CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Fusarium head blight (FHB) is a devastating fungal disease threatening wheat production worldwide. Utilization of resistant wheat cultivars is generally considered as the most effective, economic and environmental friendly approach for management of the disease. This research aimed to identify and map quantitative trait loci (QTL) for FHB resistance in two spring wheat lines (ND2710 and PI 277012) and two durum wheat lines (10Ae564 and Joppa). Using a mapping population consisting of 233 recombinant inbred lines (RILs) from a cross between ND2710 (with FHB resistance derived from Sumai 3) and the spring wheat cultivar 'Bobwhite' (susceptible to FHB), four QTL (*Qfhb.ndwp-3B*, *Qfhb.ndwp-6B*, *Qfhb.ndwp-2A*, and *Qfhb.ndwp-6A*) were mapped on chromosomes 3B, 6B, 2A, and 6A, respectively, in ND2710. *Qfhb.ndwp-3B* and *Qfhb.ndwp-6B* were mapped to the same genomic regions as *Fhb1* and *Fhb2*, confirming that they originated from Sumai 3. Two FHB resistance QTL, *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*, were previously identified on chromosome 5AS and 5AL, respectively, in PI 277012. In this study, *Qfhb.rwg-5A.2* was delimited in a 1.09-Mbp genomic region, and DNA markers tightly linked to *Qfhb.rwg-5A.2* were developed using 947 RILs from the cross between PI 277012 and Grandin (susceptible to FHB). Using a mapping population consisting of 205 RILs from the cross between the durum cultivar Joppa and the durum wheat line 10Ae564 (with FHB resistance derived from PI 277012), one QTL (*Qfhb.ndwp-2A*) on chromosome 2A from Joppa and two QTL (*Qfhb.ndwp-5A* and *Qfhb.ndwp-7A*) each on 5A and 7A from 10Ae564 were detected. *Qfhb.ndwp-5A* was mapped to the same genomic region as *Qfhb.rwg-5A.2* on 5AL, and thus confirming that this QTL was derived from PI 277012. The DNA markers closely linked to the FHB resistance QTL identified in two spring wheat lines and two durum wheat lines will be useful for marker-assisted selection of FHB resistance in wheat breeding programs.

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DEDICATION

I would like to dedicate this thesis to my husband for his support and care throughout my Ph. D study.

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CHAPTER I. LITERATURE REVIEW

Wheat Crop

Wheat is a cereal crop widely cultivated all over the world (Shewry, 2009). It grows in a wide range of environments and conditions, from 67 °N in Norway, Finland, and Russia, to 45 °S in Argentina (Feldman 1995). The crop ranks third place in cereal production quantity after maize and rice, and about 729 million tons of wheat were produced in 2014 (http://faostat.fao.org). The world's main wheat producers are the European Union, China, India, Russia, the United States, and Canada. In 2016-2017, about 737 million tons of wheat were produced in the world (USDA ERS 2017). With the world's increasing population, the global crop production needs to be doubled by 2050 to meet the demands of consumption (Foley et al. 2011). However, the yields of wheat are increasing at a far lower rate than it is required to double the global production by 2050 (Ray et al. 2013). To meet the challenge, it is essential to increase yield per acre since the increase of wheat planting area is limited or even impossible. Wheat production can be affected by abiotic and biotic stresses, thus any efforts that are effective to combat these stresses are critical to improve the yield of the crop.

As a major wheat producer, the United States of America produces about 55-60 million tons of grains every year, ranked the third place in production volume (USDA ERS Wheat Data). The United States is also the world's biggest wheat exporter with 50% of its total wheat production exported (USDA ERS 2016). Wheat varieties grown in the U.S. are either winter wheat or spring wheat depending on their growth habits. Winter wheat is sown in the fall and harvested in the summer because it needs low temperatures for vernalization to flower and finally produce seeds, while spring wheat and durum wheat are primarily planted in the spring and harvested in late summer or fall. Depending on grain color and hardness, and the sowing

seasons, wheat grown in the U.S. can be further classified into five major classes: hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), white, and durum wheat. Each class has some differences in end use. HRW and HRS are mainly used to make products that need high protein flour. SRW and white wheat are used to make products that require low protein flour such as cakes, cookies, crackers, noodle products, cereals and white-crusted breads, while durum wheat is mainly used in the production of pasta. Classes of wheat grown in the United States tend to be regional-specific: HRW is grown mainly in the Great Plains (Texas north through Montana); SRW is grown in States along the Mississippi River and in the Eastern States; white wheat is grown in Washington, Oregon, Idaho, Michigan, and New York; HRS is primarily grown in Northern Plains (North Dakota, Montana, Minnesota, and South Dakota) where cold wintertime temperature would kill winter wheat in dormancy, while durum wheat is primarily cultivated in North Dakota and Montana (USDA ERS 2016).

Wheat Evolution

Wheat was first cultivated in southwest Asia about 10,000 years ago. As part of Neolithic revolution, the domestication of wheat induced a transition of human behavior from hunter-gathers to farmers. It was the principal event involved in the development of human civilization and had a profound effect on life thereafter (Shewry 2009; Riehl et al. 2013; Marcussen et al. 2014). Cultivated wheat can be divided into three main groups on the basis of their genome complement: diploid (*T. monococcum*, ssp. *monococcum* L., 2n = 2x = 14, A^mA^m) (einkorn), tetraploid wheats, including *T. timopheevii* ssp. *timopheevii* Zhuk (2n = 4x = 28, AAGG), *T. turgidum* ssp. *dicoccum*, *T. turgidum* ssp. *durum*, *T. turgidum* ssp. *parvicoccum*, *T. turgidum* ssp. *carthlicum*, *T. turgidum* ssp. *turgidum*, and *T. turgidum* ssp. *polonicum* (2n = 4x = 28, AABB), and hexaploid wheats consisting of *T. zhukovskyi* (2n = 6x = 42, A^mA^mAAGG), *T. aestivum* ssp.

spelta, *T. aestivum* ssp. *aestivum*, *T. aestivum* ssp. *compactum*, and *T. aestivum* ssp. *sphaerococcum* (2n = 6x = 42, AABBDD) species.

Triticum urartu (2n=2x=14, AA) is the donor of the A genome to all polyploidy wheat species (Dvorák et al. 1993). *Aegilops. speltoides* (2n = 2x = 14, genome SS) is proved to be the maternal donor for both tetraploid and hexaploid wheat by cytoplasmic analysis (Wang et al. 1997). The D genome is clearly derived from *Aegilops tauschii*. Hybridization has also been a major factor in wheat evolution. The initial hybridization between A and B genome ancestors occurred approximately 10,000 years ago, which produced new species including wild emmer wheat (*T. turgidum* ssp. *dicoccoides*, 2n = 4x = 28, AABB) and *T. timopheevii* ssp. *araraticum* (2n = 4x = 28, AAGG) (Nishikawa et al. 1994; Dvorak et al. 1993). The occurrence of non-brittle rachis mutation led wild emmer wheat to cultivated emmer wheat. The extinct *T. turgidum* ssp. *parvicoccum*, free-threshing tetraploid wheat, appeared shortly after domesticated emmer (Kislev 1984). Durum wheat (*T. turgidum* ssp. *durum*) evolved from domesticated emmer possibly through ssp. *parvicoccum* (Hillman 1978). Hexaploid and tetraploid wheats arose from the same evolutionary lineage. *T. aestivum* (AABBDD) is the most important hexaploid wheat, derived from a hybridization of an AB genome-containing domesticated form of tetraploid, wild emmer wheat (*T. turgidum* spp. *dicoccoides*) and the wild wheat species *Ae. tauschii* (Kihara 1944; Salse et al. 2008; Marcussen et al. 2014; Petersen et al. 2006).

Fungal Pathogen: *Fusarium graminearum*

Taxonomy and Classification

The genus *Fusarium* was initially described and defined by Link in 1809. *Fusarium* taxonomy was first studied by Wollenweber and Reinking (1935), who organized approximately 1,000 named species into 16 sections, which contains 65 species, 55 varieties, and 22 forms

based on anamorphic characters. Criteria used in this organization were the presence or absence of microconidia and chlamydospores, shapes of microconidia and macroconidia, and the position of chlamydospores. All systems of *Fusarium* taxonomy published since 1935 are based on their work. However, there are some problems with this system because some of the characters used to separate species, varieties, and forms were unstable; the cultures used were not started from single spores, and a few of their species and many of their varieties might be cultural mutants of *Fusarium* species. Snyder and Hansen (1940) regrouped the 16 sections of species into nine species (*F. oxysporum, F. solani, F. moniliformae, F. roseum, F. lateritium, F. tricinctum, F. nivale, F. rifidiuscula* and *F. episphearia*). Though the simplification made the diagnosis and identification of *Fusarium* species much simpler, some species like *F. oxysporum* and *F. solani* were still species complexes (Nelson 1991). Summerell et al. (2003) described a utilitarian method, which combined morphological, biological, physiological characters, and vegetative compatibility for *Fusarium* identification (Summerell et al. 2003). More and more new species are being described with the advent of more sophisticated, more widely available and more commonly applied genetic and molecular techniques.

F. graminearum, named for the asexual stage, is a haploid homothallic ascomycota fungus, producing three types of asexual spores: macroconidia, microconidia, and chlamydospores (Leslie and Summerell 2006). *Gibberella zeae* is the name for the sexual stage (teleomorph) of *F. graminearum*. In the sexual stage, perithecia are produced and sac-like structure called asci form inside the fruiting body. Ascospores released from the asci serve as the primary inocula for plant infection (Beyer et al. 2005). According to the new international nomenclature agreement for naming fungi (one fungus, one name), *Fusarium* is conserved as the sole name for the fungal genus, and the usage of *Gibberella* is expected to be discontinued.

Recent phylogenetic study divided isolates of *F. graminearum* from around the world into nine distinct phylogenetic lineages (O'Donnell et al. 2004), which were named as different species respectively. Until now, at least 16 phylogenetically distinct species have been associated with *Fusarium* head blight (Desjardin 2006; Starkey et al. 2007).

Biogeography and Phylogeography

Outbreaks and epidemics of FHB in small grain cereals have been reported in major production areas worldwide since the re-emergence of this economically devastating disease in the U.S. in the early 1990s (McMullen 1997). The causal agent of this disease is the *F. graminearum* complex containing several related *Fusarium species*. *F. graminearum* is currently distributed globally. Several recent mycogeographic studies have shown that species of *Fusarium* causing FHB differ in their geographic distribution (Leslie 1990; Sangalang 1995). *F. graminearum* was thought to contain a single panmictic species until genealogical concordance phylogenetic species recognition (GCPSR) (Taylor et al. 2000) was used to investigate a worldwide collection of FHB strains (O'Donnell et al. 2004). Phylogenetic analyses of DNA sequences reveals that this morphospecies comprises at least 14 biogeographically structured, phylogenetically distinct species (O'Donnell et al. 2008). The current study focuses on species diversity and trichothecene toxin chemotype potential of some genetically novel FHB isolates. With the help of molecular markers, isolates of *F. graminearum* can be identified as one of three chemotypes: 3-acetyl-deoxynivalenol (3ADON), 15-acetyl- deoxynivalenol (15ADON), and nivalenol (NIV) (Ward et al. 2008; Puri and Zhong 2010). Various genetic markers have been used, including randomly amplified polymorphic DNA (RAPD), sequence characterized amplified region (SCAR) marker data, sequence related amplified polymorphism (SRAP), restriction fragment length polymorphism (RFLP), variable number of tandem repeat (VNTR)

and phylogenetic analyses of Tri101 gene sequences (Gale et al. 2011; Puri et al. 2012; Ward et al. 2008; Qiu et al. 2014). Combination of these techniques determines that the *F. graminearum* species complex (FGSC) comprises 16 phylogenetically distinct species (O'Donnell et al. 2004; O'Donnell et al. 2008; Sarver et al. 2011).

Fusarium Head Blight (FHB) on Wheat

Fusarium head blight (FHB), first described in 1884 in England, is considered a major threat to small grains like wheat and barley after its reemergence in the past two decades (Goswami and Kistler 2004). Since then, the disease has occurred worldwide and recent outbreaks have been reported in Asia (Suga et al. 2008; Yang et al. 2008), Canada, Europe (Qu et al. 2008) and South America (Gale et al. 2007; Ward et al. 2008). FHB epidemics occurred every year from 1993 to 1998 at varying intensities in the Northern Great Plains region of the United States, particularly in northeastern North Dakota, northwestern Minnesota, and southern Manitoba (Mcmullen et al. 1997, 2012). According to the data about frequency of epidemics since 1997, FHB caused less losses compared to the former epidemic years in some regions, but it continues to cause economic losses to both wheat and barley at some locations in the United States during wet years (McMullen et al. 2012). This disease has caused tremendous economic losses to wheat growers and the industry due to reduced yield and quality of wheat grain. Reduced grain quality was caused by contamination with several mycotoxins, including Deoxynivalenol (DON) and its derivatives, oestrogenic mycotoxin, aurofusarin, and zearalenone (Scott 1990; Bai and Shaner 1996; McMullen et al. 1997; Trail 2009). Mycotoxin-contamination of grains is a great threat to global food safety because the toxins have been linked to mycotoxicoses of humans and livestocks (Ward et al. 2008).

Symptoms and Disease Cycle

FHB epidemics are favored by several factors, including local and regional environmental conditions, physiological state and genetic make-up of the host, and virulence of the pathogen. High humidity for a prolonged time (48 - 72 h) coinciding with warm temperature (24 - 28 °C) at anthesis is optimum for successful disease establishment and spread (Osborne and Stein 2007). At early anthesis stage, anthers may be the first floral part to be infected under favorable environmental conditions (Ribichich et al. 2000). FHB symptoms in wheat are confined to spikes. The most obvious symptoms are water-soaked brownish necrotic lesions formed on the surface of glumes, which later become bleached, giving a symptom of partially green and partially white heads (Wiese 1987; McMullen et al. 2008). Under prolonged wet conditions, infected tissues can be filled with a typical light pink to salmon-orange fungal mass (McMullen et al. 2008; Trail 2009). At harvest, grains from infected spikes might be shriveled, light weighted with a pinkish discoloration. FHB symptoms are different between resistant and susceptible wheat germplasms. In highly resistant plants, only the lemma of inoculated or infected spikelet showed dark brown discoloration (Bai and Shaner 1994). In moderately resistant and moderately susceptible plants, the symptoms may spread to neighboring spikelets up and down the rachis two weeks after initial infection, but some other spikelets remain uninfected. However, the whole spike of the highly susceptible plants can become bleached in 7 to 10 days after initial infection (Ribichich et al. 2000). The generalized disease cycle starts when the airborne ascospores land on the flowering spikelets as the primary inoculum, followed by germinating and entering into the plant through natural openings or degenerating anther tissues (Trail 2009). As infection initiates, the fungus grows intercellularly and asymptomatically (Bushnell et al. 2003), then it grows intracellularly and rapidly colonizes the tissue. Asexual

spores (conidia) can be produced on the surface of infected spikes during wet weather, and infect plants as secondary inoculum by rain splash or the wind in short distances (Parry et al. 1995; Trail 2009). Both ascospores and conidia play an important role in FHB epidemics (Markell and Francl 2003). The fungus completes its life cycle in culture or in association with its hosts (Trail 2009). It overwinters as mycelia or as perithecia on infected crop residues, seeds, or wild plant hosts.

Disease Management

Strategies to reduce losses caused by FHB include cultural practices, fungicide spray, biological control, and utilization of resistant cultivars. Cultural practices for control of FHB are aimed to reduce the pathogen inoculum for disease development. Since *F. graminearum* survives saprophytically on residues of crops and produces both macroconidia and ascospores on these substrates, practices with impact of inoculum reduction such as crop rotation and tillage (burying or burning of infested residue) may be effective for FHB management in individual cereal fields and over broader regions of cereal production. Fungicides with active ingredients of metconazole, protioconazole, tebuconazole, and propiconazole have been proved effective for FHB management (Paul et al. 2008). Some fungal, bacterial and yeast species are reported to be potential biological control agents of *F. graminearum* (Yuen and Schoneweis 2007; Gilbert et al. 2004; Jochum et al. 2006; Khan et al. 2004). However, biocontrol agents have not been successfully commercialized. Although cultural practices and fungicides applications reduce the yield loss caused by FHB, use of resistant cultivars is a much more efficient and environmentally friendly way to control this disease (Bai and Shaner 2004). Due to the complex nature of interaction between the host, pathogen, and environment, sole dependence on a single management strategy has little or no effect in reducing FHB damage (McMullen et al. 2012). An

integrated disease management approach using a combination of host resistance, cultural practices and fungicide application is the most efficient and effective way to control FHB (McMullen et al. 1997; Bai and Scanner 2004; Wegulo et al. 2011)

Host Resistance

FHB resistance in wheat can be classified into three types: passive (morphological) and active (physiological) types of resistance, as well as mechanisms of tolerance to Fusarium infection (Mesterhazy 1995). Passive resistance may come from some morphological or developmental characteristics, such as plant height, flower opening, heading date, spike density, and the presence/ absence of awns in some genotypes (Mesterhazy 1995, 2009). Tolerance, not the same as resistance, is considered to be an active means of minimizing the damage of FHB. Tolerance to FHB in wheat can be defined that a wheat variety maintains high grain yield even when the disease severity is similar to that in susceptible varieties. Active forms of resistance are associating with some physiological response of the plant when challenged by a pathogen and are conferred by the action of one or several genes.

Two types (I and II) of active resistance to FHB in wheat were first described by Schroeder and Christensen (1963). Type I resistance restricts initial infection, while type II resistance prevents fungal spread within a spike (type II resistance). More types of resistance were proposed: type III for resistance to mycotoxin accumulation, type IV for resistance to Fusarium-damaged kernels (type IV), and type V for tolerance (Mesterhazy 1995; Mesterházy et al. 1999). Types I and II are two most widely accepted types of resistance. Type II resistance, as the most frequently studied one, is assessed by point inoculation method in which a single floret close to the middle of the spike is inoculated and the blighted spikelets caused by disease spread are counted typically at 21 days after inoculation (Bushnell et al. 2003). This technique for

screening type II resistance has been routinely used in both greenhouse and field experiments (Bai et al. 2003). Type I resistance is evaluated by spore spray inoculation, but the resistance is inconsistent although it has been reported in some cultivars. Thus, little effort has been made to select type I resistance in wheat (Bai and Shaner 2004). In recent years, some researchers focus on resistance to kernel infection and DON accumulation due to food safety concerns, but most wheat breeders still concentrate on selecting wheat lines with type II resistance to reduce FHB development in the crop.

Sources of FHB Resistance

Although no sources of germplasm are immune to FHB in wheat (Dill-Macky and Jones 2000), some wheat accessions have been identified with varying degrees of FHB resistance. Sources of FHB resistance in hexaploid spring wheat have originated from countries in Asia, primarily China and Japan, and in Latin America, and sources of FHB resistance in winter wheat have been identified in Europe and North America (Bai and Shaner 2004; Buerstmayr et al. 2009). Sources of FHB resistance have also been identified in tetraploid wheat, and in species that are related to wheat (Buerstmayr et al. 2009). The Chinese spring wheat cultivar Sumai 3 has been identified in several studies with high level of type II resistance (Buerstmayr et al. 2009). The resistance is quite stable across different environments, and thus has been extensively used in the world's wheat breeding programs (Rudd et al. 2001; Bai 1996). Many widely utilized wheat varieties or lines exhibiting high levels of FHB resistance are derivatives of Sumai 3, including Ning 7840 and Ning 8331 (Rudd et al. 2001; Bai and Shaner 2004; Buerstmayr et al. 2009). Other Chinese sources that are not derived from Sumai 3 also have been identified, such as Wangshuibai, Baishanyuehuang, Huangcandou, Huangfangzhu, and Haiyanzhong (Jia et al. 2005; Lin et al. 2006; Yu et al. 2008). Japanese wheat cultivars such as Shinchunaga,

Nobeokabouzu, and Nyu Bai were also reported to show high levels of FHB resistance (Bai and Shaner 2004). Other germplasm with FHB resistance includes: Arina, Renan, and Fundulea 201R from Europe (Gervais et al. 2003; Somers et al. 2004; Steiner et al. 2004; Paillard et al. 2004), Frontana and Ecruzilhada from Brazil (Mesterhazy 1995; Singh and Ginkel 1997), and Roane, Ernie and Freedom from the United States (Jin et al. 2013; Rudd et al. 2001). They are believed to have resistance originated differently from Sumai 3. The resistant landraces from Asia have unfavorable agronomic traits, while moderately resistant cultivars from local regions have good adaptation to the region (Waldron et al. 1999). Thus, introgression of FHB resistance from Asian sources to locally adapted cultivars with moderate resistance is an efficient way to enhance the level of FHB resistance.

Identification of novel genes for FHB resistance remains essential in wheat. Breeders make a great effort to search for additional sources through screens of wild accessions and related, alien species. FHB resistance has been identified in some wheat-related genera such as *Aegilops*, *Elymus*, *Leymus*, *Roegneria*, and *Thinopyrum*, and the resistance level is even higher than that in Sumai 3 (Buerstmayr et al. 2009; Bai and Shaner 2004; Cai et al. 2005). Large scale evaluation of accessions from different species in the genus *Triticum* lead to identification of novel sources with high FHB resistance. PI 277012 is a hexaploid wheat accession that consistently showed a high level of FHB resistance in both greenhouse and field experiments, and two QTL on chromosome 5A were identified to be significantly associated with the FHB resistance in the field and greenhouse (Chu et al. 2011).

In general, durum wheat is more susceptible compared with bread wheat, and most durum wheat cultivars are very susceptible (Clarke et al. 2010). Large-scale screening of durum wheat accessions and landraces only led to identification of limited sources with moderate FHB

resistance (Elias et al. 2005; Huhn et al. 2012; Oliver et al. 2008). Due to the lack of resistance sources, durum wheat geneticists and breeders attempted to broaden the genetic basis for durum wheat improvement by searching resistance donors in wild and cultivated relatives. Some tetraploid accessions have been identified to be moderately resistant against FHB, including wild emmer wheat *T. dicoccoides*, cultivated emmer wheat *T. dicoccum*, and Persian wheat *T. carthlicum* (Buerstmayr et al. 2003; Oliver et al. 2007). The transfer of resistance from wild relatives into durum wheat has been completed by chromosome engineering (Stack et al. 2002; Kumar et al. 2007).

Genetics and QTL Analysis of FHB Resistance

Molecular Markers

The advent of molecular marker technology plays an important role in improving the precision and efficiency of plant breeding and genetic studies. Molecular markers are certain pieces of DNA associating with specific positions in the genome, acting as landmarks for genes or QTL. Classical markers are those that were applied in breeding programs before 1980s, including morphological (visible traits), cytological (chromosome karyotypes and bands) and biochemical markers (protein isozymes) (Jiang 2013). They are either very limited or highly technical demand, and thus cannot be extensively used in breeding programs. Since 1980s, DNA markers have been widely used due to their abundancy. DNA markers can be divided into three groups: hybridization-based, PCR-based, and sequence-based markers (Caixeta et al. 2014). The hybridization-based RFLP (restriction fragment length polymorphism) markers were the most popular ones in the 1980's. There are several disadvantages for using RFLP: high cost, need of large amount of DNA, and time consuming (Caixeta et al. 2014). RFLP was replaced by PCR-based markers in the 1990's.

PCR-based markers use a molecular technique known as polymerase chain reaction (PCR) which needs a small amount of DNA, avoids radioisotopes and generates a high level of polymorphisms. Widely used PCR-based markers include RAPD (random amplified polymorphic DNA) (Williams J.G.K. 1990), AFLP (amplified fragment length polymorphism) (Vos et al. 1995), DArT (diversity arrays technology) (Jaccoud et al. 2001), and SSR (simple sequence repeats) (Akkaya et al. 1992). RAPD amplifies random DNA segments with primers of short nucleotide sequence, and polymorphisms can be detected based on presence or absence of DNA amplification. AFLP uses restriction enzymes to digest genomic DNA and the resulting fragments are ligated to specific sequence adapters. Selective fragments are amplified with two PCR primers that have the corresponding adaptor and restriction site specific sequences. The selected fragments are analyzed following gel electrophoresis. This technique not only has higher reproducibility, resolution, and sensitivity in whole genome level compared to RAPD markers but also allows for analysis of a large number of fragments at one time. DArT is another PCR-based marker technology based on microarray hybridizations that detect the presence versus absence of individual fragments in genomic representations, and genotype large numbers of loci in a single assay. SSR makers, also known as microsatellites, are short DNA sequences with 1 to 6 base tandem repeats. They are ubiquitously spread through genomes of all species (Zietkiewicz et al. 1994). SSR markers have been used in QTL mapping and marker-assisted selection for a long time due to the high throughput and reproducibility.

With the advent of DNA sequencing, the sequence-based markers emerged allowing for higher throughput and greater genome coverage. These markers include SNP (single nucleotide polymorphism) (Jordan and Humphries 1994), STSs (sequence tag sites) and markers developed from ESTs (expressed sequence tags) (Gupta et al. 1999), which were also developed in the

1990s. STS is a short DNA sequence whose location and base sequence are known. STS markers serve as landmarks in developing physical map of a genome. EST is a short sub-sequence of a cDNA sequence. SNP marker is variation in a single DNA nucleotide base (A, G, C, or T) at a specific location in the genome. As the newest type of markers, SNPs have an unlimited number and are ready for high throughput genotyping and have already been broadly used in genetic research and breeding programs. Compared to Sanger's method, next-generation sequencing technology has allowed for the development of an unprecedented amount of sequencing data and made it less expensive for SNP discovery (Morozova and Marra 2008). Recently, genotyping by sequencing (GBS) technique has been developed, enabling discovery of a large number of SNPs in maize, sorghum, and wheat (Mammadov et al. 2012). The high throughput GBS approach is a powerful tool for SNP discovery in species that lack reference genomes (Poland et al. 2012). More recently, high-density SNP genotyping arrays with about 90,000 gene-associated SNPs have been developed as a powerful tool to characterize genetic variations in allohexaploid and allotetraploid wheat populations (Wang et al. 2014).

Genetic Map and QTL Mapping

Genetic maps are linkage maps generated for specific populations that show the relative position and order of markers, genes, and QTL based on the recombination frequency between them. Linkage maps are constructed using all of the markers discussed in the previous section and usually developed using bi-parental populations. The frequently used population types are recombinant inbred lines (RIL) in more or less advanced selfing generations, doubled haploid populations (DH), populations derived from backcrosses, and F_2 populations (Buerstmayr et al. 2009). Marker positions and intervals may be different in linkage maps constructed using different populations. Thus, a consensus map combining all the map information from different

populations can lead to a more accurate reference for marker and QTL positions. A wheat SNP consensus map was constructed using high density 90K SNP arrays and a total of 46,977 SNPs were genetically mapped in eight DH populations (Wang et al. 2014). This map is a useful genetic resource for SNP mapping projects, providing a high resolution dissection of complex traits in wheat.

QTL mapping (Sax 1923; Thoday 1961) is a tool based on DNA markers that can be used to dissect quantitative traits, and map QTL for traits of interest in genetic maps and determine the QTL effects and interactions (Kearsey 1998). Mapping population is the start point for QTL mapping. Usually two parents having contrasting phenotypes for the trait of interest are chosen to make a cross to generate the mapping population (Collards et al. 2005). For example, when studying FHB resistance, it is common to select a resistant parent and a susceptible parent in making a cross (Kolb et al. 2001). Factors influencing the power to detect QTL include population size (Tanksley 1993), marker density, and accuracy of phenotypic and genotypic data (Hackett and Luo 2003; Kolb et al. 2001; Cuthbert et al. 2006). Environmental effects also affect the identification of QTL especially the minor QTL. Thus, FHB resistance QTL mapping experiments are usually conducted with replications under multiple years and environments (Collard et al. 2005; Haley and Knott 1992; Kolb et al. 2001).

Several different methods for QTL mapping have been developed, including single marker analysis (SMA) (Tanksley et al. 1982), simple interval mapping (SIM) (Lander & Botstein 1989), composite interval mapping (CIM) (Jansen 1993, 1994; Zeng 1993, 1994), and multiple interval mapping (MIM) (Wang et al. 2006). SMA is the easiest method for QTL detection with individual markers, but there are several disadvantages of using SMA: it is difficult to detect the QTL if a marker is far from a QTL, and effects of QTL may be

underestimated due to recombination between the marker and QTL (Tian 2015). SIM overcomes some of the weakness associated with SMA but it is still limited to looking at a single QTL per chromosome. When two QTL are located in close marker intervals, SIM cannot separate them (Manly and Olson 1999). CIM combines interval mapping with multiple regression, and thus can detect closely linked QTL (Zeng 1994), though it also has limitations on dealing with genetic factors such as epistasis. However, the MIM method is powerful in detecting QTL interactions (Wang et al. 2006).

QTL associated with FHB resistance types I-IV have been studied in more than 50 wheat cultivars and mapped on all 21 wheat chromosomes (Buerstmayr et al. 2009; Liu et al. 2009). Some wheat accessions harbor multiple QTL on different chromosomes (Buerstmaryr et al. 2009; Cativelli et al. 2013). Sumai 3, as a major FHB resistance source used in many breeding programs, carries a major QTL *Qfhs.ndsu*-3BS or *Fhb1* for type II resistance on the short arm of chromosome 3B (Waldron et al. 1999), and a minor QTL *Fhb2* on chromosome 6B (Anderson et al. 2001; Cuthbert et al. 2007). These two QTL have also been verified in different studies (Anderson et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002). A number of FHB resistance QTL have been identified from various sources on different chromosomes (Mardi et al. 2006; Xue et al. 2010; Xue et al. 2011; Chu et al. 2011; Cainong et al. 2015; Guo et al. 2015). However, *Fhb1*, originally derived from Sumai 3, is the only one that has been reported to maintain large and stable effects across different genetic backgrounds (Anderson et al. 2001; Bai et al. 1999; Buerstmayr et al. 2003; Chen et al. 2006; Cuthbert et al. 2006; Jayatilake et al. 2011; Jiang et al. 2007a; Jiang et al. 2007b; Lemmens et al. 2005; Shen et al. 2003; Somers et al. 2003; Yu et al. 2008).

Fhb1 has been introgressed into durum wheat by recurrent backcrossing and a resistant line DBC-480 was developed, which led to the development of novel FHB-resistant breeding lines that are agronomically close to European germplasm (Prat et al. 2017). Although some FHB resistance sources from worldwide are being used in wheat breeding programs, to diversify sources of resistance, breeders keep looking for resistance sources through screening wheat germplasm collections. PI 277012 is one of the FHB resistant wheat accessions identified from a large-scale evaluation of germplasm. It consistently showed a high level of resistance in different environments (Chu et al. 2011). Two major QTL for FHB resistance were identified on chromosome 5AS and 5AL, explaining up to 20 and 32% of phenotypic variation respectively (Chu et al. 2011). Large-scale of evaluation of both cultivated and wild tetraploid relatives of durum wheat only leads to identification of limited resistance sources in several tetraploid species: *T. dicoccoides*, *T. dicoccum*, *T. cathlicum*, and *T. durum* (Otto et al. 2002; Somers et al. 2006; Kumar et al. 2007; Buerstmayr et al. 2012). QTL with small to moderate effect have been mapped to 11 chromosomes in tetraploid wheat, but most of them are located at the same regions previously reported in common wheat (Prat et al. 2014).

Breeding for FHB Resistance

Breeding for varieties with increased FHB resistance has proven to be an efficient method to reduce the risk of yield and quality loss to the disease in wheat growing regions around the world (Ruckenbauer et al. 2001). The goal of breeding is to transfer high levels of FHB resistance to locally adapted backgrounds (Bai et al. 2000). It can be realized through two ways: classical breeding and genetic transformation. Once FHB resistance candidate genes become available, the application of genetic transformation plays a major part in the elucidation of their function. Successful classical breeding for FHB resistance requires three preconditions:

genetic variation for FHB resistance, introduction of the resistance trait into breeding material, and effective selection tools to track the resistance (Burstmayr et al. 2009). Large genetic variations for FHB resistance exist in the hexaploid wheat gene pool, and corresponding resistance QTL have already been identified. Due to the additive effect of major components of the FHB resistance, pyramiding FHB resistance QTL from diverse gene pools into locally adapted cultivars may significantly improve FHB resistance in commercial wheat cultivars (Kolb et al. 2001). However, most FHB highly resistant sources have un-adapted agronomic traits (Bai et al. 2000). To transfer the resistance QTL into adapted genetic backgrounds, phenotypic selection is a useful and successful approach, but it is time consuming, and difficult to operate. Marker-assisted selection (MAS) enables the incorporation of desired QTL being accomplished in relatively short time because selection can be started very early in the selection process (BC_1 or F_2) (Burstmayr et al. 2009).

Many elite wheat lines and cultivars in U.S. were reported to show moderate resistance to FHB, including ‘Bacup’, ‘Sabin’, ‘Alsen’, ‘Steele’, ‘Everest’, ‘Overland’, ‘ND2710’ and ‘Glenn’ (Mergoum et al. 2007). Some of these lines may carry QTL from Asian resistant sources, while others may contain native resistance QTL only. To further improve FHB resistance in these wheat cultivars, they can serve as recurrent parents to transfer QTLs from other resistance sources. Besides, transgressive segregation is also a good way to create FHB resistant cultivars (Bai et al. 2000). Elite resistant lines selected from transgressive segregation possess a high level of FHB resistance than their breeding parents. Most of the durum cultivars are susceptible to FHB, thus there is an urgent need to develop resistant cultivars. Resistance sources that can be used in durum breeding include resistant relatives of durum wheat and resistant hexaploid wheat. Backcrossing strategies, advanced phenotypic selection, MAS for validated resistance QTL, and

genomic selection associated with selection against resistance suppressors are promising approaches used to combine resistance alleles to enhance FHB resistance in durum wheat breeding programs (Prat et al. 2014).

References

Akkaya, M. S., Bhagwat, A. A., & Cregan, P. B. (1992). Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics*, *132*, 1131-1139.

Anderson, J. A., Stack, R. W., Liu, S., Waldron, B. L., Fjeld, A. D., Coyne, C., Moreno-Sevilla, B., Fetch, J.M., Song, Q.J., Cregan, P.B. & Frohberg, R. C. (2001). DNA markers for Fusarium head blight resistance QTLs in two wheat populations. *Theoretical and Applied Genetics*, *102*, 1164-1168.

Avise, J. C. (2000). Phylogeography: the history and formation of species. Harvard university press.

Bai, G. H., & Shaner, G. (1996). Variation in *Fusarium graminearum* and cultivar resistance to wheat scab. *Plant disease*, *80*, 975-979.

Bai, G. H., & Shaner, G. E. (1994). Wheat scab: perspective and control. *Plant Disease*, *78*, 760-766.

Bai, G., Kolb, F. L., Shaner, G., & Domier, L. L. (1999). Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. *Phytopathology*, *89*, 343-348.

Bai, G., & Shaner, G. (2004). Management and resistance in wheat and barley to Fusarium head blight. Annu. Rev. *Phytopathology*, *42*, 135-161.

Bai, G., Guo, P., & Kolb, F. L. (2003). Genetic relationships among head blight resistant cultivars of wheat assessed on the basis of molecular markers. *Crop Science*, *43*, 498-507.

Bai, G. H., Shaner, G., & Ohm, H. (2000). Inheritance of resistance to Fusarium graminearum in wheat. *Theoretical and Applied Genetics*, *100*, 1-8.

Beyer, M., Verreet, J. A., & Ragab, W. S. (2005). Effect of relative humidity on germination of ascospores and macroconidia of Gibberella zeae and deoxynivalenol production. *International journal of food microbiology*, *98*, 233-240.

Bockus, W. W., Fritz, A. K., Martin, T. J. (2009). Reaction of the 2008 Kansas Intrastate Nursery to Fusarium head blight. Plant Disease Management Reports. Report 3:CF009. The Ameri-can Phytopathological Society, St Paul, MN

Buerstmayr, H., Stierschneider, M., Steiner, B., Lemmens, M., Griesser, M., Nevo, E., & Fahima, T. (2003). Variation for resistance to head blight caused by Fusarium graminearum in wild emmer (Triticum dicoccoides) originating from Israel. *Euphytica*, *130*, 17-23.

Buerstmayr, H., Ban, T., & Anderson, J. A. (2009). QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. *Plant breeding*, *128*, 1-26.

Buerstmayr, H., Lemmens, M., Hartl, L., Doldi, L., Steiner, B., Stierschneider, M., & Ruckenbauer, P. (2002). Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). *Theoretical and Applied Genetics*, *104*, 84-91.

Buerstmayr, M., Huber, K., Heckmann, J., Steiner, B., Nelson, J. C., & Buerstmayr, H. (2012). Mapping of QTL for Fusarium head blight resistance and morphological and developmental traits in three backcross populations derived from Triticum dicoccum× Triticum durum. *Theoretical and Applied Genetics*, *125*, 1751-1765.

Bushnell, W. R., Hazen, B. E. and Pritsch, C. (2003). Histology and physiology of Fusarium head blight. In KJ Leonard, WR Bushnell (Eds.). In: Fusarium Head Blight of Wheat and Barley. APS Press, St. Paul, MN, pp 44-83.

Cai, X., Chen, P. D., Xu, S. S., Oliver, R. E., & Chen, X. (2005). Utilization of alien genes to enhance Fusarium head blight resistance in wheat–A review. *Euphytica*, *142*, 309-318.

Cainong, J. C., Bockus, W. W., Feng, Y., Chen, P., Qi, L., Sehgal, S. K., Danilova, T.V., Koo, D.H., Friebe, B. & Gill, B. S. (2015). Chromosome engineering, mapping, and transferring of resistance to Fusarium head blight disease from Elymus tsukushiensis into wheat. *Theoretical and Applied Genetics*, *128*, 1019-1027.

Caixeta, E. T., FerrÃ£o, L.,Felipe Ventorim, Maciel-Zambolim, E., & Zambolim, L. (2014). Chapter 2 - molecular markers. In A. Borem, & R. Fritsche-Neto (Eds.), Biotechnology and plant breeding (pp. 19-45). San Diego: Academic Press.

Cativelli, M., Lewis, S., & Appendino, M. L. (2013). A Fusarium head blight resistance quantitative trait locus on chromosome 7D of the spring wheat cultivar Catbird. *Crop Science*, *53*, 1464-1471.

Chen, J., Griffey, C. A., Maroof, S., Stromberg, E. L., Biyashev, R. M., Zhao, W., Chappell, M.R., Pridgen, T.H., Dong, Y. & Zeng, Z. (2006). Validation of two major quantitative trait loci for fusarium head blight resistance in Chinese wheat line W14. *Plant breeding*, *125*, 99-101.

Chu, C., Niu, Z., Zhong, S., Chao, S., Friesen, T. L., Halley, S., Elias, E.M., Dong, Y., Faris, J.D. and Xu, S. S. (2011). Identification and molecular mapping of two QTLs with major effects for resistance to Fusarium head blight in wheat. *Theoretical and applied genetics*, *123*, 1107-1119.

Clarke, J. M., Clarke, F. R., & Pozniak, C. J. (2010). Forty-six years of genetic improvement in Canadian durum wheat cultivars. *Canadian Journal of Plant Science, 90*, 791-801.

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica*, *142*, 169-196.

Cuthbert, P. A., Somers, D. J., Thomas, J., Cloutier, S., & Brulé-Babel, A. (2006). Fine mapping Fhb1, a major gene controlling Fusarium head blight resistance in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *112*, 1465.

Cuthbert, P. A., Somers, D. J., & Brulé-Babel, A. (2007). Mapping of *Fhb2* on chromosome 6BS: a gene controlling Fusarium head blight field resistance in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics, 114*, 429-437.

Desjardins, A. E. (2006). *Fusarium mycotoxins: chemistry, genetics, and biology*. American Phytopathological Society (APS Press).

Dill-Macky, R., & Jones, R. K. (2000). The effect of previous crop residues and tillage on Fusarium head blight of wheat. *Plant disease*, *84*, 71-76.

Dvořák, J., Terlizzi, P. D., Zhang, H. B., & Resta, P. (1993). The evolution of polyploid wheats: identification of the A genome donor species. *Genome*, *36*, 21-31.

Elias, E. (2005). U.S. Patent Application No. 11/071,272.

Feldman, M., & Millet, E. (1995). Methodologies for identification, allocation and transfer of quantitative genes from wild emmer into cultivated wheat. In *Proceedings of 8th international wheat genetic symposium, Beijing, China* (pp. 19-27).

Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O'Connell, C., Ray, D.K., West, P.C. and Balzer, C. (2011). Solutions for a cultivated planet. *Nature, 478*, 337-342.

Gale, L. R., Harrison, S. A., Ward, T. J., O'Donnell, K., Milus, E. A., Gale, S. W., & Kistler, H. C. (2011). Nivalenol-type populations of Fusarium graminearum and F. asiaticum are prevalent on wheat in southern Louisiana. *Phytopathology, 101*, 124-134.

Gale, L. R., Ward, T. J., Balmas, V., & Kistler, H. C. (2007). Population subdivision of Fusarium graminearum sensu stricto in the upper Midwestern United States. *Phytopathology, 97*, 1434-1439.

Gervais, L., Dedryver, F., Morlais, J. Y., Bodusseau, V., Negre, S., Bilous, M., Groos, C. & Trottet, M. (2003). Mapping of quantitative trait loci for field resistance to Fusarium head blight in an European winter wheat. *Theoretical and Applied Genetics*, *106*, 961-970.

Gilbert, J., & Fernando, W. G. D. (2004). Epidemiology and biological control of Gibberella zeae/Fusarium graminearum. *Canadian Journal of Plant Pathology*, *26*, 464-472.

Goswami, R. S., & Kistler, H. C. (2004). Heading for disaster: Fusarium graminearum on cereal crops. *Molecular plant pathology*, *5*, 515-525.

Guo, J., Zhang, X., Hou, Y., Cai, J., Shen, X., Zhou, T., Xu, H., Ohm, H.W., Wang, H., Li, A. & Kong, L. (2015). High-density mapping of the major FHB resistance gene Fhb7 derived from Thinopyrum ponticum and its pyramiding with Fhb1 by marker-assisted selection. *Theoretical and Applied Genetics*, *128*, 2301–2316.

Gupta, P. K., Varshney, R. K., Sharma, P. C., & Ramesh, B. (1999). Molecular markers and their applications in wheat breeding. *Plant breeding*, *118*, 369-390.

Hackett, C. A., Pande, B., & Bryan, G. J. (2003). Constructing linkage maps in autotetraploid species using simulated annealing. *Theoretical and Applied Genetics*, 106, 1107-1115.

Haley, C. S., & Knott, S. A. (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*, *69*, 315-324.

Hillman, G. (1978) On the origins of domestic rye—Secale cereale: the finds from aceramic Can Hasan III in Turkey." Anatolian Studies 28, 157-174.

Huhn, M. R., Elias, E. M., Ghavami, F., Kianian, S. F., Chao, S., Zhong, S., Alamri, M.S., Yahyaoui, A. & Mergoum, M. (2012). Tetraploid Tunisian wheat germplasm as a new source of Fusarium head blight resistance. *Crop science*, *52*, 136-145.

Jaccoud, D. (2001). Diversity Arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Research*, *29*, 25e–25.

Jansen, R. C. (1993). Interval mapping of multiple quantitative trait loci. *Genetics*, *135*, 205-211.

Jansen, R. C., & Stam, P. (1994). High resolution of quantitative traits into multiple loci via interval mapping. *Genetics*, *136*, 1447-1455.

Jayatilake, D. V., Bai, G. H., & Dong, Y. H. (2011). A novel quantitative trait locus for Fusarium head blight resistance in chromosome 7A of wheat. *Theoretical and applied genetics*, *122*, 1189-1198.

Jia, G., Chen, P., Qin, G., Bai, G., Wang, X., Wang, S., Zhou, B., Zhang, S. & Liu, D. (2005). QTLs for Fusarium head blight response in a wheat DH population of Wangshuibai/Alondra‘s’. *Euphytica*, *146*, 183-191.

Jiang, G. L., Shi, J., & Ward, R. W. (2007a). QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. I. Resistance to fungal spread. *Theoretical and Applied Genetics*, *116*, 3-13.

Jiang, G. L., Dong, Y., Shi, J., & Ward, R. W. (2007b). QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. II. Resistance to deoxynivalenol accumulation and grain yield loss. *Theoretical and Applied Genetics*, *115*, 1043-1052.

Jiang, G. L. (2013). Molecular markers and marker-assisted breeding in plants. In *Plant breeding from laboratories to fields*. Intech.

Jin, F., Zhang, D., Bockus, W., Baenziger, P. S., Carver, B., & Bai, G. (2013). Fusarium head blight resistance in US winter wheat cultivars and elite breeding lines. *Crop Science*, *53*, 2006-2013.

Jochum, C. C., Osborne, L. E., & Yuen, G. Y. (2006). Fusarium head blight biological control with Lysobacter enzymogenes strain C3. *Biological Control*, *39*, 336-344.

Jordan, S. A., & Humphries, P. (1994). Single nucleotide polymorphism in exon 2 of the BCP gene on 7q31-q35. *Human molecular genetics*, *3*, 1915-1915.

Kearsey, M. J., & Pooni, H. S. (1998). *The genetical analysis of quantitative traits*. Stanley Thornes (Publishers) Ltd.

Khan, N. I., Schisler, D. A., Boehm, M. J., Lipps, P. E., & Slininger, P. J. (2004). Field testing of antagonists of Fusarium head blight incited by Gibberella zeae. *Biological Control*, *29*, 245-255.

Kihara, H. (1944). Die Entdeckung der DD-Analysatoren beim Weizen. *Agric Hort (Tokyo)* 19, 889–890 (in German).

Kislev, M. E. (1984) Emergence of wheat agriculture. *Paleorient*, 61-70.

Kolb, F. L., Bai, G. H., Muehlbauer, G. J., Anderson, J. A., Smith, K. P., & Fedak, G. (2001). Host plant resistance genes for Fusarium head blight. *Crop Science*, *41*, 611-619.

Kumar, S., Stack, R. W., Friesen, T. L., & Faris, J. D. (2007). Identification of a novel Fusarium head blight resistance quantitative trait locus on chromosome 7A in tetraploid wheat. *Phytopathology*, *97*, 592-597.

Lander, E. S., & Botstein, S. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, *121*, 185.

Lemmens, M., Scholz, U., Berthiller, F., Dall'Asta, C., Koutnik, A., Schuhmacher, R., Adam, G., Buerstmayr, H., Mesterházy, Á., Krska, R. & Ruckenbauer, P. (2005). The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for Fusarium head blight resistance in wheat. *Molecular Plant-Microbe Interactions*, *18*, 1318-1324.

Leslie, J. F., Pearson, C. A., Nelson, P. E., & Toussoun, T. A. (1990). Fusarium spp. from corn, sorghum, and soybean fields in the central and eastern United States. *Ecological studies*, *44*, 66.

Leslie, J. F., & Summerell, B. A. (2006). Fusarium laboratory workshops, a recent history. *Mycotoxin Research*, *22*, 73–74.

Li, H. P., Zhang, J. B., Shi, R. P., Huang, T., Fischer, R., & Liao, Y. C. (2008). Engineering Fusarium head blight resistance in wheat by expression of a fusion protein containing a Fusarium-specific antibody and an antifungal peptide. *Molecular plant-microbe interactions*, *21*, 1242-1248.

Lin, F., Xue, S. L., Zhang, Z. Z., Zhang, C. Q., Kong, Z. X., Yao, G. Q., Tian, D.G., Zhu, H.L., Li, C.J., Cao, Y. & Wei, J. B. (2006). Mapping QTL associated with resistance to Fusarium head blight in the Nanda2419× Wangshuibai population. II: Type I resistance. *Theoretical and Applied Genetics*, *112*, 528-535.

Link, H. F. (1809). Observationes in Ordines plantarum naturales. Dlss: I, Magaz. d. Ges. naturf, Freunde Berlin, III, 1, 23.

Liu, S., Hall, M. D., Griffey, C. A., & McKendry, A. L. (2009). Meta-analysis of QTL associated with Fusarium head blight resistance in wheat. Crop Science, *49*, 1955-1968.

Mammadov, J., Aggarwal, R., Buyyarapu, R., & Kumpatla, S. (2012). SNP markers and their impact on plant breeding. International journal of plant genomics, 2012.

Manly, K. F., & Olson, J. M. (1999). Overview of QTL mapping software and introduction to Map Manager QT. *Mammalian Genome*, *10*, 327-334.

Marcussen, T., Sandve, S. R., Heier, L., Wulff, B. B., Steuernagel, B., Mayer, K. F., & Olsen, O. A. (2014). A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. *Sci*, *345*, 1250092-1250092.

Mardi, M., Pazouki, L., Delavar, H., Kazemi, M. B., Ghareyazie, B., Steiner, B., Nolz, R., Lemmens, M. & Buerstmayr, H. (2006). QTL analysis of resistance to Fusarium head blight in wheat using a 'Frontana'-derived population. *Plant Breeding*, *125*, 313-317.

Markell, S. G., & Francl, L. J. (2003). Fusarium head blight inoculum: species prevalence and Gibberella zeae spore type. *Plant Disease*, *87*, 814-820.

McMullen, M., Bergstrom, G., De Wolf, E., Dill-macky, R., Hershman, D., Shaner, G., & Van Sanford, D. (2012). Fusarium Head Blight Disease Cycle , Symptoms , and Impact on Grain Yield and Quality Frequency and Magnitude of Epidemics Since 1997. *Plant Disease*, *96*.

McMullen, M. P., Schatz, B., Stover, R., & Gregoire, T. (1997). Studies of fungicide efficacy, application timing, and application technologies to reduce Fusarium head blight and deoxynivalenol. *Cereal Research Communications*, 779-780.

McMullen, M., Halley, S., Schatz, B., Meyer, S., Jordahl, J., & Ransom, J. (2008). Integrated strategies for Fusarium head blight management in the United States. *Cereal Research Communications*, *36*, 563-568.

Mergoum, M., Frohberg, R. C., & Stack, R. W. (2007). Breeding hard red spring wheat for Fusarium head blight resistance. *Wheat production in stressed environments*, 161-167.

Mesterhazy, A. (1995). Types and components of resistance to Fusarium head blight of wheat. *Plant breeding*, *114*, 377-386.

Mesterházy, Á., Bartók, T., Mirocha, C. G., & Komoroczy, R. (1999). Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. *Plant breeding*, *118*, 97-110.

Morozova, O., & Marra, M. A. (2008). Applications of next-generation sequencing technologies in functional genomics. *Genomics*, *92*, 255-264.

Nelson, P. E., Plattner, R. D., Shackelford, D. D., & Desjardins, A. E. (1991). Production of fumonisins by Fusarium moniliforme strains from various substrates and geographic areas. *Applied and environmental microbiology*, *57*, 2410-2412.

NISHIKAWA, Kozo, Shima MIZUNO, and Yoshihiko FURUTA (1994). Identification of chromosomes involved in translocations in wild Emmer. *The Japanese Journal of Genetics*, *69*, 371-376.

O'Donnell, K., Ward, T. J., Aberra, D., Kistler, H. C., Aoki, T., Orwig, N., Kimura, M., Bjørnstad, Å. & Klemsdal, S. S. (2008). Multilocus genotyping and molecular phylogenetics resolve a novel head blight pathogen within the Fusarium graminearum species complex from Ethiopia. *Fungal Genetics and Biology*, *45*, 1514-1522.

O'Donnell, K., Ward, T. J., Geiser, D. M., Kistler, H. C., & Aoki, T. (2004). Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the Fusarium graminearum clade. *Fungal genetics and biology*, *41*, 600-623.

Oliver, R. E., Cai, X., Friesen, T. L., Halley, S., Stack, R. W., & Xu, S. S. (2008). Evaluation of Fusarium head blight resistance in tetraploid wheat (L.). *Crop Science*, *48*, 213-222.

Oliver, R. E., Stack, R. W., Miller, J. D., & Cai, X. (2007). Reaction of wild emmer wheat accessions to Fusarium head blight. *Crop science*, *47*, 893-897.

Osborne, L. E., & Stein, J. M. (2007). Epidemiology of Fusarium head blight on small-grain cereals. *International journal of food microbiology*, *119*, 103-108.

Otto, C. D., Kianian, S. F., Elias, E. M., Stack, R. W., & Joppa, L. R. (2002). Genetic dissection of a major Fusarium head blight QTL in tetraploid wheat. *Plant molecular biology*, *48*, 625-632.

Paillard, S., Schnurbusch, T., Tiwari, R., Messmer, M., Winzeler, M., Keller, B., & Schachermayr, G. (2004). QTL analysis of resistance to Fusarium head blight in Swiss winter wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *109*, 323-332.

Parry, D. W., Jenkinson, P., & McLeod, L. (1995). Fusarium ear blight (scab) in small grain cereals—a review. *Plant pathology*, *44*, 207-238.

Paul, P. A., Lipps, P. E., Hershman, D. E., McMullen, M. P., Draper, M. A., & Madden, L. V. (2008). Efficacy of triazole-based fungicides for Fusarium head blight and deoxynivalenol control in wheat: a multivariate meta-analysis. *Phytopathology*, *98*, 999-1011.

Petersen, G., Seberg, O., Yde, M., & Berthelsen, K. (2006). Phylogenetic relationships of Triticum and Aegilops and evidence for the origin of the A, B, and D genomes of common wheat (Triticum aestivum). *Molecular phylogenetics and evolution*, *39*, 70-82.

Poland, J. A., Brown, P. J., Sorrells, M. E., & Jannink, J. L. (2012). Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *PloS one*, *7*, e32253.

Prat, N., Buerstmayr, M., Steiner, B., Robert, O., & Buerstmayr, H. (2014). Current knowledge on resistance to Fusarium head blight in tetraploid wheat. *Molecular Breeding*, *34*, 1689-1699.

Prat, N., Guilbert, C., Prah, U., Wachter, E., Steiner, B., Langin, T., Robert, O. & Buerstmayr, H. (2017). QTL mapping of Fusarium head blight resistance in three related durum wheat populations. *Theoretical and Applied Genetics*, *130*, 13-27.

Puri, K. D., Saucedo, E. S., & Zhong, S. (2012). Molecular Characterization of Fusarium Head Blight Pathogens Sampled from a Naturally Infected Disease Nursery Used for Wheat Breeding Programs in China. *Plant Disease*, *96*, 1280-1285.

Puri, K. D., & Zhong, S. (2010). The 3ADON population of *Fusarium graminearum* found in North Dakota is more aggressive and produces a higher level of DON than the prevalent 15ADON population in spring wheat. *Phytopathology*, *100*, 1007-1014.

Qiu, J., Xu, J., & Shi, J. (2014). Molecular characterization of the Fusarium graminearum species complex in Eastern China. *European Journal of Plant Pathology*, *139*, 811-823.

Qu, B., Li, H. P., Zhang, J. B., Xu, Y. B., Huang, T., Wu, A. B., Zhao, C.S., Carter, J., Nicholson, P. & Liao, Y. C. (2008). Geographic distribution and genetic diversity of Fusarium graminearum and F. asiaticum on wheat spikes throughout China. *Plant Pathology*, *57*, 15-24.

Ray, D. K., Mueller, N. D., West, P. C., & Foley, J. A. (2013). Yield trends are insufficient to double global crop production by 2050. *PloS one*, *8*, e66428.

Ribichich, K. F., Lopez, S. E., & Vegetti, A. C. (2000). Histopathological spikelet changes produced by Fusarium graminearum in susceptible and resistant wheat cultivars. *Plant Disease*, *84*, 794-802.

Riehl, S., Zeidi, M., & Conard, N. J. (2013). Emergence of Agriculture in the Foothills of the Zagros Mountains of Iran. *Science*, *341*, 65–67.

Ruckenbauer, P., Buerstmayr, H., & Lemmens, M. (2001). Present strategies in resistance breeding against scab (Fusarium spp.). In *Wheat in a Global Environment* (pp. 85-95). Springer Netherlands.

Rudd, J. C., Horsley, R. D., McKendry, A. L., & Elias, E. M. (2001). Host plant resistance genes for Fusarium head blight. *Crop Science*, *41*, 620-627.

Salse, J., Bolot, S., Throude, M., Jouffe, V., Piegu, B. et al. (2008). Identification and characterization of shared duplications between rice and wheat provide new insight into grass genome evolution. *The Plant Cell*, 20, 11-24.

Sangalang, A. E., Burgess, L. W., Backhouse, D., Duff, J., & Wurst, M. (1995). Mycogeography of Fusarium species in soils from tropical, arid and mediterranean regions of Australia. *Mycological Research*, *99*, 523-528.

Sarver, B. A. J., Ward, T. J., Gale, L. R., Broz, K., Corby Kistler, H., Aoki, T., & O'Donnell, K. (2011). Novel Fusarium head blight pathogens from Nepal and Louisiana revealed by multilocus genealogical concordance. *Fungal Genetics and Biology*, *48*, 1096–1107.

Sax, K. (1923). The association of size differences with seed-coat pattern and pigmentation in Phaseolus vulgaris. *Genetics*, *8*, 552.

Schroeder, H. W., & Christensen, J. J. (1963). Factors affecting resistance of wheat to scab caused by Gibberella zeae. *Phytopathology*, *53*, 831-838.

Scott, P. M. (1990). Mycotoxigenic fungal contaminants of cheese and other dairy products.

Shewry, P. R. (2009). Wheat. *Journal of Experimental Botany*, *60*, 1537–1553.

Shen, X., Zhou, M., Lu, W., & Ohm, H. (2003). Detection of Fusarium head blight resistance QTL in a wheat population using bulked segregant analysis. *Theoretical and Applied Genetics*, *106*, 1041-1047.

Singh, R. P., & Van Ginkel, M. (1997). Breeding strategies for introgressing diverse scab resistances into adapted wheats. *Fusarium Head Scab: Global Status and Future Prospects. HJ Dubin, L. Gilchrist, J. Reeves, and A. McNab (eds.). Mexico, DF: CIMMYT*, 86-92.

Snyder, W. C., & Hansen, H. N. (1940). The species concept in Fusarium. *American Journal of Botany*, 64-67.

Somers, D. J., Fedak, G., Clarke, J., & Cao, W. (2006). Mapping of FHB resistance QTLs in tetraploid wheat. *Genome / National Research Council Canada = Genome / Conseil National de Recherches Canada*, *49*, 1586–1593.

Somers, D. J., Isaac, P., & Edwards, K. (2004). A high-density microsatellite consensus map for bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *109*, 1105-1114.

Somers, D. J., Fedak, G., & Savard, M. (2003). Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. *Genome*, *46*, 555-564.

Stack, R. W., Elias, E. M., Fetch, J. M., Miller, J. D., & Joppa, L. R. (2002). Fusarium Head Blight Reaction of Langdon Durum-Chromosome Substitution Lines. *Crop science*, *42*, 637-642.

Starkey, D. E., Ward, T. J., Aoki, T., Gale, L. R., Kistler, H. C., Geiser, D. M., Suga, H., Toth, B., Varga, J. & O'Donnell, K. (2007). Global molecular surveillance reveals novel Fusarium head blight species and trichothecene toxin diversity. *Fungal genetics and biology*, *44*, 1191-1204.

Steiner, B., Lemmens, M., Griesser, M., Scholz, U., Schondelmaier, J., & Buerstmayr, H. (2004). Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. *Theoretical and Applied Genetics*, *109*, 215-224.

Suga, H., Karugia, G. W., Ward, T., Gale, L. R., Tomimura, K., Nakajima, T., Miyasaka, A., Koizumi, S., Kageyama, K. & Hyakumachi, M. (2008). Molecular characterization of the Fusarium graminearum species complex in Japan. *Phytopathology*, *98*, 159-166.

Summerell, B. A., Salleh, B., & Leslie, J. F. (2003). A Utilitarian Approach to *Fusarium* Identification. *Plant Disease*, *87*, 117–128.

Tanksley, S. D. (1993). Mapping polygenes. *Annual review of genetics*, *27*, 205-233.

Taylor, J. W., Jacobson, D. J., Kroken, S., Kasuga, T., Geiser, D. M., Hibbett, D. S., & Fisher, M. C. (2000). Phylogenetic species recognition and species concepts in fungi. *Fungal genetics and biology*, *31*, 21-32.

Thoday, J. M. (1961). Location of polygenes. *Nature*, *191*, 368-370.

Tian, W., Hao, C., Fan, Z., Weng, X., Qin, H., Wu, X., Fang, M., Chen, Q., Shen, A. & Xu, Y. (2015). Myocardin related transcription factor A programs epigenetic activation of hepatic stellate cells. *Journal of hepatology*, *62*, 165-174.

Trail, F. (2009). For blighted waves of grain: Fusarium graminearum in the postgenomics era. *Plant physiology*, *149*, 103-110.

USDA ERS (2017) Wheat data https://www.ers.usda.gov/data-products/wheat-data/. Date accessed: 07/13/2017

USDA ERS (2016) Wheat background. https://www.ers.usda.gov/topics/crops/wheat/background/. Date accessed: 07/13/2017

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T. V. D., Hornes, M., Friters, A., Pot, J., Paleman, J., Kuiper, M. & Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic acids research*, *23*, 4407-4414.

Waldron, B. L., Moreno-Sevilla, B., Anderson, J. A., Stack, R. W., & Frohberg, R. C. (1999). RFLP mapping of QTL for Fusarium head blight resistance in wheat. *Crop Science*, *39*, 805-811.

Wang, G. Z., Miyashita, N. T., & Tsunewaki, K. (1997). Plasmon analyses of Triticum (wheat) and Aegilops: PCR-single-strand conformational polymorphism (PCR-SSCP) analyses of organellar DNAs. *Proceedings of the National Academy of Sciences of the United States of America*, *94*, 14570-14577.

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S., Milner, S.G., Cattivelli, L., Mastrangelo, A. M. Whan, A., Stephen, S., Barker, G., Wieseke, R., Plieske, J., International Wheat Genome Sequencing Consortium, Lillemo, M., Mather, D., Appels, R., Dolferus, R., Brown-Guedira, G., Korol, A., Akhunova, A. R., Feuillet, C., Salse, J., Morgante, M., Pozniak, C., Luo, M.-C., Dvorak, J., Morell, M., Dubcovsky, J., Ganal, M., Tuberosa, R., Lawley, C., Mikoulitch, I., Cavanagh, C., Edwards, K. J., Hayden, M. and Akhunov, E. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant biotechnology journal*, *12*, 787-796.

Wang S, et al. Windows QTL Cartographer 2.5., 2006 Department of Statistics, North Carolina State University, Raleigh, NC. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Ward, T. J., Clear, R. M., Rooney, A. P., O'Donnell, K., Gaba, D., Patrick, S., Starkey, D.E., Gilbert, J., Geiser, D.M. & Nowicki, T. W. (2008). An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic Fusarium graminearum in North America. *Fungal Genetics and Biology*, *45*, 473-484.

Wegulo, S. N., Bockus, W. W., Nopsa, J. H., De Wolf, E. D., Eskridge, K. M., Peiris, K. H., & Dowell, F. E. (2011). Effects of integrating cultivar resistance and fungicide application on Fusarium head blight and deoxynivalenol in winter wheat. *Plant disease*, *95*, 554-560.

Wiese, M.V. (1987). Compendium of wheat diseases. 2nd ed. American Phytopathological Society, St. Paul, Minn.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A., & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research*, *18*, 6531-6535.

Wollenweber, H. W., & Reinking, O. A. (1935). *Die fusarien, ihre beschreibung, schadwirkung und bekämpfung*. P. Parey.

Xue, S., Li, G., Jia, H., Xu, F., Lin, F., Tang, M., Wang, Y., An, X., Xu, H., Zhang, L. & Kong, Z. (2010). Fine mapping Fhb4, a major QTL conditioning resistance to Fusarium infection in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *121*, 147-156.

Xue, S., Xu, F., Tang, M., Zhou, Y., Li, G., An, X., Lin, F., Xu, H., Jia, H., Zhang, L., Kong Z. & Kong, Z. (2011). Precise mapping Fhb5, a major QTL conditioning resistance to Fusarium infection in bread wheat (Triticum aestivum L.). *Theoretical and applied genetics*, *123*, 1055-1063.

Yang, L., Van der Lee, T., Yang, X., Yu, D., & Waalwijk, C. (2008). Fusarium populations on Chinese barley show a dramatic gradient in mycotoxin profiles. *Phytopathology*, *98*, 719-727.

Yu, J. B., Bai, G. H., Zhou, W. C., Dong, Y. H., & Kolb, F. L. (2008). Quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population of wangshuibai/Wheaton. *Phytopathology*, *98*, 87-94.

Yuen, G. Y., & Schoneweis, S. D. (2007). Strategies for managing Fusarium head blight and deoxynivalenol accumulation in wheat. *International journal of food microbiology*, *119*, 126-130.

Zeng, Z. B. (1993). Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. *Proceedings of the National Academy of Sciences*, *90*, 10972-10976.

Zeng, Z. B. (1994). Precision mapping of quantitative trait loci. *Genetics*, *136*, 1457-1468.

Zhou, W., Kolb, F. L., Bai, G., Shaner, G., & Domier, L. L. (2002). Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. *Genome*, *45*, 719-727.

Zietkiewicz, E., Rafalski, A., & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, *20*, 176-183.

CHAPTER II. MOLECULAR MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN THE SPRING WHEAT LINE ND2710

Abstract

ND2710 is a hard red spring wheat line with a very high level of resistance to Fusarium head blight (FHB). It was selected from the progeny of a cross between ND2603 (an advanced breeding line derived from the Sumai 3/Wheaton cross) and Grandin (a spring wheat cultivar). The FHB resistance of ND2710 is presumably derived from Sumai 3 since the other parents Grandin and Wheaton are very susceptible to FHB. To identify and map the quantitative trait loci (QTL) for FHB resistance in ND2710, we developed a mapping population consisting of 233 recombinant inbred lines (RILs) from the cross between ND2710 and the spring wheat cultivar 'Bobwhite'. These RILs along with their parents and checks were evaluated for reactions to FHB in three greenhouse experiments and one field experiment during 2013 to 2014. The population was also genotyped with the wheat 90K SNP iSelect assay, and a genetic linkage map was developed with 1373 non-co-segregating SNP markers, which were distributed on 19 of the 21 wheat chromosomes spanning 914.98 cM of genetic distance. Genetic analyses using both phenotype and genotype data identified one major QTL (*Qfhb.ndwp-3B*) on chromosome 3BS, explaining up to 20% of the phenotypic variation in all experiments, and minor QTL (*Qfhb.ndwp-6B*, *Qfhb.ndwp-2A*, and *Qfhb.ndwp-6A*) on 6B, 2A, and 6A, respectively, explaining 5 to 12% phenotypic variation in at least two experiments, except for *Qfhb.ndwp-2A*, which was only detected in the field experiment. *Qfhb.ndwp-3B* and *Qfhb.ndwp-6B* were mapped to the genomic regions containing *Fhb1* and *Fhb2*, respectively, confirming that they were originated from Sumai 3. The additive effect of the major and minor QTL may contribute to the high level

of FHB resistance in ND2710. The SNP markers closely linked to the FHB resistance QTL will be useful for marker-assisted selection of FHB resistance in wheat breeding programs.

Introduction

Fusarium head blight (FHB), also known as scab, is a devastating fungal disease affecting all classes of wheat in North America and many other regions of the world (Goswami and Kistler 2004). In North America, *Fusarium graminearum* Schwabe [= *Gibberella zeae* (Schw) Petch] is the primary causal agent of the disease (McMullen et al. 1997). Severe FHB epidemics caused huge economic losses in wheat production by reducing yield and downgrading grain quality due to mycotoxins contamination (McMullen et al. 1997; Bai and Shaner 2004). Mycotoxins-contaminated food and feed pose high risk to both humans and animals (Parry et al. 1995; Korosteleva et al. 2007; Pestka 2010). To protect consumers from mycotoxicosis, many countries such as European Unions and the United States have established advisory levels for the most prevalent *Fusarium* mycotoxins in food (Poppenberger et al. 2003).

No single measure is effective for management of the FHB disease. Conventional agronomical methods such as adjusting planting date, crop rotations, tillage or the use of fungicides, are only partly effective to minimize damages caused by FHB. Utilization of FHB-resistant wheat varieties together with fungicides application is generally considered as the most practical and effective strategy to control this disease (McMullen and Stack 2011). Therefore, developing wheat cultivars with a relatively high level of FHB resistance has been of high priority in wheat breeding programs worldwide over the last decade (Buerstmayr et al. 2009). Although no wheats are immune to FHB, genotypes with relatively high levels of FHB resistance have been identified in different germplasm pools from Asia, Europe and Latin America (Buerstmayr et al. 2009). Examples include Chinese cultivar ‘Sumai 3’ and its derivatives, Swiss

cultivar ‘Arina’, and Brazilian cultivar ‘Frontana’ (Schroeder and Christensen 1963; Ruckenbauer et al. 2001; Paillard et al. 2004; Buerstmayr et al. 2009). Sumai 3 and its derivatives consistently showed high level of FHB resistance across different genetic backgrounds and environments and have been widely used in wheat breeding programs worldwide (Anderson et al. 2001; Buerstmayr et al. 2002, 2003; Zhou et al. 2002, 2004; Yang et al. 2005). Indeed, most of the recently released spring wheat varieties in the North Central Region contain the FHB resistance derived from Sumai 3 (Anderson et al. 2011).

FHB resistance in wheat is a complex and quantitative trait controlled by a few major genes and some modifying genes (Buerstmayr et al. 2009; Liu et al. 2009). The development of various molecular markers has greatly facilitated the genetic study and detection of QTL (quantitative trait locus/loci) for FHB resistance. In the past years, more than 200 QTL have been identified in a broad range of resistance sources on all 21 chromosomes of hexaploid wheat, but only 22 of them have been identified in multiple mapping populations and are considered stable (Buerstmayr et al. 2009). The major QTL on 3BS (syn. *Qfhs.ndsu-3BS*, designated as *Fhb1*) from Sumai 3 is the strongest and most reliable and has been proved to be consistently expressed in different genetic backgrounds (Waldron et al. 1999; Anderson et al. 2007; McCartney et al. 2007).

In the breeding process, phenotypic selection is a very useful approach for selecting lines with improved resistance (Collard et al. 2008). However, FHB phenotyping is time consuming and laborious, and often complicated by strong genotype-by-environment (G×E) interactions associated with FHB resistance. In recent years, marker assisted selection (MAS) of FHB resistance in wheat have been conducted in many labs worldwide (Agostinelli et al. 2012; Balut et al. 2013; Eckard et al. 2015). DNA markers closely linked to the resistance locus predict

presence or absence of the desired allele and can be applied in the selection process. MAS saves time since it can be started early at seedling stage and thus enhances selection efficiency. DNA markers tightly linked to *Fhb1* (syn. *Qfhs.ndsu-3BS*) such as *UMN10* flanked by markers *Xgwm533* and *Xgwm493* have been routinely used for MAS of the Sumai 3-derived FHB resistance (Liu et al. 2008). *Fhb1* was further fine mapped to a 1.1 Mb genomic region with twenty-eight candidate genes identified (Schweiger et al. 2016). The gene encoding chimeric lectin with agglutinin domains and a pore-forming toxin-like domain has been confirmed to be *Fhb1* conferring resistance to FHB (Rawat et al. 2016). The *Fhb2* region on chromosome 6B was also dissected (Cuthbert et al. 2007) and six putative resistance genes were identified using flanking marker sequences (Dhokane et al. 2016). However, effective markers have not been developed for most of the known FHB resistance QTL in wheat.

ND2710 was the first North Dakota hard red spring wheat (HRSW) experimental line generated in the process of introducing FHB resistance from Sumai 3 into adapted wheat varieties in the northern spring wheat region (del Blanco et al. 2003; Frohberg et al. 2004). It combines a high level of FHB resistance with relatively acceptable agronomic traits (Frohberg et al. 2004). ND2710 has been used as a FHB resistant parent in the NDSU spring wheat breeding program since 1994 (del Blanco et al. 2003). The objectives of this study were to verify the QTL for FHB resistance in ND2710, quantify the QTL effects using recombinant inbred lines (RILs) from the ND2710/Bobwhite cross, and develop user-friendly markers for marker-assisted selection of the FHB resistance in wheat breeding programs.

Materials and Methods

Plant Materials

To identify and map QTL for resistance to FHB in ND2710 (PI 633976), a population (designated as BN) containing 233 recombinant inbred lines (RILs) was developed from the cross between ND2710 and the FHB-susceptible spring wheat cultivar Bobwhite (PI 520368) using the single seed descent method. ND2710 is a hard red spring wheat (*Triticum aestivum* L.) line combining a high level of FHB resistance derived from Sumai 3 with relatively acceptable agronomic traits (Frohberg et al. 2004). Grandin (PI 531005) and Alsen (PI 615543) were used as susceptible and moderately resistant checks.

Evaluation of Reaction to FHB

The BN population and its parents (Bobwhite and ND2710) along with the checks were evaluated for type II resistance (resistance to fungal spread in the spikes) in three greenhouse experiments and one field environment using the procedures described by Chu et al. (2011). Greenhouse evaluations were conducted in the spring and winter seasons of 2013 (13GH1 and 13GH2), and the spring season of 2014 (14GH). Field evaluation was carried out in FHB nursery located at Fargo, North Dakota in 2014 (14FAR). In all experiments, the RILs were evaluated using randomized complete block design (RCBD) with three replicates.

In greenhouse environments, the RILs and their parents were grown in clay pots with three plants per pot. The greenhouse was supplemented with artificial light for a 14 h photoperiod with the temperature maintained between 22 and 25 °C. The inoculum was prepared at a concentration of 100,000 spores mL^{-1} by mixing spores equally from four pathogenic *F. graminearum* strains collected from North Dakota (Puri et al. 2010). Inoculation was performed using the single-spikelet inoculation method described by Stack et al. (2002). Ten microliters of

spore suspension was injected into the central spikelet of a spike using an inoculation syringe at anthesis. In each pot, eight to ten spikes at similar developmental stage were inoculated. Inoculated plants were placed in a room with misting system (1 min misting in every half hour) to facilitate disease development. After 48 hours of incubation, the plants were moved back to the greenhouse benches. In the field experiment, each line/genotype was planted in a hill plot with 8 to 10 seeds. At anthesis, at least eight spikes at similar developmental stage in a hill were inoculated. The nursery was misted for 5 min in 15 min intervals for 12 h daily (4:00 p.m. to 4:00 a.m.), until 14 days after anthesis of the latest maturing lines.

For both greenhouse and field experiments, disease ratings were conducted at 21 days post inoculation. The percentage of infected spikelets on each head was recorded as FHB severity using the scale of nine categories (0, 7, 14, 21, 33, 50, 67, 80, and 100%) described by Stack and McMullen (2011). The disease severity for each replicate (pot or hill) was calculated by averaging the severities of all heads.

The content of deoxynivalenol (DON) produced by *F. graminearum* in grains was determined in two greenhouse experiments (13GH2 and 14GH), designated as 13GH2-DON and 14GH-DON, respectively. To prepare the seed samples used for DON test, all the inoculated spikes of each line were harvested at maturity, and threshed carefully to keep scabby seeds. The threshed kernels of all three replicates from each line were combined, ground into powder, and sent to the Veterinary Diagnostic Laboratory of NDSU (Fargo, ND) for DON analysis.

Genotyping and Marker Development

Genomic DNA was extracted from fresh leaf tissues of the parents and RIL population (F6:7) with TissueLyser (Qiagen) using a simplified SDS-based procedure modified from Ahmed et al. (2009). The DNA extraction buffer consists of 20% SDS, 1M Tris-HCl (pH 8.0),

0.5M EDTA (pH 8.0), and 5M NaCl. DNA was diluted to ~50ng/µl and used for genotyping with the wheat 90K iSelect assay (Wang et al. 2014). Genotypic clusters for every SNP were determined using the manual option of GenomeStudio version 2011.1 with the polyploid clustering module v1.0 (Illumina), on basis of the data from all the described genotypes. DNA markers (*UMN10*, *Xgwm493*, *Xgwm533*, *Xgwm644*) linked to *Fhb*1 or *Fhb*2 (Röder et al. 1998; Liu et al. 2008) were also used to phenotype the BN population. Additional markers were developed for *Fhb2* using the six candidate genes (GENE1, GENE2, GENE3, GENE4, GENE5, and GENE6) for 4-Coumarate CoA ligase (4CL), basic Helix Loop Helix (bHLH041) transcription factor, glutathione S-transferase (GST), ABC transporter-4 (ABC4), callose synthase (CS), and cinnamyl alcohol dehydrogenase (CAD) respectively (Dhokane et al. 2016). Gene sequences were retrieved from wheat survey sequence annotation browser on the website of URGI (https://urgi.versailles.inra.fr/gb2/gbrowse/wheat_survey_sequence_annotation/) and used for designing primer pairs using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/). These primer pairs were used in PCR to amplify DNA sequences from the two parental genotypes (ND2710 and Bobwhite). PCR products amplified from two parental genotypes were sequenced by Genscript (Piscataway, NJ). Sequences from two parental wheat genotypes were aligned against each other to identify SNPs (http://multalin.toulouse.inra.fr/multalin/), which were transformed into CAPS markers. These markers together with the SNPs showing polymorphism between two parents identified from 90K iSelect assay, were used to construct a genetic linkage map.

Statistical Analysis, Linkage Map Construction and QTL Analysis

For testing distribution of disease severity and DON content in the BN population, the Shapiro-Wilk normality test was performed using PROC UNIVARIATE (SAS Institute 2011).

Levene's test (Levene 1960) under the general linear model (GLM) procedure was used to test homogeneity of disease severity and DON content variances among all experiments using SAS program version 9.3 (SAS Institute 2011). Correlation coefficients between disease severity and DON content were calculated using the PROC CORR procedure (SAS Institute 2011).

Genetic linkage map was constructed using the computer program MapDisto (v1.7.7) (Lorieux 2012) based on the 233 RILs from the BN population. The Kosambi mapping function was used and the threshold value of logarithm of odds (LOD) score was set at 3.0 to claim linkage between markers with a maximum fraction of recombination at 0.30.

The entire marker dataset was used to identify genomic regions associated with FHB resistance in the RIL population. According to the results of Levene's test, six phenotypic datasets were separately analyzed for QTL detection. Composite interval mapping (CIM) was used to identify significant QTL using software QGene v.4.3.10 (Joehanes and Nelson 2008). A critical LOD threshold was set as 3.0 by 1,000-iteration permutation test for the 0.05 level of probability. The percentage of phenotypic variance explained by a QTL and its additive effect were calculated. For the purpose of presentation, linkage groups possessing significant QTL were reevaluated using a set of non-redundant markers to generate the QTL figures for this paper. The results were confirmed to be identical to the initial results with the entire marker dataset.

To determine whether resistance QTL, environment, and interactions between them affect FHB disease severity, genotypic data of the peak marker of each QTL along with disease severity data of BN population collected from each environment were used for variance analysis through GLM procedure in SAS with the environment set as the random factor.

Results

FHB Phenotyping across Different Environments

The 233 RILs in the BN population and their parents Bobwhite and ND2710 had variable reactions to FHB in different experiments (Figure 2.1). The disease severities in 13GH2 were much higher than those in other experiments. In the greenhouse experiments, Bobwhite had disease severities of 91.4% in 13GH1, 74.2% in 13GH2, and 62.5% in 14GH, with the average being 76.0%, whereas ND2710 had disease severities of 7.8% in 13GH1, 12.5% in 13GH2, and 9.7% in 14GH, with the average being 10.0% (Figure 2.1). In the field nursery (14FAR), the average disease severities for Bobwhite and ND2710 were 51.8% and 10.9%, respectively (Figure 2.1). The distribution of disease severity of the BN population varied among different environments as well. The average disease severity for the population was 30.4, 49.9, 32.4, and 25.1% in the experiments 13GH1, 13GH2, 14GH, and 14FAR, respectively (Figure 2.1). Over 80% of the RILs had a disease severity of 50% or less in all experiments except for 13GH2 with 50% lines having a disease severity of 50% or less (Figure 2.1). DON tests (13GH2-DON and 14GH-DON) performed in the two greenhouse experiments (13GH2 and 14GH) indicated that ND2710 had a much lower DON content than Bobwhite and the RILs showed distribution patterns similar to those for the disease severity (Figure 2.2).

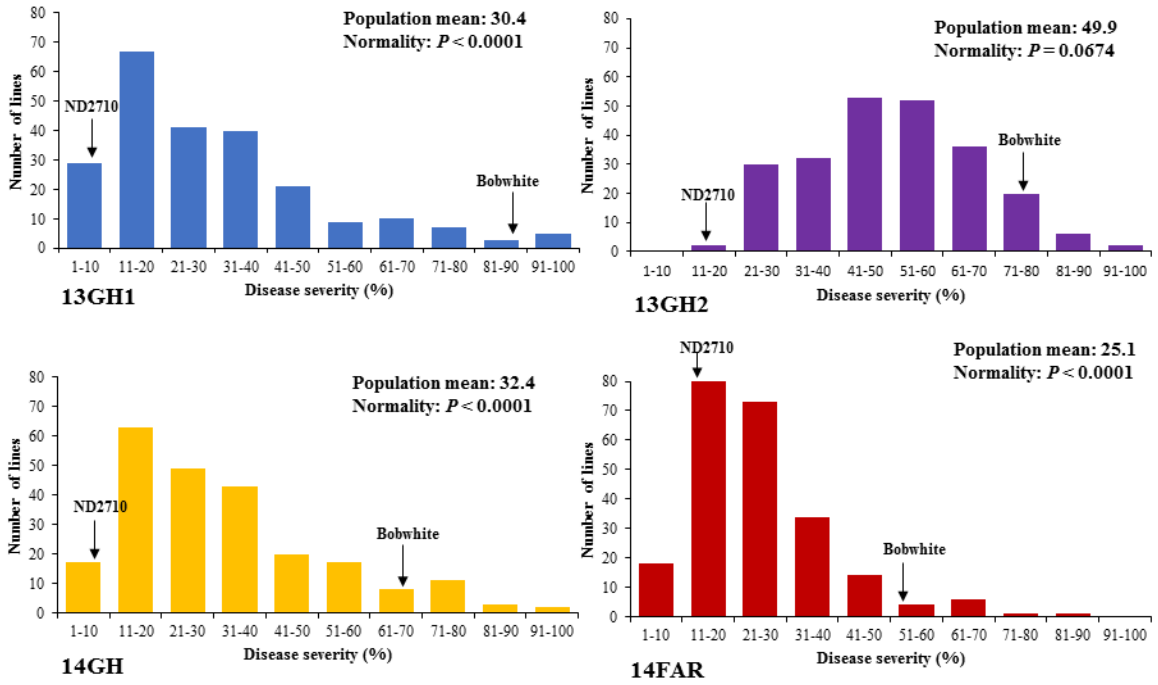

Figure 2.1. Frequency distribution of 233 recombinant inbred lines (RILs) derived from the Bobwhite×ND2710 cross for Fusarium head blight (FHB) disease severity in the four environments. 13GH1, 13GH2, and 14GH represent the greenhouse experiments performed in the spring and winter of 2013 and the spring of 2014, respectively, while 14FAR indicates field experiment performed in the FHB nursery at Fargo, North Dakota in 2014. Arrows indicate the disease severities of parents. Normality test was performed using PROC UNIVARIATE procedure and Shapiro-Wilk test (SAS Institute 2011).

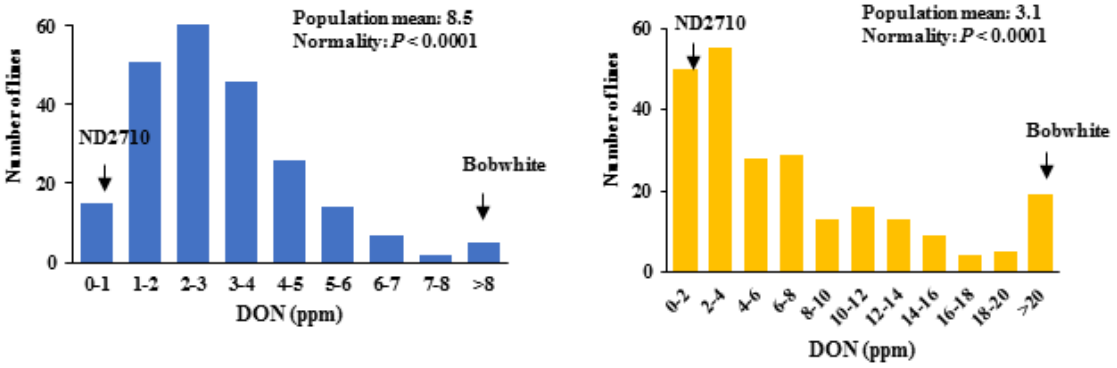

Figure 2.2. Frequency distribution of 233 recombinant inbred lines (RILs) derived from the cross of Bobwhite×ND2710 for deoxynivalenol (DON) content in two greenhouse experiments. 13GH2-DON and 14GH-DON represent DON tests from the experiments 13GH2 and 14GH, respectively. The DON content of parents were indicated by arrows. Normality test was performed using PROC UNIVARIATE procedure and Shapiro-Wilk test (SAS Institute 2011).

The normality test revealed that the distribution of disease severity and DON concentration in the RIL population was significant in all experiments except for 13GH2 (Figure 2.1), suggesting that the disease severity of the population in most of the FHB inoculation experiments deviated from a normal distribution. Thus, Levene's test (Levene 1960), which was less sensitive to non-normal distribution, was used to test homogeneity of disease severity variances across all experiments. The results showed that the variances among the three greenhouse experiments and the field nursery are heterogeneous ($P < 0.01$, $df = 3$), and variances among three greenhouse experiments are also heterogeneous ($P < 0.01$, $df = 2$). Only the variance between 13GH1 and 14GH is homogeneous ($P = 0.36$, $df = 1$). The variance between the two DON tests for greenhouse experiments is heterogeneous. There was a low to moderate correlation between measured FHB traits among environments, ranging from 0.38 to 0.76 (Table 2.1). The correlation coefficient was 0.67 between disease severity of 13GH2 and 13GH2-DON, and 0.76 between disease severity of 14GH and 14GH-DON, each being highly significant ($P < 0.0001$), suggesting that RILs with a low disease severity usually had a low DON concentration in the greenhouse.

Table 2.1. Correlation coefficients among Fusarium head blight (FHB) traits from field and greenhouse environments of the BN (Bobwhite × ND2710) population

	13GH1	13GH2	14GH	14FAR	13GH2-DON	14GH-DON
13GH	-					
13GH_2	0.64***	-				
14GH	0.68***	0.67***	-			
14FAR	0.43***	0.54***	0.45***	-		
13GH2-DON	0.47***	0.67***	0.49***	0.38***	-	
14GH-DON	0.55***	0.49***	0.76***	0.43***	0.44***	-

Note: *, **, *** significant at P < 0.05, P < 0.01, and P < 0.001, respectively. GH, greenhouse; ns, not significant

Linkage Map Construction

A total of 7413 polymorphic SNP markers were identified in the BN population using the 90K iSelect Assays. After removal of co-segregating markers, 1370 unique SNP markers were used for further genetic analysis. In addition, three SSR markers (*Xumn10*, *Xgwm493*, and *Xgwm533*) associated with *Fhb1*, and one SSR marker (*Xgwm644*) linked to *Fhb2* and two CAPS markers (*Fhb2-CAPS3* and *Fhb2-CAPS6*) developed from two candidate genes (GENE3, GENE6) for Fhb2 were used to genotype the mapping population. With a total of 1376 markers, a genetic linkage map was developed, which consisted of 35 linkage groups. Eight of the linkage groups were assigned to genome A, 11 to genome B, and 16 to genome D. The total genetic map length was 914.98 cM, partitioned into 385.43 cM for genome A, 384.74 cM for genome B, and 144.82 cM for genome D. Average distance between markers was 0.66 cM.

QTL Analysis for FHB Resistance and DON Content

QTL analysis of six datasets (13GH1, 13GH2, 14GH, 14FAR, 13GH2-DON, and 14GH-DON) led to identification of four ND2710-derived QTL for FHB resistance on chromosome 3B, 6B, 2A, and 6A, respectively (Table 2.2). *Qfhb.ndwp-3B* peaked at marker *SNP8962* was detected from all datasets, explaining 5% to 20% of the phenotypic variation with LOD value between 2.8 and 11.5 (Figure 2.3; Table 2.2). *Qfhb.ndwp-6B* peaked at marker *Fhb2-CAPS3* was detected from 13GH1, 14GH, and 14GH-DON, explaining 6%, 12% and 7% of the phenotypic variation, respectively. *Qfhb.ndwp-2A* peaked at *SNP79083* was only detected from the field experiment (14FAR), explaining 6% of phenotypic variation. *Qfhb.ndwp-6A* peaked at marker *SNP74620* was detected from 13GH2 and 13GH2-DON, explaining 5% and 8% of phenotypic variation. The QTL for resistance to FHB and DON accumulation on 3BS, 6BS, and 6AL were coincident (Figure 2.3; Table 2.2).

Table 2.2. Quantitative trait loci (QTL) associated with FHB resistance in the BN (Bobwhite × ND2710) population

	Qfhb.ndwp-3B			*Qfhb.ndwp-6B*			*Qfhb.ndwp-2A*			*Qfhb.ndwp-6A*		
Experiment[a]	LOD[b]	R^2	Add. effect	LOD	R^2	Add. effect	LOD	R^2	Add. effect	LOD	R^2	Add. effect
13GH1	6.3	0.12	-7.2	3.3	0.06	-5.7	-	-	-	-	-	-
13GH2	11.5	0.20	-7.3	-	-	-	-	-	-	2.7	0.05	-3.9
14GH	9.8	0.18	-8.2	6.3	0.12	-7.1	-	-	-	-	-	-
14FAR	4.9	0.09	-4.0	-	-	-	3.3	0.06	-3.5	-	-	-
13GH2-DON	3.9	0.07	-0.5	-	-	-	-	-	-	4.1	0.08	-0.5
14GH-DON	2.8	0.05	-3.3	3.9	0.07	-4.1	-	-	-	-	-	-

[a] 13GH1, 13GH2 and 14GH were experiments conducted in greenhouse in the spring and winter of 2013 and the spring of 2014, respectively; 14FAR was experiment conducted in the field FHB nursery at Fargo, North Dakota in 2014; 13GH2-DON and 14GH-DON represent the content of DON produced by *F. graminearum* calculated from two greenhouse experiments 13GH2 and 14GH

[b] The critical LOD threshold of 3.0 for the 0.05 level of probability was obtained through 1,000 –iteration permutation test

[c] A negative value indicates an increasing allele for disease severity derived from Bobwhite, then the resistance effects were derived from ND2710

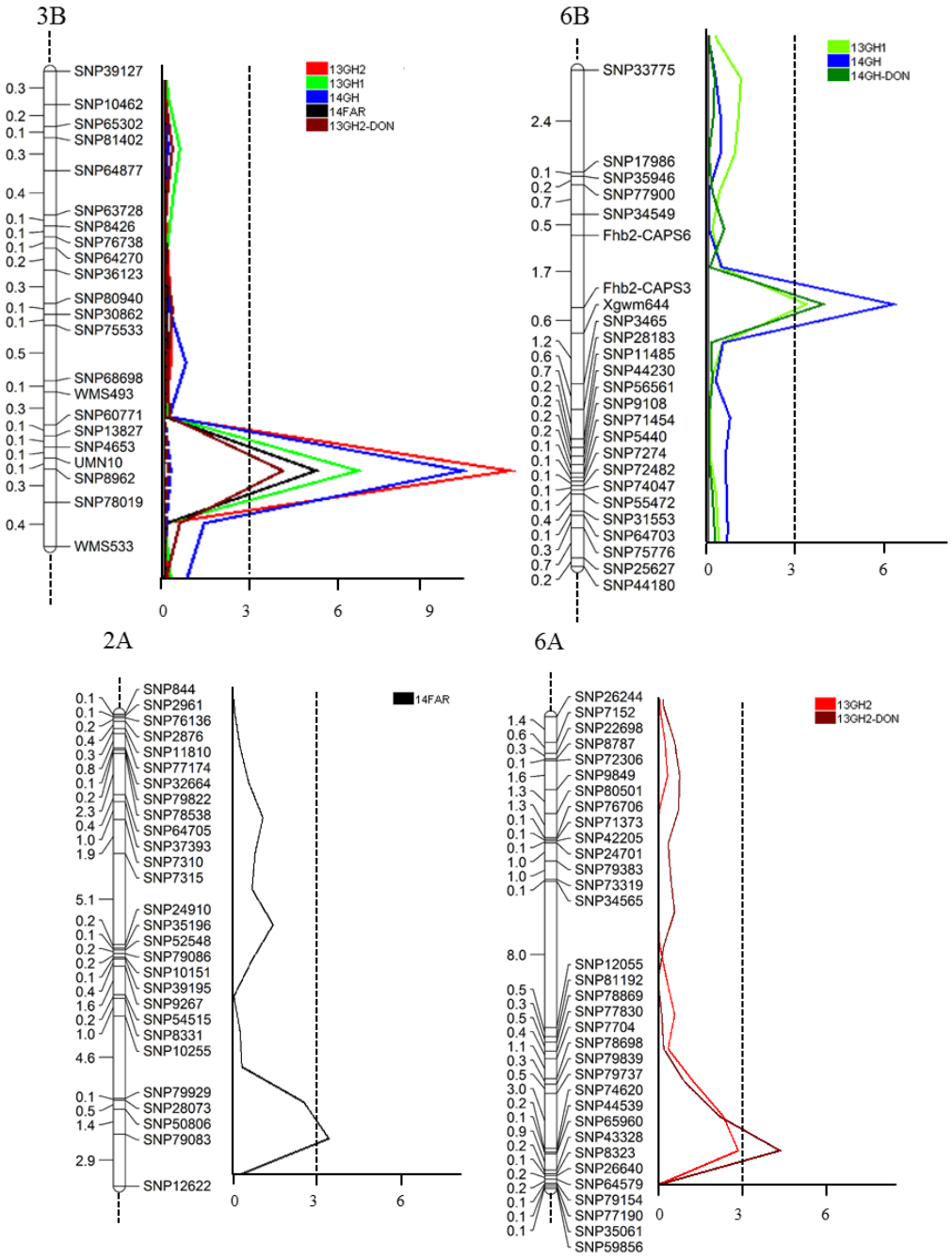

Figure 2.3. Regions of linkage maps for chromosome 3B, 6B, 2A, and 6A harboring QTL for FHB resistance detected in the BN (Bobwhite × ND2710) population. All QTL were derived from the resistant parent ND2710. The centiMorgan (cM) distances between marker loci are indicated on the left sides, while the positions of marker loci are marked on the right sides of the linkage maps. The logarithm of the odds (LOD) significance threshold of 3.0 is represented by a vertical dotted line. The red, green, and blue solid lines indicate greenhouse seasons (13GH2, 13GH1 and 14GH), black line for field experiment 14FAR,brown and dark green lines for 13GH2-DON and 14GH-DON, respectively.

Effects of QTL on Type II Resistance and DON accumulation

Under greenhouse (13GH1, 13GH2, 14GH) and field (14FAR) conditions, the average disease severity of the RILs carrying the ND2710 alleles at *SNP8962* on 3B and *Fhb1-CAPS3* on 6B were 17.4 and 19.6%, respectively, while the average disease severity of the RILs carrying the Bobwhite alleles were 45.4 and 30.2%, respectively. In the two DON tests, the average DON content for RILs carrying ND2710 alleles at *SNP8962* on 3B and *Fhb1-CAPS3* on 6B were 2.3 and 3.7 ppm, respectively, while the average DON content of RILs carrying the Bobwhite alleles were 3.7 and 17.1 ppm, respectively. For the other two markers on 2A and 6A, the average disease severity of the RILs with ND2710 alleles in greenhouse and field experiments were 27.9 and 20.3%, compared with 34.9 and 30.1% for those carrying the corresponding Bobwhite alleles. The average DON content of the RILs with ND2710 alleles were 2.4 and 6.8 ppm compared with 3.9 and 13.1 ppm for those with the Bobwhite alleles (Figure 2.4). The lower average disease severity and DON content in RILs with ND2710 alleles than in those with Bobwhite alleles confirmed that all favorable alleles for FHB resistance were contributed by ND2710. *Qfhb.ndwp-3B* contributed the largest effect on FHB resistance followed by the *Qfhb.ndwp-6B.*

To elucidate the effect of single and combined QTL on FHB response, the RILs were divided into 5 groups: group 1 (G1) carried the ND2710 alleles at the peak marker loci associated with all four QTL detected; group 2 (G2) contained the ND2710 alleles associated with the *Qfhb.ndwp-3B* and *Qfhb.ndwp-6B*; group 3 (G3) carried the ND2710 allele associated with the *Qfhb.ndwp-3B* only; group 4 (G4) carried the ND2710 allele associated with the *Qfhb.ndwp-6B* only; group 5 (G5) carried the ND2710 alleles associated with the two minor QTL *Qfhb.ndwp-2A* and *Qfhb.ndwp-6A*; and group 6 (G6) carried the Bobwhite alleles for all

four QTL. For greenhouse experiments (GH_MEAN), the average disease severity of groups 1 to 2 were significantly lower (LSD, $\alpha = 0.05$) than that of groups 4 to 6, while there was no significant difference between groups 1 to 3. (Figure 2.4). Group 6 showed significantly higher disease severity than the other groups. For the field experiment, group 1 and 2 showed significantly lower disease severity than group 4 and 6, but there was no significant difference either between group 1 and 2, or between group 4 and 6. Group 3 and 5 showed a similar level of disease severity. For the DON tests, group 1 and 2 had a significantly lower DON content than group 6, but the differences among group 1, 2 and 3 were non-significant, while the difference between group 5 and 6 was significant.

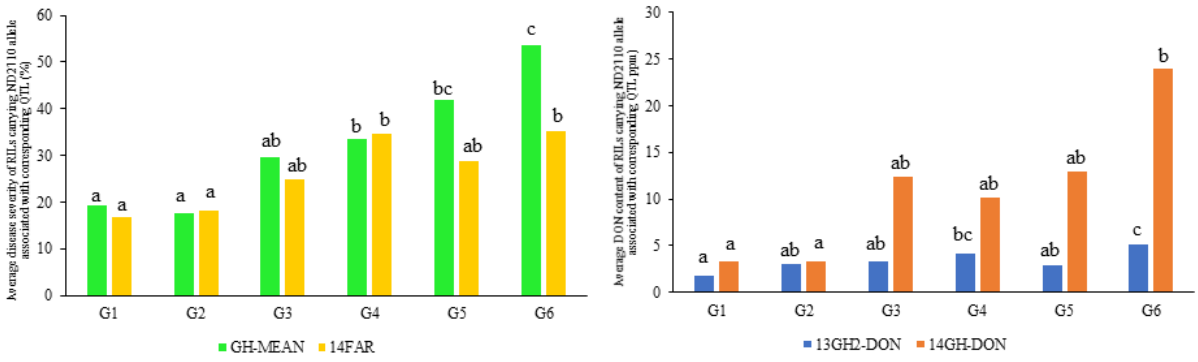

Figure 2.4. QTL effects on FHB severity and DON content. To elucidate the effect of single and combined QTL on FHB response, the RILs were divided into 6 groups: group 1 (G1) contained the ND2710 alleles associated with all four QTL; group 2 (G2) carried the ND2710 allele associated with the 3B and 6B QTL only; group 3 (G3) carried the ND2710 allele associated with the 3B QTL; group 4 (G4) carried the ND2710 alleles associated with 6B; group 5 (G5) carried the two minor QTL on 2A and 6A; and group 6 (G6) carried the Bobwhite alleles for all four QTL. Fisher's least significant difference (LSD) test was used to determine significant differences among means of different groups. Same letters above histogram indicate no significant difference at the 0.05 level of probability.

Discussion

In this study, we genotyped and phenotyped the RIL population derived from the cross between ND2710 and Bobwhite, and identified four QTL for FHB resistance contributed by the hard red spring wheat line ND2710. These QTL were mapped to chromosomes 3BS, 6BS, 2AS, and 6AL, respectively, and saturated with SNP markers, which will be useful for MAS of the FHB resistance.

The QTL on 3BS and 6BS in ND2710 are mapped to the same genomic regions as *Fhb1* and *Fhb2*, respectively. Considering the facts that ND2710 was a progeny with Sumai 3 in the pedigree (Frohberg et al. 2004) and SSR markers for *Fhb1* and *Fhb2* mapped to the QTL regions in the BN population, the high level of FHB resistance in ND2710 are mainly due to these two QTL derived from Sumai 3. *Fhb1* and *Fhb2* were first reported in Sumai 3 (Waldron et al. 1999) and Ning 7840 (Bai et al. 1999), and also detected in many different wheat genotypes related to Sumai 3 (Buerstmayr et al. 2009; Liu et al. 2009). Due to the large effect on FHB response, *Fhb1* was fine mapped as a single Mendelian gene within a 1.2 cM interval (Cuthbert et al. 2006). Liu et al. (2008) developed a SSR marker (*Xumn10*), which has been widely used to predict the presence of *Fhb1* in various genetic studies and in breeding programs. Recently, DNA sequencing, gene annotation, and function characterization of the *Fhb1* region have allowed the identification and cloning of the gene responsible for the FHB resistance (Schweiger et al. 2016; Rawat et al. 2016). These studies provide valuable resources for further understanding of the mechanism of the resistance gene and accelerate its application in wheat breeding programs.

In our study, the *Qfhb.ndwp-6B* was not significant in CIM analysis of the field experiment data. This may be due to the unfavorable environmental conditions for disease development in the summer of 2014. Among the six candidate genes identified by Dhokane et al.

(2016) for *Fhb2*, two (*Fhb2-CAPS3* and *Fhb2-CAPS6*) were mapped to the *Qfhb.ndwp-6B* region in the population derived from Bobwhite and ND2710. *Fhb2-CAPS3* from the gene for Glutathione S-transferase (GST) is at the peak of QTL-*Fhb2* and should be a useful marker for MAS of the QTL in wheat breeding programs. GSTs catalyze the conjugation of electrophilic molecules to glutathione (GSH) and have been implicated for detoxification of DON in barley (Gardiner et al. 2010). Several other studies also indicated that GST genes play a role in resistance to fungal plant pathogens (Dean et al. 2003, 2005; Han et al. 2016). Dhokane et al. (2016) speculate that GST is involved in FHB resistance by detoxification of DON and reducing the pathogenicity of the pathogen. However, further studies are needed to characterize the function of GST in FHB resistance, and to clone and characterize the gene(s) for *Fhb2*.

The QTL on 2A was only detected in the field inoculation experiment, and *Qfhb.ndwp-6A* was detected from one greenhouse experiment and the corresponding DON test. They are considered minor QTL, which are inconsistent among different environmental conditions. QTL for FHB on chromosome 2A have been reported in several sources; they were associated with either type II or DON content and explained 3 to 26.7% of the phenotypic variation in different experiments (Waldron et al. 1999; Anderson et al. 2001; Zhou et al. 2002; Steiner et al. 2004; Paillard et al. 2004; Gervais et al. 2003). *Qfhb.ndwp-2A* in ND2710 peaked at 41.7 cM on the genetic map of this study, which is approximately 38.7 cM on the 9k consensus SNP map (Cavanagh et al. 2013); it may be the same as the 2A QTL identified from winter wheat cultivar 'Freedom' of which confidence intervals (CI) starts at 39.5 cM and peaks at 43.6 cM (Gupta et al. 2001), and the 2A QTL from Chinese spring wheat cultivar 'Ning7840' with CI between 29.1 and 53.6 cM (Zhou et al. 2002). All the three QTL previously identified on 2A condition type II resistance. Four QTL on 6AS were previously identified from US wheat cultivar ND2603

(Anderson et al. 2001), French bread wheat cultivar ‘Apache’ (Holzapfel et al. 2008), German winter wheat cultivar ‘Dream’ (Schmolke et al. 2005; Haberle et al. 2007), and UK winter wheat cultivar ‘Spark’ (Gosman et al., 2007), respectively. These reported 6AS QTL condition type II resistance and explained 7.8 to 19% of phenotypic variation. ND2710 has a pedigree with ND2603, but no QTL was identified on 6AS in this study, instead a QTL on 6AL was detected from dataset 13GH2 and the corresponding DON test 13GH2-DON. The 6AL QTL may be novel.

Over 200 QTL for FHB resistance have been identified from different resistance sources worldwide conditioning FHB resistance type I, II, III, and IV (Buerstmayr et al. 2009). Most of them are responsible for single trait, and 62% of them condition type II resistance while about only 10% for the other types of FHB resistance (Buerstmayr et al. 2009; Liu et al. 2009). However, some of them confers resistance to more than one trait. For example, QTL identified from Sumai3, CJ9306, wangshuibai, Ernie, and Arina condition resistance to both type II and III resistance (Handa et al. 2008; Jiang et al. 2007a, b; Yu et al. 2008; Abate et al. 2008; Draeger et al. 2007). In the present study, *Qfhb.ndwp-2A* conditions type II resistance. We also detected the coincidence between QTL for FHB resistance and DON content on chromosome 3B, 6A, and 6B.

Although gene transformation has been widely used in functional analysis of genes of interest and for generating genetically modified plants for crop improvement, the transformation efficiency is relatively low in wheat compared to other major crops (Harwood et al. 2011). The principle element for establishing a highly efficient transformation system is the availability of efficient embryogenic cultures, because the regeneration of transgenic plants relies on the formation of somatic embryos (Rao et al. 2009). It is believed that plant regeneration ability is

under genetic control which could be either qualitative (Reisch and Bingham 1980) or quantitative (Taguchi-Shiobara et al. 2006). Immature and mature embryos are sources of explants used in wheat transformation. QTL for immature embryo tissue culture response (TCR) have been mapped on wheat chromosomes 2A, 2B, 2D, 3A, 3B, and 7D using chromosome recombinant lines (Ben Amer et al. 1997; Henry et al. 1994; Jia et al. 2009). Mature embryo, as an alternative of immature embryo, is easy to be obtained without limitations of growing seasons and development stages (Yin et al. 2011). Ma et al. (2016) studied the TCR of mature wheat embryos using a recombinant inbred lines (RILs) and identified three QTL for callus rate which were located on chromosomes 1D, 5A, 6D, and two QTL for emergence rate on 3B and 4A. Bobwhite has a high transformability and is widely used in gene transformation in wheat (Pellegrineschi et al. 2002). However, the genes or QTL responsible for this trait has not been identified. With the genetic map available in the BN population, it is possible to dissect the genetics of gene associated with the tissue culture response (TCR) of Bobwhite.

In summary, our data showed that FHB resistance in ND2710 was contributed by a combination of four QTL. Three of them were reported previously in different germplasms. The effects of these QTL were additive. The QTL on chromosome 3B showing major effects on FHB resistance and DON reduction was consistently detected in multiple experiments. The RILs combining ND2710 alleles at the QTL on 3B and 6B had significantly lower disease severity and DON content than RIL without these alleles in both greenhouse and field experiments. ND2710 is a useful source for improving wheat FHB resistance, and the identified DNA markers associated with the four QTL can be used in selection of FHB resistance in breeding programs.

References

Abate, Z. A., Liu, S., & McKendry, A. L. (2008). Quantitative trait loci associated with deoxynivalenol content and kernel quality in the soft red winter wheat 'Ernie'. *Crop science*, *48*, 1408-1418.

Ahmed, I., Islam, M., Arshad, W., Mannan, A., Ahmad, W., & Mirza, B. (2009). High-quality plant DNA extraction for PCR: an easy approach. *Journal of applied genetics*, *50*, 105-107.

Agostinelli, A. M., Clark, A. J., Brown-Guedira, G., & Van Sanford, D. A. (2012). Optimizing phenotypic and genotypic selection for Fusarium head blight resistance in wheat. *Euphytica*, *186*, 115-126.

Amer, I. B., Worland, A. J., Korzun, V., & Börner, A. (1997). Genetic mapping of QTL controlling tissue-culture response on chromosome 2B of wheat (Triticum aestivum L.) in relation to major genes and RFLP markers. *Theoretical and Applied Genetics*, *94*, 1047-1052.

Anderson, J. A., Stack, R. W., Liu, S., Waldron, B. L., Fjeld, A. D., Coyne, C., Moreno-Sevilla, B., Fetch, J.M., Song, Q.J., Cregan, P.B. & Frohberg, R. C. (2001). DNA markers for Fusarium head blight resistance QTLs in two wheat populations. *Theoretical and Applied Genetics*, *102*, 1164-1168.

Anderson, J. A., Chao, S., & Liu, S. (2007). Molecular breeding using a major QTL for Fusarium head blight resistance in wheat. *Crop Science*, *47*, S-112.

Anderson, J.A., Glover, K. & Mergoum, M. Successful adoption of spring wheat cultivars with moderate resistance to FHB by growers in the North Central Region. in Proc. 2011 Natl. Fusarium Head Blight Forum (eds. Canty, S., Clark, A., Anderson-Scully, A., Ellis, D. & Van Sanford, D.) 3 (US Wheat and Barley Scab Initiative, 2011).

Bai, G., Kolb, F. L., Shaner, G., & Domier, L. L. (1999). Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. *Phytopathology*, *89*, 343-348.

Bai, G., & Shaner, G. (2004). Management and resistance in wheat and barley to Fusarium head blight. *Annu. Rev. Phytopathology*, *42*, 135-161.

Balut, A. L., Clark, A. J., Brown-Guedira, G., Souza, E., & Van Sanford, D. A. (2013). Validation of and in Several Soft Red Winter Wheat Populations. *Crop Science*, *53*, 934-945.

Buerstmayr, H., Lemmens, M., Hartl, L., Doldi, L., Steiner, B., Stierschneider, M., & Ruckenbauer, P. (2002). Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). *Theoretical and Applied Genetics*, *104*, 84-91.

Buerstmayr, H., Steiner, B., Hartl, L., Griesser, M., Angerer, N., Lengauer, D., Miedaner, T., Schneider, B. & Lemmens, M. (2003). Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. *Theoretical and Applied Genetics*, *107*, 503-508.

Buerstmayr, H., Ban, T., & Anderson, J. A. (2009). QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. *Plant breeding*, *128*, 1-26.

Cavanagh, C. R., Chao, S., Wang, S., Huang, B. E., Stephen, S., Kiani, S., Forrest, K., Saintenac, C., Brown-Guedira, G.L., Akhunova, A. & See, D. (2013). Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proceedings of the national academy of sciences*, *110*, 8057-8062.

Chu, C., Niu, Z., Zhong, S., Chao, S., Friesen, T. L., Halley, S., Elias, E.M., Dong, Y., Faris, J.D. & Xu, S. S. (2011). Identification and molecular mapping of two QTLs with major effects for resistance to Fusarium head blight in wheat. *Theoretical and applied genetics*, *123*, 1107-1119.

Collard, B. C., & Mackill, D. J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *363*, 557-572.

Cuthbert, P. A., Somers, D. J., Thomas, J., Cloutier, S., & Brulé-Babel, A. (2006). Fine mapping Fhb1, a major gene controlling Fusarium head blight resistance in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *112*, 1465-1475.

Cuthbert, P. A., Somers, D. J., & Brulé-Babel, A. (2007). Mapping of *Fhb2* on chromosome 6BS: a gene controlling Fusarium head blight field resistance in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *114*, 429-437.

Dean, J. D., Goodwin, P. H., & Hsiang, T. (2005). Induction of glutathione S-transferase genes of Nicotiana benthamiana following infection by Colletotrichum destructivum and C. orbiculare and involvement of one in resistance. *Journal of Experimental Botany*, *56*, 1525-1533.

Dean, J. D., Goodwin, P. H., & Hsiang, T. (2003). Colletotrichum gloeosporioides infection induces differential expression of glutathione S-transferase genes in Malva pusilla. *Functional plant biology*, *30*, 821-828.

Del Blanco, I., Frohberg, R., Stack, R., Berzonsky, W., & Kianian, S. (2003). Detection of QTL linked to Fusarium head blight resistance in Sumai 3-derived North Dakota bread wheat lines. *Theoretical and Applied Genetics*, *106*, 1027-1031.

Dhokane, D., Karre, S., Kushalappa, A. C., & McCartney, C. (2016). Integrated metabolo-transcriptomics reveals Fusarium head blight candidate resistance genes in wheat QTL-Fhb2. *PloS one*, *11*, e0155851.

Draeger, R., Gosman, N., Steed, A., Chandler, E., Thomsett, M., Schondelmaier, J., Buerstmayr, H., Lemmens, M., Schmolke, M., Mesterhazy, A. & Nicholson, P. (2007). Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. *Theoretical and Applied Genetics*, *115*, 617-625.

Eckard, J. T., Gonzalez-Hernandez, J. L., Caffe, M., Berzonsky, W., Bockus, W. W., Marais, G. F., & Baenziger, P. S. (2015). Native Fusarium head blight resistance from winter wheat cultivars 'Lyman,''Overland,''Ernie,'and 'Freedom'mapped and pyramided onto 'Wesley'-Fhb1 backgrounds. *Molecular breeding*, *35*, 6-15.

Frohberg, R. C., Stack, R. W., & Mergoum, M. (2004). Registration of spring wheat germplasm ND2710 resistant to Fusarium head blight. *Crop science*, *44*, 1498-1500.

Gardiner, S. A., Boddu, J., Berthiller, F., Hametner, C., Stupar, R. M., Adam, G., & Muehlbauer, G. J. (2010). Transcriptome analysis of the barley–deoxynivalenol interaction: evidence for a role of glutathione in deoxynivalenol detoxification. *Molecular plant-microbe interactions*, *23*, 962-976.

Gervais, L., Dedryver, F., Morlais, J. Y., Bodusseau, V., Negre, S., Bilous, M., Groos, C. & Trottet, M. (2003). Mapping of quantitative trait loci for field resistance to Fusarium head blight in an European winter wheat. *Theoretical and Applied Genetics*, *106*, 961-970.

Goswami, R. S., & Kistler, H. C. (2004). Heading for disaster: Fusarium graminearum on cereal crops. *Molecular plant pathology*, *5*, 515-525.

Gosman, N., Bayles, R., Jennings, P., Kirby, J., & Nicholson, P. (2007). Evaluation and characterization of resistance to fusarium head blight caused by Fusarium culmorum in UK winter wheat cultivars. *Plant pathology*, *56*, 264-276.

Gupta, A., Lipps, P. E., Campbell, K. G., & Sneller, C. H. (2001, December). Identification of QTL associated with resistance to FHB in Ning 7840 and Freedom. In *Proceedings of the 2001 national Fusarium head blight forum. Michigan State University, East Lansing* (p. 180).

Häberle, J., Schmolke, M., Schweizer, G., Korzun, V., Ebmeyer, E., Zimmermann, G., & Hartl, L. (2007). Effects of two major Fusarium head blight resistance QTL verified in a winter wheat backcross population. *Crop science*, *47*, 1823-1831.

Han, Q., Chen, R., Yang, Y., Cui, X., Ge, F., Chen, C., & Liu, D. (2016). A glutathione S-transferase gene from Lilium regale Wilson confers transgenic tobacco resistance to Fusarium oxysporum. *Scientia Horticulturae*, *198*, 370-378.

Handa, H., Namiki, N., Xu, D., & Ban, T. (2008). Dissecting of the FHB resistance QTL on the short arm of wheat chromosome 2D using a comparative genomic approach: from QTL to candidate gene. *Molecular Breeding*, *22*, 71-84.

Harwood, W. A. (2011). Advances and remaining challenges in the transformation of barley and wheat. *Journal of experimental botany*, *63*, 1791-1798.

Henry, Y., Marcotte, J. L., & De Buyser, J. (1994). Chromosomal location of genes controlling short-term and long-term somatic embryogenesis in wheat revealed by immature embryo culture of aneuploid lines. *Theoretical and Applied Genetics*, *89*, 344-350.

Holzapfel, J., Voss, H. H., Miedaner, T., Korzun, V., Häberle, J., Schweizer, G., Mohler, V., Zimmermann, G. & Hartl, L. (2008). Inheritance of resistance to Fusarium head blight in three European winter wheat populations. *Theoretical and applied genetics*, *117*, 1119-1128.

Jia, H., Yu, J., Yi, D., Cheng, Y., Xu, W., Zhang, L., & Ma, Z. (2009). Chromosomal intervals responsible for tissue culture response of wheat immature embryos. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *97*, 159-165.

Jiang, G. L., Shi, J., & Ward, R. W. (2007a). QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. I. Resistance to fungal spread. *Theoretical and Applied Genetics*, *116*, 3-13.

Jiang, G. L., Dong, Y., Shi, J., & Ward, R. W. (2007b). QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. II. Resistance to deoxynivalenol accumulation and grain yield loss. *Theoretical and Applied Genetics*, *115*, 1043-1052.

Joehanes, R., & Nelson, J. C. (2008). QGene 4.0, an extensible Java QTL-analysis platform. *Bioinformatics*, *24*, 2788-2789.

Korosteleva, S. N., Smith, T. K., & Boermans, H. J. (2007). Effects of feedborne Fusarium mycotoxins on the performance, metabolism, and immunity of dairy cows. *Journal of dairy science*, *90*, 3867-3873.

Levene, H. (1960). Robust tests for equality of variances. *Contributions to probability and statistics*, *1*, 278-292.

Liu, S., Pumphrey, M., Gill, B., Trick, H., Zhang, J., Dolezel, J., Chalhoub, B. & Anderson, J. (2008). Toward positional cloning of Fhb1, a major QTL for Fusarium head blight resistance in wheat. *Cereal Research Communications*, *36*, 195-201.

Liu, S., Hall, M. D., Griffey, C. A., & McKendry, A. L. (2009). Meta-analysis of QTL associated with Fusarium head blight resistance in wheat. *Crop Science*, *49*, 1955-1968.

Lorieux, M. (2012). MapDisto: fast and efficient computation of genetic linkage maps. *Molecular Breeding*, *30*, 1231-1235.

Ma, J., Deng, M., Lv, S. Y., Yang, Q., Jiang, Q. T., Qi, P. F., Li, W., Chen, G.Y., Lan, X.J. & Wei, Y. M. (2016). Identification of QTLs associated with tissue culture response of mature wheat embryos. *SpringerPlus*, *5*, 1552-1562.

McCartney, C. A., Somers, D. J., Fedak, G., DePauw, R. M., Thomas, J., Fox, S. L., Humphreys, D.G., Lukow, O., Savard, M.E., McCallum, B.D. & Gilbert, J. (2007). The evaluation of FHB resistance QTLs introgressed into elite Canadian spring wheat germplasm. *Molecular Breeding*, *20*, 209-221.

McMullen, M., Jones, R., & Gallenberg, D. (1997). Scab of wheat and barley: a re-emerging disease of devastating impact. *Plant disease*, *81*, 1340-1348.

McMullen, M., & Stack, R. (2011). Fusarium head blight (scab) of small grains. NDSU Extension Service, 804(September), 6. http://doi.org/10.1094/PHI-I-2003-0612-01

Paillard, S., Schnurbusch, T., Tiwari, R., Messmer, M., Winzeler, M., Keller, B., & Schachermayr, G. (2004). QTL analysis of resistance to Fusarium head blight in Swiss winter wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics, 109, 323-332.

Parry, D. W., Jenkinson, P., & McLeod, L. (1995). Fusarium ear blight (scab) in small grain cereals—a review. *Plant pathology*, *44*, 207-238.

Pellegrineschi, A., Noguera, L. M., Skovmand, B., Brito, R. M., Velazquez, L., Salgado, M. M., Hernandez, R., Warburton, M. & Hoisington, D. (2002). Identification of highly transformable wheat genotypes for mass production of fertile transgenic plants. *Genome*, *45*, 421-430.

Pestka, J. J. (2010). Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. *Archives of toxicology*, *84*, 663-679.

Poppenberger, B., Berthiller, F., Lucyshyn, D., Sieberer, T., Schuhmacher, R., Krska, R., Kuchler, K., Glössl, J., Luschnig, C. & Adam, G. (2003). Detoxification of the Fusarium mycotoxin deoxynivalenol by a UDP-glucosyltransferase from Arabidopsis thaliana. *Journal of Biological Chemistry*, *278*, 47905-47914.

Puri, K. D., & Zhong, S. (2010). The 3ADON population of Fusarium graminearum found in North Dakota is more aggressive and produces a higher level of DON than the prevalent 15ADON population in spring wheat. *Phytopathology*, *100*, 1007-1014.

Rao, A. Q., Bakhsh, A., Kiani, S., Shahzad, K., Shahid, A. A., Husnain, T., & Riazuddin, S. (2009). The myth of plant transformation. *Biotechnology advances*, *27*, 753-763.

Rawat, N., Pumphrey, M. O., Liu, S., Zhang, X., Tiwari, V. K., Ando, K., Trick, H.N., Bockus, W.W., Akhunov, E., Anderson, J.A. & Gill, B. S. (2016). Wheat Fhb1 encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium head blight. *Nature genetics*, *48*, 1576-1580.

Reisch, B., & Bingham, E. T. (1980). The genetic control of bud formation from callus cultures of diploid alfalfa. *Plant Science Letters*, *20*, 71-77.

Röder, M. S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M. H., Leroy, P., & Ganal, M. W. (1998). A microsatellite map of wheat. *Genetics*, *149*, 2007-2023.

Ruckenbauer, P., Buerstmayr, H., & Lemmens, M. (2001). Present strategies in resistance breeding against scab (Fusarium spp.). In *Wheat in a Global Environment* (pp. 85-95). Springer Netherlands.

SAS Institute. (2011). *SAS/IML 9.3 user's guide*. Sas Institute.

Schmolke, M., Zimmermann, G., Buerstmayr, H., Schweizer, G., Miedaner, T., Korzun, V., Ebmeyer, E. & Hartl, L. (2005). Molecular mapping of Fusarium head blight resistance in the winter wheat population Dream/Lynx. *Theoretical and Applied Genetics*, *111*, 747-756.

Schroeder, H. W., & Christensen, J. J. (1963). Factors affecting resistance of wheat to scab caused by Gibberella zeae. *Phytopathology*, *53*, 831-838.

Schweiger, W., Steiner, B., Ametz, C., Siegwart, G., Wiesenberger, G., Berthiller, F., Lemmens, M., Jia, H., Adam, G., Muehlbauer, G.J. & Kreil, D. P. (2013). Transcriptomic characterization of two major Fusarium resistance quantitative trait loci (QTLs), Fhb1 and Qfhs. ifa-5A, identifies novel candidate genes. *Molecular plant pathology*, *14*, 772-785.

Schweiger, W., Steiner, B., Vautrin, S., Nussbaumer, T., Siegwart, G., Zamini, M., Jungreithmeier, F., Gratl, V., Lemmens, M., Mayer, K.F.X. & Berges, H. (2016). Suppressed recombination and unique candidate genes in the divergent haplotype encoding Fhb1. *Theoretical and Applied Genetics*, *129*, 1607-1623.

Stack, R. W., Elias, E. M., Fetch, J. M., Miller, J. D., & Joppa, L. R. (2002). Fusarium Head Blight Reaction of Langdon Durum-Chromosome Substitution Lines. *Crop science*, *42*, 637-642.

Steiner, B., Lemmens, M., Griesser, M., Scholz, U., Schondelmaier, J., & Buerstmayr, H. (2004). Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. *Theoretical and Applied Genetics*, *109*, 215-224.

Taguchi-Shiobara, F., Yamamoto, T., Yano, M., & Oka, S. (2006). Mapping QTLs that control the performance of rice tissue culture and evaluation of derived near-isogenic lines. *Theoretical and applied genetics*, *112*, 968-976.

Waldron, B. L., Moreno-Sevilla, B., Anderson, J. A., Stack, R. W., & Frohberg, R. C. (1999). RFLP mapping of QTL for Fusarium head blight resistance in wheat. *Crop Science*, *39*, 805-811.

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S., Milner, S.G., Cattivelli, L., Mastrangelo, A. M. Whan, A., Stephen, S., Barker, G., Wieseke, R., Plieske, J., International Wheat Genome Sequencing Consortium, Lillemo, M., Mather, D., Appels, R., Dolferus, R., Brown-Guedira, G., Korol, A., Akhunova, A. R., Feuillet, C., Salse, J., Morgante, M., Pozniak, C., Luo, M.-C., Dvorak, J., Morell, M., Dubcovsky, J., Ganal, M., Tuberosa, R., Lawley, C., Mikoulitch, I., Cavanagh, C., Edwards, K. J., Hayden, M. and Akhunov, E. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant biotechnology journal*, *12*, 787-796.

Yang, Z., Gilbert, J., Fedak, G., & Somers, D. J. (2005). Genetic characterization of QTL associated with resistance to Fusarium head blight in a doubled-haploid spring wheat population. *Genome*, *48*, 187-196.

Yin, G. X., Wang, Y. L., She, M. Y., Du, L. P., Xu, H. J., & YE, X. G. (2011). Establishment of a highly efficient regeneration system for the mature embryo culture of wheat. *Agricultural Sciences in China*, *10*, 9-17.

Yu, J. B., Bai, G. H., Zhou, W. C., Dong, Y. H., & Kolb, F. L. (2008). Quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population of Wangshuibai/Wheaton. *Phytopathology*, *98*, 87-94.

Zhou, W., Kolb, F. L., Bai, G., Shaner, G., & Domier, L. L. (2002). Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. *Genome*, *45*, 719-727.

Zhou, W., Kolb, F. L., Yu, J., Bai, G., Boze, L. K., & Domier, L. L. (2004). Molecular characterization of Fusarium head blight resistance in Wangshuibai with simple sequence repeat and amplified fragment length polymorphism markers. *Genome*, *47*, 1137-1143.

CHAPTER III. FINE MAPPING OF A NOVEL MAJOR QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN THE WHEAT LINE PI 277012

Abstract

The hexaploid wheat line PI 277012 exhibited a high level of Fusarium head blight (FHB) resistance in both greenhouse and field experiments. Previous QTL analysis of a population consisting of 130 doubled haploid (DH) lines from a cross between PI 277012 and 'Grandin' identified two major FHB resistance QTLs located on chromosome arms 5AS and 5AL, respectively. The 5AS QTL (*Qfhb.rwg-5A.1*) peaked at marker *Xbarc40* between markers *Xcfa2104* and *Xgwm617*, while the 5AL QTL (*Qfhb.rwg-5A.2*) peaked at marker *Xcfd39* between markers *Xwmc470* and *Xbarc48*. *Qfhb.rwg-5A.2* is different from those found in other known sources of FHB resistance. To fine map *Qfhb.rwg-5A.2* with more DNA markers, the DH population (GP-DH) was first genotyped using the wheat 9K SNP iSelect assay, and a total of 4317 polymorphic SNPs between PI 277012 and Grandin were mapped to the genetic linkage map previously constructed for chromosome 5A with SSR markers. Sequences of SNP markers within the peak of *Qfhb.rwg-5A.2* were used to search for syntenic region in *Brachypodium distacheon* (Bd) genome and a Bd region containing 2500 genes were identified. The Bd genes were used as queries to search for the wheat genome survey sequences and 139 contigs were identified for primer design. Ten CAPS markers were developed from the wheat contig sequences and mapped to the *Qfhb.rwg-5A.2* region with the GP-DH population. Additional DNA markers were developed from the wheat 90K SNP iSelect assay and reference genome sequences of Chinese Spring. Using the newly developed DNA markers and 947 recombinant inbred lines from the cross between PI 277012 and Grandin, *Qfhb.rwg-5A.2* was delimited in a 1.09-Mbp genomic region. This study also provides DNA markers tightly linked to *Qfhb.rwg-*

5A.2, which can be used for marker-assisted selection and facilitate the isolation and functional characterization of the underlying resistance gene.

Introduction

Fusarium head blight (FHB) is a widespread and devastating disease of wheat worldwide. It can be caused by several *Fusarium* species, but in North America the primary causal agent of FHB in wheat is *Fusarium graminearum* Schw. (=*Giberella zeae* Schw. and Petch) (Gilbert and Tekauz 2000; Bai and Shaner 2004). FHB infection is favored by humid and warm weather conditions during wheat anthesis and early stage of kernel development (Osborne and Stein 2007). The disease not only reduces yield, but also downgrade grain quality due to contamination of mycotoxins such as deoxynivalenol (DON) and zearalenone (ZEA) produced by the pathogen. These mycotoxins have significant impact on human and animal health (Bai and Shaner 1994; Parry et al. 1995).

Although applications of fungicides and cultural practices can mitigate the FHB problem to some extent, utilization of resistant wheat cultivars is generally considered as the most effective, economic and environmental friendly approach for management of the disease (McMullen et al. 2012). Several types of resistance to FHB have been recognized in wheat, including Type I for resistance to initial infection, Type II resistance to fungal spread within the spike, Type III resistance to DON accumulation, and Type IV resistance to kernel infection (Schroeder and Christensen 1963; Mesterhazy 1995). Type I resistance is evaluated by spray inoculation method, while Type II resistance is assessed by single spikelet injection method. FHB resistance is polygenic and the expression of resistance is highly influenced by the environment (Cuthbert et al. 2006). In the past years, over 200 quantitative trait loci (QTL) associated with FHB resistance types I-IV have been identified and mapped on all 21 wheat

chromosomes in various sources of wheat germplasm (Buerstmayr et al. 2009; Liu et al. 2009). Sumai 3 is the most well studied resistance source with two major QTL identified on 3BS and 6BS, which were designated as *Fhb1* and *Fhb2*, respectively (Waldron et al. 1999; Anderson et al. 2001; Cuthbert et al. 2006; 2007). *Fhb1* is the most widely used gene for FHB resistance in wheat breeding programs due to its large and stable effect across different genetic backgrounds in various environmental conditions (Anderson et al. 2001; Bai et al. 1999; Buerstmayr et al. 2003; Chen et al. 2006; Cuthbert et al. 2006; Somers et al. 2003; Yang et al. 2005; Yu et al. 2008). Molecular cloning indicated that *Fhb1* is a gene encoding chimeric lectin with agglutinin domains and a pore-forming toxin-like domain (Rawat et al. 2016).

To develop wheat cultivars with sustainable and durable resistance to FHB, identification of novel sources of FHB resistance and introgression of the resistance QTL into adapted wheat varieties are essential for wheat breeding programs. In the past years, great efforts have been devoted to screen a large number of tetraploid (*T. turgidum* L.) accessions in the Northern Crop Science Laboratory, USDA-ARS, Fargo, ND (Xu et al. 2007; Oliver et al. 2008; Chu et al. 2010) in order to identify FHB resistance sources for durum wheat. During this screen process, a hexaploid spring wheat line (PI 277012), which was mistakenly classified as emmer wheat [*T. turgidum* subsp. *dicoccum* (Schrank ex Schübler) Thell (http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1206987), consistently exhibited a high level of FHB resistance comparable to Sumai 3 in both greenhouse and field environments (Xu et al. 2010; Chu et al. 2010; 2011). Further QTL analysis using a doubled haploid (DH) mapping population developed from the cross between PI 277012 and 'Grandin' (susceptible to FHB) identified two QTL for FHB resistance derived from PI 277012. These two QTL were designated as *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2*, and mapped to 5AS and 5AL, respectively (Chu et al. 2011). *Qfhb.rwg-5A.1*

explained up to 20% of the phenotypic variation and peaked at marker Xbarc40 on 5AS in a 40.8 cM interval flanked by markers *Xcfa2104* and *Xgwm617* (Chu et al. 2011). *Qfhb.rwg-5A.2* explained up to 32% of the phenotypic variation and peaked at marker *Xcfd39* in a 40.4 cM interval flanked by markers *Xwmc470* and *Xbarc48* (Chu et al. 2011).

For marker-assisted introgression of new FHB resistance sources into adapted durum and hard red spring wheat cultivars, DNA markers tightly associated with the FHB resistance QTL should be developed. Therefore, the objectives of the study were to saturate the *Qfhb.rwg-5A.2* region with more DNA markers using a large mapping population consisting of 947 recombinant inbred lines (RILs) derived from a cross between PI 277012 and Grandin, and to develop user friendly DNA markers for marker-assisted selection (MAS) of the FHB resistance in PI 277012.

Materials and Methods

Plant Materials

Two mapping populations derived from the cross between PI 277012 and Grandin were used in this study. One population (designated as GP-DH) consisted of 130 doubled haploid (DH) lines and the other population (designated as GP-RIL) contained 947 recombinant inbred lines (RILs). The GP-DH population was previously developed and used to identify *Qfhb.rwg-5A.1* and *Qfhb.rwg-5A.2* for FHB resistance in PI 277012 (Chu et al. 2011). The GP-RIL population was developed by the single seed descent method. In addition, the parents (Grandin and PI 277012), and genotypes Wheaton (FHB-susceptible) and ND2710 (FHB-resistant) were also included as controls in the FHB inoculation experiments.

Evaluation of FHB Resistance and Statistical Analysis

The RILs, parents and controls were evaluated for Type II resistance using the single-spikelet inoculation method (Stack et al. 2002) in seven experiments, including five experiments

in greenhouse and two experiments in field FHB nurseries. Greenhouse evaluations were conducted in the spring season of 2014 (14GH), the spring season of 2015 (15GH), and the spring season of 2016 (16GH1), and in the winter seasons of 2016 (16GH2 and 16GH3). In each growing season, plants from each line or genotype were grown in clay pots with three plants per pot. The greenhouse was supplemented with artificial light provided by 600 watt High-Pressure Sodium Lamps (P.L. Light Systems Inc, Beamsville, Canada) for a 14 h photoperiod and the temperature was maintained between 22 and 25 °C. To prepare inoculum, four pathogenic *F. graminearum* strains isolated from North Dakota (Puri et al. 2010) were cultured on mung bean agar media. The spores (macroconidia) were collected and quantified using a hemocytometer under a light microscope. A spore suspension at a concentration of 100,000 spore mL^{-1} was prepared by mixing spores equally from the four *F. graminearum* strains and used as inoculum. Inoculations were performed using the single-spikelet inoculation method described by Stack et al. (2002), by injecting 10 µl of spore suspension into the central spikelet of a spike at anthesis using a syringe. In each pot, eight to ten spikes at similar developmental stage were inoculated. Inoculated plants were placed in a greenhouse room with misting system (1 minute misting in every half hour) to facilitate disease development. After 48 hours of incubation, the plants were returned to the original greenhouse benches at 22 $\pm$ 5 °C with 12 h supplemental light.

Field evaluations were carried out in the FHB nursery located in Fargo, North Dakota in 2014 and 2016 (14FAR and 16FAR). Each wheat line/genotype was planted in a hill plot with 8-10 seeds. The inoculation method used in the field was the same as in greenhouse experiments. At anthesis, at least eight spikes at similar developmental stage in a hill were inoculated. The nursery was misted for 12 hours in a setting of 5 min misting in 15 min intervals from 4:00 p.m. to 4:00 a.m., until 14 days after anthesis of the latest wheat lines.

For both greenhouse and field experiments, disease ratings were conducted at 21 days post inoculation. The percentage of infected spikelets (PIS) in each spike was estimated based on a 0-100% scale described by Stack and McMullen (2011), and the mean FHB severity for each line was calculated by averaging PISs of all spikes evaluated.

The Shapiro-Wilk normality test was performed for the distribution of disease severity in the RIL population using PROC UNIVARIATE (SAS Institute 2011). Homogeneity of disease severity variances among all experiments was tested by the Levene's test (Levene 1960) under the general linear model (GLM) procedure using SAS program version 9.3 (SAS Institute 2011). Correlation coefficients between phenotypic experiments were calculated using the PROC CORR procedure (SAS Institute 2011).

DNA Extraction and Marker Development

Genomic DNA were extracted from seedling leaves of the GP-DH and GP-RIL populations with TissueLyser (Qiagen) using a simplified SDS-based procedure modified from Ahmed et al. (2009). DNA extraction buffer consists of 20% SDS, 1M Tris-HCl (pH 8.0), 0.5M EDTA (pH 8.0), and 5M NaCl. The DNA concentration was diluted to ~50ng/µl and used for the wheat SNP iSelect genotyping assays (Cavanagh et al. 2013; Wang et al. 2014). The SNP genotyping data for the GP-DH population were provided by Steven Xu at USDA-ARS. The SNP markers were integrated into the genetic map with *Qfhb.rwg-5A.2* previously constructed using SSR markers (Chu et al. 2011). To saturate the *Qfhb.rwg-5A.2* region with more markers, a comparative genomics approach was used. First, the sequences of five SNPs in the QTL region were used as queries to perform BLASTn search against the *Brachypodium* database (http://www.plantgdb.org/BdGDB) in order to identify the syntenic region in the *B. distachyon* genome. Homologous sequences of *B. distachyon* were selected using an expected value of $1E^{-10}$

and identity ≥80% as cutoff points. The genes of *B. distachyon* located in the collinear region were used as queries to search for the homologous genes in the wheat survey sequence (https://urgi.versailles.inra.fr/blast/) using BLASTn. Wheat contigs that are homologous to the *B. distachyon* genes were retrieved and used for designing primer pairs using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/). These primer pairs were used in PCR to amplify DNA sequences from the two parental genotypes (Grandin and PI277012).

PCR was performed in an Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, Waltham, MA) for each DNA sample in a volume of 50µl containing 30 ng of the template DNA, 5 pmol of each primer, 200 µM of each dNTP, 1× reaction buffer (20mM Tris-Cl, 2.0mM $MgSO_4$, 50mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, pH 8.8), and 2.5U of *Taq* DNA polymerase (New England Biolabs, Ipswich, MA). The PCR conditions were: denaturation at 95 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min and a final extension for 5 min at 72 °C. PCR products were purified using Wizard® SV Gel and PCR Clean-Up Kit (Promega, Madison, WI) and then sequenced by Genscript (Piscataway, NJ). Sequences from the two parental wheat genotypes were aligned against each other to identify SNPs (http://multalin.toulouse.inra.fr/multalin/). In addition, 31 RILs including 10 susceptible and 21 resistant lines from the GP-RIL population, were selected and genotyped along with the parents using the 90K iSelect assay, and 648 SNPs between the two parental genotypes were identified. The SNPs on 5A chromosome between the parental genotypes were then transformed into either Cleaved Amplified Polymorphic Sequence (CAPS) markers or STARP markers (Long et al. 2017).

To develop CAPS markers, NEBcutter v.2.0 tool (http://www.tools.neb.com/NEBcutter2) were used to detect restriction enzyme sites at SNP positions of the PCR amplified sequences.

Restriction enzyme digestion was carried out in a total of 25 ul volume including 1 U of the restriction enzyme (New England Biolabs, Ipswich, MA) and appropriate buffer according to the manufacturer's manual. CAPS marker for the *Q* gene (Simons et al. 2006) was also developed according to the SNPs between the sequences of *Q* and *q* alleles. STARP markers were developed using the protocol described by Long et al. (2017).

To further saturate the QTL with more markers, sequences of markers closely linked to *Qfhb.rwg-5A.2* were used as queries to search against the Chinese Spring wheat genome sequence (Wheat_WGA_v0.4_scaffolds) (https://urgi.versailles.inra.fr/blast_iwgsc/blast.php) using BLASTn. A large wheat scaffold (scaffold6791) from chromosome 5AL was retrieved and used to develop CAPS and STARP markers as described above. These markers were used to genotype the whole GP-RIL population and construct a genetic linkage map.

Genetic Map Construction and QTL Analysis

Linkage analysis was performed using MapDisto (Lorieux 2012) with the LOD threshold of 3.0 and theta value of 0.3. Recombination of fraction was converted into map distance using the Kosambi function. The genetic linkage maps were generated using the software MapChart version 2.1 (Voorrips 2002).

According to the result of homogeneous test, the phenotypic data was grouped into three datasets: the mean of 14GH and 16GH2 (designated as GH-Mean1), the mean of 15GH, 16GH1, and 16GH3 (designated as GH-Mean2), the mean of 14FAR and 16FAR (designated as Field-Mean). Composite interval mapping (CIM, Zeng 1994) was used to detect QTL with the software QGene v.4.3.10 (Joehanes and Nelson 2008). The LOD threshold of 3.0 was set as significant for the 0.05 level of probability by a 1,000-iteration permutation test. The percentage

of phenotypic variance explained by a QTL and its additive effect were calculated using single-marker regression (SMR, Jiang and Zeng 1997).

Results

FHB Reaction in the GP-RIL Population

FHB disease severities in the GP-RIL population varied among different experiments and transgressive segregation was observed in all experiments (Figure 3.1). Among all the experiments, Grandin was very susceptible to FHB with a mean disease severity larger than 50%, whereas PI 277012 exhibited resistance to FHB with a mean disease severity of 10% ranging from 7 to 30% (Figure 3.1).

The normality test indicated that the GP-RIL population deviated from a normal distribution for disease severity. Thus, Levene's test (Levene 1960) was used to test homogeneity of disease severity variances across all experiments. The results showed that the variances were homogeneous between 14GH and 16GH2 ($P = 0.09$, $df = 1$), among 15GH, 16GH1, and 16GH3 ($P = 0.40$, $df = 2$), and between the two field experiments (14FAR and 16FAR) ($P = 0.50$, $df = 1$). Low to moderate correlation efficiencies were observed between measured FHB traits among environments (Table 3.1).

Table 3.1. Correlation coefficients among Fusarium head blight (FHB) traits from greenhouse and field environments of the GP population

	14GH	15GH	16GH1	16GH2	16GH3	14FAR	16FAR
14GH	-						
15GH	0.44***	-					
16GH1	0.55***	0.54***	-				
16GH2	0.50***	0.55***	0.68***	-			
16GH3	0.50***	0.54***	0.63***	0.62***	-		
14FAR	0.53***	0.42***	0.59***	0.51***	0.49***	-	
16FAR	0.30***	0.19***	0.38***	0.33***	0.30***	0.35***	-

Note: *, **, *** significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. GH, greenhouse; ns, not significant

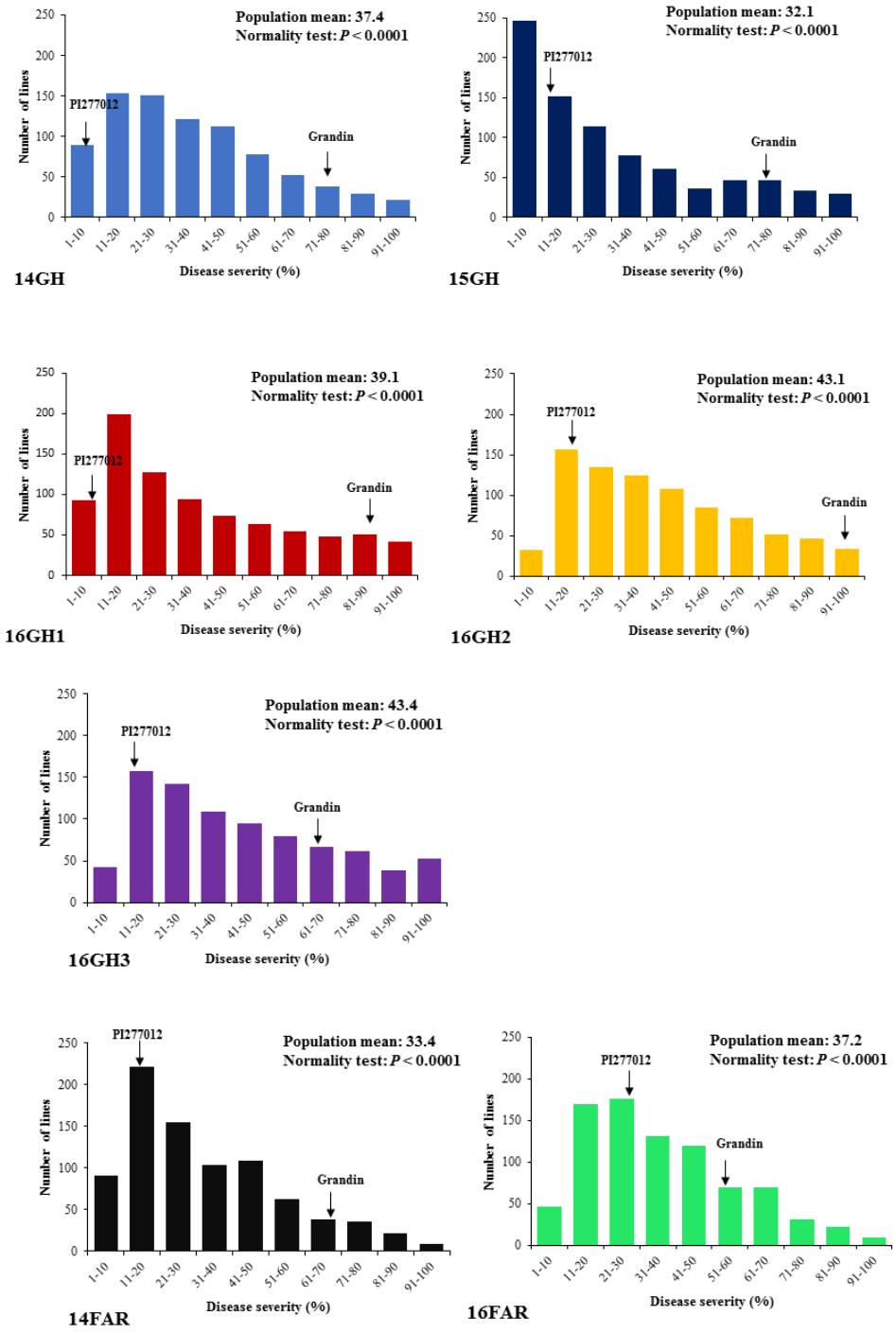

Figure 3.1. Distributions of 947 recombinant inbred lines (RILs) derived from the cross of Grandin × PI277012 for Fusarium head blight (FHB) disease severity in seven experiments. The experiments performed in the greenhouse in the spring season of 2014 and 2015, the spring season of 2016 and in the winter season of 2016 were designated as 14GH, 15GH, 16GH1, 16GH2, and 16GH3, respectively, while the experiments performed in the field FHB nursery at Fargo, North Dakota in 2014 and 2016 were designated as 14FAR and 16FAR, respectively. The arrows indicate the disease severities of parents. Normality test was performed using PROC UNIVARIATE procedure and Shapiro-Wilk test (SAS Institute 2011).

Development of Markers from Wheat 9K and 90K SNP iSelect Assays

With the data from the 9K iSelect assay, 4317 polymorphic SNPs were identified in the GP-DH population. Among them, 224 SNP markers were mapped to the genetic linkage map of 5A originally developed by Chu et al. (2011). Genotyping 31 RILs from the GP-RIL population using the wheat 90K iSelect assay identified 648 polymorphic SNPs, with 126 of them mapped on the chromosome 5AL based on the 90K consensus map (Wang et al. 2014). Thirteen of the SNPs mapped around *Qfhb.rwg-5A.2* were first converted to STARP markers, and then added to the linkage map developed using the GP-DH population (Figure 3.2). According to their positions on the genetic map, nine of them were selected to genotype the RIL population (Table 3.2).

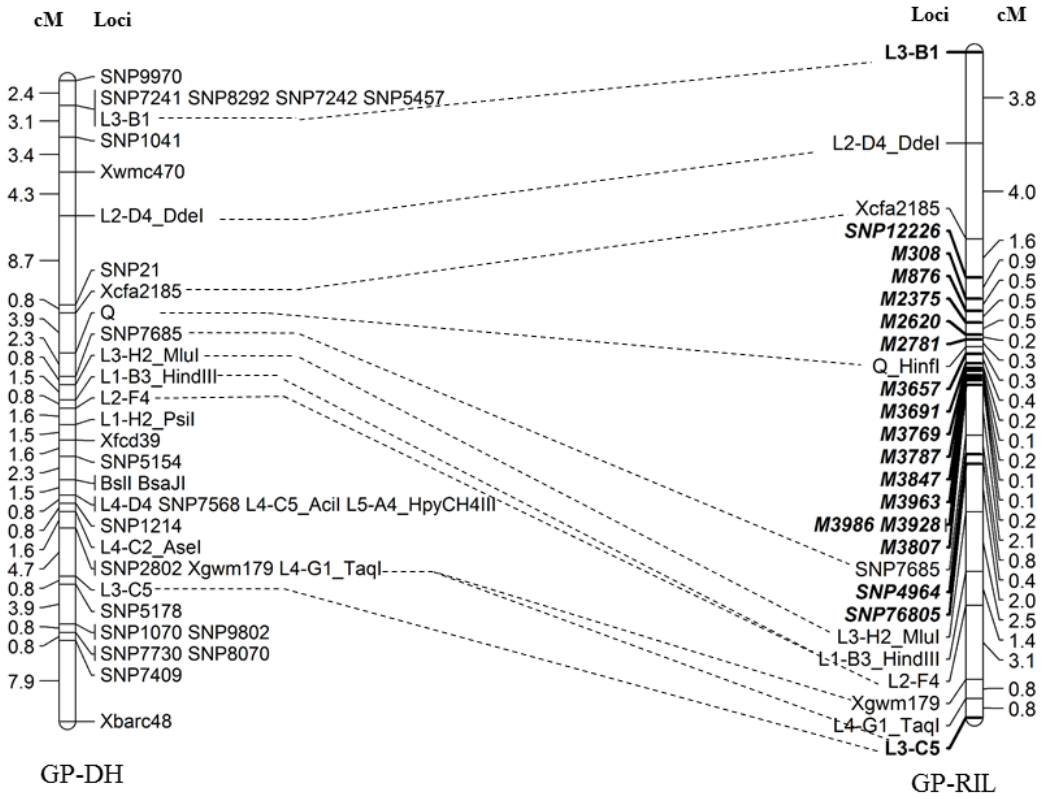

Figure 3.2. Linkage maps for chromosome 5AL constructed using the GP-DH and GP-RIL populations, respectively. The dotted lines indicate the markers used in phenotyping both populations.

Table 3.2. Markers used to genotype 947 RILs

Markers	Sources	Types	Digestion	Primer F	Primer R
L3-B1	Bd	Codominant	-	GTCGAGGTCCTGCTGTCTT	AGTTGCTGCTTCATCACGTC
L2-D4_DdeI	Bd	CAPS	DdeI	TGTGTCCAGACCAAACCAGT	TGTGACACGCCCTAGAAACT
SNP21	9K	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***CTTTAACACTTGATTTTATAA AS-2: ***GACGCAAGTGAGCAGTATGAC***CTTTAACACTTGATTTTGCAG	CACTTGATGAATTTTTTCCTG
Xcfa2185	SSR	SSR	-	TTCTTCAGTTGTTTTGGGGG	TTTGGTCGACAAGCAAATCA
SNP12226	90K	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***AGGGTTTTGTAGCTCCCGAA AS-2: ***GACGCAAGTGAGCAGTATGAC***AGGGTTTTGTAGCTCCTAAC	CCTTTTGTCACGCATTTAGC
M308	scaffold	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***CCGGAAGATAAGCCCCCGCA AS-2: ***GACGCAAGTGAGCAGTATGAC***CCGGAAGATAAGCCCCTACC	CGCCTCCTCTATCTTCATCTTCA
M876	scaffold	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***CGTTGTCTGGTCAGCCACCAA AS-2: ***GACGCAAGTGAGCAGTATGAC***CGTTGTCTGGTCAGCCAATAG	TCATGATCTCCATGCCATCC
M2375	scaffold	CAPS	RsaI	CATGAACCCACAATTCATCAGT	TACGAGAAGGTCTTGGAGAAGC
M2620	scaffold	CAPS	DdeI	TACGATGGTGTGCTTCATGATAA	GTCACTGGACAGGATCACTACAAG
M2781	scaffold	CAPS	PciI	GACCGAAACTCCTCAAAGACTG	ATGGACTGGTCTGAATTGGACT
Q	SSR	CAPS	HinfI	CACGCCTGCTCTCCTAAAAC	ATGGCGGACTGCTGTAAAAC
M3657	scaffold	CAPS	SacII	GGGAGATCGTTTCATCTGTGA	CTTTCTGCATCCAAAGACAGC
M3691	scaffold	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***CTTCCGCTAAGTTGGGACCT AS-2: ***GACGCAAGTGAGCAGTATGAC***CTTCCGCTAAGTTGGGCTCG	TCAAACCGGGACTAATGTGTCT
M3769	scaffold	CAPS	SnaI	GAAACTCCCCCTCAACACTC	TAGAACATGCCAGACCAAGGAT
M3787	scaffold	CAPS	AlwNI	ATCTCTTGTGGTGGCTCTTGAT	TCTTCTGGTATCGTGAATTGGA
M3847	scaffold	CAPS	DraIII	GACGCGGCTATGATGATTACTA	GGCAATGCGAATCATTGTAAC
M3928	scaffold	CAPS	BstUI	CGACAAGTCCCTTTAATCGTTC	CTTCTTTGGGAGTTGTCCTTTG
M3963	scaffold	CAPS	DdeI	ACCCGCACGTTACTGTAGAAAT	CCGAGGGTATGATCAGGTTTAC
M3986	scaffold	CAPS	RsaI, PsiI	CCGTACATACAGTACCGCGTAA	CGAATATGATCGGTGTATGTGG
M3807	scaffold	CAPS	XbaI	CTATAAGATGGCGGGAAACAGT	GAGGATGACAAACCCTGAACTC
SNP1942	9K	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***TATGCGTAAGGCACCTTCTA AS-2: ***GACGCAAGTGAGCAGTATGAC***TATGCGTAAGGCACCTCTTG	AGCATTCCTGATGACCAAATC

Table 3.2. Markers used to genotype 947 RILs (continued)

Markers	Sources	Types	Digestion	Primer F	Primer R
SNP7685	90K	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***CAGTAGAGGATCTAGTCCCCCA AS-2: ***GACGCAAGTGAGCAGTATGAC***CAGTAGAGGATCTAGTCCATCG	CAAATGGTAAGATCACTGATG
SNP4964	90K	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***CACAGGCAGCCAAAAAAACTG AS-2: ***GACGCAAGTGAGCAGTATGAC***CACAGGCAGCCAAAAAAACATA	TTGGGTCAACAAGAGTTCAAGG
SNP76805	90K	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***CGACAGGACAGACTCAAGCCAT AS-2: ***GACGCAAGTGAGCAGTATGAC***CGACAGGACAGACTCAAGTAAC	GTCCAGTGCATGTTTACACC
L3-H2_MluI	Bd	CAPS	MluI	GGAAGGTGTCGGGGATGTAC	GCATCAGTTTCTACCCCTGC
L1-B3_HindIII	Bd	CAPS	HindIII	CCCGAGTTACCAGTTAGCGA	CTCCCGTTGAAACCAAAGCA
L3-C5	Bd	Codominant	-	AATACGCACGTTGGCTGATG	GGACCAGCAAGAGACCCTAA
L5-F2_BslI	Bd	CAPS	BslI	TCTTCGGTAGTTTCCTCGCC	CGAAATCACCTGTGCTGCAT
L4-C2_AseI	Bd	CAPS	AseI	CCCTCCTCTCACCCAGAAAT	TGTGCAAATAGTCCATAGCTGC
SNP1214	9K	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***TCTCTGCCGACATCTCAAAT AS-2: ***GACGCAAGTGAGCAGTATGAC***TCTCTGCCGACATCTCCGAC	AGAGAGATGATTGCGACGAC
Xgwm179	SSR	SSR	-	AAGTTGAGTTGATGCGGGAG	CCATGACCAGCATCCACTC
L2-F4	Bd	Dominant	-	AAACATCCGTGGTGCAACTC	ATCCCGGTCCTGATGATGC
L4-G1_TaqI	Bd	CAPS	TaqI	AAGGCTGGTCATAGTGGGAG	CATTCCGGAAAGCACCACAA
SNP5178	90K	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***GTCTGCGTCACCGGCGCCCCT AS-2: ***GACGCAAGTGAGCAGTATGAC***GTCTGCGTCACCGGCGCTTCC	TGTGGGCACGTGGGAGGATAG
SNP8070	90K	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***GGTGTGCTGCAACTGCAGAAT AS-2: ***GACGCAAGTGAGCAGTATGAC***GGTGTGCTGCAACTGCAAGAC	CACAACAACAAACGAACGGTC
SNP7730	90K	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***TATAATGCATTGTTTCCACCT AS-2: ***GACGCAAGTGAGCAGTATGAC***TATAATGCATTGTTTCCCACC	AGTGACGCAATCGTCTTTTCG
SNP61150	90K	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***GGGTGACTCCATCTTCCTCA AS-2: ***GACGCAAGTGAGCAGTATGAC***GGGTGACTCCATCTTCTCCG	TCGCTTTGAGAACAATGCTGC

Table 3.2. Markers used to genotype 947 RILs (continued)

Markers	Sources	Types	Digestion	Primer F	Primer R
SNP79318	90K	STARP	-	AS-1: ***GCAACAGGAACCAGCTATGAC***GAACAACATACCATCATTAT AS-2: ***GACGCAAGTGAGCAGTATGAC***GAACAACATACCATCACCAG	GTCGGTAGTGAGAGCATTGG

Development of Markers through Comparative Genomics of Wheat with *B. distachyon*

To develop more markers for *Qfhb.rwg-5A.2*, sequences of five SNPs (*SNP21*, *SNP5154*, *SNP7568*, *SNP1214*, and *SNP2802*) were used to identify their homologous sequences in *B. distachyon*. The result indicated a synteny existed between the wheat chromosome 5AL region and a 62.4 Mb region (*Bradi1g13850 – Bradi1g76480*) of *B. distachyon* chromosome 1. The collinearity region in *B. distachyon* contains 2500 genes. All the genes in the collinear region were used as queries to search for orthologous wheat survey sequences on the IWGSC website (https://urgi.versailles.inra.fr/blast_iwgsc/blast.php) and 139 wheat sequence contigs were identified. From these contigs, 223 primer pairs were designed and used to amplify DNA sequences from Grandin and PI 277012. DNA fragments amplified from 144 primers in these two parental genotypes were sequenced, and sequences were aligned against each other to identify SNPs. Ten CAPS markers were converted from the SNPs between two parental genotypes. Two dominant and two co-dominant markers were also identified. These fourteen markers were added to the linkage map developed using the GP-DH population (Figure 3.2).

Marker Saturation of the *Qfhb.rwg-5A.2* interval and QTL Analysis

Nine SNP markers from the 90k iSelect assay, 9 markers developed from comparative genomic approach, together with 3 SSR and 3 SNP markers from the 9K iSelect assay, were used to genotype the 947 RILs derived from the cross between Grandin and PI 277012 (Table 3.2). In addition, a 5.0 Mb scaffold (scaffold 6791) sequence was identified from the reference genome sequence of Chinese Spring by BLASTn search with sequences of the markers (*L3-H2_MluI*, *L1-B3_HindIII*, *L2-F4*) mapped to the *Qfhb.rwg-5A.2* region. From scaffold 6791, three STARP (*M308*, *M876*, and *M3691*) and 11 CAPS markers were developed. A fine genetic map of 5AL was constructed, which consisted of 38 markers, with the GP-RIL population (Figure 3.3).

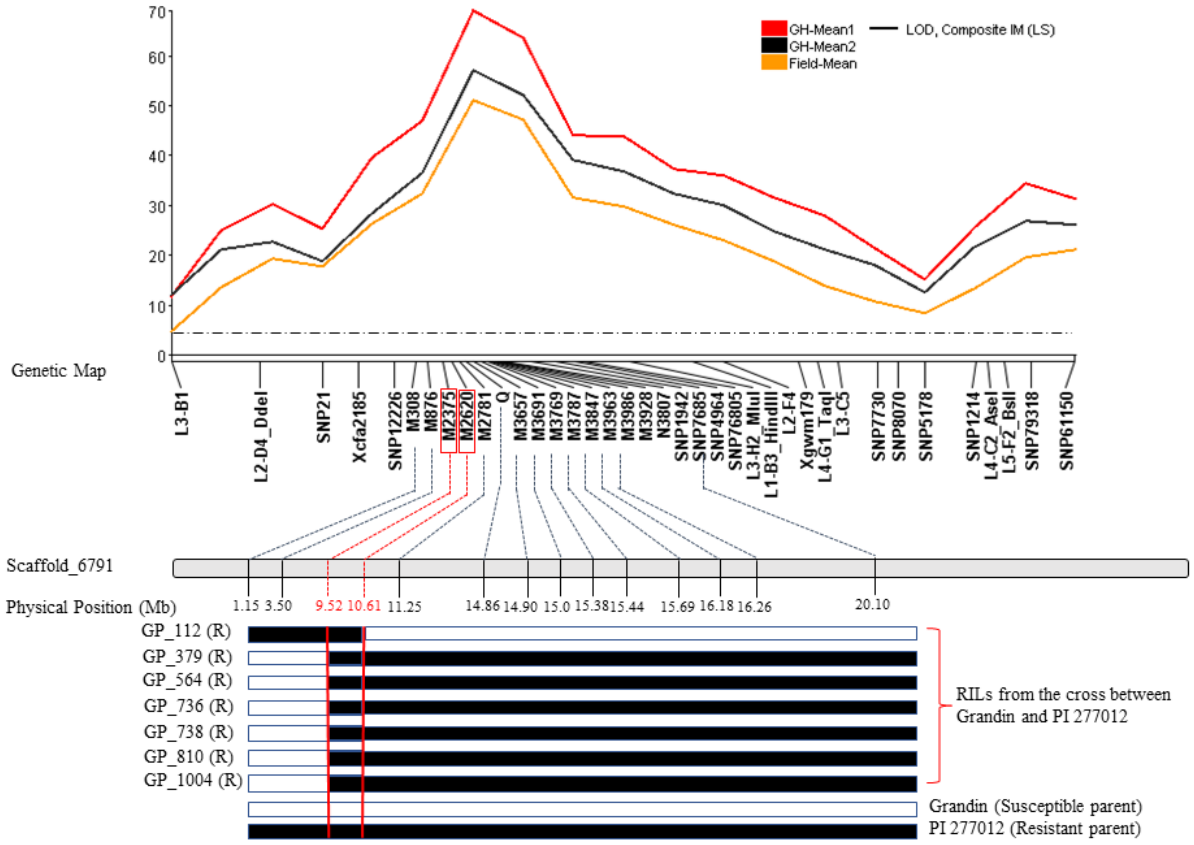

Figure 3.3. Linkage map for chromosome 5AL harboring the QTL *Qfhb.rwg-5A.2*, physical position of markers in the 25 Mb scaffold 6791 of Chinese Spring, and graphical illustration of the genotype types of FHB-resistant (R) recombinant inbred lines (RIL) at marker loci around the QTL region. The black and open rectangles represent genomic regions from PI 277012 and Grandin, respectively. The black dotted lines indicate the physical positions of DNA markers in scaffold 6791. The red dotted lines define the interval harboring *Qfhb.rwg-5A.2*.

Table 3.3. Single marker regression in the GP recombinant inbred line population associated with FHB resistance

		Field-Mean			GH-Mean2			GH-Mean1		
Locus name	Position	Add effect	LOD	R^2	Add effect	LOD	R^2	Add effect	LOD	R^2
L3-B1	0	2.655	4.787	0.023	5.275	11.983	0.057	4.86	11.842	0.056
L2-D4_DdeI	3.7	5.119	18.202	0.085	7.077	21.863	0.101	7.482	28.953	0.131
SNP21	6.3	4.859	16.513	0.077	6.262	17.115	0.08	6.677	22.99	0.106
Xcfa2185	7.8	5.823	24.108	0.111	7.645	26.019	0.119	8.338	37	0.165
SNP12226	9.3	6.525	30.905	0.14	8.478	32.65	0.147	8.666	40.477	0.179
M308	10.2	6.479	30.438	0.138	8.748	34.95	0.156	9.125	45.399	0.198
M876	10.7	6.904	34.85	0.156	9.47	41.505	0.183	9.642	51.273	0.221
M2375	11.3	7.518	41.899	0.184	9.932	46.006	0.2	10.272	59.084	0.25
M2620	11.7	8.038	48.523	0.21	10.485	51.835	0.223	10.675	64.449	0.269
M2781	12	8.222	51.083	0.22	10.928	57.003	0.242	10.987	69.01	0.285
Q	12.3	8.193	50.559	0.218	10.622	53.288	0.228	10.668	64.209	0.268
M3657	12.6	8.059	48.875	0.212	10.52	52.315	0.225	10.666	64.434	0.269
M3691	12.9	7.908	46.948	0.204	10.495	52.146	0.224	10.673	64.67	0.27
M3769	13.2	8.243	51.431	0.221	10.609	53.322	0.228	10.817	66.582	0.277
M3787	13.3	8.176	50.527	0.218	10.559	52.788	0.226	10.741	65.531	0.273
M3847	13.4	7.883	46.549	0.203	10.452	51.594	0.222	10.617	63.791	0.267
M3963	13.5	8.076	49.143	0.213	10.524	52.395	0.225	10.756	65.748	0.274
M3986	13.6	7.932	47.259	0.205	10.363	50.669	0.218	10.564	63.134	0.264
M3928	13.7	8.059	48.908	0.212	10.505	52.175	0.224	10.743	65.563	0.273
M3807	13.8	8.015	48.207	0.209	10.509	52.114	0.224	10.636	63.914	0.267
SNP1942	15	6.544	30.966	0.14	9.2	38.862	0.172	9.141	45.373	0.198
SNP7685	16	6.61	31.563	0.142	9.222	38.954	0.173	9.032	44.05	0.193
SNP4964	16.8	6.535	30.885	0.139	9.019	37.203	0.165	9.149	45.477	0.198
SNP76805	17.2	6.402	29.418	0.133	8.968	36.589	0.163	9.093	44.657	0.195
L3-H2_MluI	19.1	5.612	22.29	0.103	7.806	27.185	0.124	7.664	30.788	0.139
L1-B3_HindIII	21.7	5.726	23.139	0.106	8.225	30.254	0.137	8.307	36.478	0.163
L2-F4	23	4.952	17.241	0.08	6.858	20.784	0.096	7.041	25.827	0.118
Xgwm179	26.1	4.334	12.952	0.061	6.948	21.156	0.098	7.318	27.777	0.126
L4-G1_TaqI	26.9	4.21	12.278	0.058	6.842	20.616	0.095	7.062	25.904	0.118
L3-C5	27.8	3.973	10.903	0.052	6.488	18.455	0.086	6.53	21.956	0.101
SNP7730	29.4	3.076	6.456	0.031	4.718	9.537	0.045	4.739	11.251	0.053
SNP8070	30.3	3.52	8.525	0.041	5.534	13.283	0.063	5.584	15.845	0.074
SNP5178	31.4	3.894	10.478	0.05	6.393	17.916	0.083	6.336	20.634	0.095
SNP1214	33.5	4.831	16.302	0.076	7.073	22.074	0.102	7.39	28.505	0.129
L4-C2_AseI	34	5.277	19.644	0.091	7.73	26.716	0.122	8.051	34.371	0.154
L5-F2_BslI	34.7	5.642	22.592	0.104	8.077	29.323	0.133	8.344	37.132	0.165
SNP79318	35.8	5.257	19.445	0.09	7.356	23.986	0.11	7.41	28.663	0.13
SNP61150	37.6	5.967	25.501	0.117	7.999	28.776	0.131	8.081	34.691	0.155

Based on the result of homogeneity test, phenotypic data collected from five greenhouse experiments and three field experiments were divided into three datasets: GH-Mean1 representing for the mean of 14GH and 16GH2, GH-Mean2 for the mean of 15GH, 16GH1, and 16GH3, and Field-Mean for the mean of 14FAR and 16FAR. QTL analysis with these three datasets detected a significant QTL on chromosome 5AL (Figure 3.3). All markers on the linkage map were associated with FHB resistance, and each of them explained more than 5% of phenotypic variation (Table 3.3). The single-marker analysis and composite interval mapping indicated that the QTL peaked at marker M2781, explaining 28.5, 24.2, and 22% of phenotypic variation in GH-Mean1, and GH-Mean2, and Field-Mean, respectively (Table 3.3, Figure 3.3). Marker density in this region is high, with 4.2 markers per cM (Table 3.3). Analysis of phenotype data and genotypes of RILs at marker loci in the QTL region placed *Qfhb.rwg-5A.2* in the interval between markers *M2375* and *M2620* (Figure 3.3).

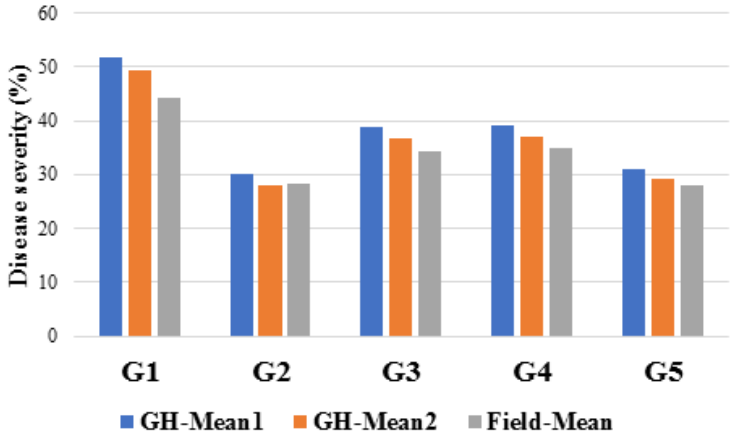

Figure 3.4. Effects of QTL on FHB severity of RILs. To select markers used for MAS, phenotypic data (the mean of greenhouse experiments 14GH and 16GH2 was designated as GH-Mean1, the mean of greenhouse experiments 15GH, 16GH1, and 16GH3 was designated as GH-Mean2, the mean of field experiments designated as Field-Mean) was classified into five groups according to the genotypes of RILs at marker loci *M2375*, *M2620*, and *M2781*. Group 1 (G1) contained RILs with Grandin alleles at all three marker loci, group 2 (G2) contained RILs with PI 277012 allele at marker locus *M2375*, group 3 (G3) contained RILs with PI 277012 allele at marker locus *M2620*, group 4 (G4) contained RILs with PI 277012 allele at marker locus *M2781*, while group 5 (G5) contained RILs with PI 277012 alleles at all three marker loci.

CAPS Markers for Marker-assisted Selection of *Qfhb.rwg-5A.2*

To select markers used for MAS, we analyzed the phenotyping data and genotypes of RILs at marker loci *M2375*, *M2620*, and *M2781*. Phenotypic data was classified into 5 groups: group 1 (G1) contained RILs with Grandin alleles at all three marker loci, group 2 (G2) contained RILs with PI 277012 allele at marker locus *M2375*, group 3 (G3) contained RILs with PI 277012 allele at marker locus *M2620*, group 4 (G4) contained RILs with PI 277012 allele at marker locus *M2781*, while group 5 (G5) contained RILs with PI 277012 allele at all three marker loci. The result indicates that RILs with PI 277012 alleles at the three marker loci had the lowest disease severity (less than 30%), while RILs with the corresponding Grandin alleles had the highest disease severity (Figure 3.4).

Discussion

In this study, we developed more markers to saturate the 5AL region harboring *Qfhb.rwg-5A.2* for FHB resistance in PI 277012 and identified markers that are closely linked to this FHB locus. By genotyping and phenotyping a large population consisting of 947 RILs for FHB reaction under both greenhouse and field environments, we successfully mapped *Qfhb.rwg-5A.2* to an interval of 0.4 cM or 1.0 Mbp. The information will not only facilitate the quick incorporation of this QTL into wheat cultivars by MAS in wheat breeding programs, but also provide foundation on the effort of cloning genes underlying the FHB resistance.

The large genome size of wheat and numerous repetitive DNA sequences in wheat genome make the development of reference genome sequence far behind other major crops, such as maize and rice (Mayer et al. 2011; Liu et al. 2012). However, the relatively small genome size of *B. distachyon* makes it an excellent model organism for functional genomics research in temperate cereals and forage grasses (Vogel et al. 2016). Thus, collinearity among wheat

chromosome and *B. distachyon* chromosome has been used for comparative analysis of wheat genes. For example, a high resolution map of wheat stripe rust resistance gene *Yr26* was established using markers developed using orthologs among wheat, *B. distachyon* and rice (Zhang et al. 2013). Zhong et al. (2016) also constructed a high density linkage map of the wheat powdery mildew resistance gene *Pm40* using comparative genomic analysis based on EST-STS markers. It was reported that the collinearity between *Brachypodium* and wheat is higher compared with the collinearity between wheat and rice based on comparative genomics analysis of disease resistance gene regions (Liu et al. 2012). In this study, collinear regions between the *Qfhb.rwg-5A.*2 region and *Brachypodium* genome were identified with mapped marker sequences in the GP-DH population. Genes in a region of *B. distachyon* chromosome 1 that are collinear with *Qfhb.rwg-5A.*2 region on wheat chromosome 5A were used as queries to search for orthologous wheat survey sequences. However, only a limited number of CAPS markers were developed from the wheat sequence contigs and mapped to the QTL region. This is due to the fact that most of the sequences amplified from Grandin and PI 277012 showed no SNPs, which were not segregating in the mapping population. Lack of polymorphism between the PCR sequences from the two parents suggests a high homology between Grandin and PI 277012 in the 5AL QTL region.

To map-based isolate a resistance gene, a high-resolution genetic linkage map is a prerequisite, followed by closest flanking markers on a single contig, and gene identification and functional analysis via genetic transformation or via mutagenesis (Pellio et al. 2005). A large mapping population is required to provide sufficient genetic resolution based on recombination. However, the degree of polymorphism and the recombination rate within the target gene region are largely dependent on the parents chosen to develop the population. It is well known that more

recombination occurs between more closely related genomes (Saintenac et al. 2009). Grandin and PI 277012 used in this study were originated from different geographic regions and have different pedigree. However, the recombination rate around the QTL region was high in the GP-RIL population. This may be due to the low diversity around the Q gene region where the QTL was mapped. The low polymorphism was found from the process of marker development using the Chinese Spring scaffold sequence because most of the DNA sequences amplified from Grandin and PI 277012 show no SNPs. The high recombination rate and low polymorphism in the target region make it difficult to develop unique markers for tagging the QTL. On the other hand, once a unique marker tightly linked to the QTL is identified, it should be very useful for MAS because the distance between the marker and the candidate gene for the FHB resistance may be very close. This can facilitate the map-based cloning of the FHB resistance gene.

In this study, a 5.0 Mb scaffold (scaffold 6791) from the reference genome sequence of Chinese Spring was identified for the *Qfhb.rwg-5A.2* region using BLASTn search with sequences of mapped markers as queries. With this scaffold sequence, more CAPS markers were developed. Eventually, *Qfhb.rwg-5A.2* was defined in a 1.1 Mb genomic region flanked by markers *M2375* and *M2620*. Within this interval, 22 genes were predicted (Table S). However, none of these genes encodes typical NBS-LRR resistance homolog and chimeric lectin, suggesting that the candidate gene at *Qfhb.rwg-5A.2* may be different from the disease resistance genes that have been cloned so far. However, Chinese Spring is susceptible to FHB and may not contain the candidate gene sequence. To isolate the FHB resistance candidate gene, the corresponding sequences in PI 277012 are needed for further functional characterization.

Marker-assisted selection (MAS) is a selection method based on molecular marker patterns associated with a known resistance QTL (Steiner et al. 2017). Its value in improving

FHB resistance level in adapted, high-yielding wheat germplasm has been demonstrated by many research studies (Anderson et al. 2007; Wilde et al. 2007; Von der Ohe et al. 2010; Salameh et al. 2011; Agostinelli et al. 2012; Balut et al. 2013). Effective MAS requires a resistance QTL with relatively large and stable effects and the availability of tightly linked markers. *Fhb1* is one of the few major QTL identified from numerous QTL mapping studies (Buerstmayr et al. 2009). A nearly diagnostic marker UMN10 has been developed for *Fhb1* and used worldwide in many wheat breeding programs for MAS of *Fhb1* (Liu et al. 2008; Buerstmayr et al. 2009, 2015; Schweiger et al. 2016). In this study, QTL analysis indicated that three markers (M2375, M2620, and M2781) were significantly associated with *Qfhb.rwg-5A.2*, which was fine mapped into a 0.4 cM interval. Among the three markers, *M2781* explained the highest phenotypic variation in all experiments. However, *Qfhb.rwg-5A.2* was further delimited in the interval between *M2375* and *M2620* by analysis of phenotypes and genotypes of RILs at these two marker loci. This difference may be due to some unknown factors affecting the accuracy of QTL analysis. In the process of saturating the QTL region with additional markers, we noticed that the peak of QTL shifted with increase of marker density. Further studies are required to confirm that *Qfhb.rwg-5A.2* is located in the region flanked by *M2375* and *M2620* other than outside the interval.

M2375, *M2620*, and *M2781* are the three most closely-linked markers developed in this study for *Qfhb.rwg-5A.*2 and should be useful for rapid detection of *Qfhb.rwg-5A.*2 in wheat cultivars and breeding lines, and therefore, can be used for incorporating *Qfhb.rwg-5A.*2 with other resistance genes to develop wheat cultivars with durable resistance. These markers also provide start point in identifying the FHB resistance gene by the map-based cloning approach.

Cloning of the resistance gene at *Qfhb.rwg-5A.*2 may contribute to our understanding of the mechanism of the novel resistance in PI 277012 at the molecular level.

References

Agostinelli, A. M., Clark, A. J., Brown-Guedira, G., & Van Sanford, D. A. (2012). Optimizing phenotypic and genotypic selection for Fusarium head blight resistance in wheat. *Euphytica*, *186*, 115-126.

Ahmed, I., Islam, M., Arshad, W., Mannan, A., Ahmad, W., & Mirza, B. (2009). High-quality plant DNA extraction for PCR: an easy approach. *Journal of applied genetics*, *50*, 105-107.

Anderson, J.A., Stack, R.W., Liu, S., Waldron, B.L., Fjeld, A.D., Coyne, C., Moreno-Sevilla, B., Fetch, J.M., Song, Q.J., Cregan, P.B. & Frohberg, R. C. (2001). DNA markers for Fusarium head blight resistance QTLs in two wheat populations. *Theoretical and Applied Genetics*, *102*, 1164-1168.

Anderson, J. A., Chao, S., & Liu, S. (2007). Molecular breeding using a major QTL for Fusarium head blight resistance in wheat. *Crop Science*, *47*, S-112.

Bai, G., & Shaner, G. (1994). Scab of wheat: prospects for control. *Plant Disease*, *78*, 760-766.

Bai, G., Kolb, F. L., Shaner, G., & Domier, L. L. (1999). Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. *Phytopathology*, *89*, 343-348.

Bai, G., & Shaner, G. (2004). Management and resistance in wheat and barley to Fusarium head blight. *Annu. Rev. Phytopathology*, *42*, 135-161.

Balut, A. L., Clark, A. J., Brown-Guedira, G., Souza, E., & Van Sanford, D. A. (2013). Validation of and in Several Soft Red Winter Wheat Populations. *Crop Science*, *53*, 934-945.

Buerstmayr, H., Steiner, B., Hartl, L., Griesser, M., Angerer, N., Lengauer, D., Miedaner, T., Schneider, B. & Lemmens, M. (2003). Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. *Theoretical and Applied Genetics*, *107*, 503-508.

Buerstmayr, H., Ban, T., & Anderson, J. A. (2009). QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. *Plant breeding*, *128*, 1-26.

Buerstmayr, M., & Buerstmayr, H. (2015). Comparative mapping of quantitative trait loci for Fusarium head blight resistance and anther retention in the winter wheat population Capo× Arina. *Theoretical and Applied Genetics*, *128*, 1519-1530.

Cavanagh, C.R., Chao, S., Wang, S., Huang, B.E., Stephen, S., Kiani, S., Forrest, K., Saintenac, C., Brown-Guedira, G.L., Akhunova, A. & See, D. (2013). Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proceedings of the national academy of sciences*, *110*, 8057-8062.

Chen, J., Griffey, C.A., Maroof, S., Stromberg, E.L., Biyashev, R.M., Zhao, W., Chappell, M.R., Pridgen, T.H., Dong, Y. & Zeng, Z. (2006). Validation of two major quantitative trait loci for fusarium head blight resistance in Chinese wheat line W14. *Plant breeding*, *125*, 99-101.

Chu C.G., Zhong S., Chao S., Friesen T.L., Halley S., Elias E.M., Faris J.D., Xu S.S. (2010) Fighting against FHB-an excellent novel resistance source for future wheat breeding. In: Canty S, Clark A, Anderson-Scully A, Ellis D, Van Sanford D (eds) Proceedings of the National Fusarium Head Blight Forum, Milwaukee, WI, 7–9 December, 2010, University of Kentucky, Lexington

Chu, C., Niu, Z., Zhong, S., Chao, S., Friesen, T.L., Halley, S., Elias, E.M., Dong, Y., Faris, J.D. & Xu, S. S. (2011). Identification and molecular mapping of two QTLs with major effects for resistance to Fusarium head blight in wheat. *Theoretical and applied genetics*, *123*, 1107.

Cuthbert, P. A., Somers, D. J., Thomas, J., Cloutier, S., & Brulé-Babel, A. (2006). Fine mapping Fhb1, a major gene controlling Fusarium head blight resistance in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *112*, 1465.

Cuthbert, P. A., Somers, D. J., & Brulé-Babel, A. (2007). Mapping of Fhb2 on chromosome 6BS: a gene controlling Fusarium head blight field resistance in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics*, *114*, 429-437.

Gilbert, J., & Tekauz, A. (2000). Recent developments in research on Fusarium head blight of wheat in Canada. *Canadian Journal of Plant Pathology*, *22*, 1-8.

Jiang, C., & Zeng, Z. B. (1997). Mapping quantitative trait loci with dominant and missing markers in various crosses from two inbred lines. *Genetica*, *101*, 47-58.

Joehanes, R., & Nelson, J. C. (2008). QGene 4.0, an extensible Java QTL-analysis platform. *Bioinformatics*, *24*, 2788-2789.

Levene, H. (1960). Robust tests for equality of variances. *Contributions to probability and statistics*, *1*, 278-292.

Liu, S., Pumphrey, M., Gill, B., Trick, H., Zhang, J., Dolezel, J., Chalhoub, B. & Anderson, J. (2008). Toward positional cloning of Fhb1, a major QTL for Fusarium head blight resistance in wheat. *Cereal Research Communications*, *36*, 195-201.

Liu, S., Hall, M. D., Griffey, C. A., & McKendry, A. L. (2009). Meta-analysis of QTL associated with Fusarium head blight resistance in wheat. *Crop Science*, *49*, 1955-1968.

Liu, Z., Zhu, J., Cui, Y., Liang, Y., Wu, H., Song, W., Liu, Q., Yang, T., Sun, Q. & Liu, Z. (2012). Identification and comparative mapping of a powdery mildew resistance gene derived from wild emmer (Triticum turgidum var. dicoccoides) on chromosome 2BS. *Theoretical and applied genetics*, *124*, 1041-1049.

Long, Y. M., Chao, W. S., Ma, G. J., Xu, S. S., & Qi, L. L. (2017). An innovative SNP genotyping method adapting to multiple platforms and throughputs. *Theoretical and Applied Genetics*, *130*, 597-607.

Lorieux, M. (2012). MapDisto: fast and efficient computation of genetic linkage maps. *Molecular Breeding*, *30*, 1231-1235.

Mayer, K.F., Martis, M., Hedley, P.E., Šimková, H., Liu, H., Morris, J.A., Steuernagel, B., Taudien, S., Roessner, S., Gundlach, H. & Kubaláková, M. (2011). Unlocking the barley genome by chromosomal and comparative genomics. *The Plant Cell*, *23*, 1249-1263.

McMullen, M. P., & Stack, R. W. (2011). Fusarium head blight (scab) of small grains. NDSU Extension Service, 804, 6.

McMullen, M., Bergstrom, G., De Wolf, E., Dill-Macky, R., Hershman, D., Shaner, G., & Van Sanford, D. (2012). A unified effort to fight an enemy of wheat and barley: Fusarium head blight. *Plant Disease*, *96*, 1712-1728.

Mesterhazy, A. (1995). Types and components of resistance to Fusarium head blight of wheat. *Plant breeding*, *114*, 377-386.

Oliver, R. E., Cai, X., Friesen, T. L., Halley, S., Stack, R. W., & Xu, S. S. (2008). Evaluation of Fusarium head blight resistance in tetraploid wheat (L.). *Crop Science*, *48*, 213-222.

Osborne, L. E., & Stein, J. M. (2007). Epidemiology of Fusarium head blight on small-grain cereals. *International journal of food microbiology*, *119*, 103-108.

Parry, D. W., Jenkinson, P., & McLeod, L. (1995). Fusarium ear blight (scab) in small grain cereals—a review. *Plant pathology*, *44*, 207-238.

Pellio, B., Streng, S., Bauer, E., Stein, N., Perovic, D., Schiemann, A., Friedt, W., Ordon, F. & Graner, A. (2005). High-resolution mapping of the Rym4/Rym5 locus conferring resistance to the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2) in barley (Hordeum vulgare ssp. vulgare L.). *Theoretical and applied genetics*, *110*, 283-293.

Puri, K. D., & Zhong, S. (2010). The 3ADON population of Fusarium graminearum found in North Dakota is more aggressive and produces a higher level of DON than the prevalent 15ADON population in spring wheat. *Phytopathology*, *100*, 1007-1014.

Rawat, N., Pumphrey, M.O., Liu, S., Zhang, X., Tiwari, V.K., Ando, K., Trick, H.N., Bockus, W.W., Akhunov, E., Anderson, J.A. & Gill, B. S. (2016). Wheat Fhb1 encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium head blight. *Nature genetics*, *48*, 1576-1580.

Saintenac, C., Falque, M., Martin, O. C., Paux, E., Feuillet, C., & Sourdille, P. (2009). Detailed recombination studies along chromosome 3B provide new insights on crossover distribution in wheat (Triticum aestivum L.). *Genetics*, *181*, 393-403.

Salameh, A., Buerstmayr, M., Steiner, B., Neumayer, A., Lemmens, M., & Buerstmayr, H. (2011). Effects of introgression of two QTL for fusarium head blight resistance from Asian spring wheat by marker-assisted backcrossing into European winter wheat on fusarium head blight resistance, yield and quality traits. *Molecular breeding*, *28*, 485-494.

SAS Institute. (2011). *SAS/IML 9.3 user's guide*. Sas Institute.

Schroeder, H. W., & Christensen, J. J. (1963). Factors affecting resistance of wheat to scab caused by Gibberella zeae. *Phytopathology*, *53*, 831-838.

Schweiger, W., Steiner, B., Ametz, C., Siegwart, G., Wiesenberger, G., Berthiller, F., Lemmens, M., Jia, H., Adam, G., Muehlbauer, G.J. & Kreil, D. P. (2013). Transcriptomic characterization of two major Fusarium resistance quantitative trait loci (QTLs), Fhb1 and Qfhs. ifa-5A, identifies novel candidate genes. *Molecular plant pathology*, *14*, 772-785.

Somers, D. J., Fedak, G., & Savard, M. (2003). Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. *Genome*, *46*, 555-564.

Stack, R. W., Elias, E. M., Fetch, J. M., Miller, J. D., & Joppa, L. R. (2002). Fusarium Head Blight Reaction of Langdon Durum-Chromosome Substitution Lines. *Crop science*, *42*, 637-642.

Steiner, B., Buerstmayr, M., Michel, S., Schweiger, W., Lemmens, M., & Buerstmayr, H. (2017). Breeding strategies and advances in line selection for Fusarium head blight resistance in wheat. *Tropical Plant Pathology*, 1-10.

Vogel, J.P. (ed.) (2016). Genetics and Genomics of Brachypodium. Cham Heidelberg New York Dordrecht London: Springer International Publishing AG Switzerland. 353.

Von der Ohe, C., Ebmeyer, E., Korzun, V., & Miedaner, T. (2010). Agronomic and quality performance of winter wheat backcross populations carrying non-adapted Fusarium head blight resistance QTL. *Crop science*, *50*, 2283.

Voorrips, R. E. (2002). MapChart: software for the graphical presentation of linkage maps and QTLs. *Journal of heredity*, *93*, 77-78.

Waldron, B. L., Moreno-Sevilla, B., Anderson, J. A., Stack, R. W., & Frohberg, R. C. (1999). RFLP mapping of QTL for Fusarium head blight resistance in wheat. *Crop Science*, *39*, 805-811.

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S., Milner, S.G., Cattivelli, L., Mastrangelo, A. M. Whan, A., Stephen, S., Barker, G., Wieseke, R., Plieske, J., International Wheat Genome Sequencing Consortium, Lillemo, M., Mather, D., Appels, R., Dolferus, R., Brown-Guedira, G., Korol, A., Akhunova, A. R., Feuillet, C., Salse, J., Morgante, M., Pozniak, C., Luo, M.-C., Dvorak, J., Morell, M., Dubcovsky, J., Ganal, M., Tuberosa, R., Lawley, C., Mikoulitch, I., Cavanagh, C., Edwards, K. J., Hayden, M. and Akhunov, E. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. *Plant biotechnology journal*, *12*, 787-796.

Wilde, F., Korzun, V., Ebmeyer, E., Geiger, H. H., & Miedaner, T. (2007). Comparison of phenotypic and marker-based selection for Fusarium head blight resistance and DON content in spring wheat. *Molecular Breeding*, *19*, 357-370.

Xu S.S., Oliver R.E., Cai X., Friesen T.L., Halley S., Elias E.M. (2007) Searching for new sources of FHB resistance in the relatives of wheat. In: Canty S, Clark A, Ellis D, Van Sanford D (eds) Proceedings of the National Fusarium Head Blight Forum, Kansas City, MO, 2–4 December, 2007, University of Kentucky, Lexington

Xu S.S., Chu C.G., Friesen T.L., Chao S., Zhong S., Halley S., Cai X., Elias E.M. (2010) Introgression of two major FHB-resistance QTLs into durum and hard red spring wheat. In: Canty S, Clark A, Ander- son-Scully A, Ellis D, Van Sanford D (eds) Proceedings of the National Fusarium Head Blight Forum, Milwaukee, WI, 7–9 December, 2010, University of Kentucky, Lexington

Yang, Z., Gilbert, J., Fedak, G., & Somers, D. J. (2005). Genetic characterization of QTL associated with resistance to Fusarium head blight in a doubled-haploid spring wheat population. *Genome*, *48*, 187-196.

Yu, J. B., Bai, G. H., Cai, S. B., Dong, Y. H., & Ban, T. (2008). New Fusarium head blight-resistant sources from Asian wheat germplasm. *Crop science*, *48*, 1090-1097.

Zeng, Z. B. (1994). Precision mapping of quantitative trait loci. *Genetics*, *136*, 1457-1468.

Zhang, X., Han, D., Zeng, Q., Duan, Y., Yuan, F., Shi, J., Wang, Q., Wu, J., Huang, L. & Kang, Z. (2013). Fine mapping of wheat stripe rust resistance gene Yr26 based on collinearity of wheat with Brachypodium distachyon and rice. *PLoS One*, *8*, e57885.

Zhong, S., Ma, L., Fatima, S.A., Yang, J., Chen, W., Liu, T., Hu, Y., Li, Q., Guo, J., Zhang, M. & Lei, L. (2016). Collinearity Analysis and High-Density Genetic Mapping of the Wheat Powdery Mildew Resistance Gene Pm40 in PI 672538. *PloS one*, *11*, e0164815.

CHAPTER IV. MOLECULAR MAPPING OF QTL FOR FHB RESISTANCE INTROGRESSED INTO DURUM WHEAT

Abstract

In the past years, great efforts have been devoted to introgress FHB resistance from tetraploid and hexaploid wheat accessions into adapted durum wheat cultivars. However, most of the quantitative trait loci (QTL) for FHB resistance existing in the introgression lines are not well characterized or validated. In this study, we aimed to identify and map QTLs for FHB resistance in durum line 10Ae564 and cultivar Joppa. A population of 205 recombinant inbred lines ($F_{2:7}$) was developed from a cross between Joppa and 10Ae564 and evaluated for reactions to *F. graminearum* in two field nurseries and two greenhouse experiments. Meanwhile, grains of inoculated spikes collected from the greenhouse experiments were tested for DON content. The RIL population was genotyped using the wheat 90K-SNP chips and a single nucleotide polymorphism marker-based linkage map was constructed. The disease severity, DON content data and linkage map from this population were used for QTL analysis. The results showed that one QTL on chromosome 2A from Joppa and two QTL each on 5A and 7A from 10Ae564 were associated with FHB resistance and DON content. *Qfhb.ndwp-2A* was detected in the two greenhouse experiments and mean of greenhouse DON tests, explaining 14%, 15%, and 9% of the phenotypic variation, respectively. *Qfhb.ndwp-7A* was detected only in the two greenhouse experiments, explaining 9 and 11% of the phenotypic variation, respectively. *Qfhb.ndwp-5A* was detected in one greenhouse season, mean of field experiments, and mean of greenhouse DON tests, which explained 19%, 10%, and 7% of phenotypic variation, respectively. This study further confirms that minor QTL exist in ND durum cultivars and combining major QTL from

hexaploid wheat and native durum germplasm will be useful for improving FHB resistance in durum.

Introduction

Fusarium head blight (FHB) is one of the most important fungal diseases threatening wheat production worldwide. The disease is mainly caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schw.) Petch] in North America. Huge economic losses can be caused by FHB in wheat production due to yield reduction and mycotoxins contamination of harvested grains. Mycotoxins pose a health risk to both human and livestock (McMullen et al. 1997; Bai et al. 2002; Pestka 2010; Covarelli et al. 2014), and they are of particular concern for durum wheat (*Triticum durum turgidum* ssp.) as it is predominantly consumed by human. Although cultural practices and fungicides applications can mitigate the impact of FHB on wheat to some extent, use of resistant cultivars is considered the most effective and efficient strategy for management of the disease.

Resistance to FHB is a complex and quantitatively inherited trait that is controlled by multiple genes and affected by environmental factors (Bai and Shaner 1994). Several types of resistance have been described (Schroeder and Christensen 1963; Mesterhazy 1995), including resistance to initial infection (Type I) and resistance to fungal spread within the spike (Type II), resistance to DON contamination and accumulation (Type III), as well as resistance to kernel infection and tolerance (Type IV). Type I and type II resistances are the most frequently recognized types of resistance, whereas other types of resistances are rarely studied due to the difficulty of identification and evaluation (Shaner 2002). In hexaploid common wheat, various sources of FHB resistance have been identified, genetically characterized, and successfully utilized in developing FHB resistant wheat cultivars (Buerstmayr et al. 2009). However, in

durum wheat, effective sources of FHB resistance are very limited. Most of current durum wheat cultivars are highly susceptible to FHB (Buerstmayr et al. 2003; Clarke et al. 2010; Miedaner and Longin 2014). Through screening of large germplasm collections, only few durum landraces were found to show some level of resistance to FHB (Elias et al. 2005; Talas et al. 2011; Huhn et al. 2012). Therefore, extensive efforts have been devoted to search for sources of FHB resistance in the related tetraploid wheat species of *T. turgidum* (Oliver et al. 2007, 2008). Some accessions of *T. dicoccoides*, *T. dicoccum*, and *T. carthlicum* were identified to exhibit moderate or high FHB resistance. These tetraploid species may be useful in durum wheat improvement, however, they have not been used so far in durum breeding because the resistance level is not comparable to Sumai 3 and it is difficult to incorporate the resistance of wild species in elite breeding programs (Prat et al. 2014).

QTL mapping studies in tetraploid wheat are relatively rare compared to those in hexaploid wheat (Prat et al. 2014). QTL with small or moderate effect have been identified in materials derived from accessions of *T. dicoccoides* (Otto et al. 2002; Chen et al. 2007; Gladysz et al. 2007; Buerstmayr et al. 2013), *T. dicoccum* (Buerstmayr et al. 2012; Ruan et al. 2012; Zhang et al. 2014), and *T. carthlicum* (Somers et al. 2006). These QTL have been mapped on 11 chromosomes including 2A, 2B, 3A, 3B, 4A, 4B, 5B, 6A, 6B, 7A and 7B (Prat et al. 2014). Most of the QTL detected in tetraploid wheat contributed smaller effect in reducing FHB severity, compared to *Fhb1*, the major resistance QTL identified in common wheat cultivar Sumai 3 (Waldron et al. 1999; Anderson et al. 2001). Use of the Sumai 3 resistance in durum wheat has been attempted, but the expression of *Fhb1* in the durum genetic background is unstable for some unknown reasons (Zhu et al. 2014, 2016 a, b). However, a recent report indicated that the

resistance of *Fhb1* was successfully introgressed into durum wheat, which represents a significant step forward for enhancing FHB resistance in durum wheat (Prat et al. 2017).

PI 277012 is a hexaploid wheat line with a high level of FHB resistance across different environments (Chu et al. 2011). Two major QTL were identified on chromosome 5A explaining 20 to 32% of the phenotypic variation (Chu et al. 2011). The FHB resistance of PI 277012 is not only effective in hexaploid wheat, but also expressed well in durum wheat background (Zhu et al. 2016b; S. S. Xu, unpublished data). The PI 277012 resistance has been introgressed into several adapted durum wheat cultivars and several introgression lines exhibited a good level of FHB resistance with acceptable agronomic traits (S. S. Xu, unpublished data). Among them, 10Ae564 is a BC_1F_5 durum wheat line derived from cross and backcross of the durum wheat cultivar Lebsock to PI 277012 (Lebsock/PI 277012/Lebsock). It has a moderate level of FHB resistance, presumably inherited from PI 277012. Joppa is a newly released durum wheat cultivar. It is less susceptible to FHB than other durum wheat cultivars currently grown in North Dakota (ND) (Elias and Manthey 2016), but no information is available on existence of QTL for FHB resistance in this cultivar. In this study, we aimed to identify and map QTL for FHB resistance in 10Ae564 and Joppa. Our overall goal is to identify effective FHB resistance QTL and associated DNA markers, and eventually introgress them into the cultivated durum varieties as germplasm for breeding FHB resistant durum varieties.

Materials and Methods

Plant Materials

A mapping population (designated as Jop10A) consisting of 205 recombinant inbred lines (RILs) ($F_{2:7}$) from a cross between Joppa and a BC_1F_5 line (10Ae564) derived from Lebsock/PI 277012//Lebsock was used for molecular mapping and QTL analysis. In addition to the RILs and

their parents, the wheat genotypes Wheaton (FHB-susceptible), Grandin (FHB-susceptible), ND2710 (FHB-resistant), and PI 277012 (FHB-resistant) were used as controls in the FHB inoculation experiments.

Evaluation of FHB Resistance

The mapping population, parents and controls were evaluated for Type II resistance using the single-spikelet inoculation method. The experiments were carried out in 2015 and 2016, including two experiments (15GH and 16GH) in greenhouse and two experiments (15FAR and 16FAR) in field FHB nurseries. All experiments were arranged in a randomized complete block design with three replicates.

For the greenhouse experiments, plants were grown in clay pots with three plants per pot. One pot was used as one replicate and thus each wheat line or genotype had three replicates with nine plants in total. The greenhouse was supplemented with artificial light provided by 600 watt High-Pressure Sodium Lamps (P.L. Light Systems Inc, Beamsville, Canada) for a 14 h photoperiod and the temperature was maintained between 22 and 25 °C. The inoculum was prepared as a spore suspension at a concentration of 100,000 spores mL^{-1} by mixing equal spore concentrations of four pathogenic *F. graminearum* strains. Inoculation was performed using the single-spikelet inoculation method described by Stack et al. (2002). Ten microliter of the spore suspension was injected into the central spikelet of a spike using an inoculation syringe at anthesis. In each pot, eight to ten spikes were inoculated. Inoculated plants were placed in a room with an overhead misting system set at 1 minute misting in every 30 minutes to facilitate initial fungal infection. After 48 hours of incubation, the plants were moved back to the original greenhouse benches.

In the field experiments, plants were planted in hill plots with 8-10 seeds per plot. For each wheat line or genotype, three replicates (hill plots) were planted. The inoculation method used in the field was the same as used in the greenhouse experiment. At anthesis, at least eight spikes with similar developmental stage in a hill plot were inoculated. The nursery was misted for 12 hours in a pattern of 5 min misting in 15 min intervals from 4:00 p.m. to 4:00 a.m. The misting system started on the first inoculation date and ended at 14 days after inoculation of the latest flowering wheat materials in the nursery.

The disease severity was rated at 21 days post inoculation and recorded as percentage of infected spikelets on each spike estimated based on the nine-categories scale (0, 7, 14, 21, 33, 50, 67, 80, and 100%) described by Stack and McMullen (2011). 0 indicated no infection and 100% indicated infection of all spikelets in the spike.

DON Test

The content of deoxynivalenol (DON) produced by *F. graminearum* in grains was determined for two greenhouse experiments (15GH and 16GH). These DON tests were designated as DON_15GH and DON_16GH, respectively. To prepare the seed samples for DON testing, the inoculated spikes of each line were harvested at maturity, and threshed carefully to keep scabby seeds. The threshed kernels of all three replicates from each line were combined and ground into powder. The ground samples were sent to the Veterinary Diagnostic Laboratory at North Dakota State University for DON analysis.

Marker Data

Genomic DNA was extracted from fresh leaf tissues of the parents and the RILs in the mapping population with TissueLyser (Qiagen) using a simplified SDS-based procedure modified from Ahmed et al. (2009). DNA extraction buffer consists of 20% SDS, 1M Tris-HCl

(pH 8.0), 0.5M EDTA (pH 8.0), and 5M NaCl. DNA was diluted to ~50ng/µl and used for the wheat 90K iSelect genotyping assay (Wang et al. 2014). Genotypic clusters for every SNP were determined using the manual option of GenomeStudio version 2011.1 with the polyploid clustering module v1.0 (Illumina) for all the described genotypes. SNPs showing polymorphism between two parents and segregating in the population were used to construct genetic linkage map. Four CAPS markers (*M2006*, *M3008*, *M3504*, and *M3787*) mapped in QTL region of *Qfhb.rwg-5A.2* in PI 277102 were also used to genotype the RIL population.

Statistical Analysis

Phenotypic data

The Shapiro-Wilk normality test for distribution of disease severity and DON content in the RIL population was performed using PROC UNIVARIATE (SAS Institute 2011). Homogeneity of disease severity and DON content variances among all experiments was tested by Levene's test (Levene 1960) under the general linear model (GLM) procedure using SAS program version 9.3 (SAS Institute 2011). Correlation coefficients between disease severity and DON content were calculated using the PROC CORR procedure (SAS Institute 2011).

Linkage map construction and QTL analysis

Polymorphic SNPs were used to construct genetic linkage map using the computer program MapDisto (v1.7.7) (Lorieux, 2012) based on the 205 RILs. The Kosambi mapping function was used and the threshold value of logarithm of odds (LOD) score was set at 5.0 to claim linkage between markers with a maximum fraction of recombination at 0.20.

According to the results of homogeneous test, phenotypic data were grouped into four datasets: the field mean (AVR_F), the data from the greenhouse evaluations in 2015 and 2016 (15GH and 16GH), and greenhouse DON test mean (DON_GH). The entire marker dataset was

used to identify genomic regions associated with FHB resistance in the RIL population. QTL analysis was performed using QGene v.4.3.10 (Joehanes and Nelson 2008). QTL were identified using composite interval mapping (CIM, Zeng 1994) as implemented in QGene. A critical LOD threshold was set as 3.0 by 1,000-iteration permutation test for the 0.05 level of probability. The percentage of phenotypic variance explained by a QTL and its additive effect were calculated. For the purpose of presentation, linkage groups possessing significant QTL were reevaluated using a set of non-redundant markers to generate the QTL figures for this paper. The results were confirmed to be identical to the initial results with the entire marker dataset.

To identify whether each resistance QTL, environment, and interactions between them affect FHB disease severity, analysis of variance was performed under the SAS GLM procedure with resistance QTL and FHB disease severity data of the RIL population collected from each environment. Environment was set as the random factor. Resistance QTL was represented by the genotypic data of the marker closest to the peak of QTL.

Results

FHB Evaluations of the RIL Population across Different Environments

Phenotypic data of disease severity showed that RILs and the parents exhibited variable expression of FHB resistance in different environments (Figure 4.1). 10Ae564 was less susceptible to FHB than Joppa; the average disease severity of 10Ae564 and Joppa were 30.98 and 52.35, respectively. Large variation was observed within population among different environments. The average disease severity for the population was 46.2, 50.3, 62.3, and 50.9% in the experiments 15GH, 15FAR, 16GH, and 16FAR, respectively (Figure 4.1). The percentage of lines having a disease severity of 50% or less was 61.5, 49.8, 31.7, and 49.3% in the experiments 15GH, 15FAR, 16GH, and 16FAR, respectively (Figure 4.1). Inoculated spikes of 205 RILs

harvested from experiments 15GH, 15FAR and 16GH were used for DON test designated as DON_15GH, DON_2015F and DON_16GH, respectively. 10Ae564 showed a lower percentage of DON content than Joppa in both experiments. RILs and parents had a higher DON content in field than in greenhouse experiments (Figure 4.2).

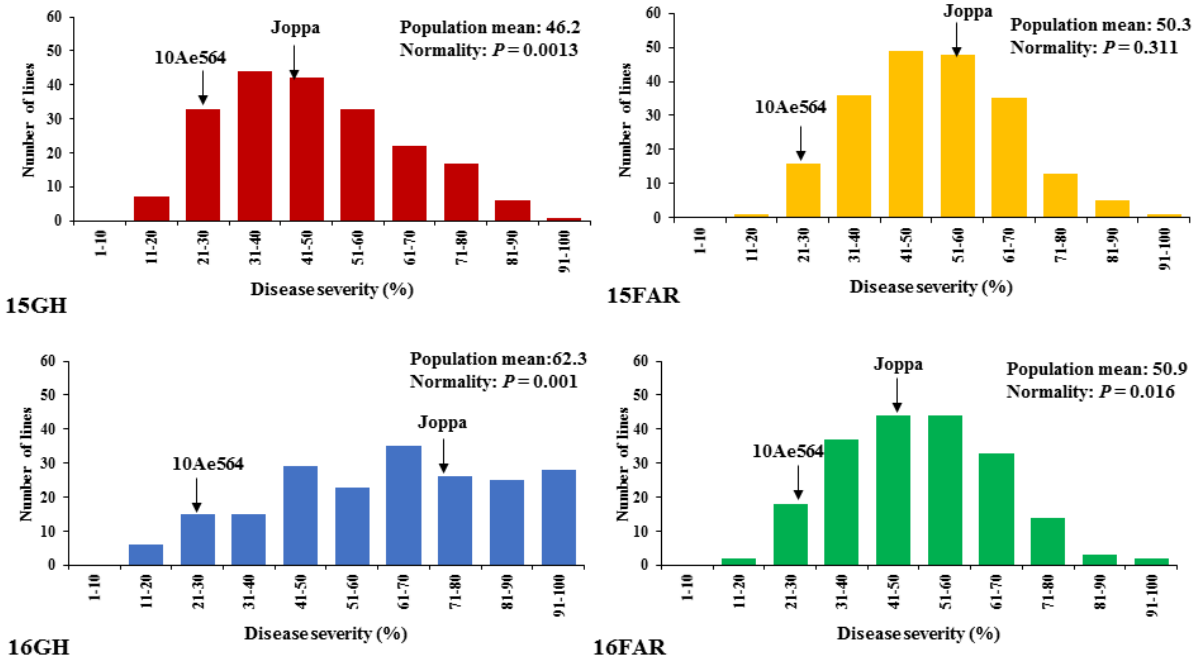

Figure 4.1. Distribution of Fusarium head blight (FHB) disease severity in the population (Jop10A) of 205 recombinant inbred lines (RILs) derived from the cross of Joppa × 10Ae564 in the five environments. The designations *15GH* and *16GH* indicate the experiments performed in the greenhouse in 2015 and 2016, respectively, while *15FAR* and *16FAR* indicate experiments performed in the field FHB nursery in Fargo, North Dakota in 2015 and 2016. The disease severities of parents were indicated by arrows. Normality test was performed using PROC UNIVARIATE procedure and Shapiro-Wilk test (SAS Institute 2011).

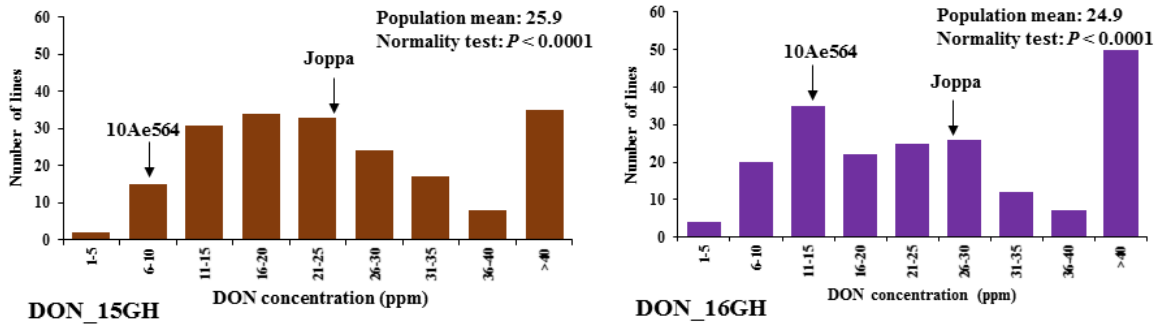

Figure 4.2. Distribution of deoxynivalenol (DON) content in the population (Jop10A) of 205 recombinant inbred lines (RILs) derived from the cross of Joppa × 10Ae564. The designation *DON_15GH* and *DON_16GH* indicate DON tests from the experiments *15GH* and *16GH*. The DON content of parents were indicated by arrows. Normality test was performed using PROC UNIVARIATE procedure and Shapiro-Wilk test (SAS Institute 2011)

The normality test revealed that the distribution of disease severity and DON concentration in the Jop10A population was significant in all experiments except for 15FAR (Figure 4.1), which means that the disease severity of the population in most of the seasons deviated from a normal distribution. Thus, Levene's test (Levene 1960) which was less sensitive to non-normal distribution, was used to test homogeneity of disease severity variances across all experiments. The results showed that the variances between the two greenhouse experiments are heterogeneous ($P < 0.01$, $df = 1$), while the variances between the two field experiments were homogeneous ($P = 0.64$, $df = 1$). The variances between the two DON tests for greenhouse experiments were homogeneous ($P = 0.17$, $df = 1$). There was a low to moderate correlation between measured FHB traits among environments, ranging from 0.14 to 0.73 (Table 4.1).

The correlation coefficient between disease severity of 15GH and DON_15GH was 0.54, while 0.73 was for disease severity of 16GH and DON_16GH (Table 4.1), which were highly significant ($P < 0.01$), suggesting that RILs with low disease severity usually have a low DON concentration.

SNP Marker Data and Linkage Group Construction

A total of 6323 polymorphic SNP markers were identified from the 90K chips. After removal of co-segregating markers, 1270 unique SNP markers together with four CAPS markers were used to construct a genetic map consisting of 37 linkage groups. Eighteen linkage groups were assigned to genome A and 19 to genome B. The total genetic map length was 399.51cM, partitioned into 234.47 cM for genome A and 255.76 cM for genome B. The average genetic distance between markers was 0.31cM.

QTL Analysis for FHB Resistance and DON Content

QTL analysis led to identification of one Joppa-derived and two 10Ae564-derived QTL associated with FHB severity and DON content. The Joppa-derived QTL (designated as *Qfhb.ndwp-2A*) was mapped to chromosome 2A, while the 10Ae564-derived QTL (designated as *Qfhb.ndwp-5A* and *Qfhb.ndwp-7A*) were mapped to chromosome 5A and 7A, respectively (Figure 4.3). Positions of these QTL and estimates of QTL effects for individual experiments as well as means over homogeneous experiments are listed in Table 4.2, and LOD profiles are shown in Figure 4.3.

Qfhb.ndwpP-5A peaked at marker *IWB26525* and spanned a 13.2-cM interval between markers *IWB71377* and *IWB8656*. It was detected in greenhouse experiment 15GH and field environment mean with a LOD value of 9.17 and 4.87, and explained 19% and 10 % of the phenotypic variation, respectively. However, this QTL was not detected in experiment 16GH. It was mapped to the same region as the major QTL *Qfhb.rwg-5A.2* in PI 277102 (Chu et al. 2011). *Qfhb.ndwp-7A* was mapped on a 6.6 cM interval between markers *IWB72301* and *IWB58523*, and only detected in the greenhouse experiments (15GH and 16GH) explaining 9 and 11% of the phenotypic variation, respectively. The Joppa-derived QTL on chromosome 2A (*Qfhb.ndwp-2A*)

was detected in both greenhouse and field experiments with LOD values of 6.69, 7.17, and 2.45, respectively, and it explained 14, 15, and 5% of the phenotypic variation in the greenhouse and field evaluations. *Qfhb.ndwp-2A* was mapped to the same region with *QFhb.rwg-2A* identified in the ND durum cultivar Ben in a previous study (Zhang et al. 2014). The three QTL on chromosome 5A, 2A and 7A were also associated with DON content, explaining 7, 9, and 6% of the phenotypic variation, respectively.

Table 4.1. Correlation coefficients among Fusarium head blight (FHB) traits from field and greenhouse environments of the population Jop10Ae

	16GH	16FAR	15GH	15FAR	DON_15GH	DON_16GH
16GH	-					
16FAR	0.18*	-				
15GH	0.52***	0.31***	-			
15FAR	0.33***	0.22**	0.30***	-		
DON_15GH	0.41***	0.33***	0.54***	0.28***	-	
DON_16GH	0.73***	0.14*	0.44***	0.29***	0.28***	-

Note: *, **, *** significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. GH, greenhouse; ns, not significant

Table 4.2. Quantitative trait loci (QTL) in the Joppa10A (Joppa × 10Ae564) recombinant inbred line population associated with FHB resistance and deoxynivalenol content

QTL	Marker interval	Peak marker	LOD value[a]				R^2 value			
			15GH	16GH	Avr_F	DON_GH	15GH	16GH	Avr_F	DON_GH
Qfhb.ndwp-2A	IWB73758-IWB65481	IWB4892	6.69	7.17	2.45	4.25	0.14	0.15	0.05	0.09
Qfhb.ndwp-5A	IWB71377-IWB8656	IWB26525	7.75	NS	4.87	3.02	0.19	-	0.10	0.07
Qfhb.ndwp-7A	IWB72301-IWB58523	IWB6895	4.39	5.02	NS	2.56	0.09	0.11	-	0.06

NS non-significant

[a] 15GH and 16GH were the experiments performed in the greenhouse in the summer of 2015 and the winter of 2016, respectively; Avr_F indicates the overall average from two field experiments; DON_GH indicates the average from two DON tests for two greenhouse experiments.

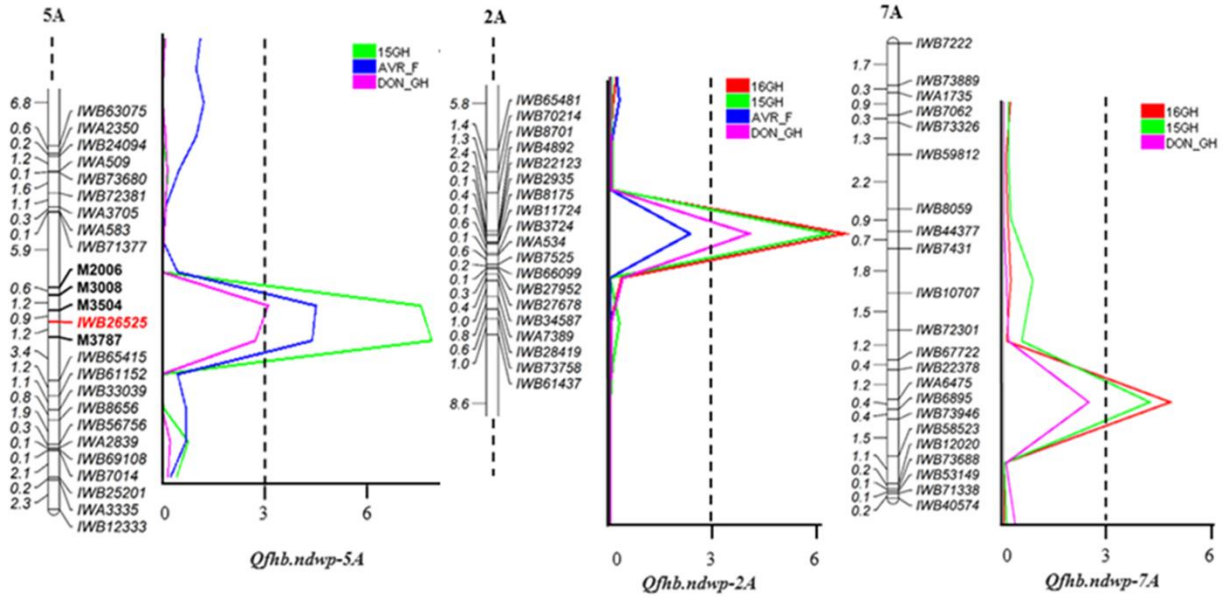

Figure 4.3. Regions of linkage maps for chromosome 2A, 5A, and 7A harboring QTL for FHB resistance detected in the Jop10A population. The QTL on chromosome 2A was derived from durum cultivar Joppa, and the QTL on 5A and 7A were derived from 10Ae564. The centiMorgan (cM) distances between marker loci and the positions of marker loci are on the left and right sides of the linkage maps, respectively. The LOD significance threshold 3.0 is represented by a *vertical dotted line*.

Table 4.3. Effect of alternative alleles at major QTL for Fusarium head blight (FHB)-related traits from greenhouse and the field for the population Joppa/10Ae564 in single years and across years.

Trait	QTL for FHB resistance[a]			2015		2016		Over 2 years	
				Mean	Reduction[b]	Mean	Reduction	Mean	Reduction
	Qfhb.ndwp-5A	*Qfhb.ndwp-2A*	*Qfhb.ndwp-7A*						
GH_SEV	-	-	-	48.7	17.2	69.8	9.1	59.2	18.2
	-	-	+	52.7	10.4	46.1	40	43.8	39.5
	-	+	-	58.8	-	76.8	-	72.4	-
	-	+	+	58.4	0.7	66.6	13.8	60.7	16.2
	+	-	-	43.8	25.5	50.7	33.9	50.7	29.9
	+	-	+	40.9	30.4	40.5	47.2	34.6	52.2
	+	+	-	50.1	14.8	79.9	-0.04	67.1	7.3
	+	+	+	46.5	20.9	62.0	19.3	47.7	34.1
FLD_SEV	-	-	-	54.1	12.5	48.5	13.7	51.3	12.8
	-	-	+	47.9	22.5	50.4	10.3	49.3	16.2
	-	+	-	61.8	-	56.2	-	58.8	-
	-	+	+	58.2	5.8	58.5	-4.0	58.4	0.7
	+	-	-	40.3	34.8	47.4	15.7	50.9	13.4
	+	-	+	37.4	39.5	45.5	19.0	40.9	30.4
	+	+	-	51.8	16.2	48.3	14.1	50.1	14.8
	+	+	+	44.4	28.2	48.3	14.1	46.6	20.7
DON_GH	-	-	-	30.1	12.2	25.7	16.3	27.6	15.9
	-	-	+	26.2	23.6	17.0	44.6	21.4	34.8
	-	+	-	34.3	-	30.7	-	32.8	-
	-	+	+	35.0	-2.0	28.7	6.5	31.9	2.7
	+	-	-	18.9	44.9	20.1	34.5	25.8	21.3
	+	-	+	18.3	46.6	19.6	36.2	18.9	42.4
	+	+	-	28.3	17.5	32.6	-6.0	30.5	7.0
	+	+	+	14.3	58.3	26.4	14.0	20.3	38.1

Note: GH, greenhouse; SEV, severity; FLD, field; DON, deoxynivalenol.

[a] 2A QTL is derived from Joppa; 5AQTL and 7AQTL are derived from 10Ae564; '+' represents 10Ae564 allele, '-' represents Joppa allele.

[b] Reduction (%) in each FHB trait compared with lines not carrying resistant alleles at the QTL.

To investigate the combined effects of detected QTL on FHB severity and DON content, the RILs were classified in subgroups according to their allele status at FHB resistance loci as illustrated in Figure 4.4 and Figure 4.5. The resistance level was then compared among different subgroups. FHB disease severity of lines carrying all three QTL showed a reduction of 30.4-52.2% in greenhouse experiments (GH_SEV) and 19.0-39.5% for field experiments (FLD_SEV) (Table 4.3). RIL lines carrying all three QTL showed the most reduction of DON content across environments not in individual environments. The disease severity and DON content of lines carrying any two QTL showed a significant reduction (10.4-50.9% for disease severity, 14.0-58.3% for DON content). Reduction was lower for those lines carrying only one QTL (0.7-18.2% for disease severity; 2.7-15.9% for DON content). Taken together, our findings demonstrate that all three QTL conferred resistance to FHB and their effects were additive.

Variance of QTL, environments, and their interactions was analyzed using genotype data of the markers that were closest to the peak of each QTL (i.e., *IWB4892*, *IWB26525*, and *IWB6895* for *Qfhb.ndwp-2A*, *Qfhb.ndwp-5A*, and *Qfhb.ndwp-7A*, respectively) and the FHB disease severity data from greenhouse experiments and field nurseries. Disease severity variance due to marker genotype for the three QTL and environments was highly significant ($P < 0.01$). Disease severity variances due to the interactions among three QTL were nonsignificant ($P = 0.11$). Disease severity variances caused by the interactions between the QTL and environments were highly significant ($P = 0.002$).

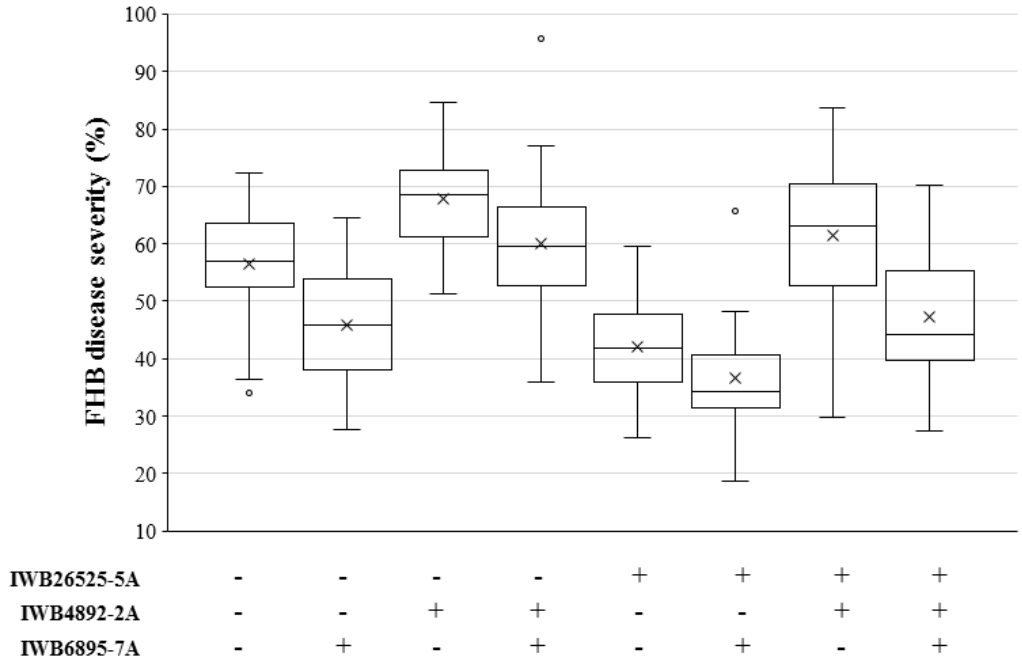

Figure 4.4. Box plot distributions of RILs according to their allele combinations at the FHB resistance loci for the Jop10A population. Medians are indicated by solid lines, × represents mean, circles represent outliers, - represents Joppa allele, + represents 10Ae564 allele.

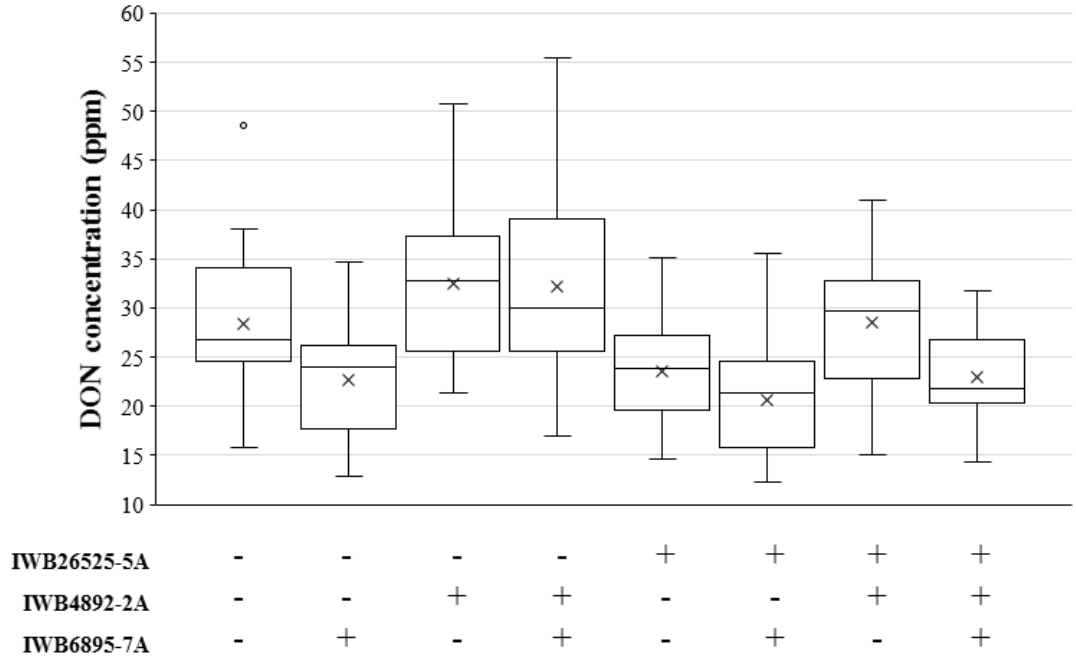

Figure 4.5. Box plot distributions of RILs according to their allele combinations at the resistance loci for DON accumulation for the Jop10A population. Medians are indicated by solid lines, × represents mean, circles represent outliers, - represents Joppa allele, + represents 10Ae564 allele.

Discussion

Since limited sources of resistance are available in durum wheat germplasm, finding effective sources of FHB resistance and DNA markers associated with FHB resistance QTL has been of top priority in durum wheat breeding programs in the regions where serious FHB epidemics occur (Prat et al. 2014). In this study, we detected two QTL (*Qfhb.ndwp-5A* and *Qfhb.ndwp-7A*) and one QTL (*Qfhb.ndwp-2A*) for FHB resistance in 10Ae564, the durum wheat line with FHB resistance from PI 277012, and Joppa, a newly released durum wheat cultivar, respectively, using a mapping population derived from the cross between 10Ae564 and Joppa. SNP markers closely linked to these QTL were also identified using the 90K SNP iSelect assay.

Among the three QTL identified, *Qfhb.ndwp-5A* derived from 10Ae564 contributed the largest effects on FHB resistance. It was detected in all field and greenhouse experiments except in 16GH. The distribution of disease severity of the Jop10A population in the experiment 16GH was much different from the other experiments. In 16GH, the average disease severity of the population was higher, and there were more lines having a disease severity of 70% or more compared to the other experiments. The high disease pressure in 16GH might reduce phenotypic variations among the individual RILs and thus masked the expression of the QTL.

To date, more than 15 QTLs for FHB resistance have been reported on chromosome 5A with 4.5~32% of the phenotypic variation under different experiments (Buerstmayr et al. 2002; Chu et al. 2011; Lin et al. 2006; Steiner et al. 2004; Yu et al. 2008; Zhang et al. 2012). *Qfhb.ndwp-5A* identified in this study is located on the same region as the major QTL *Qfhb.rwg-5A.2* identified in the wheat accession PI277012 (Chu et al. 2011) and a major QTL (*QFhb.rwg-5A.3*) identified in the cultivated emmer wheat accession PI41025 (Zhang et al. 2014). Buerstmayr et al. (2011) also reported a major QTL on 5AL identified in a *T. macha* line. All

these four QTL are very near the domestication gene *Q* (Faris et al. 2005) and may represent the same FHB resistance gene or are closely linked genes localized at the same locus.

Qfhb.ndwp-2A derived from Joppa spans an 8.8-cM interval between SNP markers *IWB73758* and *IWB65481*. This QTL is mapped to the same region where *QFhb.rwg-2A* was identified from Ben, a FHB-susceptible durum cultivar released in North Dakota in 1996 (Zhang et al. 2014; Elias and Miller 1998), according to the 90K wheat consensus map (Wang et al., 2014). Zhang et al. (2014) confirmed that the *QFhb.rwg*-2A interval is within the QTL region which confers susceptibility to FHB derived from *T. dicoccoides* Israel A (Garvin et al. 2009). Our mapping result showed that *Qfhb.ndwp-2A* conferred a moderate effect for the resistance to FHB (5-15% of phenotypic variation) in the field and greenhouse experiments. Compared to other durum cultivars released before, Joppa has much less susceptibility to FHB (Elias and Mathey 2016), indicating that minor FHB resistance QTL from old cultivars or lines might have been accumulated during the breeding process in the NDSU durum wheat breeding program. It was reported that QTL alone in the durum background apparently is ineffective to combat FHB from artificial infection or severe epidemics (Zhang et al. 2014; Somers et al. 2006). Somers et al. (2006) found that 2AL and 5AS QTL region on the tetraploid genome had little or no effect alone in reducing FHB infection, while it did lower the FHB infection level by combining with FHB resistance QTL on chromosome 6BS. In this study, it was also found that the QTL alone had a limited effect on reducing FHB infection, while the disease severity was reduced significantly when two or more QTL were combined together.

Previous studies have identified genomic regions responsible for resistance to FHB on chromosome 7A in both common wheat and durum wheat. Zhou et al. (2004) identified a minor QTL on 7AL that explained 9.8% of the phenotypic variation. Kumar et al. (2007) identified

Qfhs.fcu-7AL explaining 19% of the phenotypic variation from PI 478742 which harbors chromosome 7A from the *T. dicoccoides* accession. In this study, *Qfhb.ndwp-7A* was detected from two greenhouse experiments explaining 9% and 11% of phenotypic variation. According to wheat 90k consensus map (Wang et al. 2014), this 7A QTL was located on the short arm of 7A chromosome. Thus, it is different from the other two QTL identified by Zhou et al (2004) and Kumar et al. (2007), respectively. The origin of *Qfhb.ndwp-7A* is not known, but it is probably derived from Lebsock, a parent in the pedigree of 10Ae564.

DON contents of RILs collected from experiments 15GH and 16GH were used to detect QTL for resistance to DON accumulation. The results indicated that the correlation coefficient between FHB severity and DON content was highly significant, and the same QTL associated with resistance to FHB and DON accumulation were identified. Previous studies have also shown that QTL for DON content was either closely linked to or coincide with those for FHB severity. Chu et al. (2011) found that the two QTL identified on chromosome 5A for FHB resistance also showed major effects on reducing the percentage of Fusarium damaged kernels (FDK) and deoxynivalenol (DON) accumulation in seeds. The genomic region of 3BS between SSR marker *Xgwm*533 and *Xgwm*493 was associated with both DON resistance and FHB severity in several common wheat resistance sources (Buerstmayr et al. 2003; Lemmens et al. 2005; Somers et al. 2003; Jiang et al. 2007a, b; Ma et al. 2006). The resistance to DON accumulation in infected grains, another component of resistance, was termed Type III resistance (Mesterhazy et al. 1999). Evaluation of resistance to DON accumulation is carried out postharvest, through determining the mycotoxin content using analytical tools including chromatographic and immunochemical methods (Krska et al. 2008; Berthiller et al. 2013).

Although breeders target to reduce DON concentration in grains, it remains impractical to directly select for Type III resistance due to its phenotyping costs (Sneller et al. 2012).

Breeding for FHB-resistant cultivars in durum wheat has been hindered by lack of sources with acceptable level of FHB resistance in the germplasm collections. Recently, the major QTL *Fhb1* has been successfully introgressed into durum wheat by recurrent backcrossing, and the novel FHB-resistant breeding lines developed are agronomically close to modern European germplasm (Prat et al. 2017). The 5AL QTL in PI 277012 is another effective resistance source for developing durum wheat cultivars with improved FHB resistance. Furthermore, detection of the 2A QTL (*Qfhb.ndwp-2A*) and 7A QTL (*Qfhb.ndwp-7A*) through this study further confirms that minor QTL exist in ND durum cultivars. Those minor QTL alone may not confer effective resistance to FHB, but combining or pyramiding them with the major QTL from hexaploid wheat will be useful for enhancing FHB resistance in durum wheat.

References

Ahmed, I., Islam, M., Arshad, W., Mannan, A., Ahmad, W., & Mirza, B. (2009). High-quality plant DNA extraction for PCR: an easy approach. *Journal of applied genetics*, *50*, 105-107.

Anderson, J. A., Stack, R. W., Liu, S., Waldron, B. L., Fjeld, A. D., Coyne, C., Moreno-Sevilla, B., Fetch, J.M., Song, Q.J., Cregan, P.B. & Frohberg, R. C. (2001). DNA markers for Fusarium head blight resistance QTLs in two wheat populations. *Theoretical and Applied Genetics*, *102*, 1164-1168.

Bai, G. H., Desjardins, A. E., & Plattner, R. D. (2002). Deoxynivalenol-nonproducing Fusarium graminearum causes initial infection, but does not cause DiseaseSpread in wheat spikes. *Mycopathologia*, *153*, 91-98.

Bai, G., & Shaner, G. (1994). Scab of wheat: prospects for control. *Plant Disease*, *78*, 760-766.

Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S. D., Haesaert, G., Karlovsky, P., Oswald, I.P., Seefelder, W., Speijers, G. & Stroka, J. (2013). Masked mycotoxins: A review. *Molecular nutrition & food research*, *57*, 165-186.

Buerstmayr, H., Lemmens, M., Hartl, L., Doldi, L., Steiner, B., Stierschneider, M., & Ruckenbauer, P. (2002). Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). *Theoretical and Applied Genetics*, *104*, 84-91.

Buerstmayr, H., Stierschneider, M., Steiner, B., Lemmens, M., Griesser, M., Nevo, E., & Fahima, T. (2003). Variation for resistance to head blight caused by Fusarium graminearum in wild emmer (Triticum dicoccoides) originating from Israel. *Euphytica*, *130*, 17-23.

Buerstmayr, H., Ban, T., & Anderson, J. A. (2009). QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. *Plant breeding*, *128*, 1-26.

Buerstmayr, M., Lemmens, M., Steiner, B., & Buerstmayr, H. (2011). Advanced backcross QTL mapping of resistance to Fusarium head blight and plant morphological traits in a Triticum macha× T. aestivum population. *Theoretical and applied genetics*, *123*, 293-304.

Buerstmayr, M., Huber, K., Heckmann, J., Steiner, B., Nelson, J. C., & Buerstmayr, H. (2012). Mapping of QTL for Fusarium head blight resistance and morphological and developmental traits in three backcross populations derived from Triticum dicoccum× Triticum durum. *Theoretical and Applied Genetics*, *125*, 1751-1765.

Buerstmayr, M., Alimari, A., Steiner, B., & Buerstmayr, H. (2013). Genetic mapping of QTL for resistance to Fusarium head blight spread (type 2 resistance) in a Triticum dicoccoides× Triticum durum backcross-derived population. *Theoretical and applied genetics*, *126*, 2825-2834.

Chen, X., Faris, J.D., Hu, J., Stack, R.W., Adhikari, T., Elias, E.M., Kianian, S.F. & Cai, X. (2007). Saturation and comparative mapping of a major Fusarium head blight resistance QTL in tetraploid wheat. *Molecular Breeding*, *19*, 113-124.

Chu, C., Niu, Z., Zhong, S., Chao, S., Friesen, T. L., Halley, S., Elias, E.M., Dong, Y., Faris, J.D. & Xu, S. S. (2011). Identification and molecular mapping of two QTLs with major effects for resistance to Fusarium head blight in wheat. *Theoretical and applied genetics*, *123*, 1107-1119.

Clarke, J. M., Clarke, F. R., & Pozniak, C. J. (2010). Forty-six years of genetic improvement in Canadian durum wheat cultivars. *Canadian Journal of Plant Science*, *90*, 791-801.

Covarelli, L., Beccari, G., Prodi, A., Generotti, S., Etruschi, F., Juan, C., Ferrer, E. & Mañes, J. (2015). Fusarium species, chemotype characterisation and trichothecene contamination of durum and soft wheat in an area of central Italy. *Journal of the Science of Food and Agriculture*, *95*, 540-551.

Elias, E. M., Manthey, F. A., Stack, R. W., & Kianian, S. F. (2005, December). Breeding efforts to develop Fusarium head blight resistant durum wheat in North Dakota. In *Proc* (pp. 25-26).

Elias, E. M., & Miller, J. D. (1998). Registration of 'Ben'durum wheat. *Crop Science*, *38*, 895-906.

Faris, J. D., Simons, K. J., Zhang, Z., & Gill, B. S. (2005). The wheat super domestication gene Q. *Frontiers of Wheat Bioscience, 100*, 129-148.

Elias, E.M., Manthey, F.A. (2016). Registration of 'Joppa' durum wheat. *Journal of Plant Registrations*, *10*, 139–144.

Garvin, D. F., Stack, R. W., & Hansen, J. M. (2009). Quantitative trait locus mapping of increased Fusarium head blight susceptibility associated with a wild emmer wheat chromosome. *Phytopathology*, *99*, 447-452.

Gladysz, C., Lemmens, M., Steiner, B., & Buerstmayr, H. (2007). Evaluation and genetic mapping of resistance to Fusarium head blight in Triticum dicoccoides. *Israel journal of plant sciences*, *55*, 263-266.

Huhn, M. R., Elias, E. M., Ghavami, F., Kianian, S. F., Chao, S., Zhong, S., Alamri, M.S., Yahyaoui, A. & Mergoum, M. (2012). Tetraploid Tunisian wheat germplasm as a new source of Fusarium head blight resistance. *Crop science*, *52*, 136-145.

Jiang, G. L., Shi, J., & Ward, R. W. (2007a). QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. I. Resistance to fungal spread. *Theoretical and Applied Genetics*, *116*, 3-13.

Jiang, G. L., Dong, Y., Shi, J., & Ward, R. W. (2007). QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. II. Resistance to deoxynivalenol accumulation and grain yield loss. *Theoretical and Applied Genetics*, *115*, 1043-1052.

Joehanes, R., & Nelson, J. C. (2008). QGene 4.0, an extensible Java QTL-analysis platform. *Bioinformatics*, *24*, 2788-2789.

Krska, R., Schubert-Ullrich, P., Molinelli, A., Sulyok, M., MacDonald, S., & Crews, C. (2008). Mycotoxin analysis: an update. *Food additives and contaminants*, *25*, 152-163.

Kumar, S., Stack, R. W., Friesen, T. L., & Faris, J. D. (2007). Identification of a novel Fusarium head blight resistance quantitative trait locus on chromosome 7A in tetraploid wheat. *Phytopathology*, *97*, 592-597.

Lemmens, M., Scholz, U., Berthiller, F., Dall'Asta, C., Koutnik, A., Schuhmacher, R., Adam, G., Buerstmayr, H., Mesterházy, Á., Krska, R. & Ruckenbauer, P. (2005). The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for Fusarium head blight resistance in wheat. *Molecular Plant-Microbe Interactions, 18*, 1318-1324.

Lin, F., Xue, S. L., Zhang, Z. Z., Zhang, C. Q., Kong, Z. X., Yao, G. Q., Tian, D.G., Zhu, H.L., Li, C.J., Cao, Y.& Wei, J. B. (2006). Mapping QTL associated with resistance to Fusarium head blight in the Nanda2419× Wangshuibai population. II: Type I resistance. *Theoretical and Applied Genetics, 112*, 528-535.

Lorieux, M. (2012). MapDisto: fast and efficient computation of genetic linkage maps. *Molecular Breeding*, *30*, 1231-1235.

Ma, H. X., Zhang, K. M., Gao, L., Bai, G. H., Chen, H. G., Cai, Z. X., & Lu, W. Z. (2006). Quantitative trait loci for resistance to fusarium head blight and deoxynivalenol accumulation in Wangshuibai wheat under field conditions. *Plant pathology*, *55*, 739-745.

McMullen, M., Jones, R., & Gallenberg, D. (1997). Scab of wheat and barley: a re-emerging disease of devastating impact. *Plant disease*, *81*, 1340-1348.

Mesterhazy, A. (1995). Types and components of resistance to Fusarium head blight of wheat. *Plant breeding*, *114*, 377-386.

Mesterházy, Á., Bartók, T., Mirocha, C. G., & Komoroczy, R. (1999). Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. *Plant breeding*, *118*, 97-110.

Miedaner, T., & Longin, C. F. H. (2014). Genetic variation for resistance to Fusarium head blight in winter durum material. *Crop and Pasture Science*, *65*, 46-51.

Oliver, R. E., Cai, X., Friesen, T. L., Halley, S., Stack, R. W., & Xu, S. S. (2008). Evaluation of Fusarium head blight resistance in tetraploid wheat (L.). *Crop Science*, *48*, 213-222.

Oliver, R. E., Stack, R. W., Miller, J. D., & Cai, X. (2007). Reaction of wild emmer wheat accessions to Fusarium head blight. *Crop science*, *47*, 893-897.

Otto, C. D., Kianian, S. F., Elias, E. M., Stack, R. W., & Joppa, L. R. (2002). Genetic dissection of a major Fusarium head blight QTL in tetraploid wheat. *Plant molecular biology*, *48*, 625-632.

Pestka, J. J. (2010). Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. *Archives of toxicology*, *84*, 663-679.

Prat, N., Buerstmayr, M., Steiner, B., Robert, O., & Buerstmayr, H. (2014). Current knowledge on resistance to Fusarium head blight in tetraploid wheat. *Molecular breeding*, *34*, 1689-1699.

Prat, N., Guilbert, C., Prah, U., Wachter, E., Steiner, B., Langin, T., Robert, O. & Buerstmayr, H. (2017). QTL mapping of Fusarium head blight resistance in three related durum wheat populations. *Theoretical and Applied Genetics*, *130*, 13-27.

Ruan, Y., Comeau, A., Langevin, F., Hucl, P., Clarke, J. M., Brule-Babel, A., & Pozniak, C. J. (2012). Identification of novel QTL for resistance to Fusarium head blight in a tetraploid wheat population. *Genome*, *55*, 853-864.

SAS Institute (2011) SAS/STAT 9.3 user's guide. SAS Institute, Cary

Schroeder, H. W., & Christensen, J. J. (1963). Factors affecting resistance of wheat to scab caused by Gibberella zeae. *Phytopathology*, *53*, 831-838.

Shaner, G. (2002, December). Resistance in hexaploid wheat to Fusarium head blight. In *2002 National Fusarium Head Blight Forum Proceedings* (p. 208).

Sneller, C., Guttieri, M., Paul, P., Costa, J., & Jackwood, R. (2012). Variation for resistance to kernel infection and toxin accumulation in winter wheat infected with Fusarium graminearum. *Phytopathology*, *102*, 306-314.

Somers, D. J., Fedak, G., Clarke, J., & Cao, W. (2006). Mapping of FHB resistance QTLs in tetraploid wheat. *Genome / National Research Council Canada = Genome / Conseil National de Recherches Canada*, *49*, 1586-1593.

Somers, D. J., Fedak, G., & Savard, M. (2003). Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. *Genome*, *46*, 555-564.

Stack, R. W., Elias, E. M., Fetch, J. M., Miller, J. D., & Joppa, L. R. (2002). Fusarium Head Blight Reaction of Langdon Durum-Chromosome Substitution Lines. *Crop science*, *42*, 637-642.

Steiner, B., Lemmens, M., Griesser, M., Scholz, U., Schondelmaier, J., & Buerstmayr, H. (2004). Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. *Theoretical and Applied Genetics*, *109*, 215-224.

Talas, F., Longin, F., & Miedaner, T. (2011). Sources of resistance to Fusarium head blight within Syrian durum wheat landraces. *Plant breeding*, *130*, 398-400.

Waldron, B. L., Moreno-Sevilla, B., Anderson, J. A., Stack, R. W., & Frohberg, R. C. (1999). RFLP mapping of QTL for Fusarium head blight resistance in wheat. *Crop Science*, *39*, 805-811.

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., Maccaferri, M., Salvi, S., Milner, S. G., Cattivelli, L., Mastrangelo, A. M., Whan, A., Stephen, S., Barker, G., Wieseke, R., Plieske, J., International Wheat Genome Sequencing Consortium, Lillemo, M., Mather, D., Appels, R., Dolferus, R., Brown-Guedira, G., Korol, A., Akhunova, A. R., Feuillet, C., Salse, J., Morgante, M., Pozniak, C., Luo, M.-C., Dvorak, J., Morell, M., Dubcovsky, J., Ganal, M., Tuberosa, R., Lawley, C., Mikoulitch, I., Cavanagh, C., Edwards, K. J., Hayden, M. and Akhunov, E. (2014) Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnol J, 12: 787-796.

Yu, J. B., Bai, G. H., Zhou, W. C., Dong, Y. H., & Kolb, F. L. (2008). Quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population of Wangshuibai/Wheaton. *Phytopathology*, *98*, 87-94.

Zeng, Z. B. (1994). Precision mapping of quantitative trait loci. *Genetics*, *136*, 1457-1468.

Zhang, Q., Axtman, J. E., Faris, J. D., Chao, S., Zhang, Z., Friesen, T. L., Zhong, S., Cai, X., Elias, E.M. & Xu, S. S. (2014). Identification and molecular mapping of quantitative trait loci for Fusarium head blight resistance in emmer and durum wheat using a single nucleotide polymorphism-based linkage map. *Molecular breeding*, *34*, 1677-1687.

Zhang, X., Bai, G., Bockus, W., Ji, X., & Pan, H. (2012). Quantitative trait loci for Fusarium head blight resistance in US hard winter wheat cultivar Heyne. *Crop science*, *52*, 1187-1194.

Zhou, W., Kolb, F. L., Yu, J., Bai, G., Boze, L. K., & Domier, L. L. (2004). Molecular characterization of Fusarium head blight resistance in Wangshuibai with simple sequence repeat and amplified fragment length polymorphism markers. *Genome*, *47*, 1137-1143.

Zhu, X., Zhong, S., Xu, S.S., Elias, E., Cai, X. (2014). Effects of durum wheat background on the expression of hexaploid wheat-derived Fusarium head blight resistance genes [abstract]. In: Canty, S., Clark, A., Turcott, N., Van Sanford, D., editors. Proceedings of the National Fusarium Head Blight Forum, East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative, December 7-9, 2014, St. Louis, MO. p. 104-105.

Zhu, X., Zhong, S., & Cai, X. (2016a). Effects of D-genome chromosomes and their A/B-genome homoeologs on Fusarium head blight resistance in durum wheat. *Crop Science*, *56*, 1049-1058.

Zhu, X., Zhong, S., Chao, S., Gu, Y. Q., Kianian, S. F., Elias, E., & Cai, X. (2016b). Toward a better understanding of the genomic region harboring Fusarium head blight resistance QTL Qfhs. ndsu-3AS in durum wheat. *Theoretical and applied genetics*, *129*, 31-43.