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Is approved by the final examining committee:

Christie Williams
Chair

Joseph Anderson

Kiersten Wise

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UTILIZATION OF TALL WHEATGRASS TRANSLOCATION LINES TO IMPROVE FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

A Dissertation

Submitted to the Faculty

of

Purdue University

by

Xiangye Xiao

In Partial Fulfillment of the

Requirements for the Degree

of

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West Lafayette, Indiana

For my parents

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Xiao, Xiangye. Ph.D., Purdue University, May 2015. Utilization of Tall Wheatgrass Translocation Lines to Improve Fusarium Head Blight Resistance in Wheat. Major Professor: Christie Williams.

Fusarium head blight (FHB) and leaf rust (LR) are two major fungal pathogens threatening the wheat crop, consequently identifying resistance genes from various sources is always of importance to wheat breeders. Type II FHB resistance in wheat has been improved by introgressing QTL from landraces and wild species. The present study was undertaken to (1) combine two different type II FHB resistance QTL in the backgrounds of six adapted wheat lines (2) improve the map resolution of *Qfhs.pur-7EL*.

In the first objective, the FHB resistance gene *Fhb1* originated in a Chinese wheat cultivar and is located on wheat chromosome 3BS, and *Qfhs.pur-7EL* was introgressed from tall wheatgrass onto wheat chromosome 7DL were combined in six adapted wheat lines. The effect of pyramiding resistance genes through marker-assisted selection was assessed by scoring plants for disease development after inoculating with a combination of four different local FHB isolates. The response of 6 populations of pyramided lines was evaluated in both greenhouse and transplant nursery. The pyramided lines as well as *Fhb1*-only lines exhibited high levels of resistance to the mixture of four FHB isolates.

Although *Fhb1* or *Qfhs. pur-7EL* alone is strong enough to achieve satisfactory resistance, pyramided lines may be more stable over time.

In the second objective, we identified tightly linked markers for FHB-resistance QTL *Qfhs.pur-7EL* and the LR-resistance gene *Lr19* using genotyping by sequencing in a wheat-tall wheatgrass introgression-derived recombinant inbred line (RIL) population. 216,318 SNPs were discovered for this population. After filtering, 1700 high-confidence SNPs were used to conduct the linkage and QTL analysis. *Qfhs.pur-7EL* was mapped to a 2.9 cM region within a 43.6 cM segment of wheatgrass chromosome $7el_2$ that was translocated onto wheat chromosome 7DL. The LR gene *Lr19* from $7el_1$ was mapped to a 1.21 cM region in the same area, in repulsion. Five lines were identified with the resistance-associated SNP alleles in coupling for *Qfhs.pur-7EL* and *Lr19*. Investigation of the genetic characteristics of the parental lines of this RIL population indicated that they are translocation lines in two different wheat cultivar genetic backgrounds instead of 7E-7D substitution lines in Thatcher wheat background as previously reported in the literature.

The wheat lines containing pyramided FHB resistance genes and pyramided FHB and LR resistance genes: *Qfhs.pur-7EL* and *Lr19*, developed and identified in this study, show potential as genetic resources for sustainable wheat production in areas affected by Fusarium head blight and leaf rust diseases.

CHAPTER 1. INTRODUCTION

1.1 Bread wheat

Bread wheat (*Triticum aestivum*) is one of the most important grain crops worldwide. It can be grown in a wide range of environments, accounts for 20 percent of calories consumed by humans, and ranks second only to rice (FAOSTAT 2012).

Domesticated wheat (AABBDD genomes) made its first appearance about 8500 years ago, following the hybridization and polyploidization of tetraploid durum wheat (*Triticum turgidum*, AABB genomes) and a diploid goat grass (*Aeglopis tauschii*, DD genome) (Nesbitt and Samuel 1995). The tetraploid durum wheat originated from hybridization and polyploidization of *Triticum urartu* (AA genome) and something similar to *Aeglopis Speltoides* (BB genome) about 0.4 million years ago (Liu et al. 2009; Matsuoka 2011). It is believed that the polyploidization provided high genome plasticity, which is a key factor in the success of polyploid wheat under domestication (Dubcovsky and Dvorak 2007).

Sequencing of crop genomes will greatly accelerate plant breeding and crop improvement. Fifty five plant genomes were sequenced by 2013 (Michael and Jackson 2013) and provide fundamentally important resources for understanding plant gene function and the interaction of plant genomes with the environment. A whole-genome shotgun sequence analysis of wheat has allocated more than 60% of the genes to the A, B

and D genomes and identified over 132,000 SNPs that facilitate analysis of quantitative trait loci and association studies of traits (Brenchley et al. 2012). The draft genomes of *T. urartu* and *A. tauschii*, the two diploid progenitors of wheat, have also been sequenced recently (Jia et al. 2013; Ling et al. 2013). The progenitors' genomes will be useful in finishing the wheat genome sequencing project by guiding unambiguous assignment of contigs or scaffolds. The goal of the International Wheat Genome Sequencing Consortium (IWGSC) is to obtain a high quality reference sequence using a chromosome-based approach. They isolated and sequenced each individual wheat chromosome (except 3B). This draft genome can enable researchers to target the specific chromosome of their interests (IWGSC 2014).

1.2 Fusarium Head Blight

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe (Teleomorph: *Gibberella zeae* (Schwein.) Petch), is a destructive disease of wheat and has significant economical impacts all around the world (Leslie and Summerell 2006; Trail 2009). Besides wheat, *F. graminearum* can also damage other cereal crops, such as oat, barley, wild rice, maize and sorghum (Trail 2009). FHB damage includes yield penalty, reduced grain quality, light kernel weight, low seed germination, seedling blight and poor stands (Parry et al. 1995; McMullen et al. 1997; Dexter and Nowicki 2003). The wheat spike becomes bleached after FHB infection. The diseased spikelets are sterile or contain shriveled or discolored kernels, which are commonly referred to as Fusarium-damaged kernels (Wiese 1977). Most importantly, *F. graminearum* produces trichothecene deoxynivalenol (DON) that accumulates in the grain, and when consumed

threatens human and animal health because of the toxicity (Parry et al. 1995; McMullen et al. 1997).

Currently, several different types of FHB resistance are under investigation. Schroeder and Christensen proposed two types of resistance: resistance to initial infection and resistance to spread within the spike, which are referred to as type I and type II resistance, respectively (Schroeder and Christensen 1963). Type II resistance is studied most extensively in wheat; it is more stable and appears less affected by environmental factors than type I resistance (Bai and Shaner 1994). Different inoculation techniques are required to distinguish these two types of resistance. Type I resistance is evaluated by spraying inoculum of conidia spores over the spikes when 50% of the florets in the spike are flowering and counting the percentage of diseased spikes 14 days after inoculation (Bai and Shaner 2004). Type II resistance is evaluated by injecting conidia spores into a single floret of a spike when 50% of the florets are flowering and counting the diseased spikelets per spike 20-22 days after inoculation (Bai and Shaner 2004). It may be challenging to differentiate these two types of resistance during a favorable flowering season since plants with only one type of resistance may be susceptible when the inoculum is abundant (Bai and Shaner 2004).

Three other types of resistance have been proposed: a) resistance to kernel infection; b) resistance to DON accumulation (Miller et al. 1985); c) tolerance (Mesterhazy 1995). Resistance to kernel infection is measured as percentage of infected kernels, which can possibly be reduced by type I and type II resistance. Thus, it is difficult to measure this type of resistance per se (Shaner 2002). Tolerance refers to no difference in yield when comparing the same cultivar under healthy and diseased conditions (Mesterhazy 1995).

These two types of resistance were not widely accepted by the community due to conceptual weakness (Shaner 2002). Resistance to DON accumulation, referred to as type III resistance, is the low DON content in some cultivars compared to other cultivars under the same environment (Miller and Arnison 1986). Three mechanisms will result in low DON content in kernels: fungus produces a low level of DON; plant enzymes degrade DON during kernel development; spike tissue other than kernels has high DON level but failure to move the toxin into kernels during development (Bai and Shaner 2004). Low DON content is usually associated with low FHB index due to type I and type II resistance (Bai et al. 2000). More evidence is needed to know whether these two types of resistance are controlled by the same or tightly linked QTL.

Chinese wheat cultivar Sumai 3 and its derivatives are the most commonly used and reliable type II resistance sources currently available (Bai and Shaner 2004). Many QTL have been revealed from Sumai 3 or its derivatives (Anderson et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002; Yang et al. 2003). Among these QTL, *Fhb1* is the strongest, accounting for 41.6% of the phenotypic variation in a mapping population (Anderson et al. 2001). *Fhb1* was mapped to the short arm of wheat chromosome 3B (Cuthbert et al. 2006; Hao et al. 2012). This QTL is associated with low DON accumulation (Zhou et al. 2002). Lemmens et al pointed out that there is a close relationship between the resistance to DON accumulation and high DON-3-glucoside to DON ratio (Lemmens et al. 2005b). Therefore, the authors hypothesized that *Fhb1* either encodes a DON-glucosyltransferase or regulates the expression of such an enzyme (Lemmens et al. 2005b).

In addition to wheat sources of resistance, QTL for FHB resistance was also identified in a wild relative of wheat. Shen and Ohm (Shen et al. 2004; Shen and Ohm

2007) discovered a QTL for FHB resistance when a local isolate of *F. graminearum* was used to screen the population. This new QTL is located on chromosome 7EL of tall wheatgrass and was introgressed onto the long arm of the 7D chromosome of wheat. This QTL was named *Qfhs.pur-7EL* and explained 15.1%-32.5% of the phenotypic variation in the mapping population (Shen and Ohm 2007).

1.3 Wild Relatives

The use of wild relatives to improve crops has achieved great success in several aspects of breeding, especially in disease resistance. Other important characteristics include abiotic stress resistance in barley (Ellis et al. 2000), chickpea (Singh et al. 1998), rice (Nguyen et al. 2003) and sunflower (Mercer et al. 2007) and the introduction of cytoplasmic male sterility systems in sunflower (Leclercq 1969) and rice (Dalmacio et al. 1995) to develop F_1 hybrids.

Sears started plant chromosome engineering in 1956 by transferring a leaf rust resistance gene from an alien chromosome to a wheat chromosome using X-ray irradiation and an elegant cytogenetic scheme (Sears 1956). Many additional methods have been adopted to transfer alien chromatin into a variety of crops for improvement. These methods include interploidy hybridizations, which are crosses between two different ploidy levels of the same or different species and interspecific or intergeneric crosses at the same ploidy level (Seiler 1992). Interploidy hybridization has been reported in relatively few crops, such as Arabidopsis (Scott et al. 1998) and maize (Pennington et al. 2008). In general, crosses between different ploidy levels are more recalcitrant in yielding plantlets than crosses carried out using parents of the same ploidy level, primarily due to embryo inviability. Embryo rescue has been applied to aid interploid

hybrid production. However, the success rate varies among different species. The genotype (Stewart and Hsu 1978), developmental stage of the embryo at excision (Matthys-Rochon et al. 1998), and composition of the embryo culture media (Stewart and Hsu 1978) are the three main factors affecting the success and efficiency of embryo rescue. Selection of parents is very important for successful interploid crosses (Jansky 2006). As a general rule, medium composition is more critical for in vitro growth of immature than mature embryos, and medium complexity must be increased with decreasing embryo age (Matthys-Rochon et al. 1998).

The *Ph1* (pairing homoeologous) gene, located on the long arm of chromosome 5B in wheat, is a widely used pairing regulator factor in chromosome engineering (Sears 1977; Giorgi 1978). Two deletions of this locus have long been known; one is *ph1b* in hexaploid wheat (Sears 1977) and the other is *ph1c* in tetraploid (AABB) wheat (Giorgi 1978). In hexaploid wheat the *Ph1* gene suppresses homoeologous pairing and controls diploid-like meiosis. Multivalent formation at meiotic metaphase I, which is the indicator of absence of *Ph1,* is observed between the homoeologous chromosomes of the related genomes in *Ph1* deletion lines. The deletion of *Ph1* affects the centromere structure (Aragón-Alcaide et al. 1997b), and thus the level of premeiotic association of homologues at their centromeres (Aragón-Alcaide et al. 1997a; Martínez-Pérez et al. 1999). Consequently, the timing of telomere bouquet formation and of intimate association of homologues (Martínez-Pérez et al. 1999) during meiotic prophase ultimately allow recombination between homeologous chromosomes or segments (Dubcovsky et al. 1995; Luo et al. 1996). Suppression or partial inactivation of *Ph1* leads

to enough homoeologous pairing and recombination to cause alien chromatin integration into the wheat genome.

The *ph1b* mutant stock of Chinese Spring wheat was produced by radiation treatment (Sears 1977) causing a 70 Mb deletion (Dunford et al. 1995). In genotypes that are homozygous for the *ph1b* deletion, chromosome pairing and recombination occur between wheat and homoeologous alien chromosomes. This technique has been the method of choice for directed transfer of alien genes to wheat, especially disease-resistance genes from wheat-grasses.

When a useful gene is transferred from a wild relative to a cultivar by backcrossing, undesirable genes from the donor parent (wild relative) may be retained in the recipient genome due to two causes. First, genes that are independent of the gene being transferred (not tightly linked) may still be present by chance even after several backcrosses (Allard 1975). Second, a segment of the donor chromosome surrounding the useful gene is likely to be retained due to linkage. This has been termed “linkage drag” (Brinkman and Frey 1977a). Theoretically, repeated backcrossing could remove these two types of retained segments simultaneously. On average, half of the unlinked donor DNA is replaced by recurrent parent DNA in each generation, so that by the sixth backcross generation less than 1.6% of the unlinked donor genome is expected to persist (Stam and Zeven 1981). However, many generations are required to remove donor segments linked to the target gene (Stam and Zeven 1981). In reality, the retained region could be larger or smaller than expected values due to large variance and breeder selection among progenies. If the region flanking the target gene was introgressed from a different species than the

recipient, then lack of recombination may prevent the removal of undesirable linked genes (Stam and Zeven 1981).

Selection for markers tightly linked to the gene of interest and against more distant markers could be used to shorten the alien chromatin introgressed into the cultivar. As a result, individuals that have retained the target gene and its tightly linked flanking markers, yet have a crossover event that removed the more distant markers would harbor shorter segments of alien chromatin, and could be identified rapidly (Young and Tanksley 1989a).

1.1 Micosatellites

DNA simple sequence repeats (SSR) or microsatellites are ubiquitously spread throughout the genomes of all species (Zietkiewicz et al. 1994). SSRs are short DNA sequences with one- to six-base tandem repeats. It was shown that the repeat number among individuals can be different (Jeffreys et al. 1985). These differences enable SSR detection as a versatile genetic marker with the advent of the polymerase chain reaction (PCR) technology. SSR markers can be used in various studies, such as kinship and population analysis (Lu et al. 2005), detection of gene duplications or deletions (Rocha 2003) and marker-assisted selection (Zhou et al. 2003).

The frequency and distribution pattern of these tandem repeats is quite variable among different genomes (Lawson and Zhang 2006). Among crop species, the most abundant dinucleotide repeats $(AC)_n$ and $(GA)_n$ have been extensively studied (Temnykh et al. 2001). For instance, wheat contains about 23,000 $(AC)_n$ repeats and 36,000 $(GA)_n$ repeats, with an average distance of 704 Kb and 440 Kb, respectively, between consecutive SSRs (Röder et al. 1995). However in rice, the estimated total number of

$(AC)_n$ and $(GA)_n$ are 1000 and 2000 respectively, with an average distance between consecutive SSRs of 450 Kb and 225 Kb (Wu and Tanksley 1993). Trinucleotide and tetranucleotide repeats are also present in plant genomes. The top two most frequent repeats are $(AAG)_n$, and $(AAT)_n$ (Gupta et al. 1996). A more complete survey which covers 54 species reported that a descending order based upon the abundance of the sequences is $(AT)_n$, $(A)_n$, $(AG)_n$, $(AAT)_n$, $(AAC)_n$, $(AGC)_n$, $(AAG)_n$, $(AATT)_n$, $(AAAT)_n$ and $(AC)_n$ (Wang et al. 1994).

In general, DNA SSR polymorphisms can be revealed by three approaches: hybridization-based, PCR-based and a combination of hybridization- and PCR-based approaches (Gupta and Varshney 2000). In a hybridization-based approach, fragments of restriction enzyme-digested DNA are separated by electrophoresis on agarose gels, blotted onto membrane and hybridized with radio-labeled oligonucleotide probes corresponding to the SSR sequence (Gupta and Varshney 2000). This approach reveals polymorphism due to variation in the length of the restriction fragment that carries the microsatellite, not the length variation of microsatellite itself (Gupta and Varshney 2000). In the PCR-based approach, primers flank the microsatellite site (Tautz 1989; Weber and May 1989) or are complementary to a microsatellite motif randomly distributed across the genome (Meyer et al. 1993; Wu et al. 1994; Zietkiewicz et al. 1994). This approach usually reveals a large amount of information and can be used in genetic mapping and tagging projects (Weber 1990; Morgante et al. 1994). Richardson et al. (1995) reported a method called random amplified microsatellite polymorphism that combined PCR using semispecific primers and microsatellite hybridization to produce additional polymorphic sites per gel. The first step of this approach is to use a single arbitrary PCR primer for

amplifying genomic DNA. PCR products are then separated by electrophoresis and stained. The dried gel or blotted membrane is hybridized to a radioactively labeled oligonucleotide probe complementary to the microsatellites. Lastly, autoradiography is used to detect the polymorphic profiles. Since this combined approach is PCR-based it is sensitive to carryover contaminations (Ender et al. 1996).

1.1 Single Nucletiode Polymorphisms

Single nucleotide polymorphisms (SNPs), as the name indicates, have only one base-pair difference among individuals; usually there are two alleles per locus (Vignal et al. 2002). Due to their high genomic abundance and advances in sequencing technology, SNPs have become the primary marker system for many plant species (Chagné et al. 2008; Ganal et al. 2009). SNPs can appear in most regions of a genome, including gene-coding regions, thus they are a suitable marker system to choose for genome-wide studies (Chagné et al. 2008).

Several approaches have been developed for SNP identification. Searching the EST databases in the National Center for Biotechnology Information enables the identification of SNPs. An advantage of this approach is the large quantity of EST sequences that can be used free-of-charge to develop SNPs (Batley et al. 2003). However, there are still some limitations in this approach. Firstly, the sequence quality of many ESTs is not high, which results in a high false-positive rate (Batley et al. 2003; Ganal et al. 2009). Secondly, differentiating orthologous and paralogous sequences is difficult and mistakes will again lead to false-positive SNPs (Ganal et al. 2009).

Various sequencing technologies contribute significantly to identifying SNPs. For example, in an amplicon resequencing approach, PCR primers for single-copy genes are

designed to amplify the products in several representative lines. PCR products are fully sequenced to identify SNPs among these lines. This approach provides very reliable identification with a low false-discovery rate (Ganal et al. 2009). If the PCR fragment is very long, haplotype can also be detected (Ganal et al. 2009). The disadvantage of this approach is it takes a significant amount of effort to investigate multiple genes. Next generation sequencing technology increases the SNP identification throughput tremendously. For example, transcritpome resequencing targeting the variation within the gene offers rapid and affordable SNP discovery. This approach has been successfully applied in crops including maize (Barbazuk et al. 2007), wheat (Lai et al. 2012) and canola (Trick et al. 2009).

Although identifying SNPs in the gene region is attractive, QTL are often located within regulatory regions that may be distant from the gene regions that they control (Dean 2006). Therefore, the power of transcriptome and exon sequencing to identify SNPs is limited. It is important to employ a method to reduce the complexity of the genome and also avoid repetitive regions of the genome in order to explore the regulatory regions in the genome (Mammadov et al. 2012). Restriction Site-Associated DNA sequencing (Baird et al. 2008) and Genotyping by Sequencing (GBS) (Elshire et al. 2011) are two examples of the reduced genome complexity concept.

Although multiple approaches can be used for SNP identification, identifying SNPs in complex polyploid genomes is still a great challenge. Because most crops are not simple diploids it is very often difficult to find useful polymorphism within a single genotype since it is a challenge to resolve the paralogous sequences of the duplicated regions in the genome or the presence of the homoeologous loci from other subgenomes

which lead to false-positive SNPs (Ganal et al. 2009; Mammadov et al. 2012). All these issues mentioned above require sophisticated statistical and bioinformatics tools to resolve.

1.1 Genotyping by Sequencing

GBS is a SNP development and genotyping approach applying the next generation sequencing technology. Because crop genomes are too large to sequence in entirety for SNP discovery and mapping projects, the GBS technique provides reduced representation libraries of short barcoded amplicons that can be multiplexed for efficient sequencing (Elshire et al. 2011). Traditionally the use of SNPs requires two steps: identifying polymorphic loci and assaying these polymorphic markers across a full set of plant materials such as a mapping population (Poland and Rife 2012). The beauty of GBS is that it integrates these two steps into one step. This approach has been robustly applied to many crops such as maize (Elshire et al. 2011), wheat (Poland et al. 2012a), barley (Elshire et al. 2011; Poland et al. 2012a) and switchgrass (Lu et al. 2013) to produce tens of thousands of markers per species. The original GBS protocol utilized a one-enzyme cutting process in library construction but later was modified to use two enzymes (Elshire et al. 2011; Poland et al. 2012a). No matter how many enzymes are used when constructing the multiplexing library, the basic procedure is similar. The quantified and normalized genomic DNA is digested by the selected enzyme(s), followed by adding the barcoded adapter into the reaction system. The barcoded fragments are amplified by PCR. The PCR products are purified and the size and quality of the library are before sequencing (Elshire et al. 2011; Poland et al. 2012a).

GBS gives lower genome coverage than does RAD sequencing (Baird et al. 2008; Davey et al. 2011). The idea of GBS is to sequence as many targets as possible and use imputation techniques to infer the SNP alleles in plants with missing data. (Davey et al. 2011). The TASSEL software pipeline can be used to analyze GBS data to generate SNP calls for every individual in a mapping population, thus producing the HapMap (Glaubitz et al. 2014). Although the GBS approach does not require prior information about the genome, a reference genome of the species, which is a representative assembly of a species' set of genes, does speed up and ease the analysis process (Edward Buckler, personal communication). Another part of the TASSEL pipeline, named UNEAK, will facilitate GBS analysis if a reference genome is not available (Lu et al. 2013). In this pipeline, the author used a network-based filter strategy to remove tag pairs (two tags or SNPs with only one base pair difference) with sequencing error and leave the true tag pair for each SNP call; tag pairs with sequencing error can be detected because of their low abundance compared to tag pairs being sequenced correctly. There are still some unsolved challenges for this pipeline, including the universal issue for the polyploid species; it is difficult to differentiate paralogous and orthologous sequences from true polymorphisms at one locus. Thus, the pipeline may give a high rate of false positive SNP calls and hence require more stringent filtering strategies for downstream use. GBS has been used in various plant genetics and breeding studies, such as linkage mapping (Poland et al. 2012a), association mapping (Brachi et al. 2011; Elshire et al. 2011), genomic selection (Poland et al. 2012b) and evolution studies (Lu et al. 2013).

1.2 Marker-Assisted Selection

Marker-assisted selection (MAS) is a powerful tool that uses association between the genetic marker and target traits to indirectly select for traits that are difficult or expensive to phenotype in a short timeframe, such as before production of the next generation. MAS speeds up the conventional plant breeding cycle and facilitates the improvement of traits that cannot be enhanced easily by conventional methods (Ribaut and Hoisington 1998). In general, there are four situations in which MAS is a better approach than conventional breeding. First, when traits are difficult to select directly through phenotype, such as if they are expensive or time-consuming to measure, or have complex inheritance. Second, MAS can be applied under any environment for traits whose expression depends on specific environments or developmental stages. Third, MAS can be used to speed up backcross breeding or to maintain recessive alleles during backcrossing. And last, MAS can be adopted when pyramiding multiple monogenic traits or multiple QTL for the same target trait with complex inheritance (Xu 2002; Xu 2003; Koebner 2005; Xu et al. 2005).

The prerequisite of MAS is to identify markers that are tightly linked to the gene of interest (Kumar 1999). Therefore, the markers identified in preliminary mapping studies may not be suitable to use directly in the MAS program without further testing since the markers may be too far away from the gene of interest, which allows crossovers between the marker and the gene to break the linkage. Selection for distant markers produces a high percentage of false-positive and negatives in the screening process because of recombination between marker and gene of interest (Mohan et al. 1997; Collard et al. 2005).

In general, constructing a high density linkage map and precision phenotyping to accurately map the gene or QTL of interest is the first step in a MAS program (Collard et al. 2005). The population size is a key issue in this step since a large population will resolve recombination better. A high degree of polymorphism and marker density are essential to map the gene of interest in a relatively narrow region (Darvasi et al. 1993). The second step is maker validation. The markers identified in the first step should be tested for their effectiveness in predicting the desired phenotype in other independent populations and different genetic backgrounds to ensure broad application across different breeding programs (Sharp et al. 2001; Cakir et al. 2003; Collins et al. 2003). This step will determine whether the marker can be used routinely in MAS screening (Ogbonnaya et al. 2001; Sharp et al. 2001). Directly assuming the QTL-marker association in other genetic backgrounds without validating the result may lead to false results, especially for inheritance of complex traits such as yield (Reyna and Sneller 2001). The most useful markers for MAS should be polymorphic in divergent populations derived from a wide range of parental lines (Langridge et al. 2001). Therefore, a maker developed for a gene in one cross may not be useful in other crosses even though the same gene may be segregating in the second cross, unless the marker is from within the gene itself (Mohan et al. 1997).

As the sequencing technology advances, the quality and quantity of markers needed for high-resolution mapping studies is no longer a limiting factor. However, the quality of the phenotyping is a factor that affects the accuracy of genetic mapping studies and thus is a limiting factor for the downstream MAS, especially for complex traits (Xu and

Crouch 2008). Therefore to optimize the efficiency of large scale MAS, establishing a high throughout phenotyping platform is the key to success (Xu and Crouch 2008).

CHAPTER 2. PYRAMIDING TWO DIFFERENT QTL FOR TYPE II FUSARIUM HEAD BLIGHT RESISTANCE IN ADAPTED WHEAT LINES

2.1 Abstract

Resistance to Fusarium head blight (FHB) is an important goal of wheat breeding programs in humid and semi-humid regions of the world. Type II FHB resistance in wheat has been improved by introgressing QTL from landraces and wild species. The present study was undertaken to combine two different type II FHB resistance QTL in the backgrounds of six adapted wheat lines. The FHB resistance gene *Fhb1* originated in a Chinese wheat cultivar and is located on wheat chromosome 3BS, and *Qfhs.pur-7EL* was introgressed from tall wheatgrass onto wheat chromosome 7DL. The effect of pyramiding resistance genes through marker-assisted selection was assessed by scoring plants for disease development after inoculating with a combination of four different local FHB isolates. The response of 6 populations of pyramided lines was evaluated in both greenhouse and transplant nursery. The pyramided lines as well as *Fhb1*-only lines exhibited high levels of resistance to the mixture of four FHB isolates. Although *Fhb1* or *Qfhs.pur-7EL* alone is strong enough to achieve satisfactory resistance, pyramided lines may be more stable over time. The adapted wheat lines containing pyramided FHB resistance genes, developed in this study, show potential as genetic resources for sustainable wheat production in areas affected by Fusarium head blight disease

2.2 Introduction

Fusarium head blight (FHB) or scab, caused by *Fusarium graminearum* is a destructive fungal disease of wheat and other small-grain cereals (Mesterhazy 1995). The disease causes yield loss and grain quality reduction. More importantly, the pathogen produces deoxynivalenol (DON) during development, which is a toxin when consume by humans and animals (McMullen et al. 1997). Although five types of resistance to FHB have been described (Schroeder and Christensen 1963; Miller et al. 1985; Mesterhazy 1995), type II resistance, which limits the severity or spread of the disease from initial point of infection to other florets on a spike, is the most effective and best understood.

QTL providing type II resistance to FHB have been identified from wheat line Sumai 3 or its derivatives (Anderson et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002; Yang et al. 2003). Among these QTL, *Fhb1* is the strongest, accounting for 41.6% of the phenotypic variation in a mapping population (Anderson et al. 2001). *Fhb1* was mapped to the short arm of wheat chromosome 3B (Cuthbert et al. 2006; Hao et al. 2012). This QTL is associated with low DON accumulation (Zhou et al. 2002). Lemmens et al. (2005a) reported a correlation between the resistance to DON accumulation and high ratio of DON-3-glucoside to DON. Therefore, the authors hypothesized that *Fhb1* either encodes a DON-glucosyltransferase or regulates the expression of such an enzyme. In addition to wheat sources of resistance, a QTL for FHB resistance was also identified in a wild relative of wheat (Shen et al. 2004; Shen and Ohm 2007), located on chromosome $7el_2$ of tall wheatgrass, *Thinopyrum ponticum*. This QTL, *Qfhs.pur-7EL,* was introgressed onto the long arm of the 7D chromosome of wheat and explained 15.1%-32.5% of the phenotypic variation in their mapping population (Shen and Ohm 2007)

Gene pyramiding is commonly used to construct superior lines with resistance to multiple diseases or to augment the strength or durability of resistance to one disease (Cao et al. 2002; Chan et al. 2005). However, not all pyramided resistance genes show an additive effect; pyramiding two genes for brown plant hopper, *Bph1 and Bph2*, resulted in a similar level of resistance as found in the stronger of the two individual gene source lines (Sharma et al. 2004).

The goal of this project was to combine two different sources of FHB type II resistance in adapted wheat backgrounds for future cultivar development. Our hypothesis was that, due to phenotypic and marker-assisted selection to pyramid resistance loci, the level of FHB resistance would increase through line advancement while plant height, straw diameter and seed yield would remain at acceptable levels.

2.3 Materials and methods

2.3.1 Populations for pyramiding two FHB resistance genes

Lines "07469" and "07117" (H. Ohm, unpublished data; pedigrees 07469=992059A1-11/INW0315//981358C1-4-2-13/97462A1-21-1-5-1-15/5/0128A1-36/3/Chinese Spr.ph1b/KS24-2(275-4)//Chinese Spr./4/0128A1-36 and 07117=INW0411/3/Chinese Spr ph1b/KS24-2-2(275-4)//Chinese Spr/4/0128A1-36/INW0411/5/99840C4-8-4-11) were segregating for a translocation (Kim et al. 1993) harboring a tall wheatgrass (*Thinopyrum ponticum*) type II FHB resistance gene, *Qfhs.pur-7EL* (also called *FhbLop*;(Zhang et al. 2011), that is within a 15 cM region flanked by SSR markers Xcfa2240 and Xbf145935 (Shen and Ohm 2007) on wheat chromosome 7DL. 07469 may carry a second 7EL-7DL translocation from intermediate wheatgrass (*Th. intermedium*) harboring the barley yellow dwarf virus resistance gene, *Bdv3*. Wheat line "Wheatear "

carries a 7EL-7DL translocation from tall wheatgrass harboring a resistance linkage block *Lr19/Sr25* (Sharma and Knott 1966). Thus, three different 7EL-7DL were segregating in the material used for this study. The wheat line "Patterson" was used as the FHB susceptible control.

Wheatear and 07469 (populations 1 to 5) plus Wheatear and 07117 (population 6) were crossed in November of 2009. In March 2010, the F_1 plants were crossed (Figure 2.1 and Figure 2.2; generation 2) with 6 different adapted winter wheat lines (Table 2.1) derived from many generations of crosses and selection, in the Purdue wheat-breeding program, to combine agronomically important traits: large spikes, moderate height and strong straw (Table 2.1). All these lines have included type II FHB resistance gene *Fhb1* and some lines may have included uncharacterized QTL for type I or type II resistance to FHB. Progeny from the 6 crosses with adapted lines were planted in July 2010 (generation 3) and allowed to self-pollinate to produce 6 populations. As these lines were advanced through four generations of self-crosses (to generation 7; Figure 2.2) in both transplant nursery and greenhouse, pedigree breeding ensured that the lineage of each plant could be traced.

2.3.2 Plant growth

Seeds for greenhouse generations were sown in flats containing soil (Sunshine Redi-earth, Sun Gro Horticulture, Seba Beach, Canada). The flats were transferred to a cold room (2.22°C, with 12 hours light) for one week to break dormancy and returned to the greenhouse for germination. When the seedlings reached the one-leaf stage, the flats were again transferred to the cold room for vernalization. Sixty-five days later, each seedling was transplanted into a separate 3-inch pot and grown to maturity. For the transplant

nurseries, plants were started in flats during the winter as described above and transplanted into field plots in the spring. Plots were three feet long and one foot wide, each planted with sibling seedlings from a single spike.

2.3.3 FHB inoculation and scoring

F. graminearum cultures were provided by Dr. Kiersten Wise (Purdue University) following the procedures of Bai and Shaner (1996). Four *F. graminearum* isolates were combined to produce the inoculum. FG1, FG2, and FG2-23 were collected from undesignated locations in Indiana in 2009, and 10INSWF P5-2 was collected from Vincennes, IN in 2010 (Nolan Anderson, personal communication). The inoculum was prepared one month prior to use and cultured in mung bean medium (Desjardins et al. 1996).

Point inoculation of florets in wheat spikes (Bai and Shaner 2004) was used in all greenhouse and transplant nursery trials in the evaluation of type II FHB resistance. Ten µl of inoculum with a concentration 50,000 spores/ml were injected into the left floret of the second or third spikelet from the tip of each spike at anthesis. The inoculated spikes were covered with plastic bags for three days to maintain high humidity. The disease symptoms were scored 21 days after inoculation. The total number of discolored spikelets from the inoculation point and below was recorded as the FHB score.

2.3.4 Agronomic trait scoring and plant selection

In early generations, agronomic traits were evaluated by observation. At generation 7, plant height, straw diameter and seed weight per plant were measured to evaluate the agronomic performance. Height was measured from soil level to the tip of the spike. Straw diameter was measured with a caliper at the base of the spike on the main tiller. All

the spikes from each plant were harvested, threshed and the seeds were weighted. When selecting plants for advancement, low FHB score (0.5 and 1) was the first criterion but resistance to other diseases seen in the field and the measured agronomic traits were also considered. The general rule for selection was pyramiding as many good features (low FHB score, good agronomic traits, resistance to other diseases) as possible.

2.3.5 DNA extraction and marker assay

Two cm of seedling leaf tissue was collected into liquid nitrogen from each plant in the greenhouse or field and stored at -80°C until use. The extraction method was described by Ata-ur-Rehman et al. (2007) as modified in Liu et al. (2013).

Each DNA sample was screened with three fluorescence-tagged SSR markers: Xumn10 for *Fhb1*, Xcfa2240 and Xbf145935 for *Qfhs.pur-7EL*. Forward primers (Table 2.2) were labeled with one of the following three fluorescines: 6-FAM™, NED™ or VIC® (Applied Biosystems, Foster City, CA).

SSRs were amplified in a 10 μL reaction volume consisting of 1 unit Taq DNA polymerase (#M0267, New England Biolabs, Ipswich, MA), 80 ng genomic DNA, 1μl of 10X buffer (100 mM Tris-HCl [pH 9.0], 500 mM KCl, 1% TritonX100), 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.15 μM florescence-labeled 6-FAM, VIC or NED forward and 0.15 μM unlabeled reverse primer. PCR was performed in a MyCycler thermal cycler (BioRad, Hercules, CA) with the following program: initial denaturation of 95°C for 3 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 45 s, extension at 72°C for 1 min; and a final extension at 72°C for 7 min, with short-term storage at 4°C.

Following amplification, 3 μL of product were mixed with 9 μL of Hi-Di Formamide (Applied Biosystems) and 0.01 μL of GeneScan-500 LIZ size standard (Applied Biosystems), denatured at 95°C for 5 min and placed on ice. Amplicons were separated by capillary electrophoresis on an ABI 3700 DNA Analyzer (Applied Biosystems) at the Purdue University Genomics Core Facility. The output files were processed using GeneMarker® v1.91 (SoftGenetics, State College, PA). SSR allele sizes for the markers were as follows: Xumn10 was 241 bp in *Fhb1*-positive wheat DNA, Xcfa2204 was 280 bp in wheat DNA and 239 bp in tall wheatgrass DNA, and Xbf145935 was 202 bp in wheat and 196 bp in tall wheatgrass. Both wheatgrass alleles of flanking markers need to present for the plant to be scored as *Qfhs.pur-7EL*-present.

2.3.6 Statistical analysis

The 6 populations of plants were separated into four genotype groups: *Fhb1* only, *Qfhs.pur-7EL* only, both *Fhb1* and *Qfhs.pur-7EL* and neither for analysis of QTL effect. Analyses were performed using SAS Version 9.2 (SAS Institute, Inc., Cary, NC, 2008). The analysis of variance (ANOVA) model was applied in the "PROC ANOVA" procedure to assess the significance of population and genotype effects. Differences in mean were considered significant at a *p*-value of 0.05.

2.4 Results

2.4.1 Progress in FHB resistance through selection

To assess improvement in resistance due to selection as the populations were advanced to generation 7, type II FHB severity scores were compared. In the 2011 transplant nursery test (generation 4; Figure 2.2) when plants from all 6 populations were combined, 71.4% received an FHB severity score between 0.5 and 1. FHB severity scores

for the 2011 fall greenhouse test (generation 5) were similar, with 65.3% of the plants receiving a score between 0.5 and 1. In the 2012 transplant nursery test (generation 6), selection resulted in a higher proportion of plants, 87.4%, with FHB severity scores between 0.5 and 1. And in the 2012 fall greenhouse test (generation 7), 85.6% of the plants had scores between 0.5 and 1. However, transgressive segregation resulted in a few plants from every generation being more susceptible to FHB than the control plants.

A comparison of the six populations for decreased FHB severity (Table 2.1) showed that the scores were lower under transplant nursery conditions than in the controlled environment of the greenhouse tests, which was more conducive to FHB spread within the spike. Nevertheless, it is evident that selection resulted in considerable decreases in FHB severity scores when comparing the 2011 versus 2012 transplant nursery except population 2 and the 2011 versus 2012 fall greenhouse scores except population 4. Among these comparisons, the greatest gain in selecting for decreased FHB severity occurred in populations 3 and 5 and the least progress was seen with populations 2 and 4 even with slight increase of the FHB severity. On average, the susceptible control, Patterson, received an FHB score between 7 and 10 in all tests, indicating effectiveness of the inoculum and consistency in scoring in the different environments and years.

Through the process of selection, the proportion of highly resistant plants containing all three SSR markers, suggesting the presence of both *Fhb1* and *Qfhs.pur-7EL* resistance loci, increased from 21.4% in the 2011 transplant nursery to 53.1% in the 2012 transplant nursery (Figure 2.3). Because the 2011 transplant nursery test sample size was small, no significant correlation was observed in comparisons of FHB resistance with any of the 6 populations. By generation 4 in the 2011 transplant nursery and generation 6 in the 2012

transplant nursery significant differences in FHB severity were evident among plants with different combinations of SSR markers (Table 2.3). Plants containing neither of three SSR markers, genotype 1, exhibited the lowest levels of FHB resistance and performed significantly worse than plants in the genotype 4 (Table 2.3). However, even genotype 1 plants (no SSR markers present) exhibited moderate FHB severity scores, indicating prevalence of FHB-resistance QTL in the six adapted lines.

2.4.2 Interactions between population and genotype

To investigate the contributions of the adapted lines to FHB resistance, interactions between the six populations and four genotypes were examined in generation 6 in the 2012 transplant nursery. FHB severity scores varied for different populations within one genotype group, indicating differences in background effect of the adapted lines (Figure 2.4). Because population 6 had a similar low FHB severity score (0.5) in genotype groups 1 (lacking any markers) and 4 (all markers present), the adapted line "Roane" appeared to carry other factors for FHB resistance. Population 6 mean FHB severity scores were stable and low across all 4 genotype groups and thus independent of marker presence for both *Qfhs.pur-7EL* and *Fhb1*.

2.4.3 Agronomic performance

Since the *Qfhs.pur-7EL* resistance locus, originating in the wild relative *Th. ponticum*, was introgressed into the six populations, linkage drag was assessed by measuring three important agronomic traits that were subjected to selection throughout the experiment. Plant height, straw diameter and seed weight per plant were measured on FHB inoculated plants in the 2012 fall greenhouse (generation 7). The average plant height of the six populations combined was 70.14 cm, which was 6.51 cm taller than the susceptible

control, Patterson (Figure 2.5a). The six populations combined performed better than Patterson for average straw diameter (2.26 mm verses 1.92 mm) and seed weight per plant (2.25 grams versus 0.98 grams) (Figure 2.5b and c). Patterson was very susceptible to FHB and thus the seed weight per plant when challenged with FHB was very low compared to its performance when FHB inoculation was absent. These data showed that the six populations performed as well as the Paterson control for these three agronomic traits, even though one of the source genes, *Qfhs.pur-7EL,* resides on a genomic segment from a wild species (Table 2.4).

2.5 Discussion

We reached our goal of combining type II FHB resistance gene *Fhb1* with *Qfhs.pur-*7EL in different adapted winter wheat backgrounds (populations 1, 5 and 6, Figure 2.4). Because of selection and the presence of unmarked FHB resistance loci in the adapted lines, a majority of the lines showed moderate (1 to 3 infected spikelets per spike) to high (0.5 and 1 infected spikelets per spike) levels of type II FHB resistance, even if none of the three markers were present in the genome (Table 2.3). The adapted lines underwent many generations of FHB phenotypic selection during the development process to increase the presence of uncharacterized QTL besides *Fhb1*. These uncharacterized FHB QTL appear to contribute to the moderate FHB resistance in plants with none of the three markers and thus support our hypothesis in that selection increased resistance. However, further analysis must include quantification of DON in the highly resistant plants before they can be considered for cultivar development. Recent work comparing DON levels in resistant wheat lines demonstrated lack of significant correlation between resistance and DON accumulation (Ji et al. 2015).

Plants with the Xumn10 marker only, linked to *Fhb1*, or plants with both flanking markers for *Qfhs.pur-7EL* showed similar FHB resistance to lines with all three markers (Table 2.3; genotype 2 and 3 versus 4). This result confirmed that *Fhb1* and *Qfhs.pur-7EL* are very strong QTL for FHB resistance (Anderson et al. 2001; Shen and Ohm 2007). When a large effect QTL is present in the genome, additional resistance effect might not be detected if pyramiding another QTL. However, the durability of the pyramided lines might be improved compared to the individual source lines. In 2012 genotyping result, we observed the recombinant genotype of two flanking markers for *Qfhs.pur-7EL*. Due to ambiguity about the presence of *Qfhs.pur-7EL* in plants where the flanking markers had recombined, those genotypes were eliminated from further analysis. The presence of the second wheatgrass 7E/7D translocation conferring *Bdv3* and the third wheatgrass 7E/7D translocation conferring *Lr19/Sr25* in the pedigree of some plants may have allowed recombination between the two markers.

Linkage drag is often a problem that makes incorporating alien chromatin in breeding lines less favorable (Young and Tanksley 1989b). Molecular markers can greatly speed up the selection of progenies with desired gene combinations while removing linkage drag, thus efficiently reducing the time and effort in a traditional breeding program (Young and Tanksley 1989b). In this study, pyramiding of *Fhb1* and *Qfhs.pur-7EL* resulted in a higher or similar level of FHB resistance compared to other types of marker combination. However, we did not detect inferior agronomic performance in this study with regarding plant height, straw diameter and seed weight per plant although yield reduction due to alien chromatin was reported in previous research (Dyck and Friebe 1993). Therefore, we can conclude that undesirable traits can be

reduced to an undetectable level with intensive phenotypic and marker-assisted selection as long as no highly deleterious traits are encoded inside the translocated alien segment. Thus these results supported our hypothesis that, due to phenotypic and marker-assisted selection to pyramid resistance loci, the level of FHB resistance would increase through line advancement while plant height, straw diameter and seed yield would remain at acceptable levels.

In this experiment, we used multiple crosses with the same crossing objective. This experiment design enabled us to study the interaction of genetic background of adapted lines and the genotype groups and also detect the best populations for later studies or breeding. Population 6, from the adapted line Roane, was by far the best in terms of the FHB score and overall agronomic traits. Although the seed weight per plant of the plants in population 6 is lower than other populations, the yield components (number of plants per area, number of spiketlets per spike and thousand kernel weight) of this population in the 2012 transplant nursery performs better than other population (data not shown). One of derivatives of this cross was advanced into the yield trial program in 2013 and ranked first in the preliminary yield test (data not shown). All populations may also show leaf rust and stem rust resistance because of the presence of Lr19/Sr25 in one of the parental lines, Wheatear. Beside Lr19/Sr25, there is also a yield bonus QTL in the translocation segment (Monneveux et al. 2003). This QTL might contribute to the high performance of the yield in the six populations.

We tested the hypothesis that pyramiding two FHB QTL can provide better FHB resistance than the single QTL lines without penalty of agronomic performance, especially yield. However with our current data we cannot detect improvement of FHB

resistance due to the pyramiding. We also identified population 6 with high FHB resistance and good agronomic traits. Therefore, we can conclude that pyramiding gene sources from landraces and wild relatives is a valid strategy in FHB resistance breeding in wheat that can be achieved without agronomical penalty. Further testing will determine whether the durability of the plants with pyramided FHB resistance genes is improved over plants with only single resistance genes.

Table 2.1 Decreased FHB severity due to selection within populations

Population	Parental adapted line	11 TN (Generation 4)		11 FGH (Generation 5)		12 TN (Generation 6)		12 FGH (Generation 7)	
		N [1]	Mean±SE [2]	N	Mean±SE	N	Mean±SE [2]	N	Mean±SE
1	1026A	13	1.19±0.22 B	201	2.02±0.22 CD	196	0.89±0.1 D	264	1.22±0.11 D
2	1065RA	8	1.06±0.15 B	112	4.16±0.45 B	35	3.33±0.69 B	35	3.63±0.86 C
3	P25R62	3	2.00±0.58 B	25	3.10±1.12 BC	41	0.69±0.05 D	118	1.13±0.12 D
4	1070RA	7	2.86±1.53 B	50	3.95±0.76 B	35	1.60±0.26 C	36	5.00±0.81 B
5	106A	2	1.50±0.50 B	61	2.59±0.43 CD	26	0.92±0.14 D	19	1.00±0.12 D
6	Roane	9	0.83±0.20 B	127	1.53±0.17 D	57	0.76±0.09 D	221	0.88±0.06 D
Patterson		3	8.50±0.86 A	10	9.70±0.91 A	5	8.6±1.88 A	10	7.38±2.07 A

[1] Number of plants in this population at each generation.

[2] Populations with different letters within the same generation are significantly different from each other at the p=0.05 level according to the mean FHB score.

Table 2.2 Primer sequences for the SSR markers

Marker	Forward primer	Reverse primer
Xumn10	5'- CGT GGT TCC ACG TCT TCT TA -3'	5'- TGA AGT TCA TGC CAC GCA TA -3'
Xcfa2240	5'- TGC AGC ATG CAT TTT AGC TT-3'	5'- TGC CGC ACT TAT TTG TTC AC -3'
Xbf145935	5'- CTT CAC CTC CAA GGA GTT CCA C -3'	5'-GCG TAC CTG ATC ACC ACC TTG AAG G -3'

Table 2.3 FHB severity at generations 4 and 6 due to selection within genotype groups

Genotype	*Fhb 1*-linked marker	*Qfhs.pur-7EL*-linked markers		11 TN		12 TN	
	Xumn10	Xcfa2240	Xbf145935	N [1]	Mean±SE [2]	N	Mean±SE
1	-	-[3]	-	5	3.10±2.24 A	43	2.88±0.59 A
2	-	+[3]	+	3	1.00±0.58 AB	118	1.08±0.11 B
3	+	-	-	23	1.26±0.17 AB	9	1.17±0.33 B
4	+	+	+	11	1.18±0.12 B	220	0.82±0.08 B

[1] Number of plants in this group

[2] Populations with different letters within the same generation are significantly different from each other at p-value of 0.05 level according to the transformed FHB score

[3] Tall wheatgrass alleles are designated "+" and wheat alleles "-" in Xcfa2240 and Xbf145935.

Table 2.4 Population agronomic trait assessments[1] at the end of selection (generation 7)

Population	Height (cm) [2]	Straw diameter (mm)	Seed weight per plant (g)
1	63.91±0.38 A	2.18±0.02 B	2.76±0.05 E
2	77.32±1.30 C	2.49±0.24 C	2.27±0.16 D
3	69.50±0.76 B	2.27±0.02 B	2.25±0.07 D
4	69.01±1.63 B	2.02±0.04 A	2.25±0.12D
5	68.91±1.65 B	2.36±0.07 B	2.12±0.23 C
6	72.16±0.76 B	2.26±0.15 B	1.82±0.11 B
Patterson	63.63±0.87 A	1.92±0.09 A	0.98±0.18 A

[1] Mean values with SEs are given for height (cm), straw diameter (mm) and seed weight per plant (g). All plants measured were infested with FHB.

[2] Values with different letters within the same trait column are significantly different from each other at p-value of 0.05 level.

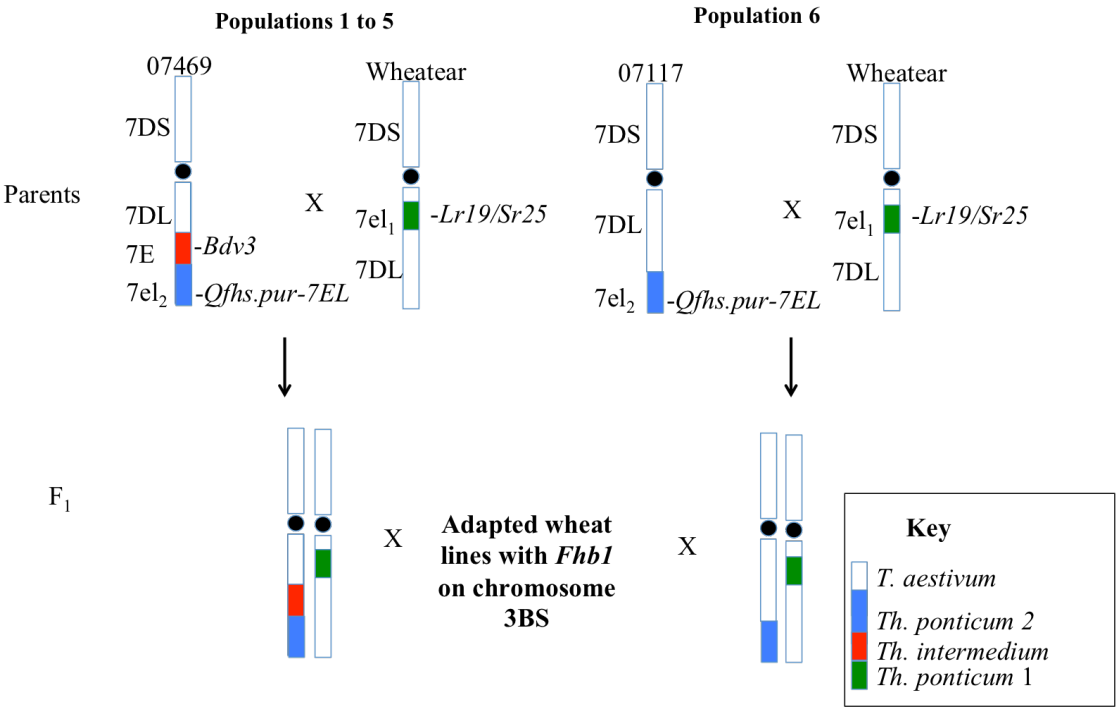


Figure 2.1 Crossing scheme

The three parents of the populations had different translocation segments for chromosome 7D/7E (marked by different colors in the figure), each carrying different resistance QTL. F_1 plants were crossed with five (populations 1-5) or one (population 6) different adapted wheat lines.

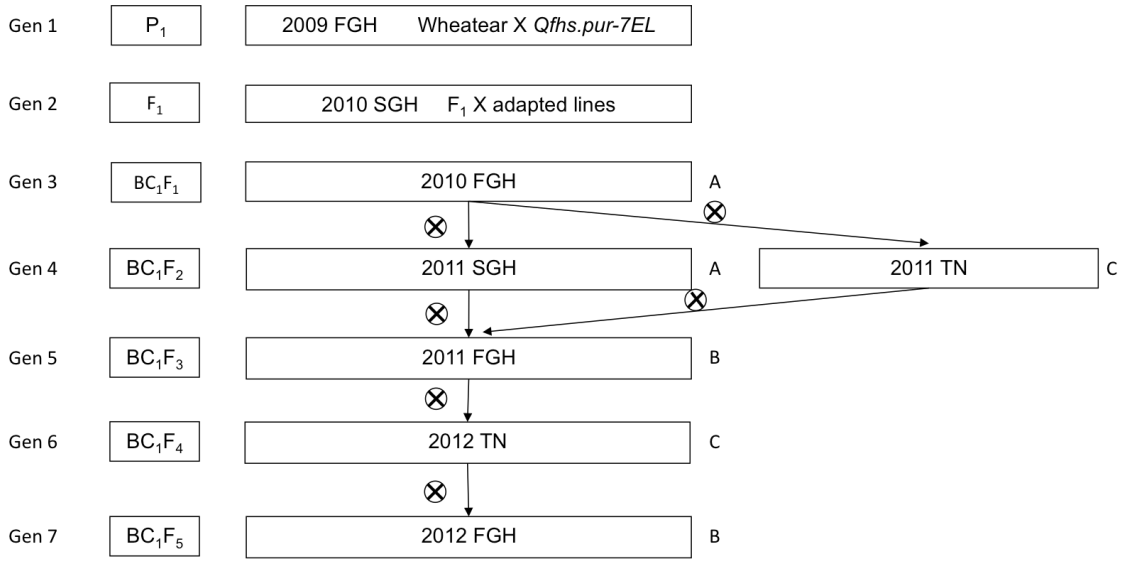
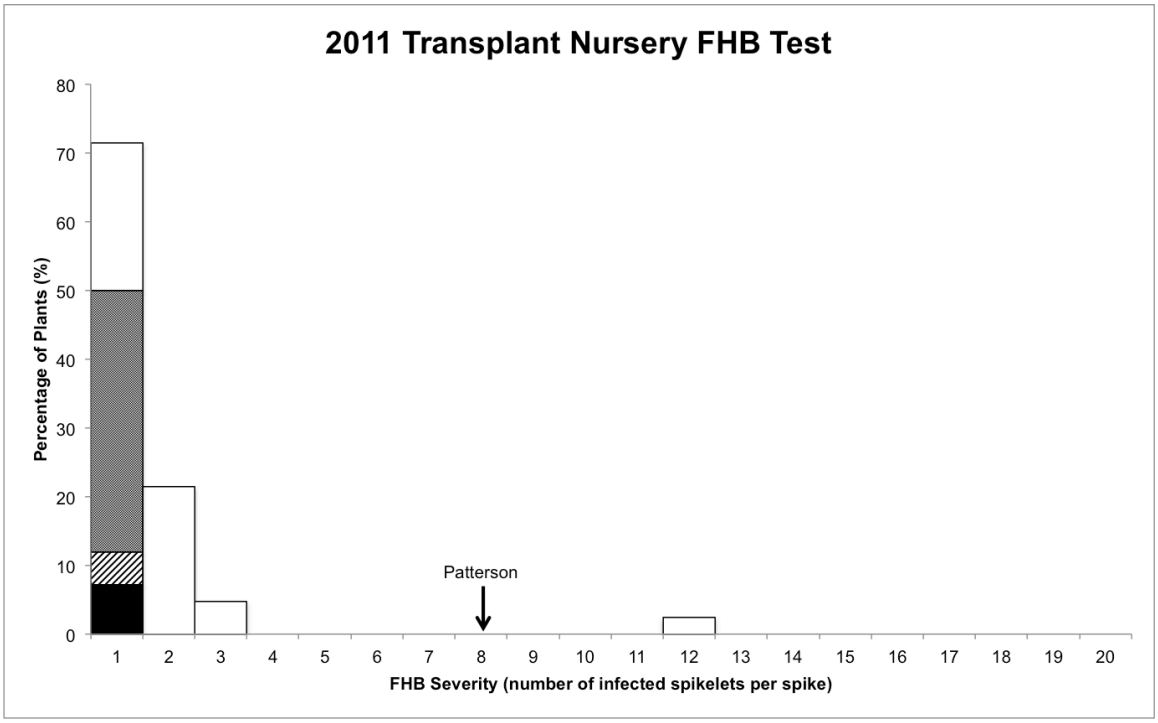


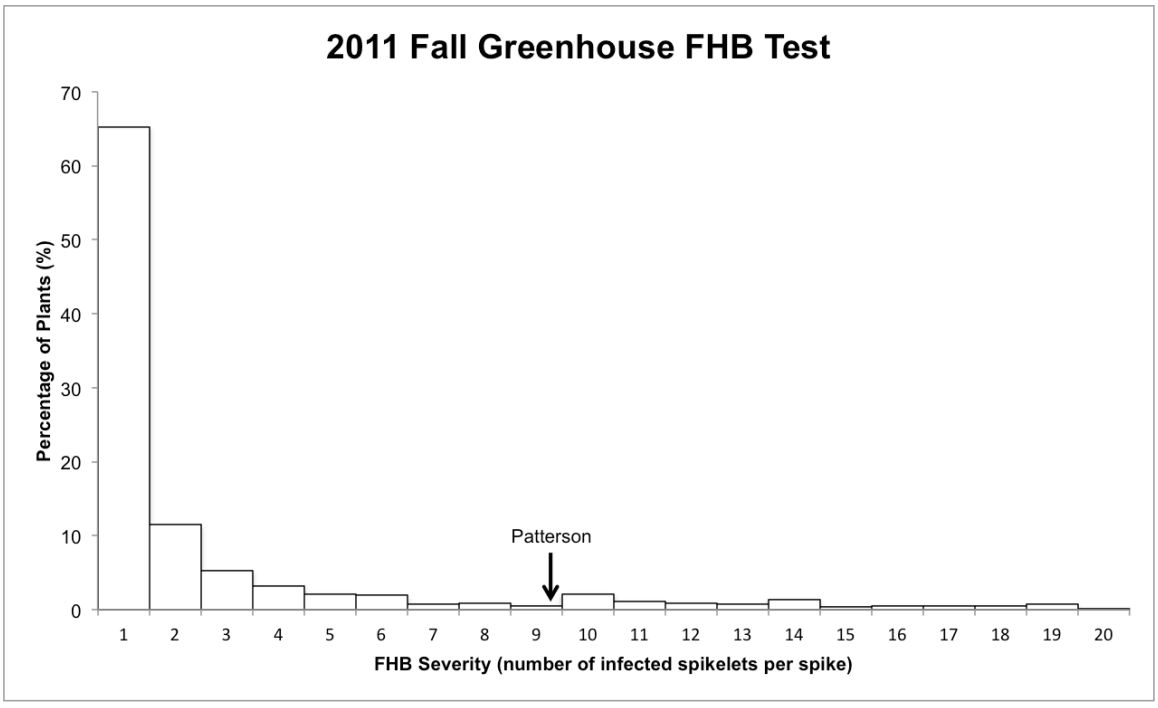
Figure 2.2 Population construction

Six populations were constructed by crossing the F_1 plants (resulting from Wheatear crosses to 07469 and 07117 which both contain FHB resistance QTL *Qfhs.pur-7EL*), to six adapted lines (containing *Fhb1*) and then self-crossing until generation 7. "A" indicates the materials were genotyped, "B" indicates the materials were phenotyped and "C" indicates the materials were both genotyped and phenotyped. TN = Transplant nursery, FGH = Fall greenhouse, SGH = Spring greenhouse.

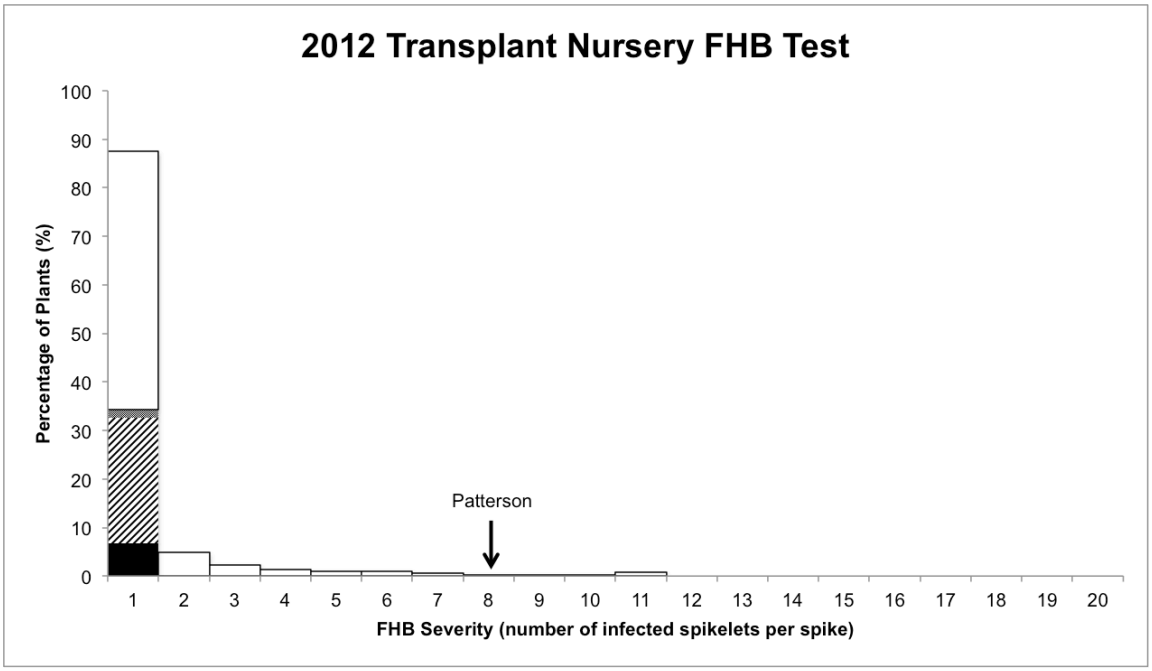
a.


2011 Transplant Nursery FHB Test
Percentage of Plants (%)
0
10
20
30
40
50
60
70
80
Patterson
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
FHB Severity (number of infected spikelets per spike)

b.


2011 Fall Greenhouse FHB Test
Percentage of Plants (%)
0
10
20
30
40
50
60
70
Patterson
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
FHB Severity (number of infected spikelets per spike)

c.



d.

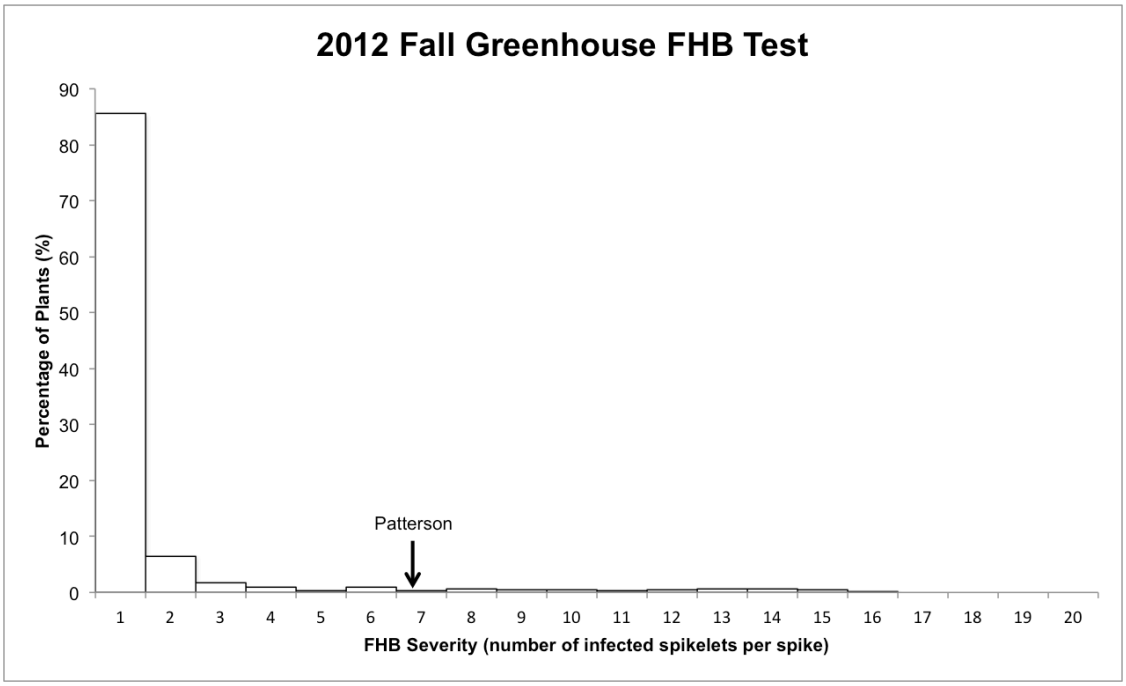


Figure 2.3 FHB score frequency distribution in four different FHB tests

The X-axis represents type II FHB severity scores and the Y-axis represents the percentage of plants from all six populations combined. The low severity bars in panels "a" and "c" are divided to display data by genotype: black indicates lines (3 in a and 26 in c) with none of three marker (Xumn10, Xcfa2240, and Xbf145935; genotype 1); stripe indicates lines (2 in a and 101 in c) with two flanking markers of *Qfhs.pur-7EL* (Xcfa2240, and Xbf145935; genotypes 2); grey indicates lines (16 in a and 7 in c) with only the Xumn10 marker (genotype 3); and white indicates the lines with all three markers (9 in a and 207 in c; genotype 4).

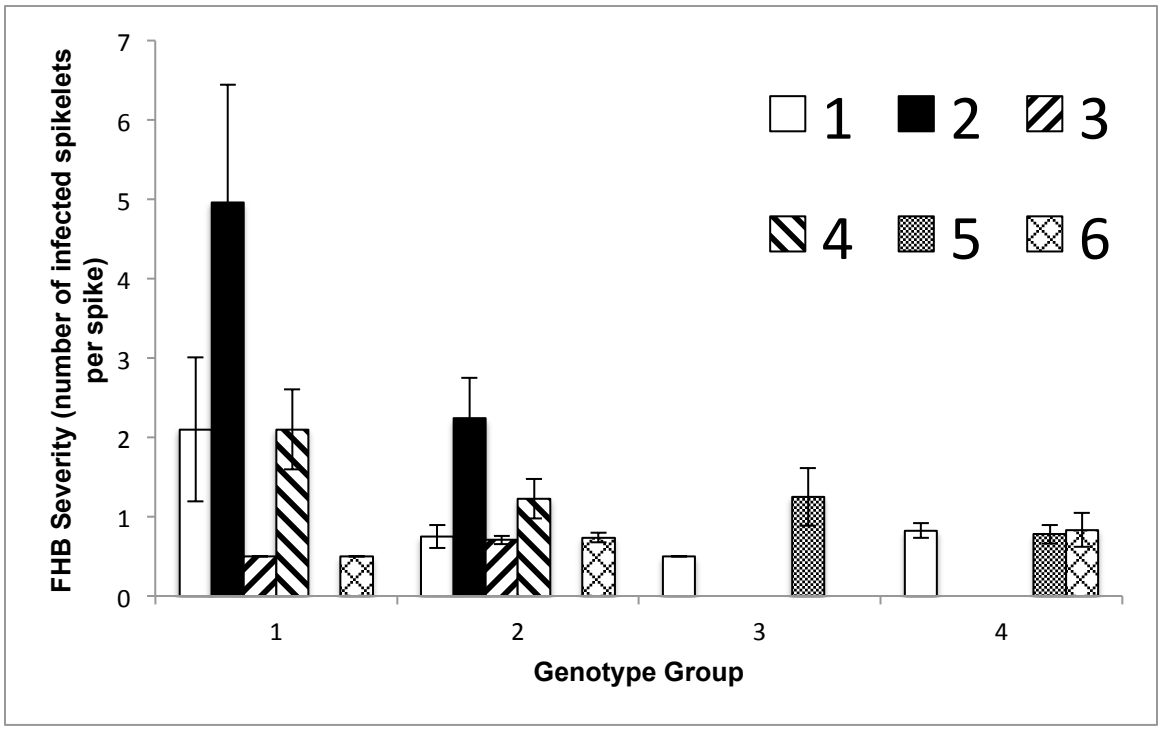
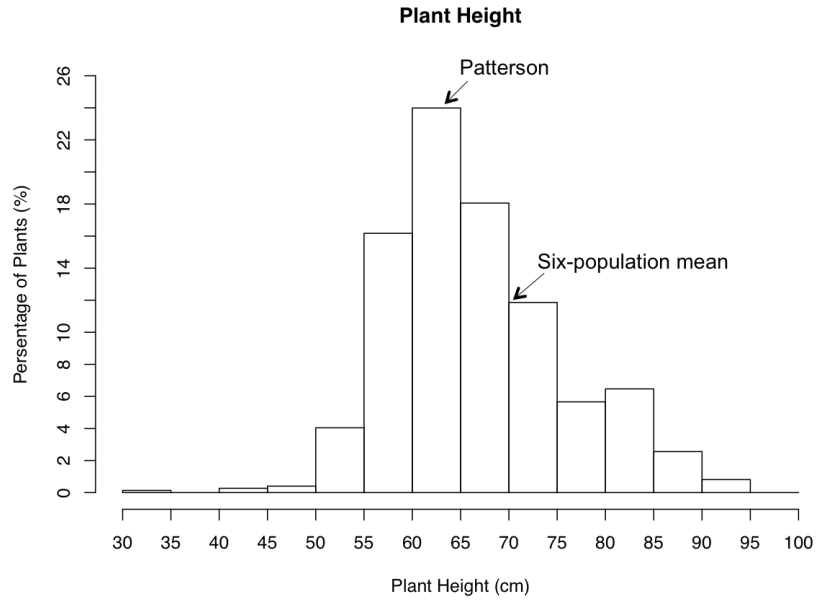


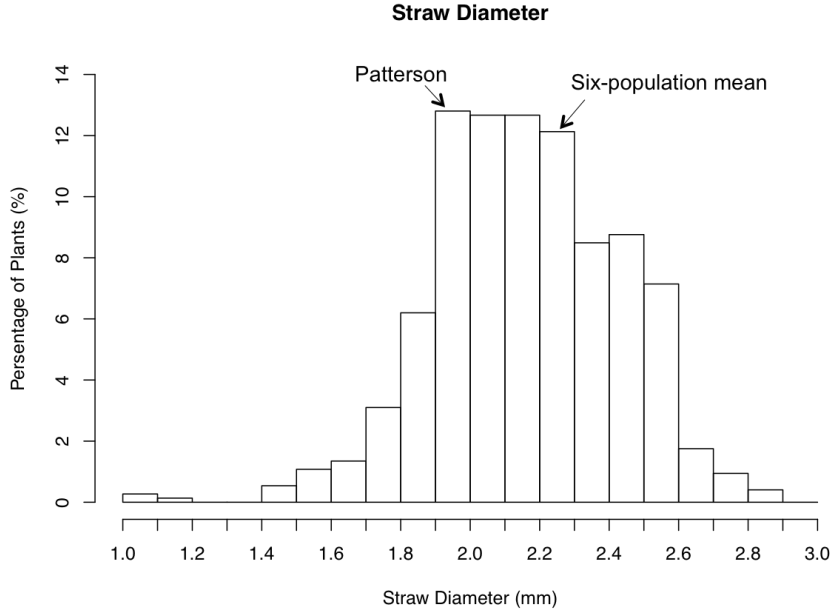
Figure 2.4 Interaction plot of genotype and population effect

The bars within each genotype group show the mean FHB severity score ± SE for all six populations. When a population is not represented for a genotype, there were no individual plants that had that genotype. Genotype 1 has none of the three markers, Xumn10, Xcfa2240 and Xbf145935. Genotype 2 has both Xcfa2240 and Xbf145935 markers linked to *Qfhs.pur-7EL*, genotype 3 has the Xumn10 marker linked to *Fhb1*, and genotype 4 has both markers linked to *Qfhs.pur-7EL* and the marker for *Fhb1*.

a


Plant Height
Patterson
Six-population mean
Persentage of Plants (%)
0
2
4
6
8
10
14
18
22
26
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
Plant Height (cm)

b


Straw Diameter
Patterson
Six-population mean
Persentage of Plants (%)
0
2
4
6
8
10
12
14
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
Straw Diameter (mm)

c

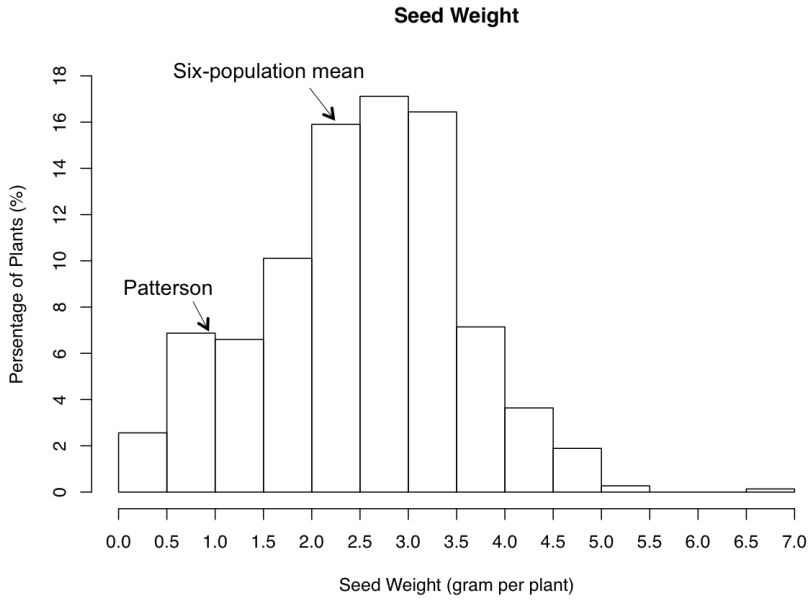


Figure 2.5 Distribution of plant height, straw diameter and seed weight per plant.

The Y-axis represents the percentage of plants from all six populations combined. Arrows indicate the combined mean for the six populations and for Patterson. All plants measured were infested with FHB.

CHAPTER 3. MAPPING QTL FOR TYPE II FHB AND LEAF RUST RESISTANCE IN A WHEAT-TALL WHEATGRASS INTROGRESSION RIL POPULATION

3.1 Abstract

Fusarium head blight (FHB) and leaf rust (LR) are two major fungal pathogens threatening the wheat crop, consequently identifying resistance genes from various sources is always of importance to wheat breeders. We identified tightly linked markers for FHB-resistance QTL *Qfhs.pur-7EL* and the LR-resistance gene *Lr19* using genotyping by sequencing in a wheat-tall wheatgrass introgression-derived recombinant inbred line (RIL) population. 216,318 SNPs were discovered for this population. After filtering, 1700 high-confidence SNPs were used to conduct the linkage and QTL analysis. *Qfhs.pur-7EL* was mapped to a 2.9 cM region within a 43.6 cM segment of wheatgrass chromosome $7el_2$ that was translocated onto wheat chromosome 7DL. The LR gene *Lr19* from $7el_1$ was mapped to a 1.21 cM region in the same area, in repulsion. Five lines were identified with the resistance-associated SNP alleles in coupling for *Qfhs.pur-7EL* and *Lr19*. Investigation of the genetic characteristics of the parental lines of this RIL population indicated that they are translocation lines in two different wheat cultivar genetic backgrounds instead of 7E-7D substitution lines in Thatcher wheat background as previously reported in the literature.

3.2 Introduction

Fusarium head blight (FHB) or scab, caused by *Fusarium graminearum* is a destructive fungal disease of wheat (Mesterhazy 1995). The pathogen not only affects wheat, but also other small grain cereals causing reduction in yield and grain quality. Most importantly, *F. graminearum* produces the toxin trichothecene deoxynivalenol that accumulates in the grain, and when consumed threatens human and animal health (Parry et al. 1995; McMullen et al. 1997). Although the commonly used type II FHB resistance gene *Fhb1,* from the wheat cultivar Sumai 3, provides strong resistance, identifying resistance in other cultivars, landraces or related species is essential to maintain diversity for FHB resistance in breeding programs. *Qfhs.pur-7EL* (syn. *FhbLop;* Zhang et al. 2011) is a quantitative trait locus (QTL) from tall wheatgrass (*Thinopyrum ponticum)* chromosome $7el_2$ conferring strong resistance to FHB (Shen and Ohm (2007).

Leaf rust, caused by *Puccinia recondite* Roberge ex Desmaz. f. sp. *tritici* Eriks. & E. Henn is the most common rust disease of wheat and appears wherever wheat is grown (Samborski 1985). Susceptible wheat cultivars suffer a 5% - 15% yield loss, depending on the developmental stage at initial infection (Samborski and Peturson 1960; Samborski 1985). *Lr19*, transferred from tall wheatgrass (*Th. ponticum)* chromosome $7el_1$ to wheat chromosome 7D (Sharma and Knott 1966), confers highly effective resistance to LR worldwide, regardless of reported virulence (Huerta-Espino and Singh 1994). The translocated *Lr19* segment is homoeologous with a segment of wheat chromosome 7DL, however the regions cannot pair and recombine in the presence of the recombination inhibitor *Ph1* (Knott 1980; Marais and Marais 1990). Thus, *Lr19* cannot be mapped through approaches based on recombination frequencies in common wheat.

Tall wheatgrass has been an important genetic resource for improving resistance to biotic (Cox et al. 2002) and abiotic (Chen et al. 2004) stresses of wheat. Alien chromatin conferring resistance can be incorporated as a whole-chromosome addition or substitution, but a short translocation is preferable for wheat improvement because it minimizes linkage drag (Brinkman and Frey 1977b; Wang 2011). The use of wheat lines containing *Ph1* deletions allows homoeologous recombination between wheat and alien chromatin for the introduction of beneficial genes (Riley 1958; Sears and Okamoto 1958). In addition, overlapping translocations can be used to reduce the length of an alien translocation or introduce another gene through recombination if tightly linked markers throughout the alien segments are available (Young and Tanksley 1989b).

Accurate QTL mapping is the foundation of marker-assisted selection in a breeding program (Kumar 1999). However, map resolution is limited by the number of markers available, which affects the ability to detect and to accurately map the QTL (Beavis 1997). Genotyping-by-sequencing (GBS) is an efficient method for discovering tens of thousands of single nucleotide polymorphism (SNP) markers while simultaneously genotyping in genetics studies (Poland and Rife 2012). GBS has been used for linkage mapping in wheat and barley (Poland et al. 2012a), association mapping in maize and barley (Brachi et al. 2011; Elshire et al. 2011), genomic selection in wheat (Poland et al. 2012b) and evolution studies in switchgrass (Lu et al. 2013).

The population in this study was used previously to map the FHB resistance QTL, *Qfhs.pur-7EL,* to a 15.3 cM region flanked by SSR markers (Shen and Ohm (2007). This same population was used with an additional year of FHB data to refine the location to a 3.71 cM region while also mapping the LR resistance gene, *Lr19* (Zhang et al. 2011).

Both publications reported that the parental lines contained *Th. ponticum* chromosomes 7E substituting for wheat 7D in 'Thatcher' wheat background. With the advent of GBS, we were able to obtain dense coverage of the introgressed segments plus the rest of the genome with SNP markers, and thus more accurately characterize the germplasm for use in breeding.

The goals of the present study were 1) to provide dense coverage of SNP molecular markers for marker-assisted breeding and 2) to identify lines with the FHB-resistance QTL *Qfhs.pur-7EL* and the LR-resistance gene *Lr19* in coupling. In addition, we determined that the parental lines (resistance donors) differed from what was reported previously by (Shen and Ohm 2007; Zhang et al. 2011).

3.3 Materials and methods

3.3.1 Population construction

The exact origins of parents used for constructing the mapping population are difficult to trace due to the lines being renamed in different publications. We assumed that the names given in Kim et al. (1993) and Knott et al. (1977) apply to these lines since those were cited in previous publications associated with construction of the mapping population (Shen and Ohm, 2007; Zhang et al., 2011). K2620 is a disomic substitution line, showing moderate resistance to stem rust races 15B and 56 and carrying FHB-resistance QTL *Qfhs.pur-7EL*. K2620 has the *Th. ponticum* (tall wheatgrass) chromosome $7el_2$ substituting for wheat chromosome 7D (Knott et al. 1977; Kim et al. 1993) in what we believe is a wheat background of cultivar "Marquis" rather than "Thatcher" as cited previously (Shen and Ohm, 2007 and Zhang et al., 2011). K11463 is a substitution line showing LR resistance with wheat 7D replaced by *Th. ponticum* $7el_1$ in

"Thatcher" wheat background (Knott 1968; Kim et al. 1993). Chromosome $7el_1$ and $7el_2$ are able to recombine, so QTL on those chromosomes can be mapped when the lines are used to construct a mapping population (Dvořák 1975).

The RIL population used here was constructed by Shen and Ohm (2007) as described below. The initial cross was made between K2620 and K11463 in March 2003 in a greenhouse at Purdue University, West Lafayette, Indiana. The population was evaluated at the F_2 generation for type II FHB resistance by point inoculation in the greenhouse in March 2004. After self-crossing to produce $F_{2:3}$ families, ten plants per family were evaluated with FHB in the greenhouse in November 2004. A random plant from each $F_{2:3}$ family was harvested to represent its family during RIL construction through single-seed descent. In October 2005 and March 2006 the $F_{4:5}$ and $F_{5:6}$ generations, respectively, were evaluated for type II FHB resistance in the greenhouse. The final $F_{5:6}$ population consisted of 274 lines. In addition to the current study, the FHB-resistance QTL was mapped previously with SSR markers in this same RIL population by two different research groups (Shen and Ohm 2007; Zhang et al. 2011). *Lr19* was also mapped in this same RIL population with SSR markers by Zhang et al. (2011).

3.3.2 Plant growth and phenotyping

The RIL population used here for mapping was scored at Purdue University for type II FHB resistance as described below and the disease data were published by Zhang et al. (2011; Figure 2 and Table 2). For scoring resistance to FHB, ten seeds from each F_5 and six seeds from each F_6 RIL were sown in flats and at the 2-leaf seedling stage were transplanted individually into 3-inch pots in the greenhouse. Plants were artificially point inoculated with *F. graminearum* during anthesis. Ten µl of inoculum with a

concentration of 50,000 spores/ml were injected into the left floret of the second or third spikelet from the spike tip. The inoculated spikes were covered with plastic bags for three days to maintain high humidity. Disease symptoms were scored 21 days after inoculation. The total number of discolored spikelets per spike from the inoculation point and below was counted and the average was calculated for the ten or six plants to represent the FHB score for their RIL.

The LR evaluation, conducted at Shandong Agricultural University, Taian, China in 2008, included 237 RILs from the $F_{6:7}$ generation plus both parents, K11463 and K2620. When the plants were at heading stage, one of the most virulent LR isolates in southeast Asia, named 09-10-2, was used to infest the plants, as published in Pathan and Park (2006). The infection type of the plants was scored 14 days after inoculation using a score from 0 to 4 (Bushnell 1984). Plants with infection type scores of 0 through 2 were considered resistant whereas 3 and 4 were susceptible. LR data for this RIL population were published by Zhang et al. (2011).

3.3.3 DNA extraction for GBS

Young leaf tissue collected from each of the 274 RILs at the $F_{6:7}$ generation, plus the two parental lines, K2620 and K11463, was frozen in liquid nitrogen and stored at -80℃. DNA was extracted using the GenElute Plant Genomic DNA Miniprep kit (Sigma-Aldrich, St. Louis , MO) according to the manufacturer's instructions. DNA concentrations were quantified with Quant-iT™ PicoGreen® (Molecular Probes/Invitrogen, Eugene, OR) on a fluorescence-based microplate reader and diluted to 20ng/µl.

3.3.4 Library construction and genotyping-by-sequencing

A two-enzyme *PstI-MspI* protocol was used (Poland et al., 2012b) and barcode adapters were applied to DNA during library construction. The 274 RIL plus two parental samples were pooled into three 96-plex libraries, PCR-amplified, and each library was sequenced on a lane of an Illumina HiSeq 2000 (San Diego, CA).

3.3.5 SNP calling and location assignment

The SNP reads were processed using the default parameters of the Universal Network Enabled Analysis Kit (UNEAK) pipeline (Lu et al., 2013) of the program Trait Analysis by aSSociation, Evolution, and Linkage (TASSEL) 4.0 (Bradbury et al., 2007).

The Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990) was used to assign the 64bp tag sequence, containing each SNP, to wheat chromosomes by searching the wheat genome sequence published by The International Wheat Genome Sequence Consortium (IWGSC 2014; http://www.wheatgenome.org). The top hit of the BLAST results, with alignment length larger than 50bp, was used to designate the putative chromosome location for each SNP.

3.3.6 Filtering, linkage analysis and QTL mapping

During data filtering steps, a SNP was removed from the dataset if more than 20% of the RIL population had missing data for that sequence, if more than 5% of RILs were heterozygous for the SNP, or if the sequence was not polymorphic between the two parents. SNPs were clustered into linkage groups using JoinMap 4.0 software (Van Ooijen 2006) with a threshold value of 3.0 for minimum logarithm of the odds (LOD) score for linkage. The maximum likelihood algorithm of JoinMap was used to order the markers (Haley and Knott 1992). Map distance between SNP loci was estimated using

multipoint analyses with the regression algorithm and Kosambi mapping function, but using the order determined by maximum likelihood mapping (Kosambi 1943). The linkage group information was loaded into MapQTL 6.0 software (Van Ooijen 2009) and interval mapping identified the QTL. The FHB phenotypes were coded as continuous numeric data for QTL mapping and LR phenotypes were coded as R (resistant) or S (susceptible). A 1000-permutation test was used to calculate the empirical significant LOD threshold across the target linkage group to detect the underlying putative QTL at the p=0.05 level (Churchill and Doerge 1994). SNP markers closely linked to the FHB resistance locus within the approximate 95% confidence interval for QTL position were identified based on a LOD-1.5 support interval (Dupuis and Siegmund 1999).

3.4 Results

3.4.1 SNP identification

The UNEAK pipeline identified 216,318 SNP sequences and BLAST analysis assigned 45,849, 63,772 and 32,899 to the A (21.19%), B (29.48%) and D (15.21%) subgenomes, respectively, with 73,798 (34.12%) remaining unassigned. After filtering to remove sequences missing in more than 20% of the RILs, the number dropped dramatically to 12,513 SNPs, which is about 5.8% of the raw calls. The second filtering criterion, requiring that a SNP be removed if more than 5% of the RIL plants were heterozygous at that site, brought the number down to 1,741 markers. Lastly, only sequences that were polymorphic between the parental lines were used, resulting in 1700 SNPs, genome-wide, for the downstream analysis. After filtering, the D subgenome was under represented (Table 3.1).

3.4.2 Linkage analysis

Linkage analysis resulted in 24 linkage groups (Table S1) and BLAST analysis assigned them to parts of 16 chromosomes (Table 3.1). Linkage group 9 was not included in summary Table 3.1 because BLAST assigned 31 and 22 markers to chromosomes 1AL and 7AS, respectively. Since markers from these two chromosomes were interspersed, we were unable to infer a location for the linkage group. Due to its small number of markers, linkage group 24 was not included. No linkage groups were assigned to chromosomes 1A, 2D, 3D, 4B, 4D or 5A. Many chromosomes were represented by markers on only one arm. Incomplete coverage of the wheat genome by the draft sequence resulted in the mapping by linkage analysis not always supporting the chromosomal location assigned in BLAST; most linkage groups contained a mixture of SNPs assigned to homoeologous and non-homoeologous chromosomes. All linkage groups contained a large proportion of markers that BLAST could not align to any wheat sequence in the database. Consequently, each linkage group was placed on its chromosome based on where BLAST assigned the majority of its SNP sequences (Table S1 and Table 3.1).

BLAST analysis yielded unexpected results for markers within the linkage group that defined the chromosome containing the FHB-resistance QTL *Qfhs.pur-7EL* and the *Lr19* resistance gene: chromosome 7E from *Th. ponticum*. The linkage group appeared to comprise two distinct regions. Within the first 13.16 cM of the linkage group (Table S2), which contained the two resistance loci, the marker density was lower, 2.05 SNPs per cM, than in the remaining 32.62 cM where the density was 4.69 SNPs per cM. Twenty-five out of 27 markers within the first 13.16 cM had no corresponding homoeologous locus in the wheat genome; however, 54 out of 153 markers within the remaining 32.62 cM had

high similarity to sequences in the homoeologous group 7 chromosomes of the wheat genome (Table S2). Within the first 13.16 cM the two markers with similarity to group 7 of wheat had relatively large e-values of 3.00E-12 and 4.00E-15 (indicative of only moderate similarity to the best match in the wheat genome), whereas the average e-value for group 7 markers in the remaining 32.62 cM was more significant at E-25 for 7DS and E-20 for 7DL. The identification of these two regions of the linkage group raised questions as to whether the parental lines contained 7E-7D translocations rather than the previously reported 7E-7D whole chromosome substitutions (Zhang et al., 2011). As expected, the majority of the SNPs assigned to this chromosome had no match in the database (115 SNPs with no hits out of 187 total SNPs; Table S1 and Table S2) since *Th. ponticum* has not been sequenced and since the wheat genome assembly contains gaps (IWGSC 2014). Because previous research with this RIL population indicated that both parental lines were in a Thatcher wheat background (Zhang et al., 2011), it was unexpected to identify so many SNPs throughout the other chromosomes of the wheat genome (Table 3.1).

3.4.3 Mapping resistance loci

Interval mapping, using phenotypic data for both the $F_{4:5}$ and $F_{5:6}$ generations, placed FHB resistance QTL *Qfhs.pur-7EL* in identical locations spanning 2.9 cM near the end of the short arm of a group 7 chromosome (Figure 3.1). Sequences for the four SNPs located within the LOD-1.5 support intervals are shown in Table 3.2. The leaf rust resistance gene, *Lr19*, mapped to position 8.48 cM within a 1.21 cM region flanked by SNPs TP136477 and TP328971 (sequences in Table 3.2), which is 5.09 cM proximal to *Qfhs.pur-7EL* marker TP31888.

3.4.4 Pyramided lines with *Qfhs.pur-7EL* and *Lr19*

RILs with all four resistance-linked SNP alleles had significantly lower FHB scores (higher FHB resistance) than RILs with four susceptibility-linked SNP alleles (Table 3.3). Using that information, we identified five lines (line ID 303, 328, 346, 390 and 465) in the $F_{6:7}$ RIL population that resulted from a recombination between the two parental *Th. ponticum* segments and thus carried all four SNPs located in the LOD-1.5 confidence interval for *Qfhs.pur-7EL* plus the two SNPs flanking *Lr19*. All five lines were resistant to leaf rust (Table 3.4). However, the FHB scores of lines 390 and 465 in the $F_{4:5}$ generation (mean FHB score 8 and mean 7, respectively) were higher than the mean FHB score of the 107 RILs with all four resistance-linked SNPs (mean 5.09 SD 2.88, p= 0.16 and p= 0.25 respectively). In the $F_{5:6}$ generation the lines containing all markers for both resistance loci, except for line 390, exhibited acceptable levels of type II FHB resistance (Table 3.4). Line 346 was the most resistant to FHB of the five lines with all markers, and it presumably carried FHB- and leaf rust-resistance in coupling.

3.5 Discussion

We used GBS along with QTL mapping to develop improved molecular markers for a RIL population used previously (Shen and Ohm 2007; Zhang et al. 2011) to map loci for type II FHB and LR resistance. This high-throughput approach mapped the FHB resistance QTL *Qfhs.pur-7EL* to a 2.9 cM LOD 26 interval compared to the 15.3 cM interval of Shen and Ohm (2007) and 3.71 cM of Zhang et al. (2011). We also improved the resolution of the map for *Lr19* from 3.97 cM (Zhang et al. (2011) to 1.21 cM. Thus we achieved our first goal to provide dense coverage of SNP molecular markers for marker-assisted breeding.

Four SNPs were identified in the LOD-1.5 support interval for *Qfhs.pur-7EL* and two flanking SNPs were identified for *Lr19*. With these six SNPs, five lines in the RIL population were identified that had all of the markers. Resistance data indicated that at least two of the lines, 328 and 346, were highly resistant to both diseases and carried *Qfhs.pur-7EL* and *Lr19* in coupling (Table 3.4). Although line 390 had all four *Qfhs.pur-7EL*-linked markers plus was resistant to LR, it was not very resistant to FHB; the recombination that put all 6 markers in coupling may have been within the marker-deficient proximal 1cM tail of the LOD-1.5 confidence interval and thus distal to the FHB resistance QTL, resulting in its loss. The pyramided lines exhibiting FHB and LR resistance in coupling, our second goal, show promise as resources for improving the resistance of wheat cultivars to two economically important diseases.

Since Zhang et al. (2011) used the same RIL population and provided us with their mapping data plus the FHB and LR phenotypic data for construction of our SNP map, we attempted to integrate their SSR data with the SNP data from this study. However, many of the SSRs expanded the integrated SNP/SSR map dramatically and showed very high stress values in JoinMap 4.0 analysis (Table S3). Markers with high stress values (greater than ± 5.000) do not have a good fit in the map (Van Ooijen 2006). When we used JoinMap with their SSR data alone, the markers with high stress values in the integrated SNP/SSR map still caused map expansion. *Xswes19*, which is one of the flanking SSR markers for *Qfhs.pur-7EL* Zhang et al. (2011), was among these high stress value markers. Consequently, we were unable to integrate our SNP-based map with published wheat SSR markers. The SNPs that we identified did not expand the map and thus are considered reliable markers (Table S3).

Our data suggested that the parental lines contained 7E/7D translocations rather than full chromosome substitution lines. The parental lines of this RIL population were reported to contain the entire *Th. ponticum* chromosome $7el_2$ replacing wheat 7D in parent K2620 and chromosome $7el_1$ replacing wheat 7D in parent K11463 (Sharma and Knott 1966; Dvořák 1975; Knott et al. 1977). Both parents were reported to have Thatcher wheat background (Shen and Ohm 2007; Zhang et al. 2011). The presence of 7e chromosomes replacing 7D in the original K2620 and K11463 lines was verified through fluorescent in situ hybridization using markers specific to tall wheatgrass as probes (Kim et al. 1993). However, this understanding of the parental lines of our RIL population was not verified through our BLAST assignments of SNPs to wheat chromosomes.

The 64bp sequences containing SNPs (GBS data) mapping to the target linkage group containing *Qfhs.pur-7EL* were aligned (BLAST) to the draft wheat genome to identify their chromosomal locations (Table S2). Using the regression linkage map, twenty-one of the first 34 markers of the target linkage group corresponded to sequences on the wheat 7DS chromosome with significant E-values lower than E-23 in the BLAST result. This good match to wheat chromosome 7DS sequences would not be expected if the parental lines contained entire 7e chromosomes substituting for the wheat 7D chromosomes, as reported by Shen and Ohm (2007); Zhang et al. (2011). These markers covered only 3 cM of the short arm of 7DS. The next 126 markers, mapping to the middle of the target linkage group, constituted a region where 25 markers corresponded to wheat 7DL sequences with the majority of them having E-values around E-20 and a few around E-23. This central region also contained 79 markers, interspersed with the others, that did not align to the wheat genome. Finally, within the 13.16 cM at the end of

the target linkage group, 25 of the 27 markers surrounding *Qfhs.pur-7EL* did not match wheat sequences. The other two markers corresponded to 7AL and 7DL with E-values of 3.00E-12 and 4.00E-15, respectively, which is much less significant than the markers that corresponded to wheat sequences throughout the rest of the chromosome (average E-value E-23). The unusual structure of this chromosome, 13.16 cM that appeared to not match wheat sequences attached to 32.62 cM that had high similarity with wheat 7DL and 7DS chromosome arms, suggests that the region containing resistance loci *Qfhs.pur-7EL* and *LR19* is a translocation rather than a chromosome substitution, as reported earlier (Shen and Ohm 2007; Zhang et al. 2011).

Additional evidence that the parental lines are not as previously reported is that a large proportion of SNPs (1513 out of the total 1700) were assigned by BLAST to wheat chromosomes other than 7D/7E. This high polymorphism was unexpected since the parental lines were both reported to be in a Thatcher wheat background (Shen and Ohm 2007; Zhang et al. 2011). Therefore, we speculate that the parental lines were not in the same genetic background. Following the production of these lines through the literature, it is clear that many 7E/7D translocation lines and substitution lines were generated in the 1960s and 1970s, some in Thatcher backgrounds and some in Marquis (Sharma and Knott, 1966; Knott 1968; Knott et al 1977). Kim et al, 1993 appeared to use some of these lines but gave them different names. It seems plausible that the lines used to make the mapping population used by (Shen and Ohm 2007; Zhang et al. 2011) and our group is not what we thought it was.

In summary, we achieved our goals to increase the map resolution of the QTL region and identify lines with *Qfhs,pur-7EL* and *Lr19* combined in coupling. This germplasm is

a resource for future cultivar development. Currently the SNP markers flanking *Qfhs,pur-7EL* and *Lr19* are being redesigned to produce KASP markers (KBioscience, Hoddesdon, UK) which can be easily used by plant breeders. In addition, by combining the high throughput GBS method for generating many markers in a mapping population with the BLAST approach, we reevaluated the chromosome constitution of the parental lines used for constructing the RIL population and suggest that they are neither 7E-7D substitution lines nor in the same genetic background.

Table 3.1 Chromosome locations of SNP markers

Chromosome [1]	Mapped Markers [2]	Assigned Markers [3]	Length (cM)
1B	69	45	72.9
1DS	26	21	28.45
2A	151	125	90.36
2B	197	167	101.36
3AL	48	28	29.4
3B	115	77	114.51
4A	71	55	75.35
4BS	12	9	30.79
5BL	84	66	41.16
5DL	16	15	7.02
6AL	40	32	39.13
6B	123	67	57.16
6DS	15	13	32.19
7AL	28	20	16.65
7BL	45	29	18.85
7D/7E	187	47	45.78
Total	1227	816	801.06
Genome	Percentage of markers mapped	Percentage of markers assigned	Length (cM)
Genome A	27.55	31.86	250.89
Genome B	52.57	56.37	436.73
Genome D	19.88	11.77	113.44

[1] Chromosome location was inferred by the majority of BLAST hits for markers in each linkage group.
[2] Number of SNPs after filtering mapped to each chromosomes by JoinMap, based on a minimum logarithm of the odds threshold value of 3.0. Linkage groups with fewer than 12 markers mapping to one chromosome were not represented.
[3] Number of SNPs among the mapped markers assigned to the indicated wheat chromosome based on BLAST results. Some markers mapped to locations not predicted by BLAST, resulting in more markers placed on the chromosome map than were assigned by BLAST.

Table 3.2 Sequences of SNPs linked to *Qfhs-pur.7EL* and *Lr19*

Location	Marker	Marker Sequences (R/S [1])
Qfhs.pur-7EL		
1.61	TP83101	TGCAGCAGATGGCCTTCTTTGTTTGTTCGCCCCC(**T/C**)GTCCACCTCCCTACGCCGAGATCCATCTG
2.77	TP340234	TGCAGTTTTGCAAGCGTTAGTAGTGCTCACCATGAAAAGGAAAAGAGAGTGAGGAATTA(**A/G**)TACA
2.95	TP114709	TGCAGCCCCAGCGACCCTCCCCAATACAA(**C/A**)GCCAGCGCCGCCACCAGGTTCAGCCGAGATCGGA
3.38	TP31888	TGCAGAGATCTGCACCTGCCGAAGTTGAAGTGTTGTCATTTGTGATTACTGACATGTATTT(**A/G**)TA
Lr19		
7.93	TP136477	TGCAGCGACGACCCCACCGCGAACCGCAAAACGAATCTGTTGCCGAC(**A/C**)ACCGTGTGCGCGAGGT
9.14	TP328971	TGCAGTTCGATCATGGGACAAGTTCATCGATGTACGCAG(**C/A**)TACCCCTCCTTCCTTCCGAGATCG

[1]*Qfhs.pur-7EL:* K2620 resistant parent SNP allele/ K11463 susceptible parent SNP allele

Lr19: K11463 resistant parent SNP allele/ K2620 susceptible parent SNP allele

Table 3.3 Mean FHB scores for RILs with resistance- or susceptibility-linked SNP alleles

Test	SNPs in LOD-1.5 support interval				N [1]	Mean ±SD [2]
	TP 83101	TP340234	TP114709	TP31888		
$F_{4:5}$	+	+	+	+	107	5.09 ± 2.88 [A]
	-	-	-	-	116	8.47 ± 2.89 [B]
$F_{5:6}$	+	+	+	+	107	2.94 ± 1.76 [A]
	-	-	-	-	116	6.88 ± 2.50 [B]

+ indicates resistance-linked SNP alleles from K2620
- indicates susceptibility-linked SNP alleles from K11463
[1] Number of RILs with the marker combination indicated
[2] Groups within a test generation with different letters are significantly different from each other at p=0.05

Table 3.4 Mean FHB resistance score and leaf rust resistance response[1] for lines with all the resistance-linked SNP alleles for *Qfhs.pur-7EL* and *Lr19*

Line	Mean FHB Score		Leaf rust
	$F_{4:5}$	$F_{5:6}$	$F_{6:7}$
303	5	2.8	R
328	4	1.7	R
346	3	1.1	R
390	8	7.7	R
465	7	2.2	R
Average [2]	5.09	2.94	

[1] FHB scores from Shen and Ohm (2007) and leaf rust response data from Zhang et al. (2011)

[2] Mean FHB score of RILs with all four resistance-linked SNP alleles within the LOD-1.5 support interval of *Qfhs.pur-7EL* and two flanking SNPs for LR resistance-linked alleles.

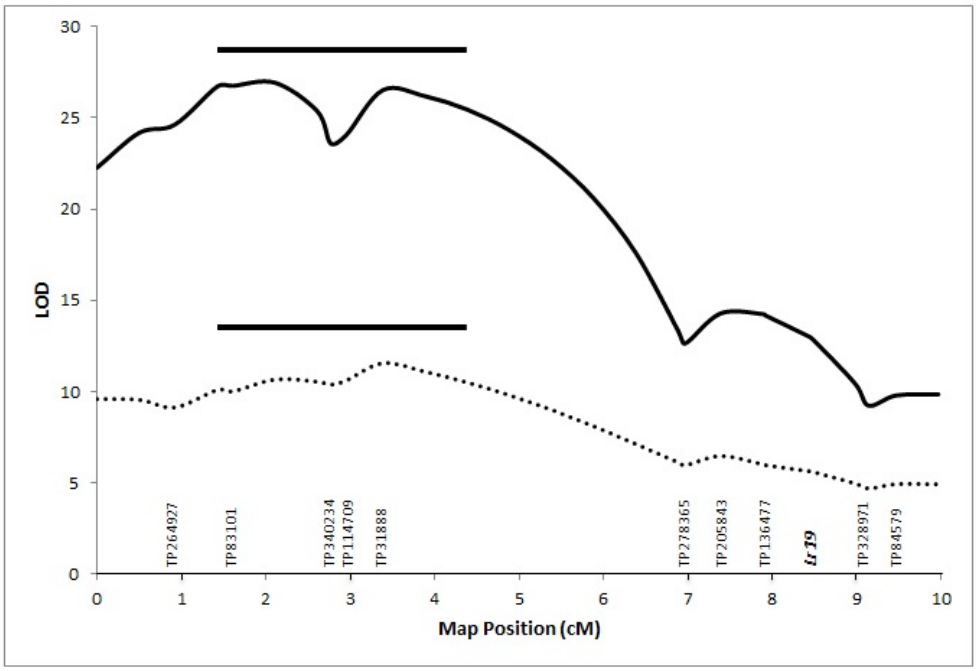


Figure 3.1 Location of resistance loci on chromosome 7DL/7EL.

Type II FHB-resistance data were combined with GBS SNP data to detect the *Qfhs.pur-7EL* QTL. Separate LOD scans were calculated for the $F_{4:5}$ (lower scan, dotted line) and $F_{5:6}$ (upper scan, solid line) generations of the RIL population using interval mapping. The map positions of the LOD-1.5 support interval (an approximate representation of the 95% confidence interval) for *Qfhs.pur-7EL* are defined by the heavy bars above each scan. The end points for the $F_{4:5}$ LOD-1.5 support interval are 1.4 and 4.3 cM and for $F_{5:6}$ are 1.4 and 4.3 cM. The position of the leaf rust resistance gene, *Lr19*, is indicated along with its flanking markers. Exact marker positions are designated in table 3.3.

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APPENDICES

APPENDICES

Table S 1 BLAST assignment of markers and linkage groups to chromosomal locations

Linkage Group	Chr.	Number of hits
1	4AL	44
	no_hit	10
	3AL	1
	4DL	1
	2DL	1
2	3B	64
	no_hit	16
	4BS	1
	5BS	1
	4BL	1
	3DL	1
	4AS	1
	2AS	1
3	5BL	66
	no_hit	12
	5DL	2
	4BS	1
4	2BL	84
	2BS	73
	no_hit	18
	4BS	4
	2AL	1
	2AS	1
	6BL	1

Table S1 continued

Linkage Group	Chr.	Number of hits
4 cont.	6BS	1
	6AL	1
	2DL	1
5	1BS	11
	no_hit	4
6	2AL	78
	2AS	47
	no_hit	18
	2BL	2
	4AS	1
	2DS	1
	3AS	1
	2DL	1
	3AL	1
	4DS	1
7	2BL	10
	2AL	1
	no_hit	1
8 Target linkage group	no_hit	115
	7DL	26
	7DS	21
	7AL	5
	2DL	3
	7BL	3
	1AL	2
	4AL	2
	7AS	2
	1DL	1
	2BL	1
	3AL	1
	3AS	1
	4DS	1
	5BL	1
	5DL	1
	6AS	1

Table S1 continued

Linkage Group	Chr.	Number of hits
8 Target 7DS	7DS, 7AS, 7BS	21, 1, 0
	no hit	11
	Other	1
8 Target 7DL proximal	7DL,7AL, 7BL	27, 3, 5
	no hit	77
	Other	12
8 Target 7E distal	no hit	27
	7AL	1
	7DL	1
9	1AL	31
	7AS	22
	no_hit	20
	1AS	4
	1BL	2
	5AS	1
	4AS	1
	5BL	1
	2BS	1
10	6BS	48
	no_hit	38
	6BL	19
	6DS	4
	6AL	3
	6AS	2
	6DL	2
	5BL	1
	2DL	1
	3AL	1
	4BS	1
	3DL	1
	3B	1
	1AS	1
11	7BL	29
	no_hit	12
	4BS	2

Table S1 continued

Linkage Group	Chr.	Number of hits
11 cont.	7DL	1
	7AL	1
12	3AL	22
	no_hit	9
	3B	1
	7BS	1
	1AL	1
13	6AL	24
	no_hit	3
	6DL	2
	6AS	1
14	4AS	11
	no_hit	3
15	4BS	9
	no_hit	3
16	1BL	34
	no_hit	12
	1AL	3
	5BL	2
	1DL	2
	4AL	1
17	3B	13
	7BL	8
	no_hit	6
	7AL	1
	2DS	1
18	7AL	20
	no_hit	5
	5DL	1
	7DL	1
	5AS	1
19	3AL	6
	no_hit	5
	3B	2
	3DL	1

Table S1 continued

Linkage Group	Chr.	Number of hits
20	6AL	8
	no_hit	2
21	1DS	21
	no_hit	5
22	5DL	15
	no_hit	1
23	6DS	13
	no_hit	2
24	no_hit	3
	5BL	2
	1BL	2
	1AL	1
	4BS	1

Table S 2 BLAST result for the target linkage group

Locus	Position	Hit	Percent Identity	Alignment Length	Mismatch Count	Gap Count	Query Start	Query End	E Value
TP239787	0	no_hit							
TP264927	0.922	no_hit							
TP83101	1.612	no_hit							
TP340234	2.77	no_hit							
TP114709	2.955	no_hit							
TP31888	3.387	no_hit							
TP278365	6.967	no_hit							
TP205843	7.389	no_hit							
TP136477	7.93	no_hit							
TP328971	9.141	no_hit							
TP84579	9.467	no_hit							
TP186207	10.365	7AL	90.16	61	3	1	7	64	3.00E-12
TP293818	11.054	no_hit							
TP147138	11.225	no_hit							
TP211581	11.502	7DL	90.62	64	6	0	1	64	4.00E-15
TP313803	11.87	no_hit							
TP288979	12.113	no_hit							
TP17639	12.335	no_hit							
TP182704	12.429	no_hit							
TP185861	12.551	no_hit							
TP7601	12.618	no_hit							

Table S2 continued

Locus	Position	Hit	Percent Identity	Alignment Length	Mismatch Count	Gap Count	Query Start	Query End	E Value
TP319409	12.619	no_hit							
TP145389	12.762	no_hit							
TP330245	12.843	no_hit							
TP133139	12.937	no_hit							
TP115666	13.017	no_hit							
TP268527	13.162	no_hit							
TP338664	13.235	7DL	95.31	64	3	0	1	64	4.00E-20
TP291695	13.314	1AL	91.8	61	5	0	4	64	4.00E-15
TP252436	13.38	no_hit							
TP251777	13.46	no_hit							
TP268397	13.604	7AL	96.83	63	1	1	1	62	1.00E-20
TP60637	13.654	no_hit							
TP145604	13.725	7DL	98.44	64	1	0	1	64	2.00E-23
TP210900	13.797	7DL	96.23	53	2	0	4	56	1.00E-15
TP232288	13.819	no_hit							
TP177370	13.849	2DL	92.98	57	4	0	5	61	2.00E-14
TP135154	13.889	no_hit							
TP310222	13.933	no_hit							
TP280931	14	7DL	95.31	64	3	0	1	64	4.00E-20
TP151239	14.03	no_hit							
TP60307	14.102	no_hit							
TP339660	14.156	no_hit							
TP196676	14.218	no_hit							

Table S2 continued

Locus	Position	Hit	Percent Identity	Alignment Length	Mismatch Count	Gap Count	Query Start	Query End	E Value
TP249577	14.243	no_hit							
TP72228	14.334	no_hit							
TP5622	14.395	3AL	98.39	62	1	0	3	64	3.00E-22
TP313326	14.442	no_hit							
TP658	14.486	no_hit							
TP255814	14.57	7DL	95.31	64	3	0	1	64	4.00E-20
TP2610	14.687	no_hit							
TP116092	14.896	1DL	96.72	61	2	0	4	64	4.00E-20
TP318032	15.069	no_hit							
TP33095	15.405	no_hit							
TP119639	15.651	2DL	93.75	64	4	0	1	64	2.00E-18
TP283828	15.879	7DL	95.31	64	3	0	1	64	4.00E-20
TP163999	16.308	no_hit							
TP93108	17.198	no_hit							
TP47168	17.379	no_hit							
TP247164	17.671	no_hit							
TP125166	17.835	no_hit							
TP320664	17.964	no_hit							
TP111804	18.104	no_hit							
TP163716	18.49	7DL	100	56	0	0	3	58	1.00E-20
TP219203	18.586	no_hit							
TP251801	18.73	7AL	96.88	64	2	0	1	64	9.00E-22
TP111331	18.849	5DL	92.73	55	3	1	4	57	7.00E-13

Table S2 continued

Locus	Position	Hit	Percent Identity	Alignment Length	Mismatch Count	Gap Count	Query Start	Query End	E Value
TP328695	19.016	no_hit							
TP281652	19.291	7DL	94.23	52	3	0	1	52	2.00E-13
TP134745	19.423	no_hit							
TP312519	19.744	no_hit							
TP273225	19.83	no_hit							
TP295741	19.946	no_hit							
TP22357	20.343	no_hit							
TP216898	20.589	7DL	96.83	63	2	0	2	64	3.00E-21
TP161581	20.897	no_hit							
TP8339	21.048	3AS	98.36	61	1	0	1	61	9.00E-22
TP121404	21.207	4AL	96	50	2	0	15	64	6.00E-14
TP122889	21.326	no_hit							
TP57748	21.794	no_hit							
TP151956	22.062	no_hit							
TP73370	22.341	no_hit							
TP290192	22.51	7DL	95	60	3	0	5	64	7.00E-18
TP81034	23.442	no_hit							
TP2377	23.92	7DL	100	63	0	0	2	64	2.00E-24
TP280158	24.308	no_hit							
TP318687	24.504	no_hit							
TP324357	24.623	no_hit							
TP7439	24.854	7DL	98.39	62	1	0	3	64	3.00E-22
TP84593	25.163	7BL	96.83	63	2	0	1	63	3.00E-21

Table S2 continued

Locus	Position	Hit	Percent Identity	Alignment Length	Mismatch Count	Gap Count	Query Start	Query End	E Value
TP328841	25.614	no_hit							
TP264551	25.769	7AL	95.31	64	3	0	1	64	4.00E-20
TP38108	26.074	no_hit							
TP114817	26.521	no_hit							
TP283528	26.566	7DL	93.75	64	2	1	1	62	7.00E-18
TP203526	26.728	no_hit							
TP131657	26.884	7DL	96.55	58	2	0	7	64	2.00E-18
TP125259	27.251	2BL	96.88	64	2	0	1	64	9.00E-22
TP74525	27.566	no_hit							
TP306891	28.015	no_hit							
TP25593	28.229	7AS	100	51	0	0	4	54	7.00E-18
TP338371	29.716	no_hit							
TP331711	30.327	no_hit							
TP321775	30.647	no_hit							
TP227498	30.887	7DL	95.31	64	3	0	1	64	4.00E-20
TP240326	31.306	no_hit							
TP78596	31.756	no_hit							
TP153539	31.813	6AS	91.23	57	5	0	1	57	7.00E-13
TP138366	31.867	no_hit							
TP23242	31.966	no_hit							
TP5640	32.247	1AL	98.18	55	1	0	1	55	2.00E-18
TP124164	32.448	4DS	95.08	61	3	0	1	61	2.00E-18
TP136150	32.808	no_hit							

Table S2 continued

Locus	Position	Hit	Percent Identity	Alignment Length	Mismatch Count	Gap Count	Query Start	Query End	E Value
TP73060	33.504	no_hit							
TP119614	34.115	no_hit							
TP252591	34.655	no_hit							
TP262451	34.773	7DL	93.75	64	4	0	1	64	2.00E-18
TP276120	34.941	7BL	93.65	63	4	0	1	63	7.00E-18
TP262721	35.202	7DL	96.88	64	2	0	1	64	9.00E-22
TP120282	35.366	no_hit							
TP147337	35.445	7DL	96.67	60	2	0	5	64	2.00E-19
TP74423	35.634	no_hit							
TP146148	36.965	7DL	100	56	0	0	1	56	1.00E-20
TP327141	37.543	no_hit							
TP198802	37.854	no_hit							
TP110973	38.813	no_hit							
TP292906	38.968	7DL	95.31	64	3	0	1	64	4.00E-20
TP236639	39.139	no_hit							
TP177263	39.533	no_hit							
TP35306	39.868	no_hit							
TP97642	40.04	7DL	98.41	63	1	0	2	64	7.00E-23
TP317254	40.149	no_hit							
TP170327	40.215	no_hit							
TP170546	40.415	no_hit							
TP2555	40.656	no_hit							
TP296994	40.818	7AL	93.75	64	4	0	1	64	2.00E-18

Table S2 continued

Locus	Position	Hit	Percent Identity	Alignment Length	Mismatch Count	Gap Count	Query Start	Query End	E Value
TP93994	40.885	no_hit							
TP295102	41.014	no_hit							
TP82658	41.108	7BL	98.44	64	1	0	1	64	2.00E-23
TP50674	41.148	no_hit							
TP134711	41.254	no_hit							
TP329123	41.345	no_hit							
TP35985	41.452	no_hit							
TP46958	41.604	no_hit							
TP221147	41.779	7DL	95.31	64	3	0	1	64	4.00E-20
TP38686	41.896	7DL	98.44	64	1	0	1	64	2.00E-23
TP326044	42.015	2DL	94.64	56	3	0	1	56	1.00E-15
TP109708	42.53	no_hit							
TP192301	42.788	5BL	92.19	64	5	0	1	64	9.00E-17
TP23253	43.035	7DL	100	60	0	0	2	61	7.00E-23
TP118783	43.179	7DL	98.44	64	1	0	1	64	2.00E-23
TP148067	43.497	no_hit							
TP32407	43.603	no_hit							
TP110608	43.726	7DS	100	64	0	0	1	64	4.00E-25
TP155703	43.789	4AL	96.15	52	2	0	1	52	4.00E-15
TP226361	43.871	no_hit							
TP6115	43.92	7DS	100	64	0	0	1	64	4.00E-25
TP171315	43.947	7DS	100	64	0	0	1	64	4.00E-25
TP50965	43.976	no_hit							

Table S2 continued

Locus	Position	Hit	Percent Identity	Alignment Length	Mismatch Count	Gap Count	Query Start	Query End	E Value
TP130287	44.023	no_hit							
TP312927	44.076	no_hit							
TP222642	44.115	7AS	98.41	63	1	0	2	64	7.00E-23
TP219350	44.158	7DS	100	64	0	0	1	64	4.00E-25
TP249831	44.187	no_hit							
TP420	44.206	7DS	100	64	0	0	1	64	4.00E-25
TP166912	44.25	7DS	100	64	0	0	1	64	4.00E-25
TP98007	44.287	7DS	98.44	64	1	0	1	64	2.00E-23
TP119730	44.309	7DS	98.44	64	1	0	1	64	2.00E-23
TP248306	44.353	no_hit							
TP151065	44.416	no_hit							
TP331763	44.472	7DS	98.39	62	1	0	1	62	3.00E-22
TP97562	44.508	7DS	100	53	0	0	1	53	6.00E-19
TP90083	44.555	7DS	98.44	64	1	0	1	64	2.00E-23
TP259621	44.592	7DS	100	64	0	0	1	64	4.00E-25
TP110682	44.682	no_hit							
TP49064	44.742	7DS	100	64	0	0	1	64	4.00E-25
TP325924	44.771	7DS	100	64	0	0	1	64	4.00E-25
TP229909	44.852	no_hit							
TP245850	44.899	7DS	100	64	0	0	1	64	4.00E-25
TP85166	44.943	7DS	98.44	64	1	0	1	64	2.00E-23
TP267695	45.035	no_hit							
TP3117	45.088	7DS	100	64	0	0	1	64	4.00E-25

Table S2 continued

Locus	Position	Hit	Percent Identity	Alignment Length	Mismatch Count	Gap Count	Query Start	Query End	E Value
TP282522	45.217	7DS	98.44	64	1	0	1	64	2.00E-23
TP329756	45.266	7DS	100	64	0	0	1	64	4.00E-25
TP262498	45.379	no_hit							
TP31664	45.481	7DS	100	64	0	0	1	64	4.00E-25
TP212624	45.778	7DS	98.44	64	1	0	1	64	2.00E-23

Table S 3 Integrated SNP/SSR markers for target linkage group 8

Locus[1]	Position	N. N. Stress (cM)
*Xcfd25	0	0
TP181613	3.941	0.501
TP89880	4.885	0.877
TP35361	6.726	1.531
TP49076	8.052	-0.001
TP310455	8.063	-0.002
TP243263	10.664	0.223
TP204795	11.39	0.886
TP304797	12.886	1.548
TP250622	15.208	-0.19
TP277136	16.676	-0.026
TP331898	16.963	0.287
TP11772	17.479	0.715
*gwm111	41.265	-34.969
TP5640	60.865	1.615
*Xswes354	62.987	2.469
TP73060	65.982	3.188
TP240326	69.16	0.158
TP338371	70.345	-0.29
TP74525	74.176	0.286
TP306891	74.466	-0.021
TP25593	75.652	0.584
TP131657	76.244	-0.021
TP283528	76.811	-0.001
TP114817	76.828	0.011
TP203526	77.204	-0.028
TP125259	78.405	-0.286
TP227498	83.146	0.287
TP321775	83.432	0.287
*BE406148	84.756	0.295
TP331711	85.295	0.734
*Xpsp3123	90.996	9.963
TP78596	96.631	-0.053
TP138366	96.773	-0.001
TP153539	96.773	-0.001

Table S 3 continued

Locus[1]	Position	N. N. Stress (cM)
TP23242	96.916	0.29
TP124164	97.642	0.271
TP136150	98.519	0.22
TP119614	101.258	0.441
TP252591	103.301	0.944
TP276120	103.994	-0.004
TP262451	104.228	0.07
TP262721	104.753	0.447
TP74423	105.027	0
TP147337	105.04	0
TP120282	105.182	0.299
TP146148	106.948	-0.07
TP198802	107.882	0.075
TP327141	108.169	0.287
TP236639	110.127	-0.032
TP110973	110.414	0.578
TP292906	110.992	-0.052
TP177263	112.454	-0.07
TP35306	113.361	0.397
TP97642	113.939	0.289
TP170327	114.082	0
TP317254	114.093	-0.001
TP295102	115.106	0.287
TP82658	115.393	-0.002
TP50674	115.535	-0.004
TP296994	116.024	0.293
TP93994	116.263	0.07
TP170546	117.303	0.922
TP221147	118.325	0.284
TP134711	119.083	0.411
TP329123	119.515	0.227
*BG607393	125.969	1.672
*BE496854	138.102	-4.503
*Xcfa2174	159.994	25.325
*Xswes130	186.207	7.429
*Xwmc809	205.434	14.389
*Xgwm44	224.459	0.784

Table S 3 continued

Locus[1]	Position	N. N. Stress (cM)
*Xgwm635	246.263	22.528
*Xmag2931	266.295	4.853
*Xwmc653	282.974	12.855
*BE399084	295.803	2.381
*Xksum052	303.353	3.282
*Xcfe202	309.486	3.242
*Xcfd14	319.142	6.209
*Xcfa2049	324.991	0.577
*Xgwm295	335.842	11.675
*Xxfd66-	342.496	1.026
*Xbarc214	348.97	7.573
*Xswes22	354.139	1.245
*Xcfd21	358.677	6.057
*Xbarc154	364.744	1.973
*Xgwm473	369.256	6.241
*Xcfa214	377.907	8.688
*Xcfe100	384.41	2.604
*Xbarc70	390.115	5.808
*Xcfd31	396.888	5.84
*Xgwm350	402.604	3.752
*Xedm154	406.912	2.052
TP110682	409.516	0.235
TP90083	410.161	0.59
TP110608	410.831	0.453
TP155703	411.409	0.578
TP171315	411.873	-0.008
TP151065	412.159	0.526
TP331763	413.136	0.871
TP212624	414.651	2.114
TP119730	416.278	0.303
TP97562	416.443	-0.003
TP229909	416.73	0.586
TP85166	417.746	0.602
TP3117	418.71	0.428
TP32407	419.558	0.5
TP312927	420.287	0.578
TP130287	420.868	0.284

Table S 3 continued

Locus[1]	Position	N. N. Stress (cM)
TP50965	421.299	0.438
TP6115	421.87	0.29
TP226361	422.467	0.581
TP166912	422.994	-0.004
TP325924	423.308	0.051
TP219350	423.769	0.21
TP259621	424.076	0.287
TP222642	424.345	-0.005
TP248306	424.631	0.404
TP249831	424.901	-0.006
TP420	425.251	0.267
*Xcfe19	431.255	1.688
*cfa2106	447.725	0.998
*wmc606	451.691	4.256
*Xswes157	471.682	4.596
TP245850	478.128	0.549
TP262498	479.118	0.965
TP148067	480.143	0.566
TP329756	481.171	0.277
TP267695	481.728	0.24
TP31664	482.624	0.076
TP49064	483.019	0.005
TP282522	483.684	0.663
TP98007	484.785	0.283
TP118783	485.144	-0.006
TP23253	485.43	0.287
TP192301	486.467	0.731
TP109708	487.584	1.343
TP326044	489.002	0.556
TP38686	490.009	0.561
TP35985	491.375	0.267
TP2555	492.107	0.271
TP46958	493.323	-0.373
*Xgwm333	498.084	3.864
*Xswes376	505.915	6.598
*Xswes375	519.14	12.824
*Xwmc121	538.266	16.876

Table S 3 continued

Locus[1]	Position	N. N. Stress (cM)
*Xmag2934	555.661	-4.518
*psr129	587.952	-6.276
TP216898	597.65	0.378
TP161581	598.555	0.283
TP8339	598.943	0.284
TP121404	599.577	0.389
TP122889	600.082	0.599
TP57748	601.237	-0.025
TP151956	601.677	0.545
TP73370	602.567	-0.009
TP290192	602.727	0.299
TP81034	604.602	0.287
TP2377	604.921	0.288
TP280158	605.949	0.236
TP324357	606.091	-0.002
TP318687	606.381	0.286
TP84593	607.6	0.877
TP7439	608.346	0.506
TP264551	609.145	0
TP38108	609.393	0.286
TP328841	609.538	-0.069
TP22357	616.531	-0.425
TP273225	617.418	0.286
TP312519	617.71	-0.003
TP295741	617.856	0.292
TP134745	618.742	0.007
TP281652	619.776	0.671
TP111331	621.834	2.687
*BE605194	626.302	5.691
TP328695	629.672	-0.101
TP251801	630.221	-0.016
TP219203	630.69	0.478
TP320664	631.411	0.77
TP247164	632.1	-0.015
TP111804	632.678	0.447
TP163716	633.855	0.882
TP125166	634.286	-0.017

Table S 3 continued

Locus[1]	Position	N. N. Stress (cM)
TP93108	634.938	0.022
TP47168	635.059	-0.002
TP283828	636.979	0.05
TP163999	637.835	0.586
TP119639	638.304	-0.02
TP33095	638.304	-0.02
TP318032	639.098	0.178
TP145604	640.509	0.04
TP268527	641.038	-0.481
*Xpsp3003	658.551	28.179
*XL3	677.197	-1.509
*BE604744	681.443	1.419
*HX29	683.853	1.005
*BF483039	689.437	2.244
*BE445506	692.18	-0.31
TP211581	694.087	0.706
TP330245	694.727	-0.028
TP182704	695.453	0.581
TP7601	695.74	0.092
TP319409	695.74	0.092
TP288979	695.973	0.293
TP133139	696.476	0
TP17639	696.482	0
TP185861	696.666	-0.015
TP313803	697.997	0.351
TP147138	698.646	0.405
TP293818	699.483	-0.018
TP186207	700.762	0.454
*BG607810	704.964	0.249
*Xpsr121	707.734	0.981
*BE445653	710.899	4.696
*BF145935	715.806	0.866
TP84579	719.459	-0.074
TP328971	719.775	-0.051
TP278365	722.359	0.574
TP205843	723.132	0.575
TP136477	723.935	0.511

Table S 3 continued

Locus[1]	Position	N. N. Stress (cM)
TP340234	729.397	-0.081
TP31888	729.731	-0.003
TP114709	729.877	0.307
TP83101	731.822	0.117
TP239787	733.925	2.12
TP264927	735.034	0.073
*Xcfa2240	739.537	6.15
*Xmag1932	774.423	18.453
TP60637	786.989	-0.37
TP291695	787.543	0.08
TP252436	788.038	0.598
TP72228	789.049	0.292
TP60307	789.49	0.287
TP268397	789.779	-0.008
TP151239	790.222	0.577
TP135154	790.66	-0.004
TP210900	790.808	-0.003
TP232288	790.808	-0.003
TP658	791.188	0.004
TP196676	791.25	0.054
TP116092	791.92	-0.068
TP115666	793.616	-0.029
TP145389	793.919	-0.01
*Xbarc76	800.54	10.46
*Lr19	809.443	5.75
*Xwmc273	817.359	5.312
*BE637476	821.681	0.908
*BM137749	824.216	2.017
TP2610	827.459	0.025
TP5622	827.952	0.001
TP249577	828.106	0
TP339660	828.149	-0.001
TP313326	828.563	0.245
TP338664	828.967	-0.005
TP177370	829.166	0.098
TP310222	829.481	0.047
TP255814	829.794	-0.008

Table S 3 continued

Locus[1]	Position	N. N. Stress (cM)
TP280931	830.221	0.315
TP251777	830.993	-0.264
*Xswes19	847.928	0

[1] Asterisks indicate SSR markers and markers starting with “TP” are SNPs.

VITA

VITA

Xiangye Xiao graduated from Northwest A&F University in Yangling, China and received her Bachelor of Science in Agriculture in June 2008. She started her Ph.D. in wheat genetics and breeding in August 2009 at Purdue University. Her research focus was to improve wheat disease performance through utilization of wheat wild relatives. While in her Ph.D., she was active in innovative activities. She was one of four people team in Purdue Corn and Soybean innovation competition in 2012. Their team proposed to use corn starch to produce a special padding insert in football helmet to reduce the concussion and ranked second in corn division in the annual competition.

She presented her research in several national conferences, including Fursarium Head Blight Forum and Plant and Animal genome conference.