

While triazole and strobilurin fungicides can be used to control leaf rust, their application can be expensive and have negative environmental impacts (Osborne & Stein, 2009). Breeding resistant cultivars is the best strategy for leaf rust management and has largely relied on the identification of and selection for leaf rust resistance (*Lr*) genes. The first leaf rust resistance in wheat was described by Mains et al. (1926), with the first *Lr* gene being designated by Ausemus (1946). Since then, over 60 *Lr* genes have been designated (Cereal Disease Laboratory, 2013). Wheat

leaf rust resistance genes are classified into 2 categories: seedling resistance genes and adult plant resistance (APR) genes.

Seedling resistance can be manifested in the seedling growth stages, although it can also be expressed in adult plants (Bhavani et al., 2011). Many of these genes have been shown to have race specificity, operating on a gene-for-gene relationship with *P. triticina* (Kolmer, 1996). In this system there is an avirulence (*avr*) gene in the pathogen corresponding to the resistance gene in the host, the gene-for-gene hypothesis (Flor, 1971). To date, 3 seedling resistance *Lr* genes have been cloned and characterized. *Lr1*, *Lr10*, and *Lr21* have all been found to belong to the coiled coil nucleotide binding site leucine-rich repeat (CC-NBS-LRR) plant resistance gene family (Cloutier et al., 2007). The targets for these genes are not well understood and to date no *P. triticina avr* genes have been cloned. When deployed alone, seedling resistance *Lr* genes tend to last only a few years before mutations accumulated in *avr* genes alter the interaction of *Lr-avr*, allowing leaf rust to overcome host resistance. The clonal nature of *P. triticina* reproduction and extensive varietal monoculture in wheat cultivation make this threat more pronounced.

Adult plant resistance is manifested after emergence of the flag leaf. APR genes are also known as partial resistance or slow rusting genes. They are largely race non-specific (Caldwell, 1968). The slow rusting genes reduce the number and size of uredinia and lengthen the latent period before uredinia are formed (Kuhn et al., 1978). Furthermore, several APR genes have been found to have pleiotropic effects, conferring resistance to other rusts and fungal pathogens (Spielmeyer et al., 2013) To

date four APR genes have been described: *Lr34*, *Lr46*, *Lr67*, and *Lr68* (Singh et al., 1998; Lagudah et al., 2009; Hiebert et al., 2010; Herrera-Foessel et al., 2012). Of these, *Lr34* has been cloned and is predicted to be an ATP binding cassette transporter, though its substrate remains unknown (Krattinger et al., 2009). While APR genes can provide durable resistance there is still a risk of the development of virulent races with overreliance on a single gene of resistance.

The best breeding strategy to control leaf rust is to pyramid, or stack, multiple *Lr* genes within a single cultivar. While phenotypic selection for multiple sources of resistance can be difficult, the advent of DNA markers has allowed the application of marker assisted selection (MAS) in breeding for leaf rust resistance (Vida et al., 2009) and many other traits. MAS relies on genetic mapping of traits of interest and the development of tightly linked diagnostic markers for use in selection. The objective of this study was to map leaf rust resistance in the soft red winter wheat SS8641, which has been postulated to have *Lr37* and an additional source of resistance. Identifying this potentially novel source may provide a new *Lr* gene with diagnostic markers for use by US wheat breeders.

Chapter 2: Mapping *Fusarium* Head Blight Resistance QTL

Introduction

Fusarium head blight (FHB) or wheat scab caused by *Fusarium graminearum* Schwabe poses a major threat to wheat (*Triticum aestivum* L. ssp. *aestivum*) production in the United States and abroad. FHB infects wheat spikes and can cause reduced grain fill, production of low-quality "scabby" kernels or "tombstones", and accumulation of deoxynivalenol (DON) and other trichothecene mycotoxins. FHB infection reduces grain yield and quality and leads to the accumulation of DON in diseased kernels. *F. graminearum* is also a pathogen of maize (*Zea mays* L.) and can survive as a saprophyte on crop residues left in the field to infect subsequent crops. Historically, conventional tillage practices reincorporated these residues into the soil, suppressing FHB inoculum in the process. In recent decades, there has been a shift away from conventional tillage, with 35.5% of US cropland sown with no-till practices (Horowitz et al., 2010). The prevalence of decreased tillage practices that leave *F. graminearum* inoculum on the surface and cropping systems with wheat planted after maize is extremely conducive to FHB infection, leading to regional epidemics causing estimated economic losses as high as $3 billion from 1990 to 2000 (Windels, 2000).

The main FHB control strategies for this cropping system are spraying with triazole fungicides and planting of wheat cultivars with moderate resistance. Triazole fungicides have been found to be the most effective means of chemical control but are

still incapable of suppressing disease completely in a conducive environment with a susceptible wheat cultivar (Mesterházy et al., 2003). The single most effective strategy for control has been the development and deployment of resistant cultivars (Beyer et al., 2006). FHB resistance is inherited as a quantitative trait, with many loci contributing to the resistance phenotype. Many quantitative trait loci (QTL) have been identified (Buerstmayr et al., 2009). The most significant and consistent QTL identified is *Fhb1* on chromosome 3BS. *Fhb1* was identified in the Chinese spring wheat cultivar Sumai 3 and its derivative Ning 7840, explaining over 40% of the FHB resistance in the population in which it was mapped (Anderson et al., 2001). While this major QTL has been an important source of FHB resistance, incorporating this exotic material into US wheat breeding programs has been challenging, due to the unintended introduction of unfavorable traits (Brown-Guedira et al., 2008). Recent breeding efforts in the US have sought to identify "native" resistance in adapted germplasm that will supplement known exotic FHB resistance QTL. Soft red winter wheat (SRWW) is grown east of the Mississippi River, often following maize, faces a significant threat from FHB. Native resistance has been identified and characterized in several SRWW cultivars including Ernie (McKendry et al., 1995; Liu et al., 2005; Liu et al., 2007; Liu et al., 2013) and Massey (Liu et al., 2013). Other moderately resistant cultivars reported include McCormick (Griffey, 2005), Truman (McKendry et al., 2005), Bess (McKendry et al., 2007), Roane (Griffey et al., 2001), Tribute (Griffey et al., 2005), and Jamestown (Griffey et al., 2010). FHB resistance from McCormick has not yet been characterized. The SRWW breeding line MD01W233-06-1 was derived from a cross of McCormick and Choptank. This line has been

shown to have superior FHB resistance in the field and is reported to lack the alleles associated with *Fhb1* and other known resistance QTL (Costa et al., 2010). The objective of this experiment was to map the source of FHB resistance in MD01W233-06-1 using a doubled haploid mapping population derived from the F_1 cross of MD01W233-06-1 and SS8641, a highly susceptible SRWW cultivar. Both parents of the mapping population have been bred for the eastern US. Markers associated with any resistance QTL identified from this work will be immediately applicable to the region and supplement current breeding resources in SRWW.

Materials and Methods

Plant Materials

A soft red winter wheat doubled haploid (DH) mapping population of 135 lines was developed by Dr. J. Paul Murphy of North Carolina State University in 2009 using the wheat x maize wide cross method. The population was generated from F_1 progeny from the cross MD01W233-06-1 (Hereafter referred to as MD233; pedigree=McCormick/Choptank) by the Southern States (SS) 8641 (pedigree=GA 881130/2*GA 881582), made in the greenhouse at the University of Maryland, College Park. MD01W233-06-1 was selected as the resistant parent based on prior data demonstrating resistance to FHB and the absence of known FHB resistance QTL (Costa et al., 2010). SS8641 is highly susceptible to FHB. During initial seed increases and preliminary genotyping, DH lines with extremely late heading dates and heterozygous marker genotypes were eliminated from the population, reducing the number of DH lines to a total of 124.

Phenotypic Evaluation for FHB Resistance

Greenhouse Experiment

In winter 2011, the DH population and parents were grown in the greenhouse at the University of Maryland College Park for a single-floret inoculation experiment to assess types II (spread) and III (DON) resistance to FHB. An isolate of *F. graminearum* was generously provided by Dr. David Van Sanford of University of Kentucky. Inoculum preparation, inoculation, and phenotypic evaluation for severity (SEV), percentage of *Fusarium*-damaged kernels (FDK), and DON content were conducted as described in Kang et al., (2011). DON analysis was performed by Dr. Yanhong Dong at the University of Minnesota, St. Paul according to the protocol followed by Fuentes et al. (2005).

Field Experiments

To evaluate types I (initial infection), II, and III resistance, the DH population and parents were grown in inoculated field nurseries. The population was evaluated in spring of 2011 and 2012 at the University of Maryland Lower Eastern Shore Research and Education Center in Salisbury, MD and Cunningham Research and Extension Center in Kinston, NC. The DH lines and parents were planted in 1.2m single-row plots in randomized complete block designs. In Salisbury, 3 blocks were planted, while in Kinston, space limitation allowed only 2 blocks to be planted. In Salisbury, corn spawn *F. graminearum* inoculum (generously provided by Dr. Arvydas Grybauskas of University of Maryland) was spread around the plots

approximately a month before heading date. In Kinston, plots were spray inoculated with *F. graminearum* conidia suspension at the start of anthesis (Feekes 10.5). Plots in all locations were evaluated for incidence (INC) of FHB (percentage of heads with FHB symptoms, a measure of type I resistance), and severity of FHB (percentage of head with FHB symptoms, a measure of type II resistance). Plots in Salisbury were also evaluated for heading date and height after flowering (Feekes 11). At full maturity (Feekes 11.3), a random sample of spikes from each plot was collected and threshed. FDK was determined based on a subsample of 200 kernels that were used to estimate DON. In both the 2011 and 2012 field seasons, plots at Kinston were inoculated before all DH lines were at flowering (Feekes 10.5). Those lines that could not be inoculated were discarded, so as to prevent conflation of resistance and disease escape. At this location, data was collected for 115 lines in 2011 and 102 lines in 2012.

DNA extraction and Marker Analysis

A set of the 124 DH lines and parents lines were planted in 96-cell planting trays to collect leaf tissue for DNA extraction. Tissue samples were cut from seedlings at the 2-leaf stage for DNA extraction. DNA extraction was performed as described in Kang et al. (2011) by Dr. Gina Brown-Guedira at the USDA Eastern Small Grains Genotyping Lab in Raleigh, NC. The population was screened with 29 short sequence repeat (SSR) markers as described in Kang et al. (2011) by Dr. Brown-Guedira. SSRs were selected from Roder et al. (1998), Somers et al. (2004), and Song et al. (2005) including: wmc474, wmc471, gwm272, gwm11, barc170,

barc45, wmc496, barc164, wmc273, barc163, barc101, wmc278, barc100, barc12, barc80, barc10, barc28, barc127, barc147, barc137, gdm136, barc59, gwm111, gwm149, gwm260, gwm261, gwm282, gwm304, and gwm319. The population was then genotyped using the wheat 9K iSelect Beadchip Assay, with the assay performed as described in Cavanagh et al. (2013) by Dr. Shiaoman Chao at the USDA Northern Central Small Grains Genotyping Lab in Fargo, ND. SNP genotyping calls were made using GenomeStudio v2011.1 software (Illumina, San Diego, CA) as described in Cavanagh et al. (2013) by Dr. Brown-Guedira. The population was also genotyped using KASP (Kompetitive Allele Specific PCR) assays (LGC Genomics, Middlesex, UK). KASP markers were selected from markers designed by the regional genotyping labs (IWB49398, TaPpdDD001, sbv5D_6060) and selected markers from Wilkinson et al. (2012) including BS00081724, BS00024094, BS00021850, BS00024015, BS00022436, BS00023944, BS00047797, BS00064002, BS00024118, BS00117841, BS00098495, BS99999954, BS00065928, BS99999957, BS00036421, BS99999964, BS00024014, BS99999971, BS00122945, BS99999998, and BS00022283. In addition to molecular markers, the population segregates for coleoptile color, a morphological marker. SS8641 has a green coleoptile and MD233 has a red coleoptile. The population was evaluated for this trait after emergence of coleoptiles, prior to emergence of the first true leaf.

Linkage Mapping

2091 SNPS (from 9k and selected KASP markers), 21 SSR markers, and the Rc-D1 (red coleoptile) morphological markers were used for initial linkage map construction. Linkage analysis was performed using ICIMapping v.3.3 (Wang, 2013)

using the Kosambi mapping function. Chi square test for goodness of fit was used to detect segregation distortion for all markers, using the 1:1 ratio expected in a doubled haploid mapping population, with markers with p-values less than 0.05 declared distorted. Mapping was performed in a stepwise fashion. In the initial map, all 2116 polymorphic markers that exhibited no segregation distortion markers were step included, using a LOD threshold for group of 6.0, the RECORD ordering algorithm, and the default rippling parameters. In the second step, existing linkage groups were anchored to wheat chromosomes using published data (Roder et al., 1998; Khlestkina et al., 2002; Somers et al., 2004; Carollo et al., 2005; Wilkinson et al., 2012; Cavanagh et al., 2013). Linkage analysis was performed a second time, using the anchoring information and the same parameters described above. For the third step, cosegregating markers were removed from the map, with a single marker representing each bin, linkage analysis was performed with the remaining 124 markers, again with the same parameters.

Statistical analysis and QTL mapping

PROC CORR of SAS version 9.3 (SAS Institute, Cary, NC) was used to calculate Pearson's correlation coefficients. PROC GLM was used to calculate least square (LS) means for each phenotypic trait, which were then used for QTL mapping. QTL mapping was performed with the ICIM-ADD mapping method of ICIMapping version 3.3 (J. Wang, 2013). Default mapping parameters were used with LOD significance threshold of 3.0.

Results

Phenotypic Traits

Phenotypic evaluation FHB (INC, SEV, FDK, DON) and agronomic traits (heading date and height) showed variation among the DH lines (Table 1). Comparisons of the parents for each trait in each environment showed significant differences ($p<0.05$) between MD233 and SS8641 for all but 3 traits (heading date in Salisbury in 2011, plant height in Salisbury in 2012, and FHB incidence in Kinston in 2012). MD01W233-06-1 had consistently lower FHB symptoms and DON concentration than SS8641 across all environments. DH lines showed transgressive segregation with the means of DH lines over- and outperforming parental means. This occurred for all traits in every environment. In 2011 in Kinston, SS8641 had 100% severity, preventing detection of transgressive segregants. In this same environment, SS8641 DON concentration was greater than the DH line range. Pearson correlation coefficients for each trait in each environment were calculated (Table 2). Heading dates measured in Salisbury in 2011 and 2012 had significant correlations with all traits except FHB traits measured in the greenhouse inoculation experiment. Heading dates in 2011 were highly correlated with heading dates, FDK, and DON from Salisbury in 2012 ($r=0.90$, 0.74, and 0.80, respectively). Heading dates in 2012 were also highly correlated with FDK and DON from Salisbury in 2012 ($r=0.71$ and 0.79, respectively). FDK and DON were highly correlated in 4 of 5 environments. In 2011, FDK and DON were highly correlated in both Salisbury ($r=0.80$) and Kinston ($r=0.93$) field experiments and in the single-floret inoculation greenhouse experiment ($r=0.81$). In Kinston in 2012 there was also a highly

significant correlation between FDK and DON (r=0.76). Greenhouse FHB ratings were not highly correlated with field disease ratings, but were highly correlated to each other, with severity correlating to FDK (r=0.81) and DON (r=0.78).

Genetic Linkage Map

There were 8686 potential markers for linkage map construction. After removing unsuccessful and monomorphic markers and testing for segregation distortion, 2116 markers (2091 SNP, 21 SSR, and Rc-D1) were used for initial linkage map construction. Markers mapped to 37 linkage groups, which were then anchored using published consensus maps and reanalyzed, with unanchored makers mapping to linkage groups based on LOD threshold. The subsequent map had 26 linkage groups, 21 corresponding to respective wheat chromosomes with at least 1 marker in each group. Many markers cosegregated, mapping to the same genetic location. A single marker was chosen to represent each locus, leaving 254 informative loci. A final round of linkage analysis with only the unique loci revealed a map with 26 linkage groups, corresponding to each wheat chromosome (Table 3) with 5 single unanchored markers. The map spanned 2334.3cM with an average distance of 9.4cM between markers.

QTL Analysis

A total of 52 significant loci were detected for the 23 traits analyzed. There were 5 regions on 3 chromosomes where QTL for a trait were mapped from multiple environments (Table 4). LOD scores for significant QTL ranged from 3.0 to 19.5, with R^2 values ranging from 6.5 to 45.2%. The only major QTL (R^2>30%) were

associated with heading date in Salisbury from 2011 (R^2=45.2%) and 2012 (R^2=43.5%).

One QTL on the short arm of chromosome 1A (1AS) mapped from 0-1cM (Figure 1), between the SSR marker wmc496 and IWA7021 (SNP index from 9K iSelect Beadchip Assay), which mapped 2.46cM apart. A total of 8 QTL mapped to this region, including repeated QTL for FHB severity from Kinston in 2011 (additive value=8.4%) and 2012 (additive value=6.1%), DON concentration from Salisbury in 2011 (additive value=0.8ppm) and 2012 (additive value=4.5ppm) and from Kinston in 2011 (additive value=7.4ppm), and FHB incidence from Kinston (additive value=6.6%) and Salisbury in 2012 (additive value=8.6%).

On chromosome 3B, there were 3 regions that had QTL for FHB-related traits across environments (Figure 2). There were repeated QTL for FDK that mapped between IWA2493 and IWA3426 from 37-40cM: from Kinston in 2011 (additive value=5.8%) and the greenhouse (additive value=6.2%). On the long arm of chromosome 3B (3BL), 9 QTL mapped to a region between barc164 and IWA1683, spanning from 63-66cM. There were 3 QTL for FHB severity with additive values ranging from 3.6 to 9.5%. There were another 3 QTL in this region for FDK with additive values ranging from 1.4 to 7.4%. There were 2 QTL for DON concentration at the same locus from Salisbury and Kinston in 2011 (additive values of 0.9 and 5.8ppm, respectively). On 3BL at 89cM, 2 QTL for FHB incidence from Salisbury in 2011 and Kinston in 2012 mapped between IWA8043 and IWA786 (additive values of 6.0 and 6.3%, respectively).

Another region on the short arm of chromosome 2D (2DS) between TaPpdDD001 (a KASP marker diagnostic for the photoperiod sensitivity gene Ppd-D1) and IWA3248 had repeated QTL for DON and heading date. Additive values for DON QTL were -6.9% and -3.1% from Salisbury and Kinston, respectively, in 2012.

Discussion

Mapping sources of US native FHB resistance is an important focus of breeding SRWW and other wheat classes. Crossing with exotic, unadapted germplasm has led to introduction of unfavorable agronomic and quality traits (Brown-Guedira et al., 2008), and breeding efforts have relied heavily on a few major effect QTL with use of DNA markers for marker assisted selection (Anderson, 2007). This study was designed to map US native resistance in the SRWW germplasm MD233. The DH population of 124 lines derived from the F_1 progeny from the cross MD233 by SS8641 was evaluated for resistance to FHB in four inoculated field environments and a single-floret inoculation greenhouse experiment. For all measures of FHB disease (incidence, severity, FDK, and DON concentration), MD233 was consistently significantly (except in the case of incidence measured in Kinston in 2012) lower in disease symptoms than the susceptible parent SS8641, suggesting that MD233 was likely to be the source of genetic resistance in the DH lines, as was expected based on resistance reported in Costa et al. (2010).

There were three consistent QTL for FHB and agronomic traits. The QTL on 2DS showed negative additive values for heading in both years at Salisbury, FDK, DON, and plant height in 2012 at Salisbury, and for DON at Kinston in 2012. The

negative additive values indicate that SS8641 contributed to the earlier heading, shorter height, and lower FDK and DON concentrations reported at this locus. These additive values suggest resistance alleles from SS8641. However, FDK and DON from Salisbury in 2012 were highly correlated with heading date in both 2011 and 2012 Salisbury field experiments. Additionally, one of the flanking markers of all the QTL reported is TaPpdDD001, a KASP marker developed for detecting the Ppd-D1 gene, conditioning photoperiod response, suggesting that variation in heading date in the DH lines may be explained by photoperiod response. While it is possible that a closely linked gene could condition susceptibility to FHB in MD233, the correlation of heading date with FHB resistance has long been recognized (Mesterhazy, 1995; Gervais et al., 2003; Somers et al., 2003). Colocalization of the FHB symptom QTL with QTL for heading date in multiple locations suggests that this may be a locus associated with passive resistance, with earlier plants having the SS8641 allele escaping FHB-conducive environmental conditions and appearing to be resistant.

On the short arm of chromosome 1A (AS) repeated QTL were detected for severity, incidence, DON concentration, and also a single QTL for FDK. All QTL were flanked by wmc496 and IWA7021 and mapped between 0 and 1cM. The additive values for these traits were all positive, indicating that MD233 alleles were associated with the more resistant phenotype for these traits. There were no QTL found for heading date or height in this region. Other studies have similarly reported FHB resistance QTL on chromosome 1A. Schmolke et al. (2008) reported coincident QTL for FHB resistance and plant height on chromosome 1A in the G16-92/Hussar recombinant inbred line population (RIL). In their study, the linkage group for 1A

consisted only of amplified fragment length polymorphism markers (AFLP), which prevents identifying the arm of the chromosome associated with the QTL for comparison with the QTL identified on 1AS. The FHB resistance QTL on 1A mapped to the same location as a QTL for plant height. Jiang et al. (2007) were the first to report a QTL for FHB resistance specifically on 1AS in the Veery/CJ 9306 RIL population, with the resistant parent, CJ 9306 contributing resistance alleles. This QTL mapped to a 15cM region between the SSR markers wmc024 and barc148, which mapped to the proximal end of 1AS at 48 and 56cM, respectively, according to the Somers et al. (2004) microsatellite consensus map. Unfortunately, wmc496, which was linked to the 1AS QTL found in this experiment, was not included in the Somers et al. (2004) map, preventing a comparison of the positions of these QTL. Another QTL for FHB resistance has been reported on 1AS in the Becker/Massey RIL population (Liu et al., 2013). This study used Diversity Arrays Technology (DArT) markers combined with SSRs for linkage map construction. A QTL for FHB resistance conferred by the Massey allele was detected in a single environment with a peak LOD score at 36.5cM on 1AS and designated *Qfhs.vt-1AS*. Precise position comparison beyond chromosome arm is difficult due to lack of common markers in both maps.

Another three QTL were identified on chromosome 3B. The linkage map for 3B included two SSR markers reported in the Somers et al. (2004) map: barc147 and barc164, which mapped to 7cM (3BS) and 70cM (3BL), respectively, on the consensus map. In this study, these SSR mapped to 4.0cM and 57.8cM, respectively. One QTL for FDK in Kinston in 2011, FDK from the greenhouse experiment, and

FHB severity from Salisbury in 2012 spanned 37-40cM, mapping between the SSR anchors. This QTL could potentially be on either arm of the chromosome, as there is no reference point for the centromere. FHB resistance has been associated with 3BS, with *Fhb1*, a QTL derived from Chinese spring wheat Sumai 3 representing the most important source of genetic resistance to FHB. Additional sources of resistance have been found on 3B from the SRWW cultivar Ernie (Liu et al., 2007; Abate et al., 2008; Liu et al., 2013) and Massey (Liu et al., 2013).

Costa et al. (2010) tested MD233 with diagnostic markers for the QTL mapped in Ernie and *Fhb1*. They found that it lacked the allele associated with the 3B QTL from both sources. While MD233 is not thought to have these common sources of FHB resistant on 3B, other QTL have been mapped to this same chromosome. Löffler et al. (2009) and Liu et al. (2009) performed meta-QTL analyses, aligning QTL from multiple studies on a single map using shared markers as reference points. Liu et al. (2009) found 3 regions on 3B with QTL conferring FHB resistance. The region centered on *Fhb1*, mapping to 0-21.6cM between the SSR markers fba311 and gwm493. QTL associated with types I, II, III, and IV resistance were mapped to the same region from Asian wheat cultivars including Sumai 3, Ning 7840, and Wangshuibai. The SSR marker barc147 also falls in this region on 3BS, which was not a flanking marker for the QTL identified in this study, suggesting that the resistance from MD233 is not derived from *Fhb1* or another QTL on the distal end of 3BS.

Liu et al. (2009) reported another region with QTL of type II and III resistance from Wangshuibai mapped from 65.3-71.2cM anchored near the centromere between

SSR gwm285 and fab214. A third region showed QTL for types II, III, and IV FHB resistance from SRWW cultivars Ernie and Massey, Chinese spring cultivar Wangshuibai, as well as the European winter wheat cultivars Arina and Apache. This region mapped between fab214 and barc344, spanning from 71.2-85.3cM. Barc164 mapped in this region as well, which was reported on the proximal end of 3BL (Somers et al., 2004). Barc164 was also the left flanking marker for the QTL mapped between 63-66cM in this study that was associated primarily with types II (severity), III (DON), and IV (FDK) resistance. Liu et al. (2013) reported a QTL for type II resistance in the same region named *Qfhb.vt-3BL* which mapped between DArT marker wPt4048 and barc164. This may be the same QTL found in my study.

A third QTL was mapped about 20cM toward the distal end of 3BL at 89cM between the markers IWA8043 and IWA786. MD233 alleles contributed type I resistance with significant QTL for incidence in Salisbury, 2011 and Kinston, 2012 (additive values 6.0% and 6.3%, respectively). Figure 1 suggests that this QTL is separate from the QTL at 63-66cM. Paillard et al. (2004) similarly reported a QTL for type II FHB resistance between *Qfhb.vt-3BL* and the distal end of 3BL. *QFhs.fal-3BL* was detected in 2 environments, mapping to 78cM, between the flanking SSR markers cfa2134b and gwm131b, with resistance alleles contributed by the resistant parent Arina. These flanking markers mapped to a region between 75-78cM on the microsatellite consensus map (Somers et al., 2003).

Conclusions

A total of 5 QTL that were repeated across environments mapped to three chromosomes in the DH population. No QTL were identified in the region associated with *Fhb1*, validating that MD233 does not have this source of exotic FHB resistance. QTL on 2DS were not meaningful in the context of providing active host resistance, the QTL for resistance to DON conferred by SS8641alleles colocalized with QTL for heading date and a diagnostic SNP marker for Ppd-D1. QTL on 1AS and 3B mapped to regions previously reported in other mapping populations. While no novel QTL have been identified, there may be an important contribution in breeding for FHB resistance by identifying better markers for disease resistance in SRWW. Previous studies reporting QTL at these locations have largely relied on microsatellites, AFLPs, and DArT-platform SNP markers. Integration of the publicly available 9K iSelect Beadchip Assay and KASP markers may provide reliable markers for use with marker assisted selection, in contrast to the relatively few SSRs and proprietary DArT markers.

Tables and Figures

Table 1: Mean parent values, doubled haploid population means, and ranges for FHB disease and related agronomic trait ratings. Incidence, severity, FDK, and DON and were evaluated in Salisbury, MD and Kinston, NC in 2011 and 2012. Heading date and height were measured in Salisbury, MD field experiments. Severity, FDK, and DON were measured in a greenhouse single-floret inoculation experiment in College Park, MD in 2011.

Trait	Year	Location	MD01W233-06-1	SS8641	DH Mean	Range
Heading Date (Julian Days)	2011	Salisbury, MD	124.5	125	125.3	122.3 - 131.7
	2012	Salisbury, MD	143.2	138.3***	143.1	135.3 - 157.3
Height (cm)	2011	Salisbury, MD	95.9	101.6**	94.5	67.7 - 108.4
	2012	Salisbury, MD	87.2	84.9	83.2	67.7 - 98.2
Incidence (%)	2011	Salisbury, MD	24.6	66.7***	33.1	6.7 - 80
		Kinston, NC	95	100*	96.8	75 - 100
	2012	Salisbury, MD	20.4	72.5***	36.6	6.7 - 86.7
		Kinston, NC	44.1	55.5	50.8	12.6 - 94.7
Severity (%)	2011	Salisbury, MD	4.3	35.3***	12.5	1.7 - 50
		Kinston, NC	28.8	97.5***	61	14.8 - 100
		College Park, MD	21.3	88.7***	57.2	11 - 100
	2012	Salisbury, MD	12.1	64.2***	23.1	6.7 - 66.7
		Kinston, NC	20.1	75.6***	33.6	7.5 - 95
FDK (%)	2011	Salisbury, MD	2.9	16.5**	5.7	0.8 - 24.2
		Kinston, NC	10.3	10.3***	20.5	2.1 - 89.3
		College Park, MD	27.1	90.9***	52.5	9.3 - 91.3
	2012	Salisbury, MD	11.8	40.9***	23.2	2.7 - 81.3
		Kinston, NC	7.6	37.3***	26	4.3 - 97.3
DON (ppm)	2011	Salisbury, MD	1.5	10**	3.4	0.1 - 13.2
		Kinston, NC	10.9	110.3***	26	3 - 97.3
		College Park, MD	21.1	363.8**	126.9	2.5 - 529.2
	2012	Salisbury, MD	6.7	23***	16.9	1.8 - 108.3
		Kinston, NC	10.2	26.7**	13.1	3.7 - 38.7

*Mean values of MD233 and SS8641 significantly different at p=0.05
**Mean values of MD233 and SS8641 significantly different at p=0.01
***Mean values of MD233 and SS8641 significantly different at p<0.0001

Table 2: Correlation coefficients of phenotypic traits. Correlations from Salisbury, MD and Kinston, NC field experiments and greenhouse (GH) inoculation experiment in 2011 and 2012. FHB incidence (INC), severity (SEV) *Fusarium* damaged kernel (FDK), deoxynivalenol concentration (DON), heading date (Hd), and height (ht) were analyzed. Coefficients appear in color coded boxes, with blue and red indicating higher and lower correlation, respectively. Below each coefficient is the associated p-value.

Trait	2011 MD-Hd	2011 MD-INC	2011 MD-SEV	2011 MD-Ht	2011 MD-FDK	2011 MD-DON	2011 NC-INC	2011 NC-SEV	2011 NC-FDK	2011 NC-DON	2012 MD-Hd	2012 MD-Ht	2012 MD-INC	2012 MD-SEV	2012 MD-FDK	2012 MD-DON	2012 NC-INC	2012 NC-SEV	2012 NC-FDK	2012 NC-DON	2011 GH-SEV	2011 GH-FDK
2011MD-INC	-0.23																					
	0.01																					
2011MD-SEV	-0.21	0.87																				
	0.02	<.0001																				
2011MD-Ht	0.2	-0.47	-0.47																			
	0.03	<.0001	<.0001																			
2011MD-FDK	0.43	0.45	0.47	-0.36																		
	<.0001	<.0001	<.0001	<.0001																		
2011MD-DON	0.47	0.48	0.5	-0.24	0.8																	
	<.0001	<.0001	<.0001	0.01	<.0001																	
2011NC-INC	0.19	0.23	0.24	-0.18	0.22	0.29																
	0.04	0.02	0.01	0.06	0.02	0																
2011NC-SEV	0.12	0.5	0.5	-0.22	0.38	0.5	0.63															
	0.19	<.0001	<.0001	0.02	<.0001	<.0001	<.0001															
2011NC-FDK	0.27	0.46	0.55	-0.27	0.52	0.57	0.4	0.63														
	0	<.0001	<.0001	0	<.0001	<.0001	<.0001	<.0001														
2011NC-DON	0.4	0.39	0.45	-0.19	0.54	0.59	0.42	0.67	0.93													
	<.0001	<.0001	<.0001	0.05	<.0001	<.0001	<.0001	<.0001	<.0001													
2012MD-Hd	0.9	-0.25	-0.25	0.27	0.34	0.37	0.2	0.03	0.15	0.26												
	<.0001	0.01	0	0	0	<.0001	0.03	0.74	0.11	0.01												
2012MD-Ht	0.4	-0.45	-0.45	0.63	-0.14	-0.11	-0.16	-0.31	-0.22	-0.14	0.44											
	<.0001	<.0001	<.0001	<.0001	0.11	0.21	0.1	0	0.02	0.13	<.0001											
2012MD-INC	0.4	0.38	0.41	-0.08	0.55	0.53	0.42	0.52	0.49	0.54	0.35	0.03										
	<.0001	<.0001	<.0001	0.37	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.76										
2012MD-SEV	0.14	0.38	0.38	-0.1	0.39	0.42	0.35	0.46	0.53	0.53	0.12	-0.01	0.55									
	0.12	<.0001	<.0001	0.29	<.0001	<.0001	0	<.0001	<.0001	<.0001	0.19	0.9	<.0001									
2012MD-FDK	0.74	0.15	0.18	0.07	0.61	0.62	0.41	0.5	0.63	0.69	0.71	0.19	0.73	0.49								
	<.0001	0.11	0.04	0.43	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.04	<.0001	<.0001								
2012MD-DON	0.8	-0.03	0	0.21	0.52	0.57	0.31	0.36	0.45	0.56	0.79	0.25	0.58	0.37	0.91							
	<.0001	0.71	0.96	0.02	<.0001	<.0001	0	<.0001	<.0001	<.0001	<.0001	0.01	<.0001	<.0001	<.0001							
2012NC-INC	0.33	0.52	0.5	-0.19	0.48	0.57	0.37	0.52	0.43	0.44	0.26	-0.09	0.62	0.44	0.63	0.58						
	0	<.0001	<.0001	0.06	<.0001	<.0001	0	<.0001	<.0001	<.0001	0.01	0.35	<.0001	<.0001	<.0001	<.0001						
2012NC-SEV	0.21	0.57	0.65	-0.37	0.54	0.57	0.33	0.52	0.63	0.59	0.07	-0.27	0.64	0.53	0.67	0.48	0.66					
	0.04	<.0001	<.0001	0	<.0001	<.0001	0	<.0001	<.0001	<.0001	0.51	0.01	<.0001	<.0001	<.0001	<.0001	<.0001					
2012NC-FDK	0.22	0.55	0.68	-0.47	0.62	0.63	0.32	0.5	0.64	0.6	0.1	-0.31	0.54	0.4	0.6	0.45	0.56	0.76				
	0.03	<.0001	<.0001	<.0001	<.0001	<.0001	0	<.0001	<.0001	<.0001	0.3	0	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001				
2012NC-DON	0.48	0.42	0.37	-0.25	0.48	0.55	0.31	0.37	0.39	0.42	0.52	0.07	0.61	0.32	0.71	0.7	0.58	0.43	0.55			
	<.0001	<.0001	0	0.01	<.0001	<.0001	0	0	<.0001	<.0001	<.0001	0.5	<.0001	0	<.0001	<.0001	<.0001	<.0001	<.0001			
2011GH-SEV	-0.07	0.36	0.42	-0.21	0.18	0.31	0.08	0.32	0.4	0.33	-0.15	-0.19	0.27	0.37	0.19	0.07	0.37	0.56	0.38	0.25		
	0.46	<.0001	<.0001	0.02	0.04	0	0.42	0	<.0001	0	0.09	0.04	0	<.0001	0.04	0.47	0	<.0001	0	0.01		
2011GH-FDK	0.09	0.39	0.45	-0.24	0.29	0.42	0.19	0.45	0.51	0.47	0.03	-0.23	0.39	0.4	0.34	0.24	0.41	0.57	0.46	0.29	0.81	
	0.31	<.0001	<.0001	0.01	0	<.0001	0.04	<.0001	<.0001	<.0001	0.77	0.01	<.0001	<.0001	0	0.01	<.0001	<.0001	<.0001	0	<.0001	
2011GH-DON	0.04	0.41	0.48	-0.24	0.35	0.44	0.1	0.36	0.55	0.49	-0.03	-0.2	0.33	0.41	0.28	0.17	0.32	0.55	0.45	0.25	0.78	0.8
	0.68	<.0001	<.0001	0.01	<.0001	<.0001	0.3	<.0001	<.0001	<.0001	0.7	0.03	0	<.0001	0	0.06	0	<.0001	<.0001	0.01	<.0001	<.0001

Table 3: Linkage group composition. Linkage groups with the number of markers in each group and the length of each group in centiMorgans (cM)

Chromosome	Number of Markers	Length (cM)
1A	10	71.2
2A	18	178.3
3A	17	150.5
4A	13	199.0
5A	18	175.4
6A	9	110.9
7A	18	169.2
1B	17	112.1
2B	17	111.6
3B	15	144.8
4B	13	70.3
5B	15	115.3
6B	17	118.3
7B	11	143.0
1D	8	74.1
2D	10	93.8
3D	2	66.4
4D	1	0.0
5D	6	113.5
6D	4	79.3
7D	5	37.3
Total	249	2334.3

Table 4: Significant QTL positions, with flanking markers, LOD scores, R^2 values and additive effects. FHB incidence and severity, FDK, DON concentration were measured in the field in Salsibury, MD and Kinston, NC in 2011 and 2012. Plant height and heading date were also evaluated in Salisbury. Severity, FDK and DON were evaluated in a single floret inoculation experiment performed in a greenhouse at College Park, MD.

Trait	Environment	Year	Chromosome	Position (cM)	Left Flanking Marker	Right Flanking Marker	LOD	R^2 (%)	Additive Effect
Severity	Kinston, NC	2011	1AS	0	wmc496	IWA7021	5.3	14.0	8.4
Severity	Kinston, NC	2012	1AS	0	wmc496	IWA7021	4.2	10.9	6.1
DON	Salisbury, MD	2011	1AS	0	wmc496	IWA7021	4.7	11.3	0.8
DON	Kinston, NC	2011	1AS	0	wmc496	IWA7021	6.4	17.2	7.4
DON	Salisbury, MD	2012	1AS	0	wmc496	IWA7021	4.0	7.6	4.5
Incidence	Kinston, NC	2012	1AS	0	wmc496	IWA7021	3.4	11.3	6.6
Incidence	Salisbury, MD	2012	1AS	1	wmc496	IWA7021	6.3	15.2	8.6
FDK	Salisbury, MD	2012	1AS	1	wmc496	IWA7021	7.4	12.2	5.3
FDK	Kinston, NC	2011	3B	37	IWA2493	IWA3426	3.9	17.2	5.8
FDK	College Park, MD	2011	3B	38	IWA2493	IWA3426	4.2	11.7	6.2
Severity	Salisbury, MD	2012	3B	40	IWA2493	IWA3426	3.1	9.9	3.8
Height	Salisbury, MD	2012	3BL	57	IWA4575	barc164	3.7	9.5	-1.7
Severity	Kinston, NC	2011	3BL	63	barc164	IWA1683	6.1	17.7	9.5
Severity	Salisbury, MD	2011	3BL	66	barc164	IWA1683	4.2	14.6	3.6
Severity	Kinston, NC	2012	3BL	66	barc164	IWA1683	5.1	13.4	6.8
FDK	Salisbury, MD	2011	3BL	66	barc164	IWA1683	6.1	14.6	1.4
FDK	Salisbury, MD	2012	3BL	66	barc164	IWA1683	4.3	6.5	3.9
FDK	Kinston, NC	2012	3BL	66	barc164	IWA1683	4.9	20.1	7.4
DON	Salisbury, MD	2011	3BL	66	barc164	IWA1683	5.3	13.0	0.9
DON	Kinston, NC	2011	3BL	66	barc164	IWA1683	4.1	10.4	5.8
Incidence	Salisbury, MD	2012	3BL	66	barc164	IWA1683	5.0	11.7	7.6
Incidence	Salisbury, MD	2011	3BL	89	IWA8043	IWA786	4.8	16.6	6.0
Incidence	Kinston, NC	2012	3BL	89	IWA8043	IWA786	3.0	10.2	6.3
DON	Salisbury, MD	2012	2DS	47	TaPpdDD001	IWA3248	8.4	17.9	-6.9
Height	Salisbury, MD	2012	2DS	49	TaPpdDD001	IWA3248	6.3	18.5	-2.3
FDK	Salisbury, MD	2012	2DS	49	TaPpdDD001	IWA3248	9.8	17.6	-6.4
Heading	Salisbury, MD	2012	2DS	49	TaPpdDD001	IWA3248	18.7	43.5	-4.5
Heading	Salisbury, MD	2011	2DS	50	TaPpdDD001	IWA3248	19.5	45.2	-1.7
DON	Kinston, NC	2012	2DS	62	TaPpdDD001	IWA3248	6.0	20.1	-3.1

*Positive and negative additive effects indicate resistance contributed by MD01W233-06-1and SS8641 alleles, respectively.

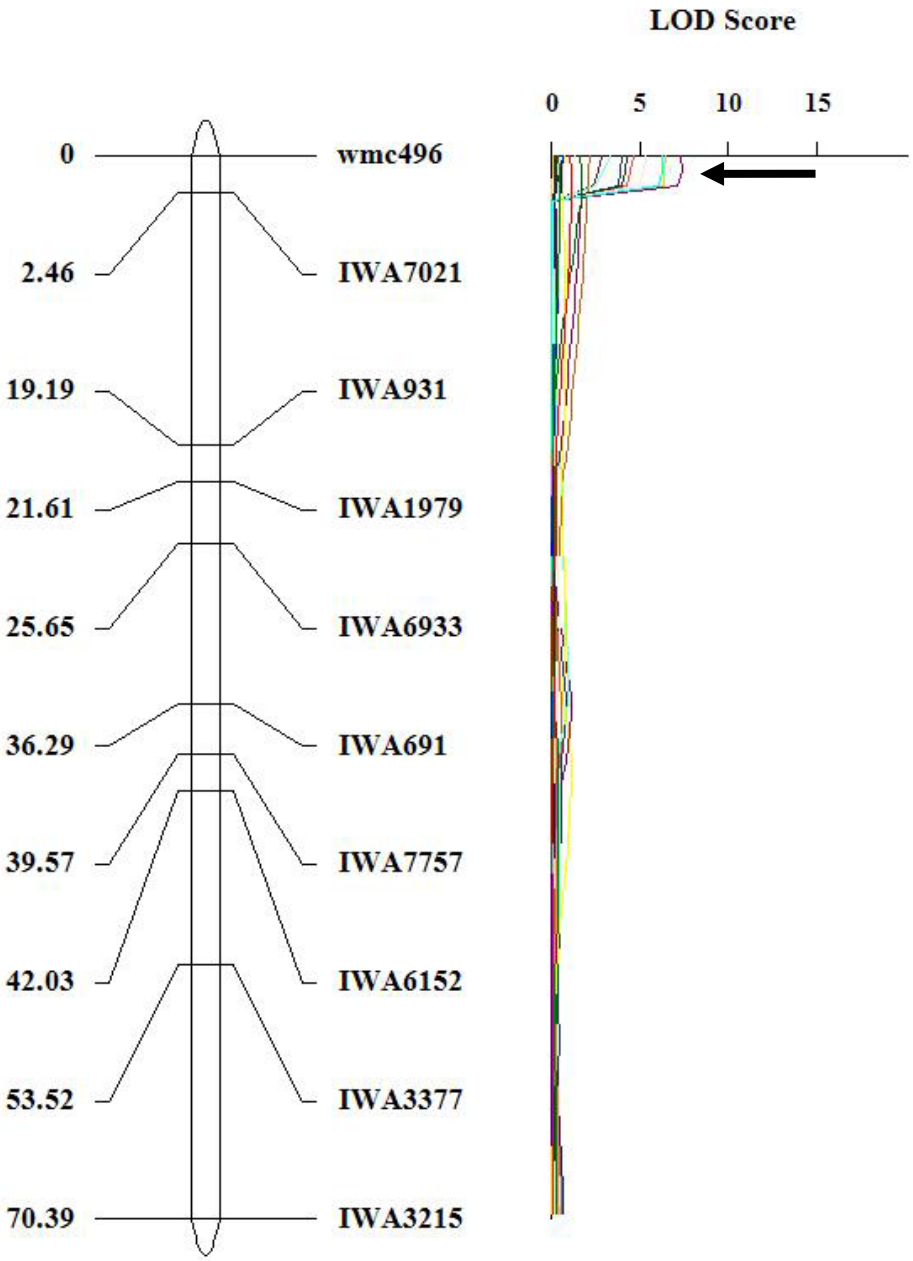

Figure 1: Chromosome 1A linkage group with LOD score plot. The black arrow indicates QTL with resistance alleles contributed by MD01W233-06-1.

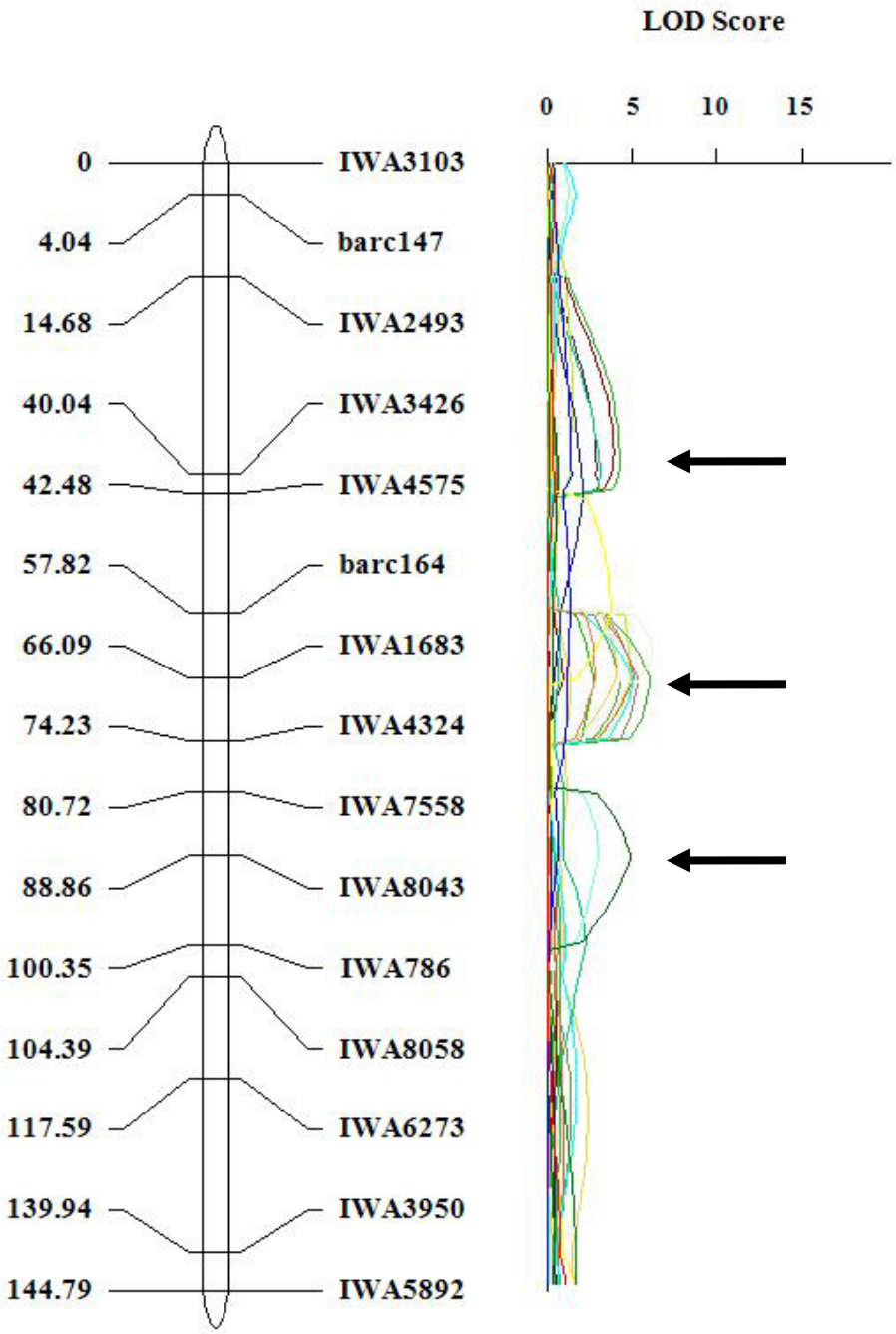

Figure 2: Chromosome 3B linkage group with LOD score plot. The black arrows indicate QTL with resistance alleles contributed by MD01W233-06-1.

Chapter 3: Mapping Leaf Rust Quantitative Resistance

Introduction

Leaf rust (*Puccinia triticina* f. sp. *tritici* Eriks.) is a common foliar pathogen of wheat (*Triticum aestivum* L. ssp. *aestivum*). This fungus was responsible for $350 million in losses in the US alone from 2000-2004 (Huerta-Espino et al., 2011). Yield reductions in susceptible cultivars typically range from 5-15% depending on host growth stage at time of initial infection (Kolmer, 1996), although losses as high as 50% have been reported (Huerta-Espino et al., 2011). Like other rusts, *P. triticina* has a complex life cycle, relying on wheat as its primary host and meadow rue (*Thalictrum speciosissimum* L.) as its alternate host, on which the sexual stages of reproduction occur (Bolton et al., 2008). The absence of suitable alternate hosts outside of Eurasia eliminates sexual reproduction for the lifecycle of *P. triticina*, and reproduction occurs clonally, with successive generations of asexual spore production on wheat plants (Ordoñez & Kolmer, 2009). Mutation occurs frequently, giving rise to new physiological races of leaf rust, defined by their virulence to known wheat leaf rust resistance genes (*Lr* genes). Leaf rust can be controlled with fungicides, although planting resistant cultivars is a more environmentally sustainable and economically practical management strategy.

Over 60 *Lr* genes have been identified (Cereal Disease Laboratory, 2013), the majority of which confer seedling resistance. Seedling resistance *Lr* genes normally behave in a gene-for-gene relationship with a complementary leaf rust avirulence (*avr*) gene. Mutations in *avr* genes can alter the interaction between *Lr* and *avr*

genes, producing new leaf rust races with virulence to previously effective *Lr* genes. In this way, *Lr1*, *Lr2a*, *Lr9*, *Lr17*, *Lr22*, *Lr24*, *Lr26*, and *Lr41* have become ineffective in many US wheat growing regions (Kolmer et al., 2009). Breeders rely on identifying new *Lr* genes and pyramiding multiple *Lr* genes within the same cultivar to make leaf rust resistance more robust and durable.

The objective of this study was to map an unknown source of leaf rust resistance in the doubled haploid mapping population derived from the F_1 cross of MD01W233-06-1 and SS8641. MD01W233-06-1 has been postulated to have *Lr1* and *Lr24* (Costa et al., 2010), while SS8641 has been postulated to have *Lr37* and an additional, unknown source of resistance (Cereal Disease Laboratory, 2014). Mapping this potentially novel source of resistance will provide breeders with markers for selection in germplasm adapted to the US soft red winter wheat growing region.

Materials and Methods

Plant Materials

A soft red winter wheat doubled haploid (DH) mapping population of 135 lines was developed from F_1 progeny of the cross MD01W233-06-1 (hereafter referred to as MD233; pedigree: McCormick/Choptank) and Southern States (SS) 8641 (pedigree: GA 881130/2*GA 881582), made in the greenhouse at the University of Maryland, College Park. The population was made by Dr. J. Paul Murphy of North Carolina State University in 2009 using the wheat x maize wide cross method. The population was initially designed to map *Fusarium* head blight resistance, with MD233 as the resistant parent. In early field experiments, DH lines showed

segregation for resistance to leaf rust in the field. During initial seed increases and preliminary genotyping, DH lines with extremely late heading dates and heterozygous marker genotypes were eliminated from the population, reducing the number of DH lines to a total of 124.

Phenotypic Evaluation for Leaf Rust Field Resistance

The population was evaluated in both field and greenhouse experiments. The population was planted over 4 locations over the 2011-2012 and 2012-2013 field seasons. In 2011-2012, the population was evaluated at the University of Maryland Lower Eastern Shore Research and Education Center in Salisbury, MD; at the Louisiana State University Central Research Station in Baton Rouge, LA, and in experimental fields of DONMARIO Semillas® in Nueve de Julio, Buenos Aires, Argentina. In 2012-2013, the population was evaluated again in Baton Rouge, and at the North Carolina State University Vernon G. James Research and Extension Center in Plymouth, NC. The DH lines and parents were planted in 1.2m single-row plots in randomized complete block designs. In Salisbury and Nueve de Julio, 3 blocks were planted, while in Baton Rouge and Plymouth, space limitation allowed only 2 blocks to be planted. Plots were infected with natural inoculum and were evaluated after heading and before physiological maturity for leaf rust symptoms. A quantitative scale (0-4) as described by (Chu et al., 2009) was used to measure leaf rust severity. In Baton Rouge in 2012, leaf rust severity was measured using a 0-100% modified Cobb's scale (Peterson et al., 1948).

Phenotypic Evaluation for Seedling Resistance

The population and parents were also evaluated for seedling resistance at the Cereal Disease Laboratory in St. Paul, MN. Screening was performed by Dr. James Kolmer using isolates of leaf rust races BBBD (Race 1) and TNRJ as described in Oelke and Kolmer (2004). Infection types were evaluated as described in Long and Kolmer (1989) and classified as having either a susceptible or a resistant host response.

DNA Extraction and Marker Analysis

The DH population and parents were planted in 96-cell planting trays to collect leaf tissue for DNA extraction. Tissue samples were cut from seedlings of the 124 DH lines and parents at the 2-leaf stage. DNA extraction was performed as described in Kang et al. (2011) by Dr. Gina Brown-Guedira at the USDA Eastern Small Grains Genotyping Lab in Raleigh, NC. The population was screened with twenty-nine short sequence repeat (SSR) markers as described in Kang et al. (2011) by Dr. Brown-Guedira. The SSRs, listed in the previous chapter, were selected from Roder et al. (1998), Somers et al. (2004), and Song et al. (2005). The 9K iSelect Beadchip Assay was used to genotype the population and parents with the assay performed as described in Cavanagh et al. (2013) by Dr. Shiaoman Chao at the USDA Northern Central Small Grains Genotyping Lab in Fargo, ND. SNP genotyping calls were made using GenomeStudio v2011.1 software (Illumina, San Diego, CA) as described in Cavanagh et al. (2013) by Dr. Brown-Guedira. The population was also genotyped using KASP (Kompetitive Allele Specific PCR) assays (LGC Genomics, Middlesex, UK). KASP markers were selected from

markers designed by the regional genotyping lab (IWB49398, TaPpdDD001, sbv5D_6060) and selected markers listed in the previous chapter from Wilkinson et al. (2012). In addition to molecular markers, the population segregated for coleoptile color, a morphological marker. MD233 has a red coleoptile, while SS8641 has a green coleoptile. The population was evaluated for this trait after emergence of coleoptiles and prior to emergence of the first true leaf.

Linkage Mapping

2091 SNPS (from 9k and selected KASP markers), 21 SSR markers, and the Rc-D1 (red coleoptile) morphological markers were used for initial linkage map construction. Linkage analysis was performed using ICIMapping v.3.3 (Wang, 2013) using the Kosambi mapping function. Initial map construction incorporated the 2116 polymorphic markers using a LOD threshold for group of 6.0, the RECORD ordering algorithm, and the default rippling parameters. Linkage groups in the initial map were anchored to wheat chromosomes using published data (Roder et al., 1998; Khlestkina et al., 2002; Somers et al., 2004; Carollo et al., 2005; Wilkinson et al., 2012; Cavanagh et al., 2013). With the same mapping parameters described above, linkage analysis was performed a second time with anchoring information included. Cosegregating markers were then removed from the map, with a single marker representing each bin. Linkage analysis was subsequently performed with the resultant 124 markers, again with the same parameters.

Data Analysis and QTL Mapping

PROC GLM and PROC CORR of SAS version 9.3 (SAS Institute, Cary, NC) were used to calculate least square (LS) means and Pearson correlation coefficients,

respectively. PROC FREQ was used to perform Chi-square tests for goodness of fit of the seedling host response ratios. Ratios of resistant to susceptible DH lines were compared to expected segregation ratios for single-gene (1:1) and 2-gene (3:1) hypotheses. QTL mapping was performed using LS means with the ICIM-ADD mapping method of ICIMapping version 3.3 (Wang, 2013). Default mapping parameters were used with a LOD significance threshold of 3.0.

Results

Phenotypic Data

The population showed variation for leaf rust in all experiments (Table 5). Comparisons of the parents for each trait in each environment showed significant differences ($p<0.01$) between MD233 and SS8641 in Salisbury and Baton Rouge in 2012. With only 2 locations showing significant differences between the parents, DH lines showed transgressive segregation for leaf rust resistance in every environment. Pearson correlation coefficients for each trait in each environment were calculated (Table 2). Correlations for all traits were highly significant ($p<0.0001$). Leaf rust severities from Salisbury in 2012 were strongly correlated with those in Baton Rouge ($r=0.83$) and Plymouth ($r=0.82$) in 2013. These locations were also highly correlated ($r=0.82$). In general, the field locations were highly correlated with each other ($0.58<r<0.83$). The parents segregated for reaction to TNRJ, but both were resistant to BBBD. Seedling reaction types using single leaf rust isolates were analyzed for goodness of fit using the Chi-square test. The population did not fit the segregation ratio for resistance controlled by a single gene (1:1 resistant to susceptible) for either isolate. The DH lines did fit the segregation ratio for resistance controlled by two

genes (3:1 resistant to susceptible) for TNRJ, indicating two *Lr* genes may be responsible for segregation of resistance to leaf rust in this population.

Linkage Analysis

From the 8686 potential markers for linkage map construction, 2116 markers (2091 SNP, 21 SSR, and Rc-D1) were found to be polymorphic, with genotypes segregating in the expected 1:1 ratio. These were used for initial linkage map construction. These markers formed 37 linkage groups. SSR and SNP consensus maps were used to anchor these linkage groups to wheat chromosomes. These were reanalyzed, with unanchored makers mapping to linkage groups based on LOD threshold. The subsequent map had 26 linkage groups, 21 corresponding to respective wheat chromosomes with at least 1 marker in each group. There were many cosegregating markers, with multiple markers mapping to the same position. A single marker was chosen to represent each position 254 markers. A final round of linkage analysis with the unique loci revealed a map with 26 linkage groups, again corresponding to each chromosome (Table 3) with 5 single unanchored markers. The map spanned 2334.3cM with an average distance of 9.4cM between markers.

QTL Mapping

QTL analysis revealed 10 significant loci mapping to 2 distinct regions on the short arm of chromosome 2A (2AS) and the long arm of chromosome 5B (5BL). The QTL on 2AS was associated with leaf rust measured in all environments. The QTL detected mapped to the distal end of 2AS, between the flanking markers IWA3699 and IWA1563 at 0cM. QTL had high LOD scores, ranging from 5.82 for resistance measured in Baton Rouge in 2012 to a LOD score of 18.35 for resistance to leaf rust

in Salisbury 2012. R^2 values ranged from 14.01% to 44.78%. The QTL on 2AS had negative additive effects in every environment, indicating that l the SS8641 allele had contributed to lower leaf rust severity than the MD233 allele.

A QTL on 5BL was associated with leaf rust resistance in all environments. All additive effects for this QTL were again negative, indicating the SS8641 allele contributed resistance. The QTL spanned the distal end of 5BL from 95cM to 114cM. Leaf rust resistance from Baton Rouge in 2012 and Plymouth in 2013 both mapped to 95cM between IWA3972 and barc59, with LOD scores of 3.45 and 9.95, respectively. Resistance from Baton Rouge in 2012 mapped to 101cM, between barc59 and IWA936, with a LOD score of 12.05. Resistance measured in Salisbury mapped between IWA936 and IWA37, to 106cM. The Salisbury resistance QTL had a LOD score of 6.81 and R^2 of 13.79%. Resistance from Nueve de Julio mapped between IWA37 and IWA22 at 114cM. LOD score for the Nueve de Julio resistance was 17.53 and R^2 of 41.16%.

Discussion

The objective of this study was to map a novel source of resistance to leaf rust in the doubled haploid mapping population derived from the F1 cross of MD233 by SS8641. This population was originally designed to map *Fusarium* head blight resistance, with MD233 contributing the resistant phenotype, and SS8641 the susceptible. Costa et al. (2010) reported that MD233 was postulated to have *Lr1* and *Lr24/Sr24*, showing resistance to all leaf rust races evaluated (QFCS, QTHJ, RCRS, RKQQ, TPMK, TTTT, TTKSK, TTKST, and TTTSK), except for race TNRJ which has reported virulence for resistance genes *Lr1, 2a, 2c, 3, 3ka, 9, 10, 11, 14a, 24*, and

30. SS8641 was postulated to have *Lr37* and unknown additional sources of resistance (Cereal Disease Laboratory, 2014).

Inoculation of seedlings with TNRJ and BBBD revealed two important pieces of information. First, MD233 was again found to be susceptible to TNRJ, confirming previous results from Costa et al. (2010). Additionally, evaluation of reaction type (resistant or susceptible) in the population that indicated resistance to BBBD and TNRJ was not controlled by a single gene. The reaction type ratio of the DH lines did obey the 3:1 ratio for resistance suggesting that resistance is controlled by two genes for TNRJ.

Three *Lr* genes have been postulated in the parents of the population, with MD233 contributing *Lr1* and *Lr24* and SS8641 contributing Lr37 and an additional unidentified source of resistance. As shown in Table 5, neither parent was particularly susceptible to leaf rust in field experiments with natural inoculum. *Lr1* has been mapped to chromosome 5D, cloned, and characterized as having coiled coil (CC), nucleotide-binding site (NBS), and leucine-rich-repeat (LRR) motifs (Cloutier et al., 2007). No QTL mapped to chromosome 5D in my study. Furthermore, Kolmer et al. (2009) reported *Lr1* has been rendered ineffective due to widespread deployment of this gene and subsequent development of virulent races. Taken together, these results suggest the segregating resistance in this population is not conferred from *Lr1*.

Lr24 was originally derived from tall wheatgrass (*Thinopyrum ponticum* (Podp.) Barkworth & D. R. Dewey), the result of a natural translocation from wheatgrass which was mapped to the distal end of the long arm of wheat chromosome

3D (Schachermayr et al., 1995). Kolmer et al. (2009) also reported *Lr24* has been rendered ineffective in much of US wheat production. Additionally, Kolmer et al. (2008) specifically noted that leaf rust races were virulent to *Lr24* in the SRWW McCormick (one of the parents of MD233). There were no QTL for leaf rust resistance found on 3D in my study. This evidence, coupled with the reported widespread ineffectiveness of *Lr24* in the SRWW-growing region, suggests this gene is not responsible for segregation of leaf rust resistance in this population.

A significant QTL on chromosome 2AS was mapped between IWA3699 and IWA1563 from 0-1cM. The additive effects for the leaf rust resistance within this QTL were negative in every environment tested, indicating that resistance was conferred by the SS8641 alleles at this locus. *Lr11*, *Lr17*, *Lr37*, and *Lr65* have all been mapped to chromosome 2AS (Cereal Disease Laboratory, 2013). Of these, *Lr37* has been postulated to confer resistance in SS8641. *Lr37* is derived from *Aegilops ventricosa* (Zhuk.) Chennav located on the 2NS/2AS translocation, which has been mapped to the distal end of 2AS (Błaszczyk et al., 2004; Helguera et al., 2003). *Lr11* and *Lr17* have been reported as largely ineffective to virulent races of *P. triticina* (Kolmer et al., 2009). Y. Wang et al. (2010) and Mohler et al. (2012) mapped *Lr65*, originally derived from spelt (*Triticum aestivum* L. ssp. *spelta*), to the distal end of 2AS. Direct comparison of these gene locations was not possible due to lack of markers common to both maps. However, *Lr37* has been a reliable source of resistance throughout the southeastern US due to selection specifically for this gene based on reliable PCR markers (Helguera et al., 2003) and long term effectiveness of

this gene in combination with *Lr* genes (Goyeau & Lannou, 2011), suggesting that *Lr37* conferred resistance at the 2AS QTL in this population.

A significant QTL was found on chromosome 5BL with additive effects indicating the resistant alleles were contributed by SS8641. This QTL was associated with leaf rust resistance from all environments, mapping between IWA3972 and IWA22 from 95-114cM. To date there has been one *Lr* gene reported on 5BL. *Lr18* was derived from *Triticum timopheevii* (Zhuk.) (Friebe et al., 1996). Leonova et al. (2011) mapped leaf rust resistance in several mapping populations with *T. aestivum-T. timopheevii* introgression lines used as the resistant parents. Resistance was mapped to QTL that mapped to chromosomes 1AL, 2AL, and 5BL, explaining 8.0, 11.5, and 64% of the phenotypic variation for leaf rust resistance. Their study mapped a leaf rust resistance QTL to the same region as *Lr18*, temporarily designated *LrTt2*, as it was unclear if this locus was the same allele for *Lr18* or a novel allele. It has been reported that *Lr18* is deployed in SRWW cultivars in the southeastern US (Kolmer et al., 2009), with 15.1% of *P. triticina* isolates collected in 2007 showing virulence to this gene. The resistance QTL on 5BL in my study may be due to the previously unreported presence of *Lr18* in SS8641 or a novel gene. Precise comparison of the location within 5BL was not possible due to a lack of common markers in these two populations.

Conclusions

Two QTL for leaf rust resistance that were repeated across environments mapped to wheat chromosomes 2AS and 5BL in the DH population. There have been several reported *Lr* genes in these regions. *Lr37*, the postulated source of resistance from SS8641, has been mapped to 2AS. However, *Lr18* is the only *Lr* gene previously mapped to 5BL, and has not been postulated to be in either parent. This suggests the 5BL QTL is an allele of *Lr18*, previously unreported in either parent, or may be a novel gene mapping to the same region.

Tables and Figures

Table 5: Mean parent values, doubled haploid population means, and ranges for leaf rust resistance. Leaf rust restance was evaluated in field experiments with natural inoculum on a 0-4 scale in Salisbury, MD; Baton Rouge, LA; and Nueve de Julio, Argentina in 2012 and in Plymouth, NC and Baton Rouge, LA in 2012Leaf rust severity was rated on a 0-100% scale in Baton Rouge in 2012.

Location	Year	MD01W233-06-1	SS8641	DH Mean	DH Range
Baton Rouge, LA	2012	22.50	0.00*	14.25	0 - 80
Nueve de Julio, Buenos Aires, Argentina	2012	0.50	0.50	1.40	0 - 4
Salisbury, MD	2012	0.53	0.25*	1.17	0 - 4
Baton Rouge, LA	2013	0.56	0.00	1.06	0 - 4
Plymouth, NC	2013	0.56	0.38	1.39	0 - 4

*Mean values of MD233 and SS8641 significantly different at p=0.01

Table 6: Correlation coefficients of leaf rust resistance. Correlations for leaf rust resistance ratings from Salisbury, MD; Baton Rouge, LA; Nueve de Julio, Argentina; and Plymouth, NC field experiments.

	Salisbury 2012	Baton Rouge 2013	Plymouth 2013	Baton Rouge 2012
Baton Rouge 2013	0.83*			
Plymouth 2013	0.84	0.82		
Baton Rouge 2012	0.69	0.68	0.59	
Nueve de Julio 2012	0.70	0.76	0.77	0.52

*All correlation coefficients were significant ($p<0.0001$)

Table 7: Single isolated seedling screening reaction type qualitative analysis. Reaction types from the parents and doubled haploid line inoculated with leaf rust isolates BBBD and TNRJ. Lines were rated as resistant (R) or susceptible (S). Chi square test for goodness of fit was performed to test 1-gene or 2-gene hypotheses.

	Reaction Type		DH Reaction Types		χ^2 p-value	
Isolate	MD01W233-06-1	SS8641	R	S	1 gene (1:1)	2 gene (3:1)
BBBD	R	R	113	11	*	*
TNRJ	S	R	89	31	*	0.41

* χ^2 statistic significant at $p<0.0001$

Table 8: Significant QTL positions, with flanking markers, LOD scores, R^2 values and additive effects. Leaf rust restance was evaluated in field experiments with natural inoculum on a 0-4 scale in Salisbury, MD; Baton Rouge, LA; and Nueve de Julio, Argentina in 2012 and in Plymouth, NC and Baton Rouge, LA in 2012.

Chr.	Position (cM)	Year	Location	Left Flanking Marker	Right Flanking Marker	LOD	R^2 (%)	Additive Value
2AS	0	2012	Salisbury, MD	IWA3699	IWA1563	18.35	44.78	-0.76
2AS	0	2012	Baton Rouge, LA	IWA3699	IWA1563	5.82	15.83	-6.93
2AS	0	2012	Nueve de Julio, BA	IWA3699	IWA1563	7.31	14.01	-0.49
2AS	0	2013	Plymouth, NC	IWA3699	IWA1563	11.56	25.35	-0.62
2AS	0	2013	Baton Rouge, LA	IWA3699	IWA1563	9.94	21.37	-0.59
5BL	95	2012	Baton Rouge, LA	IWA3972	barc59	3.45	8.98	-5.18
5BL	95	2013	Plymouth, NC	IWA3972	barc59	9.95	21.16	-0.56
5BL	101	2013	Baton Rouge, LA	barc59	IWA936	12.05	27.39	-0.66
5BL	106	2012	Salisbury, MD	IWA936	IWA37	6.81	13.79	-0.42
5BL	114	2012	Nueve de Julio, BA	IWA37	IWA22	17.53	41.16	-0.85

***Positive and negative additive effects indicate resistance contributed by MD01W233-06-1and SS8641 alleles, respectively.**

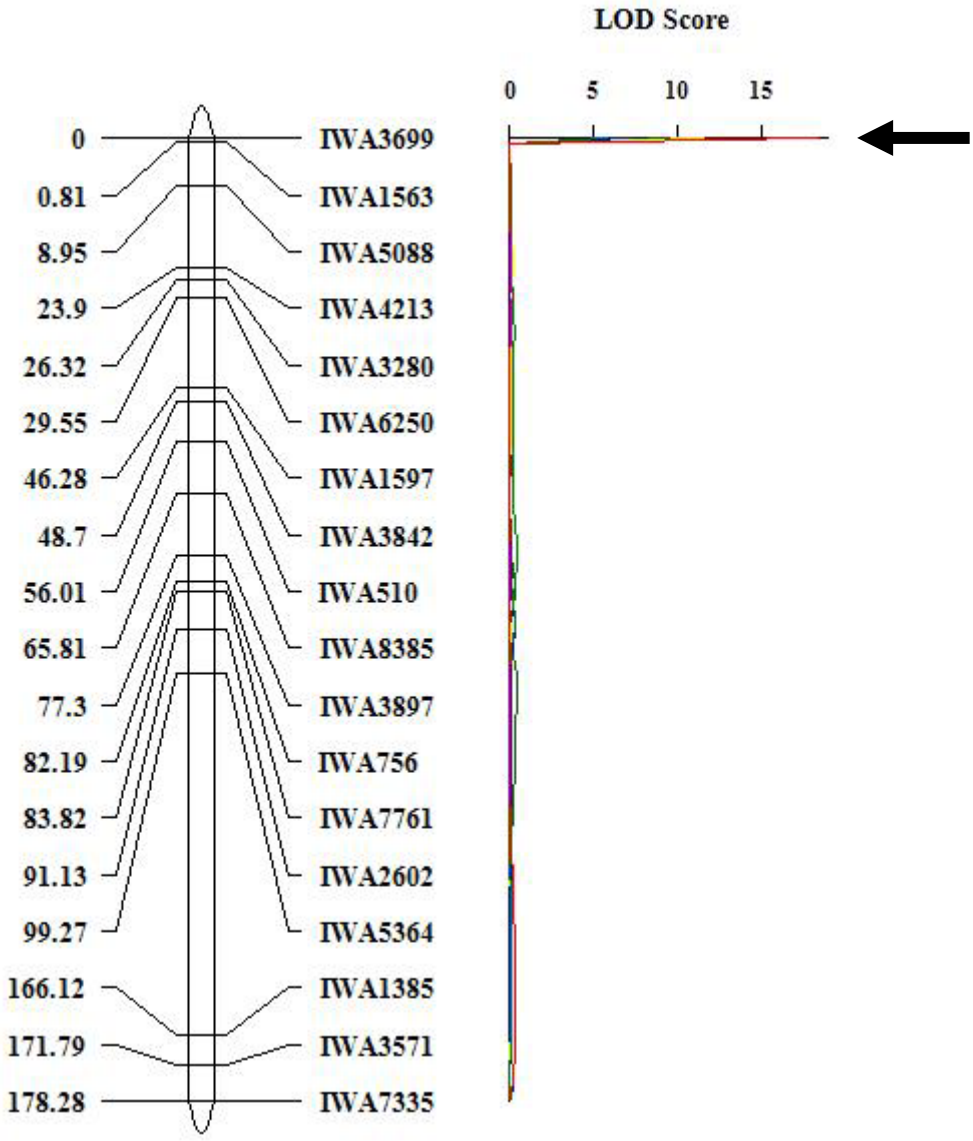

Figure 3: Chromosome 2A linkage group with LOD score plot. A black arrow indicates QTL associated with resistance alleles contributed by SS8641.

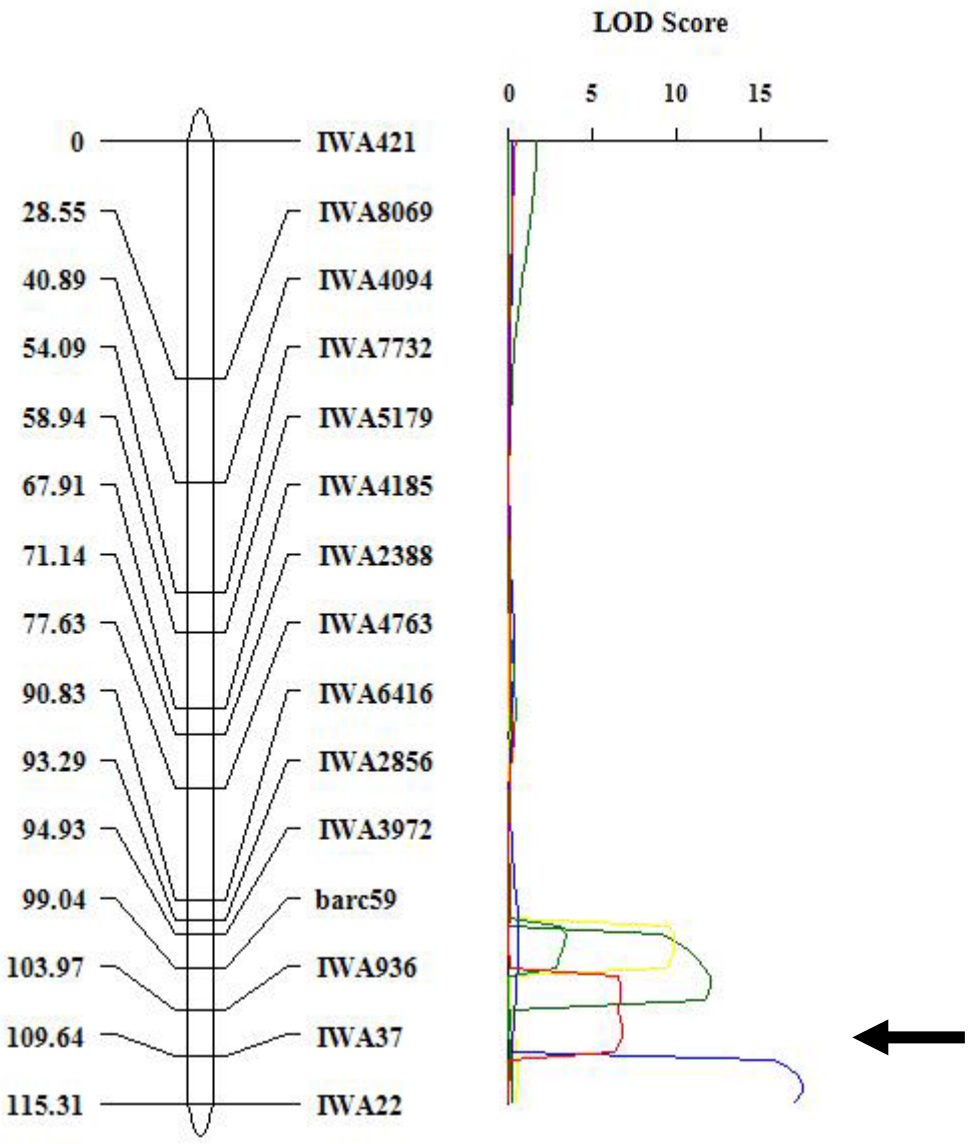

Figure 4: Chromosome 5B linkage group with LOD score plot. A black arrow indicates QTL associated with resistance alleles contributed by SS8641.

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