ABSTRACT

PETERSEN, STINE. Advancing Marker-Assisted Selection for Resistance to Powdery Mildew and *Fusarium* Head Blight in Wheat. (Under the direction of Dr. J. Paul Murphy.)

Fungal diseases of wheat (*Triticum aestivum* L.) are a problem in wheat-growing regions worldwide, impacting both availability and quality of food and feed. Development and use of wheat cultivars possessing genetic resistance is an effective strategy for disease control. Resistance to powdery mildew (*Blumeria graminis* DC f.sp. tritici) and *Fusarium* head blight (FHB) (*Fusarium graminearum*) are considered high priority traits for most wheat producers. Identification of novel sources of powdery mildew resistance for use in breeding is essential because of the rapid evolutionary potential of the pathogen. Deployment of wheat cultivars with resistance to FHB can reduce the prevalence of mycotoxins in livestock feed and wheat products for human consumption. However, the highly quantitative nature of resistance, in combination with a large environmental component, has made it a challenge to improve FHB resistance levels of adapted wheat cultivars. Phenotypic evaluations of FHB resistance can be challenging and costly, thus marker-assisted selection (MAS) is typically utilized as a complement to phenotypic selection. A key step in improving FHB resistance is the identification and mapping of quantitative trait loci (QTL) associated with resistance, particularly from adapted germplasm.

The wheat germplasm line NC09BGTS16 (NC-S16) possesses a novel powdery mildew resistance gene introgressed from *Aegilops speltoides*. Evaluations of $F_{2:3}$ families derived from the cross NC-S16 / 'Coker 68-15' indicated that a single gene, *Pm53*, conferred resistance. Molecular markers specific to chromosome 5BL segregated with the resistance gene. Another powdery mildew gene, *Pm36*, also maps to chromosome 5BL. Detached leaf

test and marker information revealed that the resistance gene in NC-S16 was different from *Pm36*. Molecular markers in close linkage with *Pm53* were identified for utilization in MAS.

Research was conducted to identify, map, and validate genomic regions associated with FHB resistance in two U.S. soft red winter wheat populations. In the first FHB study, an inbred population derived from a cross between the moderately resistant cultivar 'NC-Neuse' and the susceptible cultivar 'AGS 2000' was evaluated for FHB resistance in North Carolina and Maryland. At five of the seven FHB QTL identified, NC-Neuse alleles were associated with resistance.

In the second FHB study, an inbred population derived from a cross between 'Bess' and NC-Neuse, both moderately resistant to FHB, was evaluated for FHB resistance in North Carolina and Missouri. The objective was to validate QTL regions identified in previous studies involving NC-Neuse and the cultivar 'Truman', a full-sib of Bess. Quantitative trait loci on chromosomes 1A, 4A, and 6A with resistance contributed by NC-Neuse were validated. Additionally, QTL on chromosomes 2B and 3B with resistance contributed by Bess and Truman were validated. Marker assays were developed for QTL in the overlapping regions. Preliminary tests using these assays on recent Uniform Southern Winter Wheat Scab Nursery entries indicated that the assays developed for the QTL *Qfhb.nc-1A, Qfhb.nc-2B.1*, and *Qfhb.nc-6A* may be good candidates for use in MAS.

Advancing Marker-Assisted Selection for Resistance to Powdery Mildew and *Fusarium* Head Blight in Wheat

by
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DEDICATION

I would like to dedicate this work to my husband, John Michael Pavlecich, in recognition of his love, support, and encouragement. He has been there for me through the highs and lows of the last four years and I could not be more grateful.

BIOGRAPHY

Stine Petersen was born in 1984 of Lis Thomsen and Tage Petersen. She was raised on a farm close to Bylderup-Bov in southwest Denmark. After completing a B.S. in Natural Resources at the University of Copenhagen in 2008, she went on exchange at Purdue University in West Lafayette, IN for two semesters. During this time, she also worked as a research assistant in Dr. Herb Ohm's Small Grains Breeding Program. In 2009, she completed a six months internship in Maize Research and Product Development with Dupont-Pioneer in Janesville, WI. Stine then went back to Denmark and completed her M.S. degree in Agriculture in 2010. Her research was focused on investigating the function of a family of transcription activators in susceptibility to barley powdery mildew. She began her doctoral research at North Carolina State University in 2011. After graduation, Stine will begin work as a tomato breeder for Monsanto in Bergschenhoek near Rotterdam in the Netherlands.

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CHAPTER 1 - Literature Review

Introduction

Common wheat (*Triticum aestivum* L.) is consistently one of the largest crops in global annual acres planted and tonnage harvested. According to the 2015 Food and Agricultural Organization estimates, the production of wheat worldwide has increased significantly from about 263 million tons per year in the 1960's to a total of 716 million tons in 2013 (www.faostat3.fao.org). Wheat is ranked first in global grain production and is estimated to supply more than 20% of the total calories and protein in the human diet (Shiferaw et al., 2013). The greatest production occurs in China, India, the U.S., and Russia. The estimated annual production since 2000 was 106 million tons in China, 78 million tons in India, 58 million tons in the U.S., and 48 million tons in Russia (www.faostat3.fao.org). The average wheat yield across the world was 3 tons per hectare in 2000, and had risen to 3.6 tons per hectare in 2013 (www.faostat3.fao.org).

Demands for increased global wheat production have generally been met by development and adoption of good management practices including use of fertilizers and pesticides, and new higher yielding cultivars with increased resistance to biotic and abiotic stresses (Shiferaw et al., 2013). As the world population grows, pressure to increase production and improve wheat cultivars will continue to rise. Wheat breeders must select for a great number of important traits such as grain yield, test weight, maturity, milling and baking qualities, resistance to diseases and pests, plant height, and straw strength. Genetic resistance to diseases can significantly improve yields, grain quality, and in some cases, food safety. Traditional breeding methods combined with use of molecular markers can facilitate

substantial advancements in the development of cultivars with greater levels of disease resistance.

Wheat

Origin and Evolution of Wheat

Wheat was domesticated about 10,000 years ago in the Fertile Crescent, which is a region in the Middle East including the Tigris and Euphrates river basins. The modern-day countries with significant territory within the Fertile Crescent include Iraq, Syria, Kuwait, Lebanon, Israel, Palestine, Egypt, and Jordan.

Wheat is a self-pollinated allohexaploid (2n = 6x = 42), comprised of three independent genomes (AABBDD) derived from related wild species. About 500,000 years ago wild emmer wheat (*Triticum turgidum* subsp. *dicococides* Koern. Ex Asch & Graebn.) (AABB; 2n = 4x = 28) originated following an interspecific cross between two related diploid species with subsequent chromosome doubling. The A genome donor was *T. urartu* Tum. Ex Grand (Dvorak et al., 1993), while the actual B genome donor is unknown. The B genome of emmer and common wheat most closely resembles the S genome of the extant *Aegilops speltoides* (Tausch.) (Talbert et al., 1991; Wang et al., 1997). Hexaploid common wheat originated from a spontaneous chromosome doubling following hybridization between tetraploid emmer wheat and diploid *Ae. tauschii*, the D genome donor (Kihara, 1954; McFadden and Sears, 1946; Dvorak et al., 2012).

Production of Wheat in the United States

According to the USDA Economic Research Service (www.ers.usda.gov), wheat ranks third among U.S. field crops in both planted acreage and gross farm receipts, behind corn (*Zea mays* L.) and soybeans (*Glycine max* L.). In 2012-2013, a total of 55.3 million acres were planted with wheat in the U.S. (www.ers.usda.gov). Kansas and North Dakota are the largest producers. The national average yield across all market types of wheat is about 45 bushels per acre. Nearly 50% of the wheat produced in the U.S. is exported and about 10% is used for animal feed.

Wheat can be divided into five major market classes based on milling and baking characteristics and geographical production region. The market classes also reflect gross phenotypes. These characteristics include growth habit (spring/winter), bran coat color (red/white), and milling properties (hard/soft). Winter wheat represents 70-80% of the total U.S. wheat production. In terms of acres planted, hard red winter wheat (HRWW) is the biggest of the market classes, followed by hard red spring wheat (HRSW), soft red winter wheat (SRWW), white wheat, and durum wheat.

HRWW is grown primarily in the Great Plains of the mid-western U.S., east of the Rocky Mountains and west of the Mississippi river. Of the 55.3 million acres planted with wheat in 2012-2013, 29.6 million acres were planted with HRWW.

HRSW is grown in Minnesota, Wisconsin, North and South Dakota, and Montana. The cold, harsh winters necessitate use of spring wheat because winter wheat would not withstand the winter climate following fall planting. This region is well-known for producing wheat with high protein and good bread making attributes.

SRWW accounts for 15-20 percent of total production and is grown primarily in States along the Mississippi River and in the Eastern States, including North Carolina. The softer seed coat and lower protein content has different milling and baking qualities than hard wheats, and flour from SRW is used for crackers, cookies, cakes, and pretzels.

White wheat is regionally grown around the great lakes, primarily New York and Michigan, or the Northern great basin of Idaho, Washington, and Oregon. Whole grain soft white wheat is often used for bakery products and crackers.

Durum wheat (*Triticum turgidum* subsp. *durum*) is a hard-grained tetraploid wheat (2n = 4x = 28) with high protein. It is raised in North Dakota and Montana on roughly 3.2 million acres specifically for pastas.

Genetics of Wheat

The genome of common bread wheat is estimated to be 16 Gb in size and 2,500 cM in genetic map length (Somers et al., 2004). Each of the ancestral diploid parents of bread wheat contributed seven chromosomes. The three wheat genomes are highly collinear except for few major rearrangements, and retrotransposons (Devos et al., 2009).

An estimated 80-90% of the wheat DNA is repetitive (Smith and Flawell, 1975). Physical mapping of molecular markers using a series of wheat deletion lines has demonstrated the existence of gene-rich regions in the wheat genome, indicating that genes are not randomly distributed (Gill et al., 1996). The euchromatic DNA is isolated in a few gene islands between abundant amounts of repetitive DNA. Coding DNA is thought to be 4-8% of the total genome. A large quantity of retrotransposons is incorporated in the genome (Devos et al., 2009). Common wheat is considered to have a moderate to low level of

nucleotide diversity compared to some other plant species, particularly in the D genome (Akhunov et al., 2010; Chen et al., 2012). Linkage disequilibrium (LD), or non-random association of alleles between linked or unlinked loci, is expected to be higher in hexaploid wheat compared to species like maize and sorghum because of the rapid rate of inbreeding in wheat with a high degree of self-pollination (Flint-Garcia et al., 2003).

Wheat is a determinate annual, which is grown on several continents (Kilian et al., 2009). The crop's ability to grow in a great range of climates is due to a number of traits that alter heading date to protect the plant from environmental extremes. Wheat has evolved refined mechanisms capable of integrating photoperiod and vernalization (extended exposure to low temperatures) signals associated with seasonal variation to optimize flowering time and seed production. The transition from a vegetative growth phase to a reproductive growth phase is triggered by photoperiod (PPD) and vernalization (VRN) genes. Three homoelogous loci associated with major photoperiod effects are on chromosomes 2A (*Ppd-A1*), 2B (*Ppd-B1*), and 2D (*Ppd-D1*). Several VRN genes have been identified and mapped: *Vrn-1* (Yan et al., 2003), *Vrn-2* (Yan et al., 2004), and *Vrn-3* (Yan et al., 2006), of which *Vrn-1* controls most of the variation in vernalization requirement in wheat. The *Vrn-1* gene has been mapped in colinear regions of the long arm of chromosomes 5A (*Vrn-A1*), 5B (*Vrn-B1*), and 5D (*Vrn-D1*). A complex interaction between alleles at the *Vrn-1*, *Vrn-2*, and *Vrn-3* loci, and photoperiod dictates when an undifferentiated apex will flower (Yan et al., 2003; Yan et al., 2004; Yan et al., 2006). The vernalization genes can have the greatest effect on timing of heading date and determine growth habit classification into spring and winter annuals (Yan et al., 2003). Guedira et al. (2014) conducted a QTL mapping study to identify genetic

determinants of flowering time in a population derived from a cross between the two cultivars NC-Neuse and AGS 2000 which are adapted to the Mid-Atlantic and Southeastern U.S. regions, respectively. The biggest effect QTL were located on chromosomes 5BL and 2B, and gene-based markers for *Vrn-B1* and *Ppd-B1* confirmed that these loci are major determinants of HD in winter wheat and are important for adaptation to diverse growing environments. Additional genes associated with vernalization have been identified, but the effects have been minor in comparison to the three VRN loci. In addition to photoperiod and vernalization genes, earliness per se QTL allow for altering of maturity by a few days (Worland and Snape, 2001).

Another characteristic that contributed to an increase in wheat production was the incorporation of reduced height (*Rht*) or 'dwarfing' genes (Reynolds and Borlaug, 2006). More than 21 different alleles of several loci have been associated with reduced height (McIntosh et al., 1998), but two *Rht* genes are heavily represented in U.S. wheat varieties (Guedira et al., 2010). Commonly referred to as the Green Revolution Genes, the two homoeologous alleles *Rht-B1b* and *Rht-D1b* (previously known as *Rht1* and *Rht2*) were popularized by Norman Borlaug (Gustafson et al., 2009; Worland and Snape, 2001). The alleles were from an induced mutation in the Japanese landrace 'Daruma' (Worland and Snape, 2001). The incorporation of *Rht-B1b* and *Rht-D1b* increased straw strength (thereby reducing lodging), spikelet fertility, and yield compared to tall genotypes (Worland and Snape, 2001). More than 90% of the U.S. wheat varieties from the Midwest and East possess one of these two alleles which reduce height to around 80-90 cm depending upon the genetic background (Guedira et al., 2010). To date, the *Rht-B1* locus has seven alleles and *Rht-D1*

locus has four alleles (McIntosh et al., 2003). Another *Rht* gene, *Rht8c*, which was introduced from the Japanese cultivar Akakomugi (Gale and Youssefi, 1985), is represented in U.S. wheat varieties, although less frequently than *Rht-B1b* and *Rht-D1b*. According to Guedira et al. (2010), *Rht8c* was present in 8 and 3% of a large sample of U.S. soft winter wheat and hard winter wheats, respectively.

Molecular Breeding in Wheat

Marker-Assisted Selection

In traditional breeding, selection is based on phenotypic evaluations of lines. Evaluations are typically conducted under field conditions, but can also be conducted in greenhouses in screening for certain traits like resistance to powdery mildew (caused by *Blumeria graminis* (DC) Speer f.sp. *tritici* Em. Marchal (*Bgt*)) and leaf rust (caused by *Puccinia triticina* Erikss.). Marker-assisted selection (MAS) uses genotypic data to drive or aid in the selection of superior lines. Markers may directly or indirectly improve a trait by selecting alleles that have been statistically associated with certain phenotypic effects. When markers include a causal polymorphism for a trait, the phenotype can be changed directly when marker genotypes are selected. When a marker is in LD or associated with a gene of interest, the trait is improved indirectly by selection at the marker locus (Hospital, 2003). Marker-assisted selection can be used to select for qualitative resistance, where a single resistance gene confers complete resistance. It can also be utilized to select for quantitative resistance, where multiple small effect genes control resistance.

Use of markers can complement traditional selection for traits, which are difficult or costly to phenotype, or cannot be selected in early generations due to low heritability. MAS can be used in allele enrichment, pyramiding, marker-assisted recurrent selection, and backcrossing. The intention of applying MAS is to increase the genetic gain per cycle, reduce cost, or give greater control over donor germplasm (Collard and Mackill, 2008).
The implementation and success of MAS depends on a number of factors including heritability of the trait, relative cost of genotyping and phenotyping, LD between markers and the gene controlling the trait of interest, type of molecular marker, and statistical analysis. Miedaner and Korzun (2012) suggested that, for MAS to be feasible, quantitative trait loci (QTL) should explain at least between 10 and 20% of phenotypic variance in the original mapping population.

Molecular Markers

Molecular DNA markers are assays designed to detect differences in the DNA sequence between two or more samples. A number of marker types are available, of which Simple Sequence Repeats (SSR) (also referred to as microsatellites), Diversity Array Technology (DArT), and Single Nucleotide Polymorphism (SNP) markers are especially popular in wheat. When choosing a type of marker for a study, considerations are usually based on factors such as: cost, type of DNA polymorphism targeted, detection, visualization, inheritance pattern, ability to detect heterozygosity, reliability, technical skill, multiplex level, and ability to be automated.

SSRs are designed to anneal primers to conserved sequences flanking repeated motifs. A repeated motif of 1-8 bp in length is known as a simple sequence repeat (or

microsatellite). SSRs are PCR-based, highly reproducible, and usually co-dominant markers that can be found mainly in non-coding regions, but also within genes and in untranslated regions of coding sequences. The degree of polymorphism is affected by the source from which the microsatellite was derived. Development of SSRs is expensive due to cloning and sequencing; however once primers are developed, the assays become cost-effective. Generally, these markers are locus specific, but can amplify homoeologous loci, particularly in polyploids such as wheat. Multiplexing of SSRs into single reactions for PCR or fragment analysis makes this marker assay amenable to automation. The high level of polymorphism per locus, speed of genotyping, and relatively low cost have made SSRs a common marker type in breeding applications. Issues with homoplasmy, in which alleles are identical by state and not descent, can interfere with markers being diagnostic in MAS.

Diversity array technology (DArT) markers are assays designed to screen for thousands of markers in one reaction on a microarray platform (Jaccoud et al., 2001). This proprietary process is based upon reducing the complexity of the genome to a reproducible portion with restriction enzymes (RE) and size selection. DArT generates whole-genome fingerprints by scoring the presence versus absence of DNA fragments in genomic representations generated from samples of genomic DNA (Jaccoud et al., 2001). DArT markers are usually classified as dominant (present or absent), although it could be possible to identify co-dominance if other alleles are represented and known. Mapping studies have suggested that DArTs are more largely represented in clusters in gene rich, non-centrometic areas (Akbari et al., 2006). The limitations to using DArT markers include their dominant

inheritance, and the fact that DArTs are less amendable for development of assays targeting individual loci for use in MAS.

A single nucleotide polymorphism (SNP) is the most abundant polymorphism in the genome. Transition (C to T, G to A) or transversion (C to A, C to G) mutation events are the cause of new SNPs. Although there is a potential for four alleles, SNPs are generally bi-allelic because mutation rates are minimal. SNPs may occur in either non-coding or coding sequences and are considered evolutionarily stable due to a low mutation rate. Ravel et al. (2006) found that the frequency of SNPs in a large international collection of bread wheat was 1/335 bp. Development of SNP assays has been expensive and time consuming due to a need for sequence information. Genotyping by sequencing (GBS) (Elshire et al., 2011) is a process in which restriction enzymes are used to reduce the complexity of the genome, fragments are joined with unique barcode adapters and then pooled with multiple individuals for sequencing via next generation technologies (ie. Illumina HiSeq or Life Technologies Torrent). The DNA sequence information obtained can be used for SNP identification and genotyping in the same step.

In the past, SSR markers have been the most common type of markers in genetic studies and MAS, but SNP markers have also become popular in recent years. SNPs are even more abundant than SSRs and are often present within genes, and are functional in some cases. A 9,000 SNP chip platform and a 90,000 SNP chip platform have been developed for wheat, and consensus maps based on SNP data have been published (Cavanagh et al., 2013; Wang et al., 2014). Kompetitive Allele Specific PCR (KASP) single-tube assays (LGC

Genomics, Hoddesdon, UK) can be developed based on specific SNPs associated with a trait of interest (Chen et al., 2010), which makes SNPs very useful in genetic mapping and MAS.

Linkage Maps and QTL Mapping

In linkage-based mapping, the objective is to identify genomic regions, represented by markers, which contain genes or QTL controlling a trait of interest. This is done by combining genotypic information in the form of a linkage map with phenotypic data for the trait under investigation. A linkage map is a genetic map of a specific population that shows the positions of genes or markers relative to each other in terms of recombination frequency, rather than a specific physical distance along each chromosome. Recombination frequency is the frequency by which a single chromosomal crossover will take place between two genes (or markers) during meiosis. A centimorgan (cM) is a unit that describes the recombination frequency of 1%.

Linkage analysis can be conducted using a bi-parental mapping population, such as segregating F_2, backcross, recombinant inbred line (RIL), or doubled haploid (DH) populations. RIL and DH populations are commonly used for QTL mapping because the lines are inbred, and can be phenotyped for multiple traits in multiple environments (Buerstmayr et al., 2009). These populations are highly structured, family based, and have very high LD, requiring relatively few markers to span the genome. Generally only the two alleles in the parents are evaluated for a gene or QTL. Several linkage analysis models can be used to detect significant QTL in bi-parental populations: single marker analysis (SMA), interval mapping (IM) (Lander and Botstein, 1989), Composite Interval Mapping (CIM) (Jansen 1993), and Multiple Interval Mapping (MIM) (Jansen 1993).

A QTL can be mapped to a single marker using regression or ANOVA. Single marker analysis does not require a genetic map, but is greatly influenced by recombination. QTL effect estimates can often be misleading in this type of analysis because there can be recombination between marker and QTL. A given QTL effect may be equivalent if a large effect QTL is associated with a distant marker or a small effect QTL is associated with a nearby marker.

Interval mapping provides a method to test the effects of chromosomal positions in intervals between markers. Testing a position very near the true QTL, can provide higher power to detect the QTL. Although genotypes at positions between markers are not known, the probability of genotypes at those positions can be estimated based on the linkage map distances and the flanking marker genotypes. The optimal setting for IM is to have markers scattered evenly throughout the entire genome, allowing for many recombination events (Tanksley, 1993). By selecting the most likely position of the QTL, the QTL effects can be estimated directly at the position of the QTL. This eliminates the bias that occurs with SMA due to recombination between marker and QTL positions. When multiple QTL are being mapped, their effects can interact with the analysis and alter estimates (Jansen, 1993). Composite interval mapping and MIM use the same approach of evaluating a QTL at each position along the genome, but account for additional QTL effects by using random markers as cofactors in multiple regression for a more accurate analysis. In CIM, the genome is scanned using interval mapping to identify QTL after accounting for marker effects that are unlinked to the test position. By fitting unlinked QTL in the model, the residual variation due to other QTL is reduced, thereby increasing the power to detect QTL. In MIM, multiple QTL

models are built, fitting all QTLs at their maximum likelihood positions. This permits simultaneous estimation of QTL effects while also using the power and precision of interval mapping (Jansen, 1993).

For all these QTL mapping methods, there are limitations to the precision of position and effect estimates when using small population sizes (less than approximately 500 lines), or if a linkage map is not well-saturated. Tanksley (1993) stated that in QTL studies where populations of 100-250 individuals are used, detection is limited to major effect QTL, and Bernardo (2008) added that resolution is limited to intervals of 10-20 cM when using populations of this size. The 'Beavis effect' is an additional attribute of small populations, which can lead to overestimation of QTL effects. If QTL effects are overestimated, then the actual number of QTL controlling a trait will be underestimated (Beavis et al., 1994; Beavis, 1998).

In addition to population size and heritability, factors such as accuracy of marker order (Lehmensiek et al., 2005) and epistasis (Holland, 2001) also influence power and accuracy of QTL mapping. In a typical mapping population LD is maximized, which is why the linkage phase between markers and QTL needs to be validated in alternate populations to confirm allele associations, ensure markers are diagnostic, and then confirm the effect in different backgrounds (Collard and Mackill, 2008).

Although many QTL controlling different traits have been mapped, the implementation of these in MAS has been limited (Beavis, 1998; Bernardo, 2008). Previously, it was assumed that most markers associated with QTL from preliminary studies were directly useful in MAS. It has become apparent however that QTL confirmation, QTL

validation, and/or fine mapping of QTL regions may be required. Ideally, a confirmation step is desirable because QTL positions and effects can be inaccurate due to factors such as sampling bias and inadequate marker coverage (Collard and Mackill, 2008). Validation of QTL can include: confirmation of position and effects of QTL, verifications of QTL in independent populations and testing in different genetic backgrounds, and fine mapping. Marker validation can be done by testing markers closely linked to QTL in important breeding material, and by identifying markers that are diagnostic across a range of germplasm.

Limitations of previous marker technologies meant that chromosome coverage was relatively poor on linkage maps used for QTL mapping, causing estimation of QTL positions and effects to be imprecise. With the availability of highly parallel SNP arrays, denser maps can be developed and utilized, which should significantly improve precision in QTL estimation. It will also enable better comparisons between QTL positions identified in different populations for validation purposes.

Powdery Mildew

Wheat Powdery Mildew

Powdery mildew is a foliar disease of wheat caused by the obligate biotrophic ascomycete fungus *Bgt*. The disease typically thrives in mild, temperate climates with high relative humidity. Wheat powdery mildew is very common in Europe, parts of Asia, and the U.S., where it occurs annually and can cause significant yield losses (Cowger et al., 2012; Johnson et al., 1979; Leath and Bowen, 1989). Powdery mildew is characterized by the

development of white pustules on the leaf surface and occasionally on the stem. As the plant grows and the disease progresses, symptoms move from lower, older leaves to higher, younger leaves, and in severe cases can move up onto the wheat heads.

Powdery mildew can significantly decrease wheat yields by reducing photosynthetic capacity and competing for nutrients with the host plant. Powdery mildew infection is often associated with reduced tillering, poor tiller survival, reduced number of kernels per head, and decreased kernel weight (Johnson et al., 1979; Bowen et al., 1991; Everts and Leath, 1992). Test weight and grain protein content can also be impacted (Everts et al., 2001).

In fall-sown wheat, primary *Bgt* inoculum is thought to originate from cleistothecia (chasmothecia, or sexual fruiting bodies) produced at the end of the previous growing season (Götz et al., 1996). If winter conditions are mild, *Bgt* can also survive as mycelium and conidia.

The main period of ascospore release is thought to be fall (Turner, 1956), but this may be environmentally dependent. Prior to leaf senescence and cleistothecial production in early summer, *Bgt* undergoes multiple cycles of asexual reproduction via conidia (Tursumbaev, 1974). Under conducive conditions, *Bgt* mycelium produces chains of asexual conidia that are carried by wind and can serve as primary or secondary inoculum. Conidia land on the surface of plant leaves, mycelium grows on the surface of the host plant, a ‘peg’ is formed which penetrates the epidermal surface of the plant and facilitates development of haustoria (feeding structures) inside the plant cell.

Under optimal conditions, *Bgt* can complete its life cycle in ten days, which means several generations can be completed within a single crop season. *Bgt* has a very high

evolutionary potential due to its rapid life cycle along with the wide dispersal of airborne spores and a mixed sexual reproduction system allowing for genetic recombination among races with different virulence structures (McDonald and Linde, 2002; Wolfe and Schwarzbach, 1978).

Powdery mildew can be controlled by planting resistant cultivars or by applying fungicides such as propiconazole and strobilurin. Chemical control may be economical if the upper leaves have disease symptoms covering 5 to 10% of their leaf surface (Weisz, 2013). Foliar fungicides can delay disease epidemics and reduce yield losses, but these treatments are also costly and must be applied timely for good control. Use of cultivars with resistance to powdery mildew can provide complete resistance to the disease from seedling to adult plant stages. Genetic host resistance is often preferred over fungicide application, because it is more consistent, economical, and non-harmful to the environment.

Resistance to Powdery Mildew in Wheat

The most commonly deployed form of powdery mildew resistance is qualitative host resistance (reviewed by Cowger et al. (2012) and Huang and Roder (2004)). Most of the work on breeding for resistance to powdery mildew has been based on exploitation of race-specific major genes (*Pm* genes) that are expressed in seedlings and throughout the life cycle of the host plant. The *Pm* genes confer complete resistance, caused by a hypersensitive reaction, after infection with *Bgt* isolates carrying the corresponding avirulence gene. Over 70 *Pm* alleles at 44 loci have been formally designated (*Pm1* to *Pm54*) (Cowger et al., 2012; McIntosh et al., 2013; McIntosh et al., 2014; Hao et al., 2015). In addition, several temporarily designated powdery mildew resistance genes have been identified.

The major disadvantage of qualitative resistance is that pathogen populations can evolve to overcome widely deployed *Pm* genes over a short period of time. *Pm* genes usually do not remain completely effective for many years because of the rapid evolutionary potential of *Bgt* (McDonald and Linde, 2002; Parks et al., 2008). One example of an important virulence shift in North Carolina's powdery mildew population is the failure of *Pm4a* in 2002 and *Pm17* in 2009 (Cowger et al., 2009).

Incomplete resistance that slows down infection, growth, and reproduction of powdery mildew has also been identified in wheat. This type of resistance is commonly referred to as adult plant resistance (APR), 'slow mildewing', or partial resistance, and it behaves like a quantitative trait. APR is supposed to extend the latent period and reduce the sporulation of the fungus. According to Li et al. (2014), a total of 119 APR QTL have been identified in wheat.

Markers closely linked to *Pm* genes and APR QTL can be used in breeding with both qualitative and quantitative sources of resistance. MAS can be conducted using markers for *Pm* genes and APR QTL, allowing breeders to select resistant genotypes at an early stage, conduct targeted backcrossing, and pyramid different sources of resistance. By pyramiding multiple qualitative resistance genes or APR QTL, the durability of resistance is expected to be prolonged because the directional selection pressure on *Bgt* populations will be decreased. This will lead to an increase in the number of mutations needed for the pathogen to overcome all resistance genes and QTL present in the host plant (McDonald and Linde, 2002).

Sources of Resistance

Sources of powdery mildew resistance genes have included common wheat, rye (*Secale cereale*) (2n = 2x = 14, RR), and a number of wild relatives of wheat (Cowger et al., 2012; McIntosh et al., 2013; McIntosh et al., 2014). In many cases, resistance has been introgressed from wild relatives of wheat into elite common wheat backgrounds by crossing, occasionally requiring embryo rescue, and subsequently performing several backcrosses to the cultivated parent.

Resistance genes have been identified in species closely related to common wheat, such as the tetraploid emmer wheats (*T. dicoccum*, *T. dicoccoides*) and durum (*T. durum*) (2n = 4x = 28, AABB), and diploids *T. monococcum* (2n = 2x = 14, AA) and *T. tauschii* (2n = 2x = 14, DD), and in more distant relatives like *T. timopheevii* (2n = 4x = 28, AAGG), *Aegilops speltoides* (2n = 2x = 14, SS), *Ae. longisima* (2n = 2x = 14, SS) and *Haynaldia villosa* (2n = 2x = 14, VV) (Cowger et al., 2012; Huang and Roder, 2004).

Large germplasm collections containing hundreds of accessions are maintained by gene banks such as the USDA National Small Grains Collection (http://www.ars-grin.gov/npgs/) and The Harold and Adele Lieberman Germplasm Bank, at Tel Aviv University in Israel (http://www2.tau.ac.il/ICCI/). Information regarding species, collection year and site, and response to different *Bgt* isolates is available for numerous accessions in the Tel Aviv University germplasm bank. This is incredibly valuable, as researchers can request seed of accessions of interest, and use the resistant accessions in studies to identify and map new powdery mildew resistance genes, which can be utilized in breeding programs.

Fusarium Head Blight

Fusarium Head Blight of Wheat

Fusarium head blight (also referred to as 'ear blight' or 'head scab') is a serious fungal disease of wheat, caused by *Fusarium* species. The disease typically occurs in warm and humid climates. It can reduce grain yield and test weight significantly, but more importantly, it poses a great threat to food and feed safety due to mycotoxins produced by the fungus (Gale, 2003; McMullen et al., 2012). The mycotoxins produced by *F. graminearum* include: deoxynivalenol (DON) (also known as vomitoxin), nivalenol (NIV), zearalenon (ZEA), as well as other trichothecenes like T-2 toxin and HT-2 toxin (Gale, 2003). In addition to DON, *F. graminearum* strains may also produce modified forms of DON called 3-acetyl-DON (3-ADON) and 15-acetyl-DON (15-ADON). The toxicity of 15-ADON and 3-ADON is similar, and is about twice that of DON (Mirocha et al., 1989). In the U.S., DON is the primary mycotoxin found in *Fusarium* infected grains (McMullen et al., 1997).

In warmer regions of the world, including parts of North America, Australia, and central Europe, *F. graminearum* is generally regarded as the most important *Fusarium* species causing disease (Gale, 2003; Shaner, 2003). However, in the cooler maritime regions of Northwest Europe, *F. culmorum* tends to predominate (Snijders, 1990; Snijders and Perkowski, 1990), but *F. poae* is also of importance (Parry et al., 1995; Bottalico, 1998). *Fusarium avenaceum* has been isolated from diseased grain over a range of climatic zones (Parry et al., 1995).

The host range of *F. graminearum* includes cereals such as wheat, barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), and maize. FHB is initiated by conidia and ascospores

produced by *Fusarium* species. The primary source of inoculum is debris from previous crops, because all *Fusarium* species can survive as saprophytes (Champeil et al., 2004). The fungus survives winter as chlamydospores or hyphae (Parry et al., 1995). Conidia are initially produced in sporodochia on plant residues in the spring and early summer once the weather is warm and wet. Conidia are splash-dispersed to the heads of wheat plants, which are most susceptible to infection during flowering. Ascospores are produced in perithecia and can be dispersed by wind or water splashes. Under natural condition, maturation of perithecia takes 2-3 weeks (Champeil et al., 2004), and ascospore release typically occurs 1-3 days after a rain event. After conidia or ascospores have been dispersed to the head of a host plant, the spores will start to germinate and form a germ tube, and eventually hyphae that can penetrate the plant tissue. During flowering, anthers are pushed out from the wheat florets into an exposed, pendant position. During this elongation, the floret is open, which causes the floret interior to be vulnerable to the deposition of airborne *Fusarium* spores (Leonard and Bushnell, 2003; Kubo et al., 2013).

Although there can be differences in symptoms of FHB in wheat, the general symptoms include a water-soaked appearance of infected spikelets. The infected spikelets eventually lose the water-soaked appearance and become light brown (Bai and Shaner, 2004). The necrosis spreads from the infected spikelet to adjacent spikelets through the rachis, or to the entire wheat head. In warm, humid weather, pinkish-red mycelium and conidia develop abundantly in the infected spikelets. Small dark spots (perithecia) may also be seen on the infected spikelets. *Fusarium* infected wheat kernels often appear shriveled,

reduced in size and density, and usually have a white/gray or even pink color to them (also referred to as 'tombstones').

The production of mycotoxins by *Fusarium* species is believed to be a pathogenicity factor (Jansen et al., 2005). The association between FHB severity and DON accumulation in grain is not fully understood, but a meta-analysis showed that overall mean correlation coefficients for all relationships between DON and FHB intensity were highly significant (Paul et al., 2005). Cowger et al. (2009) found that extended post-flowering moisture can have a significant enhancing effect on both FHB severity and the accumulation of DON.

Intake of large amount of food products contaminated with mycotoxins can cause nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever in humans (Sobrova et al., 2010). Production animals, especially swine, are also sensitive to the mycotoxins. DON can cause indigestion and diarrhea in swine, and several of the other mycotoxins, especially ZEA, can have estrogen-like effects, reducing the swine's ability to reproduce. DON levels are regulated in food supplies of many countries: The European Commission limits DON levels to 1.25 ppm in unprocessed small grains, 0.5 ppm in breakfast cereals and bread, and 0.2 ppm in processed grain products used for baby food. In the U.S., advisory DON levels are limited to 1ppm for finished products for human consumption (U. S. Food and Drug Administration (FDA), http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm120184.htm). The FDA has not established an advisory level for wheat intended for milling because millers have different manufacturing practices and technologies available to them, which can substantially reduce

DON levels in the finished wheat products from those found in raw wheat (Aakre et al., 2005). In grains and grain by-products for adult cattle and for chickens, the advisory level is 10 ppm, with the added recommendation that these ingredients not exceed 50% of the diet. For swine, the advisory level is 5 ppm, with the added recommendation that these ingredients not exceed 20% of their diet. Finally, in grains and grain by-products destined for all other animals, the advisory level is 5 ppm, with the added recommendation that these ingredients not exceed 40% of their diet.

Grains contaminated with mycotoxins normally cannot be detoxified. If only a smaller part of a large portion of grain for feed is highly contaminated, it is recommended to destruct the smaller, infected part. Otherwise it can be mixed with larger portions of healthy grain to decrease the mycotoxin concentration (Aakre et al., 2005).

The occurrence and severity of FHB is influenced by a number of factors. The greatest factor is prolonged precipitation around and immediately after flowering, because it provides water splashes for conidia and ascospores to be transferred to the wheat heads (Xu, 2003). Precipitation also increases the relative humidity, which accelerates disease development. The risk of a severe FHB epidemic is increased when wheat is grown following corn or wheat, especially if reduced tillage or no-till practices are used, and inoculum is left in plant residues (Champeil et al., 2004). Planting of susceptible cultivars and irrigation can also significantly increase the risk of disease development.

Models have been developed to forecast the probability of an FHB epidemic of >10% severity (DeWolf et al., 2003) or to forecast DON concentrations in mature grain (Hooker et al., 2002). A forecasting website has been developed in the U.S.

(http://www.wheatscab.psu.edu/) which can be used to assess the risk of FHB development and severity based on weather observations. The prediction tool can help farmers decide if and when fungicides should be applied. The models behind the risk assessment tool are based on weather variables from approximately 12 days prior to anthesis to 10 days post-anthesis. Studies by Cowger and Sutton (2005) and Cowger et al. (2009) have shown that FHB severity and DON accumulation may be more strongly influenced by weather later in the grain-fill period. Daily rainfall right before harvest has also been identified as a critical factor in prediction of DON accumulation (Schaafsma and Hooker, 2007). In DON accumulation predictions, higher accuracy could be achieved by incorporating post-anthesis weather conditions in the model.

Key FHB control measures include careful crop rotation, soil tillage, planting of moderately resistant cultivars, and timely application of fungicides. Using only one of these strategies may not be efficient, and using all four at a time will not eliminate FHB development, but can significantly decrease the risk of a severe epidemic. Schaafsma and Hooker (2007) found that in wheat, environment effects accounted for 48% of the variation in DON levels across all sampled fields, followed by cultivar (27%), and previous crop (14 to 28%). Cultivation of *Fusarium* resistant cultivars plays a key role in integrated *Fusarium* control and the prevention of mycotoxin contamination. Breeding for improved FHB resistance has thus become an important breeding objective for the majority of wheat breeding programs in the Eastern U.S. and Canada.

Characterization of Resistance to FHB

Schroeder and Christensen (1963) first reported that resistance to head blight may occur at initial infection, penetration and hyphal invasion. Many studies have been conducted since then, and it is now well-known that resistance to FHB is a complex trait, highly affected by environment. Different types of resistance have been proposed: resistance to initial infection (incidence or Type I), resistance to spread within a head (severity or Type II), resistance to kernel damage, resistance to DON accumulation, and yield tolerance (Mesterhazy, 1995). Initially, many breeding programs focused on Type II resistance, but as DON threshold values have been lowered and direct economic penalties for farmers have been implemented (McMullen et al., 2012), the focus has shifted more toward resistance to accumulation of DON and reduction in *Fusarium*-damaged kernels (FDK) (Brown-Guedira et al., 2008).

Evaluation of lines for FHB resistance can be done under controlled conditions in a greenhouse or under field conditions. Greenhouse studies have most often involved point inoculation of single florets at flowering, and assessment of Type II resistance (Bai and Shaner, 2004). Field evaluations require a specialized nursery set-up with overhead irrigation, application of inoculum, and timely collection of FHB data. The advantage of conducting evaluations under field conditions is that, most often, data can be collected for all FHB resistance traits. The disadvantage of field evaluations is that FHB development may vary significantly between locations and years due to different weather and microclimate conditions, and it can therefore be a challenge to obtain consistent phenotypic data from every environment. Correlation coefficients between experiments conducted in different

environments can be used as indicators of the environment effects. Correlation coefficients for FHB severity ratings among field environments in different studies have ranged from 0.24 to 0.38 (Srinivasachary et al., 2008), 0.71 to 0.72 (Semagn et al., 2007), 0.50 to 0.84 (Schmolke et al., 2005; Holzapfel et al., 2008), while Bonin and Kolb (2009) reported a correlation coefficient for DON among two field trial environments of 0.33. In comparison, reported correlation coefficients for Type II resistance data collected over years in greenhouse studies, have ranged from 0.54 to 0.65 (Cativelli et al., 2013), 0.41 to 0.52 (Shen et al., 2003), and Liu et al. (2007) reported a correlation coefficient of 0.75 between two years.

Due to the quantitative nature of FHB resistance and the potential differences in disease development among tests, it is very important that lines are evaluated in replicated trials in multiple environments or greenhouse tests in order to obtain solid phenotypic data.

Mechanisms and Genetics of FHB Resistance

The underlying mechanisms of FHB resistance are not yet well understood. Bai and Shaner (2004) hypothesized that resistance probably involves a complex and interacting network of signaling pathways, and morphological traits such as plant height (Buerstmayr et al., 2000; Somers et al., 2003), presence or absence of awns (Mesterhazy, 1995), and cleistogamy (Kubo et al., 2013). Numerous studies have shown that inheritance of FHB resistance is quantitative, involving multiple minor effect genes (Buerstmayr et al., 2009). Additive effects play the most important role in resistance, but non-additive effects may also be significant (Snijders, 1990; Bai et al., 2000). Dominance seems to be the most important

non-additive component (Snijders, 1990), although epistatic effects have also been detected in some studies (Bai et al., 2000; Liu et al., 2013).

The most commonly used method to investigate FHB resistance has been a QTL mapping approach. Over 50 peer reviewed publications have presented results on more than 250 QTL associated with several FHB resistance traits (reviewed by Buerstmayr et al. (2009), Liu et al., (2009), and Loeffler et al. (2009)). One of these is the major effect QTL *Fhb1* (previously named *Qfhb.ndsu-3BS*) (Anderson et al., 2001; Liu et al., 2006). *Fhb1* has been reported to explain up to 60 % of the phenotypic variation in FHB severity (Anderson, et al., 2001; Bai et al., 1999; Zhou et al., 2002), and up to 93 % of variation in DON (Lemmens et al., 2005). Besides *Fhb1*, only few QTL have been reported to explain more than 20 % of variation for FHB resistance traits. Most QTL had estimated effects ranging from 3 to 29 % (Buerstmayr et al., 2009), with the majority being between 8 and 15 %.

Sources of Resistance

Fortunately, large genetic variation for FHB resistance is available in the wheat gene pool, but often the most well-adapted and highly productive cultivars are susceptible to FHB. The difficult task for wheat breeders is to create regionally adapted cultivars with high yield and good quality characteristics combined with FHB resistance. Genetic resistance to FHB has been found in germplasm native to the U.S., but especially in exotic germplasm from Asia, Europe, and South America. The majority of the resistance sources containing major effect QTL have been Asian spring wheats such as Sumai 3 (PI 481542) and its derivatives (Anderson et al., 2001; Buerstmayr et al., 2003; von der Ohe et al., 2010; Zhou et al., 2002). Many of these accessions possess *Fhb1* (Liu et al., 2006). Native U.S. sources of resistance

have included several eastern soft winter wheats such as: Ernie (PI 584525) (McKendry, et al., 1995), Truman (PI 634824) (McKendry et al., 2005), Bess (PI 642794) (McKendry et al., 2007), Freedom (PI 562382) (Gooding et al., 1997), Coker 9474 (PI 583357), Coker 9511 (PI 643095), Foster (PI 593689) (Van Sanford et al., 1997), Roane (PI 612958) (Griffey et al., 2001), McCormick (PI 632691) (Griffey et al., 2005a), Tribute (PI 632689) (Griffey et al. 2005b), NC-Neuse (PI 633037) (Murphy et al., 2004), Massey (CItr 17953) (Starling et al., 1984), and Jamestown (PI 653731) (Griffey et al., 2010).

Garvin and Anderson (2002) reported that approximately 60% of lines submitted to the Uniform Regional Scab Nursery for Spring Wheat between 1995 and 2002 had Sumai 3 or one of its derivatives in their pedigrees. In comparison, significantly fewer (20-30 %) of the entries were reported to have native sources of resistance in their pedigrees. Sumai 3 and other Asian sources of resistance may provide excellent levels of FHB resistance, but are not ideal for use in winter wheat breeding programs due to linkage drag and lack of adaption to winter wheat regions. Studies have shown that introgression of FHB resistance from Asian sources can be successful if several backcrosses are made to an elite recurrent parent. Von der Ohe et al. (2010) developed and tested two $BC_3F_{2:5}$ populations segregating for *Fhb1* and *Qfhs-ifa.5A*. The populations were evaluated for resistance to FHB as well as agronomic traits such as yield, heading date, and plant height at several locations in Germany. It was observed that the introgression of the QTL was associated with only small negative effects on agronomic traits. Similarly, Balut et al. (2013) evaluated five sets of inbred lines derived from two- and three-way crosses including the FHB-resistant parent VA01W-476 in Kentucky. VA01W-476 is a DH line derived from the cross Roane × W14, and it contains

both *Fhb1* and *QFhs.nau-2DL*. Significant negative effects of *Fhb1* and *QFhs.nau-2DL* on agronomic and quality traits were found.

Use of European resistance sources has also been limited because the lines and cultivars are not adapted to the U.S. soft red winter wheat region. Thus, the demand for use of native FHB resistance sources is strong because it can avoid complications of bad agronomics and the need for several generations of backcrossing.

QTL Mapping and MAS for FHB resistance

Considerable improvements in genetic resistance have been achieved by conventional selection resulting from phenotypic evaluations of breeding lines under greenhouse and field conditions. Phenotypic selection for FHB resistance is a resource intensive effort however, and MAS has been suggested as an alternative or complement to phenotypic selection (Griffey, 2005; Anderson, 2007). Identification of resistance QTL and tightly linked markers are vital for efficient utilization of MAS. Although MAS may not be able to replace phenotypic selection entirely, it can be very useful for selection of superior lines in years with insufficient disease development, where phenotypic selection cannot be performed. In addition, genotypic screening of advanced lines for known resistance QTL is a valuable supplement to phenotypic selection.

Among the FHB QTL mapping studies reported, most of the resistance sources have been exotic, primarily from Asia, but also from Europe (Buerstmayr et al., 2009; Liu et al., 2009). The most important QTL to date has been *Fhb1* on chromosome 3BS, which has been incorporated in most, if not all, wheat breeding programs focusing on FHB resistance. The

SSR marker most often used for screening of lines for *Fhb1* is *Xgwm533* (Anderson et al., 2001; Röder et al., 1998).

Results from FHB QTL studies have varied in terms of reliability of resistance QTL and their effects. The moderately resistant Swiss winter wheat line Arina was used in three different mapping studies (Draeger et al., 2007; Paillard et al., 2004; Semagn et al., 2007), but resulting QTL did not overlap. The QTL detected in the different Arina populations varied with the susceptible parents, which made the resistance in Arina less suitable for MAS. However, some cases of effective MAS for FHB resistance have also been reported. For example, Miedaner et al. (2006) introgressed and stacked two QTL from CM82036 (Sumai 3 / Thornbird), and one QTL from Frontana (Mardi et al., 2006), a Brazilian line, into elite European spring wheat material. They developed and evaluated 110 $F_{3:5}$ bulks with different combinations of alleles at the donor-QTL on chromosomes 3B and 5A (resistance alleles from CM82036), and chromosome 3A (resistance allele from Frontana). The bulks were evaluated for resistance to initial infection and spread (in a combined score) under field conditions after inoculation with *F. culmorum*, and for resistance to DON accumulation. All three individual donor-QTL alleles significantly reduced DON content compared to the marker class with no donor-QTL alleles. The donor-QTL alleles from CM82036 on chromosomes 3B and 5A also significantly reduced the FHB severity, whereas the donor-QTL allele from Frontana on chromosome 3A had a non-significant effect on FHB severity. In another study by Wilde et al. (2008) and Miedaner et al. (2009), three FHB resistance QTL were stacked through a double cross. The cross was made between two distinct FHB resistant sources and two elite winter wheat lines. One of the FHB resistant parents was a line

from a Dream/Lynx population (Schmolke et al., 2005). This line was crossed to Brandon. The other FHB resistant parent was a line from a G16-92/Hussar population (Schmolke et al., 2008). This line was crossed to LP235.1. The resulting F_1 lines were crossed and three different populations were developed: an unselected base population (C0), a population in which phenotypic selection had been applied (CP), and a third population in which selection based on markers associated with the FHB donor-QTL had been applied (CM). The mean FHB ratings were lower in both the CP and CM populations compared to the C0 population. The highest total selection gain for FHB was achieved with phenotypic selection, although the gain achieved with marker-based selection was very similar. The cycle length was one year shorter when using marker-based selection, which made the annual selection gain slightly higher for this method compared to phenotypic selection.

Currently, the USDA-ARS Eastern Regional Small Grains Genotyping Lab routinely screens winter wheat nurseries for the FHB resistance QTL *Fhb1* from Sumai 3, 2DL from the Chinese lines Wuhan-1 and W14 (Somers et al., 2003; Perugini, 2007), 5A from Ning 7840 (Gupta et al., 2001), 3BL from Massey (Liu et al., 2013), and 3Bc from Ernie (Liu et al., 2005; Liu et al., 2007; Liu et al., 2013). Some of the limitations to using more QTL in MAS have been a lack of mapped QTL in native sources of resistance, validation of QTL in additional populations, and identification of closely linked markers which are diagnostic in different genetic backgrounds (Brown-Guedira et al., 2008). The five QTL currently included in nursery screenings have all been identified using biparental mapping populations. The QTL *Fhb1* on chromosome 3BS was mapped and validated using Sumai 3 and related sources (Anderson et al., 2001; Balut et al., 2013; Buerstmayr et al., 2003; von der Ohe et al.,

2010; Yang et al., 2003; Zhou et al., 2002). Somers et al. (2003) mapped the QTL on chromosome 2D using a population of 91 doubled haploid lines from the cross Wuhan-1/ Maringa. The 3BL QTL from Massey was mapped in a RIL population of 152 individuals derived the cross Becker / Massey (Liu et al., 2013). A QTL associated with Type II resistance in Ernie was mapped to 3Bc, based on evaluations in the greenhouse (Liu et al., 2007), and was also found associated with resistance under field conditions (Liu et al., 2013). Buerstmayr et al. (2003) mapped the 5AS QTL in Ning 7840. Quantitative trait loci on chromosomes 3BS and 5AS from W14 were validated in a doubled haploid population with 96 individuals (Chen et al., 2006).

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CHAPTER 2 - Mapping of Powdery Mildew Resistance Gene *Pm53* Introgressed from *Aegilops speltoides* into Soft Red Winter Wheat

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Mapping of powdery mildew resistance gene *Pm53* introgressed from *Aegilops speltoides* into soft red winter wheat

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Abstract

Powdery mildew of wheat (*Triticum aestivum* L.) is a major fungal disease in many areas of the world, caused by *Blumeria graminis* f.sp. *tritici* (*Bgt*). Host plant resistance is the preferred form of disease prevention because it is both economical and environmentally sound. Identification of new resistance sources and closely linked markers enable breeders to utilize these new sources in marker-assisted selection as well as in gene pyramiding. *Aegilops speltoides* (2n=2x=14, genome SS), has been a valuable disease resistance donor. The powdery mildew resistant wheat germplasm line NC09BGTS16 (NC-S16) was developed by backcrossing an *Ae. speltoides* accession, TAU829, to the susceptible soft red winter wheat cultivar 'Saluda'. NC-S16 was crossed to the susceptible cultivar 'Coker 68-15' to develop $F_{2:3}$ families for gene mapping. Greenhouse and field evaluations of these $F_{2:3}$ families indicated that a single gene, designated *Pm53*, conferred resistance to powdery mildew. Bulked segregant analysis showed that multiple simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers specific to chromosome 5BL segregated with the resistance gene. The gene was flanked by markers *Xgwm499*, *Xwmc759*, *IWA6024* (0.7 cM proximal) and *IWA2454* (1.8 cM distal). *Pm36*, derived from a different wild wheat relative (*T. turgidum* var. *dicoccoides*), had previously been mapped to chromosome 5BL in a durum wheat cultivar. Detached leaf tests revealed that NC-S16 and a genotype carrying *Pm36* differed in their responses to each of three *Bgt* isolates. *Pm53* therefore appears to be a new source of powdery mildew resistance.

Keywords

Wheat, *Blumeria graminis* f.sp. *tritici*, plant genetic resources.

Conflict of Interest: The authors declare that they have no conflict of interest.

Author contribution statement

S. Petersen: Collected phenotypic and genotypic data, analyzed data, performed gene mapping.

J.H. Lyerly: Assisted in collection of genotypic data.

M.L. Worthington: Assisted in collection of phenotypic data.

W.R. Parks: Provided powdery mildew BGT isolates for experiments; assisted with differential leaf test.

C. Cowger: Provided powdery mildew BGT isolates for experiments; assisted with differential leaf test; provided guidance throughout this study.

D.S. Marshall: Provided guidance throughout the study.

G. Brown-Guedira: Provided guidance in collection of genotypic data.

J. P. Murphy: Developed the germplasm line NC-S16; provided guidance throughout this study.

Key message

A powdery mildew resistance gene was introgressed from *Aegilops speltoides* into winter wheat and mapped to chromosome 5BL. Closely linked markers will permit marker-assisted selection for the resistance gene.

Introduction

Powdery mildew of wheat (*Triticum aestivum* L.) is caused by the obligate, biotrophic fungal pathogen *Blumeria graminis* (syn. *Erysiphe graminis*) DC. f.sp. *tritici* (*Bgt*) Em. Marchal. This ascomycete infects the foliage, stem, and spikes of the wheat host. *Bgt* typically thrives in mild, temperate climates with high relative humidity. Wheat powdery mildew is very common in Europe, parts of Asia, and the southeastern part of the United States, where it occurs annually and can cause significant yield losses. In years with major powdery mildew epidemics, yield losses have ranged from 17 to 34 percent in Maryland and North Carolina (Johnson et al. 1979; Leath and Bowen 1989). Severe powdery mildew infection can cause yield loss by reducing number of tillers, number of grains, kernel weight, and grain protein content (Johnson et al. 1979; Parry 1990; Bowen et al. 1991; Everts et al. 2001).

Host plant resistance is often preferred over alternative disease management methods, such as fungicide application, because it is more consistent, economical, and environmentally sound. The most commonly deployed form of powdery mildew resistance is race-specific, or qualitative, host resistance (Huang and Roder 2004). Over 70 powdery mildew resistance (*Pm*) alleles at 44 loci have been formally designated (*Pm1* to *Pm49*) (Cowger et al. 2012; McIntosh et al. 2013). In addition, 18 temporarily designated powdery mildew resistance genes have been identified. These temporarily designated genes usually need to undergo further allelism tests to determine whether they are alleles at novel or previously established *Pm* loci. Several sources of resistance genes have come from wild relatives of wheat (Cowger et al. 2012; McIntosh et al. 2013). One source of resistance genes is *Aegilops*

speltoides Tausch, the closest extant relative of the wheat B genome (Luo et al. 2005). To date, two *Pm* genes have been derived from *Aegilops speltoides*: *Pm12* (Miller et al. 1988) and *Pm32* (Lapochkina et al. 1996; Hsam et al. 2003). *Pm12* has been mapped to chromosome 6B (Jia et al. 1996), and *Pm32* was mapped to chromosome 1B (Hsam et al. 2003).

As powdery mildew populations are very dynamic and continually change virulence structure, *Pm* genes in current cultivars are constantly challenged and often defeated (McDonald and Linde 2002; Parks et al. 2008). Wheat breeders and geneticists are therefore persistently searching for new sources of resistance to powdery mildew to incorporate in breeding programs (Miller et al. 1988; Lapochkina et al. 1996; Schneider et al. 2008; Gill et al. 2011).

Pyramiding several major genes into a single cultivar disrupts directional selection pressure, and therefore is expected to improve the durability of resistance. When a cultivar with one major resistance gene is deployed over large areas, it will cause directional selection, e.g. pathotypes virulent to the resistance gene will increase in frequency, whereas avirulent pathotypes will decrease in relative frequency. However, when cultivars with multiple resistance genes are deployed, it should take longer for pathogen populations to achieve virulence because of the increased number of mutations needed to overcome all resistance genes present in the host cultivar (McDonald and Linde 2002; Huang and Roder 2004). Of course, *Bgt* populations undergo frequent sexual recombination, which could facilitate the eventual defeat of pyramided *Pm* genes.

In order to optimize selection for *Pm* resistance genes, breeders can utilize molecular markers in close linkage with the genes. Marker-assisted selection (MAS) can be a faster and less expensive alternative to phenotypic selection in the greenhouse or field, it can be done in the absence of the pathogen, and it facilitates pyramiding of multiple resistance genes. To date, SSR markers have been the most commonly used markers in MAS, but recently SNP markers have become available. SNPs are even more abundant than SSRs and are often present within genes, which makes them functional or perfect (Somers et al. 2004). In the past few years, both a 9,000 SNP chip platform and a 90,000 SNP chip platform have been developed for wheat and linkage maps based on SNP data have been published (Cavanagh et al. 2013; Wang et al. 2014). The development of Kompetitive Allele Specific PCR (KASP) single-tube assays (LGC Genomics, Hoddesdon, UK) suitable for selecting for a single gene or trait on a large number of genotypes (Chen et al. 2010) makes SNPs very useful in gene mapping and MAS. KASP assays are fast to run and easy to interpret, making development of genotyping assays based on KASP technology desirable. So far, only one temporarily designated *Pm* gene, *MlUM15*, has been mapped using SNP genotyping via KASP assays (Worthington et al. 2014).

The objectives of this study were to determine the inheritance, chromosomal location, and molecular markers linked to powdery mildew resistance in the *Aegilops speltoides* derived germplasm line NC-S16.

Materials and Methods

Plant Materials

The soft red winter wheat germplasm line NC09BGTS16 (NC-S16) (PI 669386) is a BC_2F_7-derived germplasm line derived from the pedigree Saluda*3/TAU829. TAU829 is an *Aegilops speltoides* accession obtained from Gene Bank of Tel Aviv University, Israel. It was collected in Haifa (Technion) in the Mount Carmel region of Israel in 1985. Its accession number on the Tel Aviv University Gene Bank website is AEG-829-15 (http://www2.tau.ac.il/ICCI/default.asp). 'Saluda' (PI 480474) is a soft red winter wheat cultivar developed by Virginia Polytechnic Institute and State University (Starling et al. 1986). Saluda contains *Pm3a*, a defeated major gene for powdery mildew resistance (Niewoehner and Leath 1998), and is now susceptible to the naturally occurring powdery mildew population in North Carolina (Parks et al. 2008).

NC-S16 and Coker 68-15 (CItr 15291), a susceptible cultivar with no known *Pm* genes, were crossed in 2008. The F_1 hybrid was self-pollinated to produce F_2 seeds in the greenhouse during the 2009-10 winter. F_2 plants were grown in separate pots in the greenhouse and harvested individually without selection in the 2010-11 winter to produce $F_{2:3}$ families used in subsequent disease evaluations.

Disease Evaluations in Greenhouse

One hundred and forty $F_{2:3}$ families were evaluated for resistance to powdery mildew in the greenhouse during November 2011 in a randomized complete block design experiment with two replicates. Each experimental unit consisted of three 10 cm pots planted with a total

of fifteen seeds of a given $F_{2:3}$ family. Parent lines Coker 68-15 and NC-S16, as well as susceptible check Saluda, were planted in ten-pot intervals along the greenhouse bench. Greenhouse temperatures were held at 24/18 °C (day/night) and high-intensity 1000-W discharge lights supplemented natural light.

The powdery mildew *Bgt* isolate 'Arapahoe' was used as the inoculum source. It was obtained from the *Bgt* collection maintained by the USDA-ARS Plant Science Research Unit at North Carolina State University. Arapahoe was virulent to *Pm1c, Pm3a, Pm3c, Pm3e, Pm3g, Pm4a*, *Pm5a, Pm5b*, *Pm5d, Pm6, Pm7*, *Pm9*, and *Pm20* and avirulent to *Pm1a*, *Pm3b*, *Pm3d, Pm3f, Pm4b*, *Pm8, Pm12*, *Pm16, Pm17*, *Pm21*, *Pm25*, and *Pm37*, (personal observation). The inoculum was increased on Saluda plants under greenhouse conditions prior to setting up the disease evaluation experiment. $F_{2:3}$ seedlings were inoculated 19 and 20 days after planting at Zadoks growth stages 13 to 20 by gently shaking conidia from leaves of infected Saluda plants onto the foliage of the $F_{2:3}$ families, parent lines, and susceptible checks.

Disease reactions were scored 10 and 11 days after inoculation following the 0-9 rating scale developed by Leath and Heun (1990). Plants scored 0-3 were considered resistant according to this scale, where 0 = an immune reaction with no visible signs of infection, 1 = chlorotic flecks with no necrosis, 2 = chlorotic flecks with some necrosis, and 3 = mild chlorosis and barely detectable mycelium. Rating between 4 and 6 were indicative of an intermediate disease reaction, with the degree of chlorosis and visible mycelium and conidial chains increasing from slight to moderate. Plants rated between 7 and 9 were scored as susceptible with increasing amounts of mycelium and conidia. Each of the fifteen plants

within an experimental unit were scored individually and compared with the parental lines and susceptible check. The $F_{2:3}$ families were classified as homozygous resistant, homozygous susceptible or segregating depending on their phenotypic reactions. Chi-square tests were performed to test the goodness-of-fit between observed and expected segregation ratios.

Disease Evaluations in Field

Each of the 140 $F_{2:3}$ families were planted at the Lake Wheeler Road Field Laboratory south of Raleigh, NC, and the Cunningham Research Station in Kinston, NC. Seeds from each family were planted in a randomized complete block experiment, in which each experimental unit consisted of a single 1.2-meter row. Each row was planted with 40 to 60 seeds from a given $F_{2:3}$ family. The rows were spaced at 30.5 cm intervals. Two replications per family were planted at both locations. Rows of NC-S16, Coker 68-15, and Saluda were included at 60-plot intervals as resistant and susceptible controls. The experiments were bordered by rows of Saluda to promote an even spread of powdery mildew. The planting dates were October 22, 2011 at the Cunningham Research Station site and October 31, 2011 at the Lake Wheeler Road Field Laboratory site. Fertilization, irrigation, and other agronomic treatments followed standard management procedures for North Carolina (Weisz 2013).

Disease ratings were made in the middle of March 2012 when plants had reached Zadoks Growth Stage 39 to 50 and powdery mildew symptoms had developed uniformly at the two field sites. Each plot was evaluated on the 0-9 rating scale used in previous greenhouse screening (Leath and Heun 1990) and categorized as either homozygous

resistant, segregating, or homozygous susceptible. Chi-squared tests were performed to test the goodness of fit between the observed segregation ratios in the field and the expected segregation ratios. One $F_{2:3}$ line that was scored as segregating in the greenhouse was resistant in the field, but scores were otherwise consistent among trials. This difference was presumably due to seed contamination, so this line was excluded from the analysis.

A powdery mildew differential test in an adjacent field at the Lake Wheeler Road Field Laboratory showed that wheat lines carrying *Pm2*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm3f*, *Pm4a*, *Pm5a*, *Pm17*, and *Pm20* were susceptible to naturally occurring *Bgt* isolates, and lines carrying *Pm1c*, *Pm3d*, *Pm3e*, *Pm3g*, *Pm5d*, *Pm12*, *Pm13*, *Pm16*, *Pm21*, *Pm25*, *Pm34*, *Pm35*, and *Pm37* were resistant. Wheat lines carrying *Pm4b*, *Pm5b*, *Pm6*, *Pm7*, and *Pm8* displayed adult plant resistance (Cowger, personal observation).

Molecular Marker Analysis

Genomic DNA was extracted from leaf tissue collected from the F_2 plants from which the $F_{2:3}$ families originated. DNA was extracted according to the CTAB protocol described by Stein et al. (2001). Ten $F_{2:3}$ families with consistently resistant phenotypes and ten families with consistently susceptible phenotypes were chosen for bulk segregant analysis (BSA) (Michelmore et al. 1991). DNA from the ten resistant families was pooled into a resistant bulk, and DNA from the ten susceptible families was pooled into a susceptible bulk. The resistant parent, resistant bulk, susceptible bulk, and susceptible parent were then screened with genome- and chromosome specific markers from across the wheat genome. Markers polymorphic between the resistant parent and bulk, on the one hand, and the susceptible parent and bulk on the other, were considered tentatively linked to the resistance

gene. These markers were then used to screen the entire F_2 population. Once the chromosome with the resistance gene was identified, additional polymorphic markers specific to that chromosome were evaluated across the population.

The SSR primers *Xwmc537* and *Xwmc759* were ordered according to their sequences in GrainGenes (http://wheat.pw.usda.gov) with M13 sequence tags (5'-CACGACGTTGTAAACGAC-3') attached to the 5' end for universal fluorescent labeling (Schuelke 2000). The EST-derived SSR marker *BJ261635* (Blanco et al. 2008) with forward primer (5'-TAGCCTGGTACCATTCTGCC-3') and reverse primer (5'-TGTAATGGAGGTGCAGCTTG-3') was also ordered with M13 sequence tags.

SSR primers *Xgwm499, Xgwm408, Xcfd7, and Xwmc75* were direct-labeled with NED, FAM, PET, or VIC. PCR conditions were performed as described by Miranda et al. (2006). Products from markers with M13 sequence-tagged primers were run on polyacrylamide gels following procedures described in Miranda et al. (2006). Direct-labeled SSR products were run on an ABI PRISM 3730 DNA Analyzer (Applied Biosystems) and scored with Peak Scanner 2 (Life Technologies, Carlsbad, CA).

SNP markers were selected from the Illumina Infinium 9K SNP array (Cavanagh et al. 2013) based on map location and polymorphism between Coker 68-15 and NC-S16, with the NC-S16 alleles at low frequencies among 558 eastern winter wheat lines genotyped with the 9K array. Source sequences of the selected SNP markers were used for development of KASP assays. Primers were designed using Primer Picker software with default settings (LGC Genomics, Hoddesdon, UK). KASP assays included two allele-specific forward primers with tail sequences and one common reverse primer (Table 2.1). Assay mix

preparation and PCR protocols were conducted according to LGC Genomics protocols. Fluorescent endpoint genotyping was done using a LightCycler 480 (Roche Applied Sciences, Indianapolis, IN).

All markers were checked for distortion from expected segregation ratios using chi-squared tests. Linkage analysis was performed using MAPMAKER/EXP 3.0 (Lincoln et al. 1993). The Kosambi mapping function estimated centimorgan (cM) distances between markers (Kosambi 1944). The chosen marker order was selected based on maximum likelihood estimates, and compared to SNP and SSR consensus map positions (Somers et al., 2004; Cavanagh et al. 2013) and physical location of markers on the 5B wheat deletion bin map. Genetic linkage maps were drawn using MapChart 2.1 software (Voorrips 2002).

Deletion Bin Mapping

The chromosomal location of each linked marker was confirmed using Chinese Spring (CS) and chromosome 5BL aneuploid lines (provided by the Kansas State University Wheat Genetics Resource Center). The aneuploids tested included nullisomic 5B-tetrasomic 5A (N5BT5A), ditelosomic5BL (Dt5BL), and deletion lines 5BL-2 (FL 0.26), 5BL-6 (FL 0.29), 5BL-1 (FL 0.55), 5BL-14 (FL 0.75), and 5BL-16 (FL0.79). The name specifies the chromosome arm carrying the deletion followed by its arbitrary line number designation and the fraction length of the arm present in brackets.

Detached Leaf Evaluations

Detached leaf segments were used to test for differential response to 80 *Bgt* isolates between NC-S16 and '5BIL-29', a durum wheat line containing *Pm36*, located on

chromosome 5BL. *Pm36* was introgressed from wild emmer, *T. turgidum* var. *dicoccoides* (2n = 4x = 28, AABB) into durum wheat (Blanco et al. 2008). About half a million years ago hybridization between the two diploid species *T. urartu* Tumanian (2n = 2x = 14, AA) and *Ae. speltoides* gave rise to the tetraploid wheat *T. turgidum* var. *dicoccoides* (Huang et al. 2002), which is considered the wild progenitor of tetraploid and hexaploid wheats.

The lines NC-S16 and 5BIL-29 were included in detached leaf tests. Seeds of 5BIL-29 are maintained by the USDA-ARS at North Carolina State University. Cultivars Coker 68-15, Jagalene, Saluda, and Chancellor (CItr 12333) were used as susceptible controls.

The *Bgt* isolates used in this test were from powdery mildew samples collected in different years at various locations in the U.S. and abroad, and are maintained by the USDA-ARS Plant Science Research Unit at North Carolina State University. The isolates were chosen because they were known to have broad virulence spectra.

Several 1.5 cm leaf segments of each of the wheat lines under study were placed on the surface of each Petri dish containing 0.5% water agar supplemented with 50 mg/L benzimidazole. Each Petri dish was inoculated with conidia from a single *Bgt* isolate propagated on leaf segments of Chancellor. Two replicate plates were inoculated with each isolate. Four additional replicate plates were inoculated with isolates PRA-A-3-1, MOB-B-1-2, and C1-4 in a follow-up test because these were the only isolates for which NC-S16 showed an intermediate reaction in the original test. The Petri dishes were placed in a growth chamber at 85% humidity and 18 °C temperature with a 12 h photoperiod. Disease reactions were scored 10 days later according to Leath and Heun (1990), where 0 = an immune reactions with no visible signs of infection, 1 = chlorotic flecks with no necrosis, 2 =

chlorotic lesions, 3 = necrotic lesions, 4 = first signs of mycelium, 5 = 1-2 pustules per leaf segment, 6 = less than 20% of leaf area covered by pustules, 7 = 20-50% of leaf area covered in pustules, 8 = 50-90% of leaf area covered in pustules, 9 = 100% of leaf area covered in pustules. Lines with an average score of 3 or lower were considered resistant (R), while lines with an average score over 3 and under 6 were considered intermediate (I), and lines with an average score greater than 6 were considered susceptible (S).

Analyses of variance (ANOVA) were conducted using PROC GLM in SAS 9.3 (SAS 2011) using genotype as a fixed variable and replication as a random variable. Least significant differences (LSD) were also calculated using SAS 9.3.

Results

Inheritance of Resistance

Coker 68-15 and Saluda were consistently susceptible to *Bgt* isolate Arapahoe in the greenhouse, with a mean disease score of 8. NC-S16 was resistant with mean disease score of 0.2. The 140 $F_{2:3}$ families were resistant, segregating, and susceptible at a 1:2:1 ratio (χ^2 = 3.66; P = 0.16) (Table 2.2) as expected for a trait controlled by a single gene. The pooled numbers of resistant and susceptible plants within segregating lines did not fit a 3:1 ratio (1223 resistant:464 susceptible, χ^2 = 5.64; P = 0.02), suggesting the resistance in NC-S16 might be conferred by a single, partially dominant allele. NC-S16 was also highly resistant (mean disease score 0.5) to powdery mildew infection by naturally occurring *Bgt* isolates at Cunningham Research Station and Lake Wheeler Field Laboratory in the spring of 2012. Coker 68-15 (mean disease score of 8) and Saluda (mean disease score of 8) were both

susceptible to *Bgt* populations at both field locations. The $F_{2:3}$ families at both Lake Wheeler and Cunningham field sites were resistant, segregating, and susceptible in a 1:2:1 ratio (χ^2 = 4.16; P = 0.13) (Table 2.2). The single partially dominant resistance gene identified in NC-S16 was designated *Pm53*.

Molecular Mapping of Powdery Mildew Gene in NC-S16

Over 325 genome- and chromosome-specific molecular markers were initially used to screen for polymorphisms between resistant and susceptible parents and bulks. SSR marker *Xwmc537*, previously mapped to the long arm of chromosome 5B (Somers et al. 2004), was polymorphic between the resistant and susceptible bulks. Therefore, additional markers specific to chromosome 5BL were tested for polymorphism. The SSR markers *Xwmc759*, *Xgwm499*, and *Xgwm408* were polymorphic between resistant and susceptible parents and bulks. All four polymorphic SSR markers were used to screen the F_2 population. Other SSR markers previously linked to *Pm36* (Blanco et al. 2008) on chromosome 5BL (*Xcfd7*, *BJ261635*, and *Xwmc75*) were tested for polymorphism, but were not polymorphic between the resistant and susceptible parents. Six SNPs previously mapped to chromosome 5BL (Cavanagh et al. 2013) were polymorphic between NC-S16 and Coker 68-15 (*IWA2698*, *IWA1441*, *IWA6024*, *IWA2454*, *IWA5669*, and *IWA6516*). These six SNPs were also evaluated across the entire F_2 population.

Xwmc537, *IWA1441*, *Xwmc759*, *IWA6024*, *IWA2454*, and *IWA5669* were dominant markers linked to the powdery mildew resistance gene. Markers *IWA2698*, *Xgwm499*, *Xgwm408*, and *IWA6516* were scored as co-dominant. All co-dominant SSR and SNP markers segregated in the expected 1:2:1 ratio, while the dominant markers segregated in the

expected 3:1 ratio. The most likely order of the linked markers (Figure 2.1) was generally in agreement with the SNP (Cavanagh et al., 2013) and the SSR (Somers et al. 2004) consensus maps.

The powdery mildew resistance gene *Pm53* was putatively assigned to the long arm of chromosome 5B, based on the reported chromosomal locations of the four linked SSR markers and the six linked SNP markers (Somers et al. 2004; Cavanagh et al. 2013) (Figure 2.1). Using Chinese Spring deletion bin lines, its physical location on chromosome 5BL was confirmed by all ten markers. Five Chinese Spring 5BL deletion lines were used to identify the sub-chromosomal location of the loci amplified by each of the markers. *Xwmc537* was the only marker that amplified the Chinese Spring fragment in the 5BL-6 deletion line, indicating that it was physically located in the bin between fraction length 0.00 and 0.29. The other markers did not amplify any fragments in the 5BL-6 deletion line, but amplified fragments in deletion lines 5BL-1, 5BL-14, and 5BL-16, which meant they mapped to the bin 5BL-6 (FL 0.29-0.55). Due to the tight linkage between the powdery mildew resistance gene and several SSR and SNP markers, we postulated that the *Pm53* gene is located in bin 5BL-6 (FL 0.29-0.55).

The *Triticum turgidum* var. *dicoccoides* derived *Pm* gene *Pm36* was previously mapped to bin 5BL-6 (FL 0.29-0.76) (Blanco et al. 2008). Of the SSR markers closely linked to *Pm36*, three (*Xwmc75*, *Xcfd7*, and *BJ261635*) were not polymorphic between NC-S16 and Coker 68-15, so these could not be placed on the linkage map for the NC-S16 / Coker 68-15 mapping population. *Xgwm408* was polymorphic, however, and mapped 4.9 cM distal from *Pm53*.

Marker *BJ261635* was tested with NC-S16, Coker 68-15, Saluda, and 5BIL-29 (with *Pm36*). The marker produced one fragment of size 241 bp in NC-S16, Coker 68-15, and Saluda. In 5BIL-29, two fragments of sizes 241 and 248 bp were produced. This 7 bp difference between the two fragments from 5BIL-29 was consistent with results from Blanco et al. (2008) for the resistant parent, as well as homozygous resistant and heterozygous plants from their segregating population. They found the smaller fragment to be ubiquitous across samples, and the larger fragment to be co-segregating with *Pm36* (mapped 0.3cM from the gene), matching the results observed in our test.

Detached Leaf Test

Following inoculation with 3*Bgt* isolates, the disease response pattern of NC-S16 differed from the pattern of 5BIL-29 with *Pm36*, and those of the susceptible checks Coker 68-15, Saluda, Jagalene, and Chancellor (Figure 2.2). Across 12 to 18 replications, NC-S16 consistently showed intermediate responses to isolates C1-4, PRA-A-3-1, and MOB-B-1-2, with scattered sparse pustules and minor chlorosis, whereas 5BIL-29 consistently showed resistant responses to these three isolates (Table 2.3). Random placement of the various genotypes on a plate confirmed that inoculation across the plate had been even (i.e., no escapes) and reaction type was independent of proximity to a susceptible or resistant genotype (Figure 2.2). The differential responses of 5BIL-29 and NC-S16 to isolate MOB-B-1-2 illustrated in Figure 2.2 were similar for isolates C1-4 and PRA-A-3-1 as well. The mean disease score for NC-S16 was significantly different from that of 5BIL-29 for all three isolates at $P < 0.05$. Both NC-S16 and 5BIL-29 were resistant to all other *Bgt* isolates in the test.

In addition to the three isolates shown in Table 2.3, recent tests showed that several *Bgt* isolates from Missouri and at least one isolate from Alabama produced intermediate reactions in NC-S16, while 5BIL-29 showed an immune response (unpub. data).

Discussion

In the present study, a powdery mildew resistance gene was introgressed from *Aegilops speltoides* into soft red winter wheat. Greenhouse and field experiments confirmed that resistance was conferred by a single gene. The resistance gene was mapped to chromosome 5BL, in bin 5BL-6 (0.29-0.55), and tightly-linked SSR and SNP markers were identified. The resistance gene was flanked by markers *IWA6024*, *Xgwm499*, and *Xwmc759* (0.7 cM proximally) and *IWA2454* (1.8 cM distally). *Xwmc759* was scored as a dominant marker. *Xgwm499* might therefore be the preferred SSR marker to use in a genotyping assay. NC-S16 is the first soft red winter wheat line with an introgressed *Pm* gene on chromosome 5BL. It should provide breeders with an effective new source of resistance that can be tracked with SNPs and/or SSRs in MAS. Furthermore, it will allow for pyramiding of *Pm53* with *Pm* genes at other loci.

In addition to providing powdery mildew resistance, *Aegilops speltoides* has served as a source of resistance to leaf rust, including *Lr28* (McIntosch et al. 1982), *Lr35* (Kerber and Dyck 1990), *Lr36* (Dvorak and Knott 1990), *Lr47* (Dubcovsky et al. 1998), and *Lr51* (Dvorak 1977; Dvorak and Knott 1980; Helguera et al. 2005); green bug resistance (Dubcovsky et al. 1998); and stem rust resistance, including *Sr32* (McIntosh et al. 1995b; Friebe et al. 1996), *Sr39* (Kerber and Dyck 1990; McIntosh et al. 1995b), *Sr47* (Faris et al. 2008) , and *SrAes7t* (Klindworth et al. 2012). Out of nearly 200 *Aegilops speltoides*

accessions collected from five regions of Israel, almost all accessions were highly resistant to leaf rust, stripe rust, and stem rust when tested in both Israel and Minnesota, USA (Anikster et al. 2005). It is therefore likely that additional genes for disease resistance for use in wheat breeding could be found in accessions of *Aegilops speltoides*.

Only one other known *Pm* gene, *Pm36*, has been mapped to the long arm of chromosome 5B (Blanco et al. 2008). *Pm36* was introgressed from *T. turgidum* var. *dicoccoides* into durum wheat (2n = 4x = 28). It is unlikely that the same gene is shared by *T. turgidum* var. *dicoccoides* and *Aegilops speltoides* given the amount of time that each of the resistance genes has evolved independently since the polyploidy formation events hundreds of thousands years ago (Huang et al. 2002; Jauhar 2007). No example has been reported of the same resistance gene being found in wild emmer and an *Aegilops* species, except for *Aegilops tauschii* (DD genome) (McIntosh, pers. comm.). Because *Pm36* was introgressed to tetraploid durum wheat and NC-S16 is hexaploid soft red winter wheat, performing a classic allelism test was not considered practical.

Blanco et al. (2008) reported that the EST-derived SSR marker *BJ261635* was in close linkage with *Pm36* (0.3 cM). Generally, EST-SSR markers show high transferability between alien species of wheat (Gupta et al 2003). We found that NC-S16 did not amplify the fragment linked to resistance contributed by *Pm36*, indicating that the resistance gene in NC-S16 is different from *Pm36.* Also, while marker *Xgwm408* mapped 14.8 cM distal from *Pm36* according to Blanco et al. (2008), it was mapped only 4.9 cM distal from *Pm53*.
In the detached leaf test, NC-S16 consistently showed intermediate reactions to three *Bgt* isolates across 12 to 18 replications, while 5BIL-29 showed immune responses to the same

isolates. The fact that additional *Bgt* isolates from a certain geographical region conferred intermediate responses in NC-S16, but not in 5BIL-29, could be a sign of mutation to partial virulence against *Pm53*.

Taken together, the genotypic and phenotypic data lead us to conclude that *Pm53* is different from *Pm36*.

Extensive production of wheat cultivars with only one *Pm* gene places strong directional selection pressure on the pathogen population, and copious spore production provides ample potential for mutations to virulence, resulting in rapid appearance and increase of adapted isolates. The high evolutionary potential of the *Bgt* pathogen contributes to the low durability of race-specific resistance, forcing breeders and pathologists to constantly search for new sources of resistance and improved resistance deployment strategies. The identification of this novel *Pm* gene and closely linked SNPs will be useful for breeders, especially if it can be used in combination with *Pm* genes at other loci and/ or quantitative sources of resistance. NC-S16 is currently being used as a parent in the wheat breeding program at North Carolina State University, and breeding lines with *Pm53* and overall good agronomic performance are being tested in the field. Further development of these materials should result in new, powdery mildew resistant cultivars for growers in the southeastern US.

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Table 2.1. Primers for KASP assays used to screen the NC09BGTS16 (NC-S16) x Coker 68-15 population

SNP[¶] no.	SNP name	Primer A1[•]	Primer A2[•]	Primer C1
IWA1441	wsnp_Ex_c11131_18036595	TTCTTGCCCGTTCTTAGTGAGAGA	CTTGCCCGTTCTTAGTGAGAGC	TGAACTTCTCAAACTGAACGGTTCAGTTA
IWA2454	wsnp_Ex_c19724_28721128	GGAACCTTGAATGTTTCCCATTCCA	GAACCTTGAATGTTTCCCATTCCG	CCTCCCGGCAATATGCCTCCAA
IWA2698	wsnp_Ex_c2224_4171424	CATCATTAGCTTCAGGTAGTTCACC	CATCATTAGCTTCAGGTAGTTCACT	GCCATATGCAGAAATATCAGCCGCTA
IWA5669	wsnp_Ex_rep_c70120_69069699	GTTTCCCAACCTGTTCCCAGTCA	TCCCAACCTGTTCCCAGTCG	AGATCCAGAAAACCAGTCGCTGGAA
IWA6024	wsnp_JD_c37023_27225840	CGACAACATCAACCCTCCTCTAT	CGACAACATCAACCCTCCTCTAC	GTACTTTAGTCGATGAACTGCTAGCTTTA
IWA6516	wsnp_Ku_c14202_22436656	CCTCAAGCACCACAACGCCG	ACCTCAAGCACCACAACGCCA	TTCCTACACTTCCCTAGTTACAGAGAAA

[¶] SNP, single nucleotide polymorphism. The prefix *IWA* is followed by an index number.

[•] Primer sequences are given without the FAM (A1) and VIC (A2) tails.

Table 2.2. Segregation ratios for disease reaction of $F_{2:3}$ families from the NC-S16 x Coker 68-15 population evaluated in the greenhouse and two field sites in North Carolina in spring 2012

	Number of $F_{2:3}$ families					
Location	**Resistant**	**Segregating**	**Susceptible**	**Total**	**χ^2 (1:2:1)**	**P value**
Greenhouse	43	70	27	140	3.66	0.16
Cunningham	44	69	27	140	4.16	0.13
Lake Wheeler	44	69	27	140	4.16	0.13

Table 2.3. Differential reactions of NC-S16, Coker 68-15, and 5BIL-29 possessing *Pm36* previously mapped to chromosome 5BL to isolates of *Bgt* evaluated in a detached leaf test

		Isolate[•]		
Genotype	***Pm* gene**	**C1-4**	**MOB-B-1-2**	**PRA-A-3-1**
NC-S16	*Pm53*	4.1[A]	4.2[A]	3.1[A]
5BIL-29	*Pm36*	0.1[B]	0.2[B]	0.2[B]
Coker 68-15	None	8.1[C]	8.1[C]	8.0[C]
Chancellor[¶]	*Pm10, 15*	7.8[D]	7.9[C]	7.7[C]
No. of replications	-	14	18	12
LSD	-	0.32	0.53	0.82

[•] Disease reactions were scored on a 0-9 scale according to Leath and Heun (1990)

[¶] Chancellor = susceptible check; *Pm10* and *Pm15* are universally susceptible (McIntosh et al. 1995a)

[A B, C, D] Values within a column followed by different letters are significantly different at $P < 0.05$

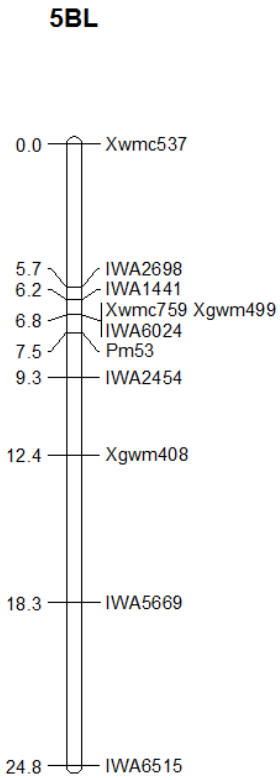

Figure 2.1. Genetic linkage map of the powdery mildew resistance gene *Pm53* on the introgressed *Ae. speltoides* segment in line NC-S16. *Xwmc537*, *Xwmc759*, *Xgwm499*, and *Xgwm408* are SSR markers; *IWA2698*, *IWA1441*, *IWA6024*, *IWA5669*, and *IWA6516* are SNP markers. Kosambi map distances (cM) are shown on the left side of the map. The software program MapChart 2.1 was used to produce this figure.

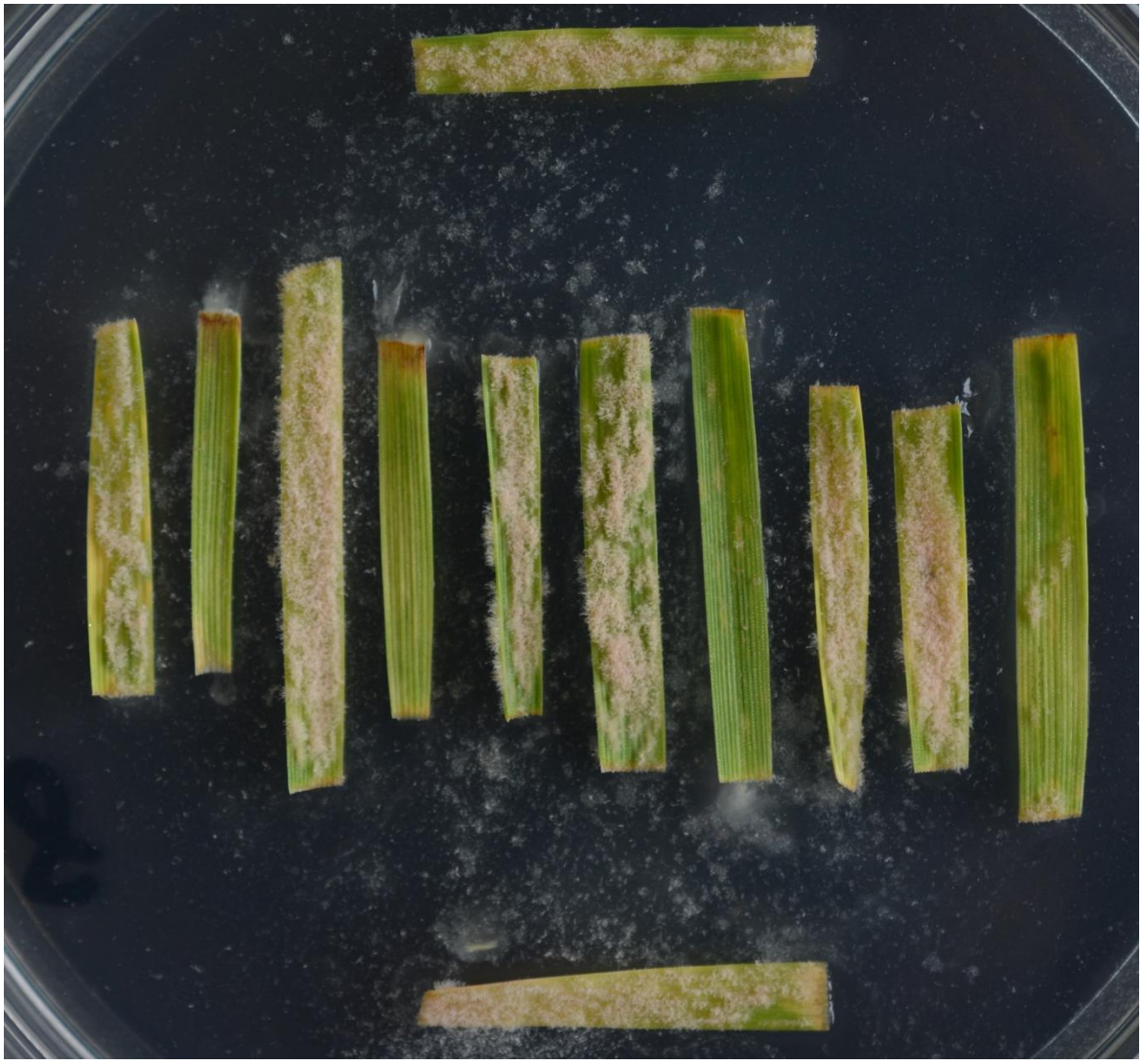

Figure 2.2. Virulence test with randomly placed detached leaf segments of genotypes (from left) Jagalene, 5BIL-29, Coker 68-15, 5BIL-29, Saluda, Coker 68-15, NC-S16, Jagalene, Saluda, and NC-S16. The segments at the top and bottom are susceptible check Chancellor. Image shows the responses to inoculation with *Bgt* isolate MOB-B-1-2 nine days after inoculation.

CHAPTER 3 - Mapping of *Fusarium* Head Blight Resistance QTL in Winter Wheat Cultivar NC-Neuse

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Abbreviations:

CIM, composite interval mapping; DArT, diversity array technology; DH, doubled haploid; DON, deoxynivalenol; FDK, *Fusarium*-damaged kernels; FHB, *Fusarium* head blight; HD, heading date; HT, height; INC, incidence; KASP, Kompetitive Allele Specific PCR; LOD, logarithm of odds; MAS, marker-assisted selection; MIM, multiple interval mapping; PCR, polymerase chain reaction; QTL, quantitative trait loci; RIL, recombinant inbred line; SEV, severity; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; USWWSN, Uniform Southern Winter Wheat Scab Nursery.

Abstract

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum*, can significantly reduce the grain quality of wheat (*Triticum aestivum* L.) due to mycotoxin contamination. The objective of this study was to identify quantitative trait loci (QTL) for FHB resistance in the moderately resistant soft red winter wheat cultivar NC-Neuse using a population developed from a cross between NC-Neuse and the susceptible cultivar AGS 2000. A total of 170 recombinant inbred lines (RILs) were evaluated following inoculation with *F. graminearum* infected corn kernels in mist-irrigated field nurseries. The lines were evaluated for FHB incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) content in seven environments between 2011 and 2014. A 3,419 cM linkage map was developed based on 1839 simple sequence repeat (SSR), diversity array technology (DArT), and single nucleotide polymorphism (SNP) markers. Composite Interval Mapping (CIM) and Multiple Interval Mapping (MIM) analyses for adjusted means within and across environments identified seven FHB resistance QTL on chromosomes 1A, 1B, 1D, 2A, 4A, 5B, and 6A. The QTL alleles conferring resistance on 1A, 1B, 2A, 4A, and 6A originated from NC-Neuse. The QTL alleles associated with resistance on chromosomes 1D and 5B originated from AGS 2000. QTL effects ranged from 9 to 12% for INC, from 6 to 11% for SEV, from 8 to 20% for FDK, and from 6 to 18% for DON. At the QTL on chromosome 1B, susceptibility was associated with the 1BL.1RS wheat-rye translocation from AGS 2000. The QTL on 5B co-localized with the *Vrn-B1* locus. Kompetitive Allele-Specific PCR (KASP) assays were developed for each NC-Neuse QTL region. A preliminary test using these assays on recent Uniform Southern Winter Wheat Nursery (USWWN) entries

indicated *Qfhb.nc-1A* and *Qfhb.nc-6A* as likely the best candidates for use in marker-assisted selection.

Introduction

Fusarium head blight (FHB), also referred to as head scab, is one of the most damaging diseases of wheat. The main pathogen causing FHB in North America is *Fusarium graminearum* (Gale, 2003; Shaner, 2003). *Fusarium* head blight reduces grain yield, grain quality, and contaminates grain with the mycotoxin deoxynivalenol (DON) and other trichothecenes (Gale, 2003). Since the 1990s, severe outbreaks of FHB have occurred in the U.S. (McMullen et al., 1997). Nganje et al. (2004) estimated that the direct economic effects from FHB in the U.S. between 1998 and 2000 came to a total of 734 million dollars, with even greater secondary economic losses. Due to financial penalties and food safety concerns associated with high mycotoxin levels in grain, it is very important to identify ways of reducing FHB in the wheat crop, particularly the accumulation of DON (McMullen et al., 2012).

One of the key FHB control measures is genetic resistance in the host plant (Bai and Shaner, 2004). Inheritance of FHB resistance has proven to be complex because it involves multiple genes whose effects may vary in different genetic backgrounds. Different types of resistance have been proposed: resistance to initial infection (incidence or Type I), resistance to spread within a head (severity or Type II), resistance to kernel damage (Type III), resistance to DON accumulation (Type IV) (Lemmens et al., 2005), and yield tolerance (Mesterhazy, 1995).

Genetic resistance to FHB has been found in germplasm native to the U.S., but especially in exotic germplasm from Asia, Europe, and South America. The majority of resistance sources have been Asian spring wheats such as Sumai 3 (PI 481542) and its derivatives (Anderson et al., 2001; Zhou et al., 2002; Buerstmayr et al., 2003; Von der Ohe et al., 2010). Many of these possess the major effect QTL *Fhb1* (Liu et al., 2006). Additionally, over 50 peer reviewed publications have presented results on more than 250 QTL associated with several FHB resistance traits (reviewed by Buerstmayr et al., 2009; Liu et al., 2009; Loeffler et al., 2009). *Fhb1* is the QTL with the biggest effect reported to date, but due to linkage drag (Balut et al., 2013), its incorporation in U.S. winter wheat cultivars has been limited. The demand for identification and mapping of resistance in native, well-adapted sources has therefore increased. Genetic studies involving U.S. soft red winter wheat sources have included the cultivars Ernie (Liu et al., 2005; Liu et al., 2007; Abate et al., 2008; Liu et al., 2013), Massey (Liu et al., 2013), Goldfield (Gilsinger et al., 2005), Freedom (Gupta et al., 2001), IL 94-1653 (Bonin and Kolb, 2009), and VA00W-38 (Liu et al., 2012).

Phenotypic selection for FHB resistance is a resource-intensive effort, and marker-assisted selection (MAS) has been suggested as an alternative or complement to it (Griffey, 2005; Anderson, 2007). Identification of resistance QTL and tightly linked markers are vital for efficient utilization of MAS.

Currently, the USDA-ARS Eastern Regional Small Grains Genotyping Laboratory routinely screens winter wheat breeding nurseries for the FHB resistance QTL *Fhb1* from Sumai 3, 2DL from Wuhan-1 or W14 (Somers et al., 2003), 5A from Ning 7840 (Gupta et al., 2001), 3BL from Massey (Liu et al., 2013), and 3Bc from Ernie (Liu et al., 2005; Liu et

al., 2007; Liu et al., 2013). One of the limitations to using more QTL in MAS has been a lack of mapped QTL in native sources of resistance (Brown-Guedira et al., 2008).

The objectives of this study were to: 1) identify and map QTL for FHB resistance in NC-Neuse by evaluating a recombinant inbred line (RIL) population from the cross NC-Neuse / AGS 2000, 2) identify closely linked markers and develop assays to use in MAS and allele enrichment in breeding programs, 3) evaluate these assays on lines from recent Uniform Southern Winter Wheat Scab Nurseries to estimate NC-Neuse resistance allele frequencies and single QTL effects, and (4) compare the identified QTL with known QTL from exotic and native sources of resistance.

Materials and Methods

Plant Materials

An F_5-derived RIL population with 170 individuals was developed from a cross between the soft red winter wheat cultivars NC-Neuse, which is moderately resistant to FHB, and AGS 2000, which is susceptible to FHB. NC-Neuse (PI 633037) was released in 2003 from North Carolina State University (Murphy et al., 2004). The cultivar possesses the height reducing allele (*b*) at the *Rht-D1* locus, a null allele at photoperiod locus *Ppd-B1*, and the allele for long vernalization at the *vrn-B1* locus, and the 2BS:2GS:2GL:2BL translocation chromosome. AGS 2000 (PI 612956) was released jointly from the University of Georgia and University of Florida in 1999 (Johnson et al., 2002). The cultivar possesses the height reducing allele (*b*) at the *Rht-D1* locus, the insensitivity allele (a) at the photoperiod locus *Ppd-B1*, the allele for short vernalization at the vernalization locus *vrn-B1*, and the 1BL.1RS

translocation. Both cultivars have the allele for photoperiod sensitivity (*b*) at the *Ppd-D1* photoperiod locus, and the allele for insensitivity (*a*) at the *Ppd-A1* photoperiod locus.

Phenotypic Evaluations

The population parents and RILs were evaluated for FHB in seven environments under mist-irrigation: Cunningham Research Station in Kinston, NC in field seasons 2010-11, 2011-12, 2012-13, and 2013-14; Lake Wheeler Field Laboratory, Raleigh, NC, in seasons 2012-13, and 2013-14; and Salisbury, Maryland in season 2011-12. The location/year combination will from here on be referred to as 'KIN', 'LW', and 'MD', followed by the last two digits of the harvest year. The entries were planted in mid to late October in 1.2 meter single-row plots, with 15 cm spacing between plots. A randomized complete block design with two or three replicates was utilized in all environments. In Kinston, NC and Salisbury, MD the plots were planted into corn debris to promote disease development. Fertilization, irrigation, and other agronomic treatments followed standard management procedures for North Carolina (Weisz, 2013) and Maryland.

Plots in Kinston and Raleigh, NC were inoculated with three applications of autoclaved corn kernels infected with *F. graminearum* isolates collected from the previous year's crop (four g $plot^{-1}$ $application^{-1}$). Applications were made at one-week intervals starting three weeks before the average heading date of entries in the nursery. In Salisbury, MD, plots were inoculated with a single application of scabby corn kernels (twelve g $plot^{-1}$) three weeks prior to heading. Overhead mist irrigation was used at all locations to promote disease development, and was terminated once FHB symptoms started appearing on the wheat heads.

Data on INC and SEV were collected about 21 days after anthesis. Incidence was scored by counting the number of infected heads from a subsample of about 35 primary heads from the middle of a plot (thereby evaluated as a percentage of infected heads in each plot). Disease severity was rated using a modified Horsfall-Barrett scale with ten categories developed by Stack and McMullen (2011). Plant height (HT) was measured for population parents and RILs in MD12, LW13, KIN13, LW14, and KIN14, and heading dates (HD) were recorded in all environments. The HD was recorded when 50% of primary heads in a plot were fully emerged from the flag leaf sheath.

At crop maturity, wheat heads were harvested from the center 60 to 90 cm of each 1.2 meter plot, dried, and threshed using an Almaco stationary thresher (Almaco; Nevada, Iowa) without a fan. Samples were cleaned by hand using sieves, in front of table-top fans set at low speed to ensure minimal loss of *Fusarium*-damaged seeds. Percent FDK was estimated by separating and counting the number of diseased kernels out of the total number of kernels in a grain sample, and subsamples underwent DON analysis at the University of Minnesota.

Data Analysis

Data were evaluated using SAS statistical software version 9.3 (SAS Institute Inc., 2012) (Appendix C). Initial evaluation included checking for violations of the assumptions for analysis of variance and linear mixed models through the use of PROC UNIVARIATE and QQPLOT. These analyses showed that the distribution of SEV, FDK, and DON were right-skewed and that a logarithmic transformation was necessary to achieve normality. The traits INC, HD, and HT were not transformed.

Experimental factors included genotype, environment, and replicate within environment. An environment was defined as a combination of location and year because some locations were not represented in all years. Analysis of variance was conducted using the SAS procedure PROC MIXED (Littell et al., 2006), with all factors (environment, replication within environment, genotype, and genotype x environment) treated as random effects.

Due to significant genotype x environment interactions, adjusted means for use in QTL analyses were estimated using PROC MIXED within and across all environments for INC, SEV, FDK, DON, HD, and HT. Genotype was treated as a fixed effect factor to obtain the phenotypic mean of the genotype adjusted for all non-genetic effects. Heading date was included as a covariate by adding HD to the model as a fixed effect factor. In the models for across environments analyses, HD was nested within environment as the relative value of HD was only explanatory within the environment where it was measured.

Heritabilities of all FHB resistance traits, HD, and HT were estimated on a per-plot basis and on an entry-mean basis according to Holland et al. (2002). SAS code from the website http://www4.ncsu.edu/~jholland/heritability/Inbreds.html was modified for this purpose.

The PROC CORR procedure in SAS was used to calculate all phenotypic correlations.

Genotypic Data Collection

Leaf tissue from 5-8 seedlings of parents and each RIL was pooled and genomic DNA was extracted using a CTAB protocol (Stein et al., 2001). DNA concentrations were quantified and diluted to 50 ng μL^{-1}. For each sample a 20 µL DNA aliquot was sent to

Diversity Arrays P/L for Diversity Array Technology (DArT) marker genotyping using the high density wheat array of 7000 probes (www.diversityarrays.com).

The population was also genotyped with 176 polymorphic SSR markers, which had been identified through a screen of 402 markers on the population parents. Sequences for SSR primers were obtained from GrainGenes 2.0 (http://wheat.pw.usda.gov/GG2/index.shtml). Polymerase chain reactions were conducted as described by Maloney et al. (2011). In PCR reactions for analysis on the ABI3730 Genetic Analyzer (Applied Biosystems, Foster City, CA), the forward primer was labeled directly with FAM, NED, PET, or VIC. In PCR reactions for analysis on the LI-COR 4300 DNA Analysis System (LI-COR Biosciences, Lincoln, NE), forward primers were modified to include the M13 sequence (5'-CACGACGTTGTAAAACGAC-3') on the 5' end for universal fluorescent labeling. For the KASP markers, assay mix preparation and PCR protocols were conducted according to LGC Genomics protocols (LGC Genomics, Hoddesdon, UK). Fluorescent endpoint genotyping was done using a LightCycler 480 (Roche Applied Sciences, Indianapolis, IN).

Finally, the population was genotyped with the Illumina 9K SNP chip (Cavanagh et al., 2013). SNP calls were made using Illumina's GenomeStudio Data Analysis Software (Illumina, San Diego, CA). Markers with more than 20% missing data were excluded, and segregation distortion was analyzed for all markers using a chi square test (expected 1:1 segregation ratio).

Map Construction and QTL Analyses

A genetic linkage map was constructed using MapMaker/Exp version 3.0 (Lincoln et al., 1993) according to the software protocol. The threshold for log-likelihood (LOD) was initially set at 15.0 to develop tight, core linkage groups. Once core linkage groups were established, the LOD was set to 5.0 to add more markers to each group. Final map orders and distances were calculated using the Kosambi function (Kosambi, 1944) and default LOD score of 3.0. Linkage groups were assigned to chromosomes using information from the wheat SSR consensus map (Somers et al., 2004) and DaRT consensus map (www.diversityarrays.com). The 9K SNP consensus map (Cavanagh et al., 2013) and the 90K SNP consensus map (Wang et al., 2014) were used to verify the positions of SNP markers.

QTL analyses for traits within environments and across environments were conducted using Composite Interval Mapping (CIM) and Multiple Interval Mapping (MIM) in Windows QTL Cartographer 2.5 (Wang et al., 2004). Result files from CIM were used for the MIM analyses. The critical LOD value to declare QTL significance was 3.2, based on 1000 permutations. Only additive effects were estimated.

KASP Assay Development

A set of KASP assays was developed based on peak markers in each of the resistance QTL regions, where NC-Neuse alleles provided resistance. Primers were designed using Primer Picker software with default settings (LGC Genomics, Hoddesdon, UK). KASP assays included two allele-specific forward primers with tail sequences and one common reverse primer. Assay mix preparation and PCR protocols were conducted according to LGC

Genomics protocols. Fluorescent endpoint genotyping was done using a LightCycler 480. The KASP assays were run on the population parents and a subset of RILs to test whether the assays would give the same results as the original SNP calls. It was thus possible to determine which KASP assays created tight, differentiated clusters for easy interpretation.

To obtain an estimate of the frequencies of SNP associated with NC-Neuse FHB resistance alleles and test QTL effects in a set of lines other than our mapping population, representative KASP assays from each NC-Neuse QTL region were run on 116 entries (including four check cultivars) from the 2013 and 2014 Uniform Southern Winter Wheat Scab Nursery (USWWSN). The USWWSN is an annual collaborative program to evaluate resistance to FHB in breeding lines submitted by public and private breeding programs. Nursery cooperators evaluate entries in mist-irrigated inoculated nurseries and record data on INC, SEV, FDK, DON, and HD. Annual reports including phenotypic data, genotypic information about previously identified QTL (*Fhb1*, 2DL from Wuhan-1 or W14, 5A from Ning7840, 5A from Ernie, and 3BL from Massey), and a list of the locations and cooperators involved are available at http://scabusa.org/pdfs_dbupload/suwwsn13_report.pdf and http://www.scabusa.org/pdfs_dbupload/suwwsn14_report.pdf.

Analysis of variance was conducted using PROC MIXED to test effects of NC-Neuse QTL and previously identified QTL from other resistance sources among the 2013 and 2014 USWWSN entries. Only disease means for each entry were available from each test location, therefore locations were treated as replications. Model terms included QTL, genotype nested within QTL, year, replication nested within year, and residual error. Similar to the analysis method described by Balut et al. (2013), QTL and genotype nested within QTL were treated

as fixed effect factors, while year and replication within year were treated as random effect factors. Adjusted means were generated and compared for each QTL and for each disease trait.

Additionally, a set of 344 inbred soft winter wheat landraces, breeding lines, and cultivars from the eastern U.S., referred to as the Eastern Soft Red Winter Wheat panel, was also utilized to estimate NC-Neuse resistance allele frequencies. Lines from most of the eastern U.S. public breeding programs and a few private programs were included in this panel. The lines were genotyped with the Infinium iSelect 9K SNP chip array (Cavanagh et al., 2013).

Results

Phenotypic Evaluations

Data were analyzed from seven environments: KIN11, KIN12, KIN13, KIN14, LW13, LW14, and MD12. The RIL population displayed normal distributions for adjusted means of INC, SEV, FDK, and DON across environments after transformation of SEV, FDK, and DON (Figure A.1).

Significant genotypic effects were found for all resistance traits in the across-environments analysis (Table A.1) and in all individual environments (data not shown). The variance components for all random-effect factors (environment, replication within environment, genotype, and genotype x environment interaction) for all four disease traits were significantly different from zero (Table A.1). The high levels of variation associated

with environment were likely due to the fact that the environments were relatively diverse – both in terms of geography, but also in timing and intensity of disease development.

NC-Neuse had significantly lower mean values for SEV, FDK, and DON compared to AGS 2000 averaged over environments, while INC was not significantly different between the two parents (Table A.2). There were transgressive segregants for all four disease traits, meaning there were several RILs with resistance significantly better than NC-Neuse, and several RILs with susceptibility significantly higher than AGS 2000.

In all individual environments, except MD12, NC-Neuse had disease ratings significantly lower than or similar to AGS 2000 (Table A.2). In the MD12 environment, NC-Neuse had a significantly higher mean INC than AGS 2000. This could suggest that early lines escaped the disease to some degree, possibly because of unfavorable conditions for disease development early in the season. Nevertheless, NC-Neuse had significantly lower SEV in comparison to AGS 2000 in this environment.

The mean HDs of NC-Neuse and AGS 2000 were significantly different averaged over environments (Table A.2). NC-Neuse had a mean HD five days later than AGS 2000. The two cultivars carry different alleles at the *Ppd-B1* and *Vrn-B1* loci (Guedira et al., 2014). The range between the earliest and the latest heading RILs was ten days averaged over all environments. AGS 2000 was taller than NC-Neuse, although not significantly, and there was significant genotypic variation within the population. AGS 2000 and NC-Neuse both carry the *Rht-B1a* and *Rht-D1b* alleles, and variation in HT between lines would therefore be expected to be due to other loci affecting HT.

The entry mean heritabilities for INC, SEV, FDK, and DON were moderate to high; INC had the lowest heritability (0.66), while DON had the highest heritability (0.82) (Table 3.1). The entry mean heritabilities for HD (0.94) and HT (0.83) were high.

Pairwise Pearson correlations between adjusted means for the four disease traits were highly significant (r = 0.70 to 0.91, $P < 0.0001$); the highest correlation was observed between FDK and DON (r = 0.91), and the lowest was between INC and SEV (r=0.70) and SEV and DON (r = 0.70) (Table 3.2). Pairwise correlations between the four disease traits and HD ranged from 0.10 to 0.47 (Table 3.2). Positive correlations ($P < 0.0001$) were found between HD and the disease traits INC, FDK, and DON. For these traits, a later heading date was associated with higher disease scores. Pairwise correlations between adjusted means for the disease traits and HT were non-significant for all but HT and DON (r = 0.29, $P = 0.0001$) (Table 3.2). A positive correlation (r = 0.41, $P < 0.0001$) between HD and HT was also observed, indicating that later lines generally were taller.

Despite our attempt to reduce the effect of HD through multiple inoculation dates, HD had significant effect on several disease traits across environments. Phenotypic means adjusted for effects of HD were generated using HD as a covariate in the model and subsequently used in the QTL analysis.

Linkage Mapping

A total of 1839 markers were placed on the genetic linkage map consisting of 27 linkage groups, covering a total map length of 3,419 cM (Table D.1). All chromosomes were represented, except chromosome 4D. Of the 1839 markers, were 83 SSRs, 302 DArTs, 1452 SNPs, and two KASP assays for the major gene loci *Vrn-B1* and *Ppd-B1*. Severe segregation

distortion and lack of recombination was observed for markers on the short arm of chromosome 1B (position 0-78.6 cM). This result was probably due to the presence in AGS 2000 of the 1BL.1RS translocation. Markers from this region were included on the linkage map despite the distortion to represent the chromosome arm. Within the 1B chromosome arm with marker distortion, the NC-Neuse alleles were overrepresented (allele frequency ~0.70). Transmission of normal 1B over 1BL.1RS has previously been observed (Rubio et al., 1999). Severe segregation distortion and lack of recombination in the RILs was observed for markers on chromosome 2B (position 0-103.8). The NC-Neuse alleles in this area were significantly overrepresented (allele frequency of ~0.85) compared to the AGS 2000 alleles. These results were probably due to the presence in NC-Neuse of the 2BS·2GS·2GL·2BL translocation chromosome. Reduced recombination and preferential transmission has been associated with this translocation in other mapping populations (Nyquist 1962; Tsilo et al., 2008). The markers with distorted segregation ratios were included on the linkage map to represent the chromosomal region. Some markers displaying distorted segregation ratios were also included on linkage groups 3D.2, 5D.2, and 7D.2 to help cover large unrepresented areas, as these groups generally had poor marker coverage.

Quantitative Trait Loci Mapping

Quantitative trait loci were considered reproducible if they were associated with resistance in multiple environments and/or with multiple disease resistance traits. A total of seven reproducible FHB resistance QTL were mapped to seven different chromosomes (Table 3.3); the NC-Neuse allele provided resistance at five of these loci, and the AGS 2000 allele was associated with resistance at two loci.

The NC-Neuse allele of the QTL *Qfhb.nc-1A* decreased INC, SEV, and DON based on adjusted means across environments, INC, FDK, and DON in KIN13, and DON in KIN14. In the analysis across environments, this QTL explained 9.3% of the variation for INC, 5.9% of the variation for SEV, and 8.7% of the variation for DON. In KIN13, it explained 10.8% of the variation for INC, 11.4% of the variation for FDK, and 10.6% of the variation for DON. In KIN14, *Qfhb.nc-1A* explained 10.9% of the variation for DON.

At the *Qfhb.nc-1B* QTL, the NC-Neuse allele decreased FDK and DON across environments, FDK and DON in MD12, and DON in LW14. This QTL explained 6.6% of the variation for FDK and 7.8% of the variation for DON in the analysis across environments. In MD12, *Qfhb.nc-1B* explained 9.5% of the variation for FDK, and 7.1% of the variation for DON. In LW14, the QTL explained 5.7% of the variation for DON. *Qfhb.nc-1B* QTL mapped within the area on chromosome 1B displaying a distorted marker segregation ratio.

The NC-Neuse allele of the QTL *Qfhb.nc-2A* decreased SEV across environments, SEV in KIN13, and DON in KIN12. The QTL explained 9.7% of the phenotypic variation for SEV across environments, 9.4% for SEV in KIN13, and 7.8% of the variation for DON in KIN12.

At the *Qfhb.nc-4A* QTL, the NC-Neuse allele decreased FDK across environments, FDK in KIN13 and LW14, and DON in LW14. The QTL explained 7.8% of the variation for FDK in the across environments analysis, 19.5% for FDK in KIN13, 11.4% for FDK in LW14, and 11.5% for DON in LW14.

The NC-Neuse allele of the QTL *Qfhb.nc-6A* decreased SEV across environments, INC in LW14, and SEV in KIN11. The QTL explained 5.9% of the phenotypic variation for SEV in the across environments analysis, 10.7% for SEV in KIN11, and 11.1% for INC in LW14.

Three RILs had the AGS 2000 (susceptible) alleles at all five NC-Neuse QTL, while six RILs had the NC-Neuse (resistant) alleles at all five loci (Table 3.4). With each additional resistance allele, INC, SEV, FDK, and DON values were generally reduced. Compared to the means of the group with no resistance alleles, INC was reduced by 28%, SEV by 33%, FDK by 56%, and DON by 59% in the group of RILs containing all five resistance alleles from NC-Neuse.

The AGS 2000 allele of the QTL *Qfhb.nc-1D.2* decreased FDK and DON across environments, and FDK and DON in LW14. The QTL explained 10.7% of the variation for FDK in LW14, 9.5% of FDK across environments, 10.8% of the variation for DON in LW14, and 8.8% for DON across environments. The AGS 2000 allele of the QTL *Qfhb.nc-5B* reduced INC in MD12, and DON in KIN12 and MD12. This QTL explained 12.0% of the variation for DON in MD12, 18.2% of the variation for DON in KIN12, and 10.3% for INC in MD12. The *Qfhb.nc-5B* QTL range includes the *Vrn-B1* locus, at which AGS 2000 carries the allele for short vernalization (Guedira et al., 2014).

Quantitative trait loci associated with HD were mapped to chromosomes 2B (the QTL interval overlapped with the *Ppd-B1* locus), 4A, 5B (the QTL interval overlapped with the *Vrn-B1* locus), and 7D.1 (Table 3.5). The QTL *Qhd.nc-4A* mapped to interval 65.9 – 86.3 cM, and did not overlap with the resistance QTL *Qfhb.nc-4A* at position interval 127.2 –

138.0 cM. The QTL associated with plant height were mapped to chromosomes 3B, 5B, and 6A (Table 3.6). The QTL *Qht.nc-6A*, associated with plant height mapped to the interval 65.7 – 118.4 cM, and did not co-localize with the resistance QTL *Qfhb.nc-6A*, which mapped to 127.2 – 138.0 cM. Since AGS 2000 and NC-Neuse both carry *Rht-B1a* and *Rht-D1b*, the RIL population was not segregating for these *Rht* genes.

Test of KASP Assays

Fifteen KASP assays were chosen to represent the QTL regions on chromosomes 1A, 1B, 2A, 4A, and 6A (Table 3.7). When genotyping the entries from the USWWSN, a line was categorized as having the resistance allele at *Qfhb.nc-1A* for example if it had the NC-Neuse allele for *IWA3805*, *IWA1587*, and *IWA886*. Similarly, lines were categorized as having the resistance allele at *Qfhb.nc-1B* if they had the NC-Neuse allele for *IWA7977*, *IWA6448*, *IWB43992*, and *IWA4987*.

Among the 116 entries from the 2013 and 2014 USWWSN, the frequencies of the NC-Neuse resistance alleles at each of the QTL regions were: 0.53 for *Qfhb.nc-1A*, 0.16 for *Qfhb.nc-1B*, 0.05 for *Qfhb.nc-2A*, 0.37 for *Qfhb.nc-4A*, and 0.13 for *Qfhb.nc-6A*. When comparing phenotypic means of USWWSN entries with and without the NC-Neuse resistance alleles identified in this study, *Qfhb.nc-1A* was associated with significantly reduced SEV, FDK, and DON (Table 3.8). *Qfhb.nc-1B* was associated with reduced INC, FDK, and DON. Of the seventeen entries with *Qfhb.nc-1B*, only one also possessed *Fhb1*. Five entries were categorized as having the *Qfhb.nc-2A* QTL, which was associated with reduced SEV, FDK, and DON. Out of the five entries, two entries had *Fhb1* and the 2DL QTL from Wuhan-1, and another entry had *Fhb1*. The small sample size and the presence of

these other FHB QTL may therefore have affected the disease means significantly. *Qfhb.nc-4A* was associated with a significant increase in SEV and FDK. *Qfhb.nc-6A* was associated with a significant reduction in SEV, FDK, and DON. Of the thirteen entries with the *Qfhb.nc-6A* QTL, one entry had *Fhb1*, the 2DL QTL from Wuhan-1, and the 5A QTL from Ning8740, while two other entries had *Fhb1*. Among the non-NC-Neuse QTL evaluated in the USWWSN, the 5A QTL from Ning8740, the 2DL QTL from Wuhan-1 or W14, and *Fhb1* were associated with significantly reduced SEV, FDK, and DON. The 3BL QTL from Massey was not associated with different group means, while the 5A QTL from Ernie was associated with a significant increase in INC, SEV, FDK, and DON. Due to the relative small sample sizes of entries with resistance alleles in each QTL group, testing combinations of QTL was not considered valid. In conclusion, these results suggested that, out of the QTL identified in this study, *Qfhb.nc-1A, Qfhb.nc-1B,* and *Qfhb.nc-6A* may have the greatest effects across different germplasm and could be good candidates for MAS, while the effect of *Qfhb.nc-2A* most likely was overestimated due a small sample size.

Amongst the 344 Eastern U.S. wheat lines previously genotyped with the 9K SNP array, the frequency of lines with resistance alleles at the *Qfhb.nc-1A* markers *IWA3805*, *IWA1587*, and *IWA886* was 0.44. The frequency of lines with resistance alleles at the *Qfhb.nc-1B* markers *IWA7977*, *IWA6448,* and *IWA4987* was 0.13. For *Qfhb.nc-2A,* the frequency of lines with resistance alleles was 0.02 based on markers *IWA412* and *IWA7947*. For *Qfhb.nc-4A,* the frequency of lines with resistance allele was 0.36 based on markers *IWA482*, *IWA2173*, *IWA2900*, and *IWA402*. Finally, for *Qfhb.nc-6A*, the frequency of lines with resistance alleles at markers IWA3483 and IWA4036 was 0.22. Zero lines had the exact

same haplotype as NC-Neuse for the fourteen markers, but several lines were very similar. The Missouri line 'MO_080104' and the North Carolina breeding line 'NC06-19896' had the same alleles as NC-Neuse at twelve out of the fourteen markers. MO_080104 is moderately resistant to FHB (A. McKendry, personal communication) and NC06-19896 is also moderately resistant to FHB.

Discussion

Multiple QTL associated with resistance were identified in this study based on phenotypic data collected from seven inoculated, mist-irrigated FHB trials. Seven of these QTL were significant in more than one environment and/or for different resistance traits, and therefore were considered reproducible. Adjusted means generated within and across environments were used in the QTL analysis so that resistance QTL with significant effects in multiple environments could be identified. The reproducible QTL mapped to chromosomes 1A, 1B, 1D, 2A, 4A, 5B, and 6A, with NC-Neuse alleles providing resistance at the QTL on chromosomes 1A, 1B, 2A, 4A, and 6A, while AGS 2000 alleles were associated with resistance at the QTL on 1D and 5B. A preliminary test using recent USWWSN entries identified *Qfhb.nc-1A, Qfhb.nc-1B,* and *Qfhb.nc-6A* as likely the best candidates for use in MAS.

Qfhb.nc-1A was especially interesting because it was effective in several environments for INC, SEV, FDK, and DON with an R^2 ranging from 5.9 – 11.4%. Other resistance QTL have been mapped to chromosome 1A, including one in the Norwegian line NK93604, which was flanked by SSR markers *Xwmc59* and *Xbarc17* (Semagn et al., 2007). Both of those markers map distally to the QTL region identified in NC-Neuse. A QTL

associated with reduced severity was identified in the Chinese line CJ 9306 (Jiang et al., 2007), and mapped between markers *Xwmc24* and *Xbarc148* on chromosome 1A, which is proximal to the region identified in NC-Neuse. Another QTL was mapped to the centromeric area of chromosome 1A in the Chinese cultivar Wangshuibai, between markers *Xbarc24* and *Xbarc148* (Yu et al., 2008). Eckard et al. (2015) mapped resistance from the hard winter wheat cultivars Lyman and Overland to chromosome 1AS. Therefore, *Qfhb.nc-1A* may be the first FHB QTL mapped to chromosome 1AL in a U.S. cultivar.

Qfhb.nc-1B mapped to chromosome 1B and was associated with FDK and DON. Several other resistance QTL have been mapped to chromosome 1B, of which some have mapped within the 1BL.1RS translocation. The lines with resistance QTL within or close to the translocation included the British cultivar Lynx (Schmolke et al., 2005), the Dutch cultivar Romanus (Holzapfel et al., 2008), the CIMMYT cultivar Seri82 (Mardi et al., 2006), and the Romanian cultivars Sincron (Ittu et al., 2000) and Fundulea201R (Shen et al., 2003). The Chinese cultivar Wangshuibai also contained a QTL on chromosome 1BS (Zhang et al., 2004). Resistance has been mapped in the cultivars Cansas (Klahr et al., 2007; Haeberle et al., 2009a), Arina (Semagn et al., 2007), Pirat, Biscay, and History (Holzapfel et al., 2008) on the distal end of chromosome 1BL. *Qfhb.nc-1B* mapped within the region on chromosome 1B that displayed distortion for the expected segregation ratio, and the NC-Neuse allele (non 1BL.1RS translocation) was associated with resistance. This is in agreement with results from Holzapfel et al. (2008), where the favorable allele originated from the line Romanus, which has the regular wheat 1B chromosome. Other studies found that QTL mapped in or near the 1RS.1BL translocation were associated with resistance (Ittu et al., 2000; Shen et al.,

2003; Zhang et al., 2004; Schmolke et al., 2005; Mardi et al., 2006; Klahr et al., 2007; Srinivasachary et al., 2008). The different results among studies suggested that the effect of 1RS.1BL depended on the genetic background.

Qfhb.nc-2A mapped close to the centromere of chromosome 2A and had an R^2 ranging between 7.8 and 9.7% for SEV and DON. Several resistance QTL have been mapped to the centromeric region of chromosome 2A, including resistance from Ning8026 (Haeberle et al., 2009b), Spark (Srinivasachary et al., 2008), Wangshuibai (Mardi et al., 2005), and Rubens (Holzapfel et al., 2008).

Qfhb.nc-4A mapped to the centromeric region of chromosome 4A and had an R^2 ranging between 7.8 and 19.5% for FDK, and an R^2 of 11.5% for DON. Three QTL have previously been mapped to chromosome 4A. A QTL mapped in the German cultivar Pirat (Holzapfel et al., 2008) and another one in the Swiss winter wheat cultivar Arina (Paillard et al., 2004) were mapped near *Xgwm160* on the distal end of chromosome 4A. A QTL in the hard winter wheat cultivar Lyman was mapped on chromosome 4A with peak marker *IWA482* (Eckard et al., 2015), which mapped within the *Qfhb.nc-4A* QTL interval identified in the present study.

Qfhb.nc-6A mapped to the centromeric region of chromosome 6A and had an R^2 ranging between 5.9 and 11.1% for INC and SEV. Quantitative trait loci from three European cultivars have been mapped on chromosome 6A; these include the German cultivar Dream (Schmolke et al., 2005), the French cultivar Apache (Holzapfel et al., 2008), and the U.K. cultivar Spark (Srinivasachary et al., 2008). The SSR marker closest to the Dream QTL was *Xgwm82*, the SSR markers closest to the Apache QTL were *Xgwm82* and *Xbarc107*, and the

SSR marker closest to the QTL in Spark was *Xbarc23*. All of these markers map to the short arm and centromeric area of chromosome 6A. The NC-Neuse QTL had a peak at marker *IWA3483* at position 126.9 cM. The two closest SSRs were *Xbarc146* at position 81.8 cM, and *Xwmc256* at position 138.5cM. The interval spans the centromeric region of chromosome 6A. A QTL in the hard winter wheat U.S. cultivar Overland was mapped between markers *IWA2812* and *IWA1282*, with peak markers *IWA3527* and *IWA2235* (Eckard et al, 2015). The marker *IWA3527* mapped to position 123.3 cM on the NC-Neuse / AGS 2000 linkage map, which was about 10 cM from the *Qfhb.nc-6A* QTL interval identified in the present study. Wright et al. (2014) reported a resistance QTL in the cultivar Jamestown which mapped on chromosome 6A, close to markers *IWB73509* and *IWB53065* (S. Malla, personal communication). According to the 90K SNP consensus map, these markers are located within the *Qfhb.nc-6A* QTL region identified in the present study. Because several cultivars have had QTL mapped in this region of chromosome 6A, it may be a good candidate region for MAS.

Qfhb.nc-1D.2 was associated with resistance to FDK and DON in LW14, and resistance to FDK and DON across environments. The AGS 2000 allele contributed resistance. The peak marker was *wPt671990*, which mapped 20 cM distal to *Xgwm337*, locating the QTL in the centromeric region of 1D. A QTL associated with resistance and plant height in Apache was previously mapped to the centromeric region of chromosome 1D (Holzapfel et al., 2008).

Qfhb.nc-5B was associated with resistance to DON in MD12 and KIN12, and resistance to INC in MD12. This QTL co-localized with the *Vrn-B1* locus. AGS 2000 has

vrn-B1 winter allele conferring short vernalization requirement, and contributed the FHB-reducing allele. It is not uncommon for maturity traits to be associated with FHB resistance because of maturity-specific timing of initial infection, and because of the weather-dependent effectiveness of inoculum. AGS 2000 headed five days earlier than NC-Neuse, on average, and significant variation in HD was found within the RIL population. *Qfhb.nc-5B* was significant only in the 2012 environments. The 2011-12 winter was unusually mild, with abundant early growth, early maturing wheat plants, and a greater range in heading dates in the population (14.5 day range in KIN12 and 12.5 day range in MD12, compared to a 10.3 day HD range across all environments). One possible scenario is that FHB resistance associated with *Qfhb.nc-5B* was due to a pleiotropic effect of the *Vrn-B1* gene. A different possibility is that early-maturing lines escaped disease to some degree in spring 2012, perhaps due to lower temperatures earlier in the epidemic or lesser inoculum availability. Despite multiple inoculation dates, HD had a significant effect on several disease traits in multiple environments. This suggested that even though inoculum is present in the nursery during the whole period of flowering, uncontrollable factors like temperature and humidity may significantly affect the effectiveness of the inoculum.

The frequencies of lines with the same alleles at SNP associated with FHB resistance as NC-Neuse were relatively high for some of the QTL based on our investigations using the USWWSN entries and the Eastern Winter Wheat panel. This may have been because only two to four KASP assays were used to determine the presence or absence of each FHB QTL. Given that the resistance in NC-Neuse is derived from eastern winter wheat germplasm, the resistance allele frequencies may be relatively high among eastern U.S. wheat lines.

Nevertheless, zero out of 344 lines possessed the same combination of resistance alleles as NC-Neuse at fourteen SNP associated with NC-Neuse FHB resistance QTL. Also, there likely are other background effects and multiple minor-effect QTL that were not identified in this study, and which influence the level of resistance in addition to the identified QTL.

Marker-assisted selection can be a valuable tool to improve resistance to FHB, especially in collaboration with phenotypic selection. It can also be helpful in pyramiding FHB QTL from different sources into one line. Markers identified in QTL mapping studies may be appropriate for immediately use in MAS, but may also need to undergo further validation before MAS can be conducted across a large range of germplasm and genetic backgrounds (Collard and Mackill, 2008). Using markers in close linkage with FHB QTL from U.S. sources can be useful in breeding populations where the FHB QTL donor has been used as a parent. Winter wheat cultivars such as NC-Neuse, Truman (PI 634824) (McKendry et al., 2005), Bess (PI 642794) (McKendry et al., 2007), and Jamestown (PI 653731) (Griffey et al., 2010) have been used extensively by many of the breeding programs in the southeastern U.S. and the number of breeding populations in which MAS could be tested and used is high. Our results will likely be most useful to breeding programs utilizing NC-Neuse as a parent. Many of the southeastern U.S. wheat programs fall in this category. The present study is the first to identify and map QTL associated with resistance in NC-Neuse. QTL positions will become more defined, and more diagnostic markers will be developed with additional validation studies. A validation study with a doubled haploid (DH) population from a cross between NC-Neuse and the cultivar Bess is underway. The objective of that study is to map QTL associated with resistance and identify regions in that population which

overlap with QTL identified in the present study and in a study from the University of Missouri involving Truman (full-sib of Bess) (Islam et al., 2011a; Islam et al., 2011b; Islam et al., 2011c).

Future studies should include investigations of QTL effects in different genetic backgrounds and the efficiency of MAS for resistance QTL from NC-Neuse and other sources of native resistance. It would be very interesting to compare the result of MAS with results of random selection and phenotypic selection.

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Table 3.1. Per plot and entry mean heritability estimates, their standard errors (SE), and harmonic means of environments (ENV) and replications (REP) used to calculate entry mean heritability incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), deoxynivalenol content (DON), heading date (HD), and plant height (HT).

	INC	SEV	FDK	DON	HD	HT
Per plot basis heritability	0.15	0.17	0.24	0.32	0.54	0.37
(SE)	(0.02)	(0.02)	(0.03)	(0.03)	(0.03)	(0.03)
Entry mean heritability	0.66	0.71	0.79	0.82	0.93	0.83
(SE)	(0.04)	(0.03)	(0.03)	(0.02)	(0.01)	(0.02)
Harmonic mean of ENV	4.99	5.83	5.80	5.79	6.84	4.99
Harmonic mean of REP	13.46	15.86	14.76	14.89	18.26	10.97

Table 3.2. Pearson correlation coefficients between across-environments adjusted means for the four disease traits: Incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON) content, and heading date (HD) and plant height (HT).

Trait	SEV	FDK	DON	HD	HT
INC	r = 0.70 *** †	r = 0.82 ***	r = 0.79 ***	r = 0.39 ***	r = 0.06 NS
SEV	…	r = 0.74 ***	r = 0.70 ***	r = 0.10 NS	r = 0.10 NS
FDK	…	…	r = 0.91 ***	r = 0.34 ***	r = 0.10 NS
DON	…	…	…	r = 0.47 ***	r = 0.29 ***
HD	…	…	…	…	r = 0.41 ***

† Significance levels of correlation coefficients are indicated as: NS ($P > 0.05$), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

Table 3.3. Reproducible quantitative trait loci (QTL) associated with reduced incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) content in the recombinant inbred line population derived from a cross between NC-Neuse and AGS 2000. QTL were determined based on adjusted phenotypic means calculated within and across seven environments.

QTL[†]	Chromosome; position; marker interval	Trait_Env[‡]	Position	Peak marker	Position interval	LOD[§]	$R^{2¶}$	Resistance source[#]
Qfhb.nc-1A	1A; 212.2 - 238.2; *IWA3805 - IWA6152*	INC_KIN13	226.61	*IWA886*	214.2 - 235.3	3.9	10.8	NC-Neuse
		INC_all	226.61	*IWA886*	223.7 - 227.6	3.3	9.3	NC-Neuse
		SEV_all	222.61	*IWA886*	218.2 - 225.7	3.4	5.9	NC-Neuse
		FDK_KIN13	231.01	*Xwmc716*	208.3 - 238.2	4.9	11.4	NC-Neuse
		DON_KIN13	214.81	*IWA3805*	211.8 - 218.2	3.6	10.6	NC-Neuse
		DON_KIN14	227.61	*wPt9938*	214.4 - 236.1	4.2	10.9	NC-Neuse
		DON_all	214.81	*IWA3805*	212.2 - 218.2	3.4	8.7	NC-Neuse
Qfhb.nc-1B	1B; 27.3 - 54.0; *IWA6290 - Xwmc419*	FDK_MD12	42.21	*IWA6107*	41.3 - 54.0	3.6	9.5	NC-Neuse
		FDK_all	28.81	*IWA7977*	27.5 - 30.6	3.7	6.6	NC-Neuse
		DON_MD12	38.51	*IWA128*	38.6 - 42.5	3.2	7.1	NC-Neuse
		DON_LW14	30.71	*IWA6448*	30.0 - 32.2	3.4	5.7	NC-Neuse
		DON_all	33.71	*IWA6448*	27.3 -42.4	3.9	7.8	NC-Neuse
Qfhb.nc-1D.2	1D.2; 56.7 - 82.0; *wPt2206 - IWA1386*	FDK_LW14	67.61	*wPt671990*	62.5 - 73.6	3.6	10.7	AGS2000
		FDK_all	67.61	*wPt671990*	57.3 - 79.3	4.3	9.5	AGS2000
		DON_LW14	66.61	*wPt671990*	56.7 - 82.0	4.9	10.8	AGS2000
		DON_all	71.61	*wPt671990*	66.0 - 77.2	3.5	8.8	AGS2000
Qfhb.nc-2A	2A; 85.9 - 128.2; *Xwmc522 - IWA2612*	SEV_KIN13	118.41	*IWA7947*	115.5 - 126.4	3.3	9.4	NC-Neuse
		SEV_all	103.81	*IWA3842*	85.9 - 128.2	5.0	9.7	NC-Neuse
		DON_KIN12	113.71	*IWA412*	112.1 - 117.4	3.3	7.8	NC-Neuse

Table 3.3. Continued

Qfhb.nc-4A	4A; 107.0 - 144.2; *Xbarc170 - IWA4480*	FDK_KIN13	121.41	*Xbarc170*	110.9 - 143.9	3.8	19.5	NC-Neuse
		FDK_LW14	134.51	*IWA2793*	114.5 - 142.1	4.9	11.4	NC-Neuse
		FDK_all	135.71	*IWA2900*	107.0 - 143.0	3.7	7.8	NC-Neuse
		DON_LW14	135.71	*IWA2900*	113.6 - 144.2	5.4	11.5	NC-Neuse
Qfhb.nc-5B	5B; 72.1 - 115.0; *IWA2500 - IWA4793*	INC_MD12	98.31	*Vrn_B1*	89.4 - 102.6	4.8	10.3	AGS 2000
		DON_KIN12	98.11	*wPt5896*	80.8 - 112.2	9.7	18.2	AGS 2000
		DON_MD12	97.11	*wPt5896*	72.1 - 115.0	7.5	12.0	AGS 2000
Qfhb.nc-6A	6A; 127.2 - 138.0; *IWA3483 - Xwmc256*	INC_LW14	138.21	*IWA4036*	134.2 - 174.1	4.2	11.1	NC-Neuse
		SEV_KIN11	138.21	*IWA4036*	135.7 - 141.9	3.5	10.7	NC-Neuse
		SEV_all	126.91	*IWA3483*	125.9 - 129.5	3.3	5.9	NC-Neuse

† QTL, quantitative trait loci. The prefix *Qfhb* is followed by the chromosomal location of the QTL.
‡ Trait_Env, Trait_Environment. The trait/environment combination with which the QTL is associated.
§ LOD, logarithm of odds.
¶ Percentage of phenotypic variation explained by the marker at the peak of the QTL.
# Parental source contributing the resistance allele.

Table 3.4. Phenotypic means and standard deviations (st.dev.) of recombinant inbred lines (RILs) with different numbers of NC-Neuse resistance alleles at the QTL identified on chromosomes 1A, 1B, 2A, 4A, and 6A.

No. of resistance alleles at QTL[‡]	No. of RILs	INC[‡§] ± st.dev.	SEV ± st.dev.	FDK ± st.dev.	DON ± st.dev.
0	3	65.5 ± 8.6	31.2 ± 3.6	19.1 ± 4.7	20.4 ± 5.5
1	35	60.6 ± 8.5	28.3 ± 5.6	16.3 ± 5.8	17.5 ± 7.1
2	57	57.6 ± 6.8	27.1 ± 5.5	14.4 ± 4.3	15.3 ± 5.0
3	44	55.7 ± 9.3	23.7 ± 4.6	13.0 ± 5.8	13.8 ± 7.6
4	18	53.5 ± 7.3	23.6 ± 3.7	11.6 ± 3.7	12.7 ± 4.3
5	6	55.7 ± 6.5	24.1 ± 3.5	11.2 ± 2.2	11.4 ± 2.1

[†] Total number of NC-Neuse resistance alleles at the QTL identified on chromosomes 1A, 1B, 2A, 4A, and 6A.
[‡] Adjusted means of RILs were calculated across all seven environments. The means across all environments for AGS 2000 and NC-Neuse were 60.3 and 52.9 for INC; 44.1 and 18.9 for SEV; 16.0 and 10.8 for FDK; and 20.4 and 11.7 for DON.
[§]INC, incidence (%); SEV, severity (%); FDK, *Fusarium*-damaged kernels (%); DON, deoxynivalenol (mg kg^{-1}).

Table 3.5. Reproducible quantitative trait loci (QTL) associated with heading date (HD) in the recombinant inbred line population derived from a cross between NC-Neuse and AGS 2000. QTL were determined based on adjusted phenotypic means calculated within and across environments.

QTL[†]	Chromosome; position; marker interval	Trait_Env[‡]	Position	Peak marker	Position interval	LOD[§]	$R^{2¶}$	Early HD allele[#]
Qhd.nc-2B	2B; 36.5 - 68.8; *IWA2441 - IWA5738*	HD_KIN11	61.21	*IWA1599*	36.5 - 68.2	5.9	17.0	AGS 2000
		HD_MD12	51.51	*IWA3213*	41.6 - 65.1	4.6	4.6	AGS 2000
		HD_KIN14	61.41	*IWA5809*	37.5 - 67.8	6.6	16.4	AGS 2000
		HD_LW14	58.31	*IWA6778*	38.0 - 65.2	5.0	8.8	AGS 2000
		HD_all	61.41	*IWA5809*	36.9 - 68.8	6.6	8.8	AGS 2000
Qhd.nc-4A	4A; 65.9 - 86.3; *wPt1091 - IWA3756*	HD_KIN13	77.91	*wPt1091*	72.2 - 77.0	3.2	7.0	NC-Neuse
		HD_LW13	77.91	*wPt1091*	71.6 - 78.0	3.2	7.1	NC-Neuse
		HD_all	77.91	*wPt1091*	65.9 - 86.3	5.0	6.6	NC-Neuse
Qhd.nc-5B	5B; 70 - 129.8; *IWA5179 - wPt3457*	HD_KIN12	96.11	*wPt1304*	71.3 - 129.8	20.3	45.7	AGS 2000
		HD_LW12	96.11	*wPt1304*	70.9 – 121	20.2	45.6	AGS 2000
		HD_MD12	97.11	*wPt1304*	70 - 129.4	21.0	39.8	AGS 2000
		HD_KIN13	96.11	*wPt1304*	82.0 – 117	9.2	19.2	AGS 2000
		HD_LW13	96.11	*wPt1304*	80.8 - 116.2	9.2	19.2	AGS 2000
		HD_LW14	85.51	*wPt1304*	73.8 - 102.6	6.0	15.8	AGS 2000
		HD_all	97.11	*wPt1304*	70.5 - 125.0	18.2	31.7	AGS 2000
Qhd.nc-7D	7D.1; 1 - 11.2; *Xbarc126 - IWA3851*	HD_KIN13	1.01	*Xbarc126*	0 - 4.9	4.0	7.9	AGS 2000
		HD_LW13	1.01	*Xbarc126*	0 - 4.8	4.02	7.9	AGS 2000
		HD_all	1.01	*Xbarc126*	0 - 11.2	5.5	6.9	AGS 2000

[†] QTL, quantitative trait loci. The prefix *Q* is followed by *hd* (heading date) and then the chromosomal location of the QTL.
[‡] Trait_Env, Trait_Environment. The trait/environment combination that the QTL is associated with.
[§] LOD, logarithm of odds.
[¶] Percentage of phenotypic variation explained by the marker at the peak of the QTL.
[#] Parental source contributing the early heading date (HD) allele.

Table 3.6. Reproducible quantitative trait loci (QTL) associated with plant height (HT) in the recombinant inbred line population derived from a cross between NC-Neuse and AGS 2000. QTL were determined based on adjusted phenotypic means calculated within and across environments.

QTL[†]	Chromosome; position; marker interval	Trait_Env[‡]	Position	Peak marker	Position interval	LOD[§]	$R^{2¶}$	Short HT allele[#]
Qht.nc-3B	3B; 109.1 - 137.6; *Xwmc762 - IWA4613*	HT_MD12	134.11	*IWA3218*	110.6 - 135.1	3.9	7.6	AGS 2000
		HT_KIN13	134.11	*IWA3218*	117.3 - 136.8	4.7	6.1	AGS 2000
		HT_all	134.11	*IWA3218*	109.1 - 137.6	4.6	7.4	AGS 2000
Qht.nc-5B	5B; 70.7 - 125.3; *IWA8262 - wPt741134*	HT_MD12	97.11	*wPt5896*	84.9 - 97.2	3.2	7.2	AGS 2000
		HT_KIN13	97.11	*wPt5896*	70.7 - 125.3	19.3	35.2	AGS 2000
		HT_LW13	98.31	*Vrn_B1*	78.3 - 103.5	4.9	12.4	AGS 2000
		HT_all	98.31	*Vrn_B1*	72.5 - 119.1	8.7	15.7	AGS 2000
Qht.nc-6A	6A; 65.7 - 118.4; *IWA731 - IWA231*	HT_MD12	75.61	*wPt731779*	66.0 - 97.2	7.0	12.5	AGS 2000
		HT_KIN13	87.41	*wPt6951*	70.7 - 115	11.3	18.8	AGS 2000
		HT_all	87.41	*wPt6951*	65.7 - 118.4	9.0	16.1	AGS 2000

[†] QTL, quantitative trait loci. The prefix *Q* is followed by *ht* (plant height) and then the chromosomal location of the QTL.
[‡] Trait_Env, Trait_Environment. The trait/environment combination that the QTL is associated with.
[§] LOD, logarithm of odds.
[¶] Percentage of phenotypic variation explained by the marker at the peak of the QTL.
[#] Parental source contributing the short plant height (HT) allele.

Table 3.7. Primers for KASP assays developed for NC-Neuse resistance QTL on chromosomes 1A, 1B, 2A, 4A, and 6A, used to genotype the 2013 and 2014 Southern Uniform Winter Wheat Scab Nurseries.

QTL	SNP[†] no.	SNP name	Primer A1[‡]	Primer A2[•]	Primer C1
Qfhb.nc-1A	*IWA3805*	wsnp_Ex_c41953_48658353	AACTTTGCTGTCAACTTTGAGGA	CTAACTTTGCTGTCAACTTTGAGGG	TTACTGCAACTGATGGGTGCACTTTATAT
Qfhb.nc-1A	*IWA1587*	wsnp_Ex_c12123_19388313	CTATCTATATTCTTTGTTCTTCAAGTCCA	CTATCTATATTCTTTGTTCTTCAAGTCCG	GATTGTTGCAACTAGCAACAGCTGTTTAT
Qfhb.nc-1A	*IWA886*	wsnp_CAP11_rep_c7847_3468669	GGAAGTGATATTCTTGCAAGAGCA	AAGTAAGCTGCTAGGTCTTGTAGCA	TACGTGCACGGTCGATCAGTTTCTA
Qfhb.nc-1B	*IWA7977*	wsnp_Ra_c49942_54740532	GGTACTGTGAATCTTTGAGAAGTGTG	GGAAGTGATATTCTTGCAAGAGCG	GCTGGAATGCAAAATGCAGCAGTCTT
Qfhb.nc-1B	*IWA6448*	wsnp_Ku_c11987_19472688	GGTACTGTGAATCTTTGAGAAGTGTG	AGGTACTGTGAATCTTTGAGAAGTGTA	TCTAGGTTGCCTGGAAGAACTGGAA
Qfhb.nc-1B	*IWB43992*	Kukri_c31554_437	ACATTACTGTCGATATGGATCTTGTGT	CATTACTGTCGATATGGATCTTGTGC	TGCTGCTTGAAAAGAAATGCAGGATACTT
Qfhb.nc-1B	*IWA4987*	wsnp_Ex_c9960_16397347	CCTGCAGAACAAGATAGCTTTGAAC	ATCCTGCAGAACAAGATAGCTTTGAAT	CCGGCCGCGCAGAGTTGCAT

Table 3.7 Continued

Qfhb.nc-2A	*IWA412*	wsnp_Ex_rep_c68493_67320068	ATGTCTAATCACCTTCTCCACATGATA	GTCTAATCACCTTCTCCACATGATG	TGCAGGCCAACAGGAGCGGAA
Qfhb.nc-2A	*IWA7947*	wsnp_Ra_c4503_8155485	CACAGTCAAAGACAACCAGCAGC	ATCACAGTCAAAGACAACCAGCAGA	CTTGAACTGAGCACACTTTCTAGGCTT
Qfhb.nc-4A	*IWA482*	wsnp_BF474615A_Ta_1_1	GATCAATTGGTTCCTGTGATATCATTC	ATGATCAATTGGTTCCTGTGATATCATTT	TGGGACAACACATTCTTGGGCCATT
Qfhb.nc-4A	*IWA2793*	wsnp_Ex_c23248_32488191	CACAATTTCCCGCTCAGCG	CCTCACAATTTCCCGCTCAGCA	GATCTCACCGATCACCTCATGAAGAT
Qfhb.nc-4A	*IWA2900*	wsnp_Ex_c24443_33687802	AGGAGGCCTGCATGCACGC	CAGGAGGCCTGCATGCACGT	CTTGCACAACCACACGCAGAGGAA
Qfhb.nc-4A	*IWA402*	wsnp_BE637808A_Ta_2_1	ATATCAATTAAATGCTACATCATGAACATAGT	ATCAATTAAATGCTACATCATGAACATAGC	TTTAGGAATGGAAGGAGTATCATTCACCA
Qfhb.nc-6A	*IWA3483*	wsnp_Ex_c34545_42833327	GGTGTTAGCACTGGTTTATCCACAAA	GTGTTAGCACTGGTTTATCCACAAG	ACGGAAATCATTGTAACCAGATGGTTGAA
Qfhb.nc-6A	*IWA4036*	wsnp_Ex_c48789_53586502	CCATTTTCCCATTGAACGAAGCATTAA	CATTTTCCCATTGAACGAAGCATTAG	CTCAGGGAGAATAACACGGGATCAT

† SNP, single nucleotide polymorphism. The prefixes *IWA* and *IWB* are followed by an index number.
‡ Primer sequences are given without the FAM (A1) and VIC (A2) tails.

Table 3.8. Mean incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) values for groups of lines from the 2013 and 2014 Uniform Southern Winter Wheat Scab Nursery with and without NC-Neuse resistance alleles at QTL.

QTL[†]	Allele[‡]	n[§]	INC		SEV		FDK		DON	
Qfhb.nc-1A	S	46	60.5	NS[¶]	36.3	***	32.1	***	10.1	**
	R	52	58.4		32.3		28.0		8.7	
Qfhb.nc-1B	S	91	59.4	**	33.7	NS	30.1	***	9.4	*
	R	17	55.8		32.3		24.6		8.0	
Qfhb.nc-2A	S	104	59.3	NS	34.4	***	30.3	***	9.5	**
	R	5	55.2		27.2		20.7		6.8	
Qfhb.nc-4A	S	68	58.1	NS	33.4	*	28.8	*	9.0	NS
	R	40	59.8		35.2		30.8		9.5	
Qfhb.nc-6A	S	98	59.0	NS	34.3	***	29.9	***	9.4	*
	R	14	57.9		30.8		25.6		7.8	
Ning_5A	S	109	59.5	*	34.5	***	30.3	***	9.5	***
	R	6	54.7		28.0		19.9		5.1	
Ernie_5A	S	108	58.9	**	33.3	***	28.9	***	8.8	***
	R	8	64.9		44.4		40.5		14.4	
Wuhan-1_2DL	S	110	59.4	NS	34.5	***	30.3	***	9.5	***
	R	6	57.8		26.5		19.0		4.8	
Massey_3BL	S	111	59.2	NS	33.9	NS	29.6	NS	9.9	NS
	R	5	56.5		36.0		29.9		9.1	
*Fhb1*_3BS	S	96	59.3	NS	35.1	***	30.6	***	9.8	***
	R	20	59.4		29.0		25.2		6.7	

[†] QTL, quantitative trait loci. The QTL identified in NC-Neuse are presented with the prefix *Qfhb.nc-* followed by the chromosome number. The other QTL are presented according to source of resistance followed by chromosome number.

[‡] Indicates presence of resistance allele (R) or susceptibility allele (S) at the respective QTL.

[§] n indicates the number of lines in the allele group.

[¶] Significance levels of mean comparisons are indicated as: NS ($P > 0.05$), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

CHAPTER 4 - Validation of *Fusarium* Head Blight Resistance QTL in U.S. Winter Wheat

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Abbreviations:

CIM, composite interval mapping; DH, doubled haploid; DON, deoxynivalenol; FDK, *Fusarium*-damaged kernels; FHB, *Fusarium* head blight; HD, heading date; HT, height; INC, incidence; KASP, Kompetitive Allele Specific PCR; LD, linkage disequilibrium; LOD, logarithm of odds; MAS, marker-assisted selection; MIM, multiple interval mapping; PCR, polymerase chain reaction; QTL, quantitative trait loci; RIL, recombinant inbred line; SEV, severity; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; USWWSN, Uniform Southern Winter Wheat Scab Nursery.

Abstract

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum*, can significantly reduce the grain quality of wheat (*Triticum aestivum* L.) due to mycotoxin contamination. Two U.S. soft red winter wheat cultivars, Bess and NC-Neuse, have moderate resistance to FHB. The objective of this study was to validate genomic regions associated with FHB resistance identified in previous studies involving NC-Neuse and the cultivar Truman, a full-sib of Bess. A total of 98 doubled haploid (DH) lines derived from the cross Bess / NC-Neuse were evaluated following inoculation with *F. graminearum* in inoculated, mist-irrigated field nurseries. The lines were evaluated for FHB incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) content in seven environments between 2011 and 2014. A 3,338 cM linkage map was developed based on 4014 simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers. Composite Interval Mapping (CIM) and Multiple Interval Mapping (MIM) analyses of adjusted means within and across environments identified twelve quantitative trait loci (QTL) associated with FHB resistance on chromosomes 1A, 1B, 2A, 2B, 3B, 4A (2 QTL), 4D, 5B, 5D, and 6A (2 QTL). NC-Neuse alleles provided resistance at QTL on five chromosomes and Bess alleles provided resistance at QTL on five other chromosomes. The QTL region on chromosome 2B included the *Ppd-B1* photoperiod locus, and the QTL on chromosome 4D included the *Rht-D1* locus for plant height. Positions of resistance QTL were compared to previous results from studies involving NC-Neuse and Truman by alignment of linkage maps. Five overlapping QTL regions were found. Quantitative trait loci on chromosomes 1A, 4A, and 6A identified in this study overlapped with QTL regions

identified in the NC-Neuse / AGS 2000 recombinant inbred line (RIL) population. The QTL identified on chromosomes 2B and 3B overlapped with QTL regions identified in the Truman / MO 94-317 RIL population. Kompetitive Allele-Specific PCR (KASP) assays were developed for QTL in the overlapping regions. A preliminary test using these assays on recent Uniform Southern Winter Wheat Scab Nursery (USWWSN) entries showed that the KASP assays developed for *Qfhb.nc-2B.1* may be good candidates for use in marker-assisted selection.

Introduction

Fusarium head blight (FHB), also referred to as head scab or ear blight, is one of the most destructive diseases of wheat. The disease is mainly caused by the fungal pathogen *Fusarium graminearium* (Gale, 2003; Shaner, 2003), which is also hosted by other cereals such as maize (*Zea mays* L.) and barley (*Hordeum vulgare* L.). *Fusarium* head blight significantly lowers grain yield and test weight, and contaminates grain with the mycotoxin deoxynivalenol (DON) (Gale, 2003). Since the 1990s, severe outbreaks of FHB have occurred in the U.S. (McMullen et al., 1997). Nganje et al. (2004) estimated that the direct economic effects from FHB in the U.S. between 1998 and 2000 came to a total of 734 million dollars, with even greater secondary economic losses. The disease is most severe in years with frequent precipitation before, during and after flowering, which is the critical time for initial infection of wheat heads (Champeil et al., 2004; Cowger et al., 2009; De Wolf et al., 2003). Due to economic penalties, in addition to food and feed safety concerns associated with high mycotoxin levels in grain, it is very important to identify ways of reducing FHB in wheat, particularly the accumulation of DON.

One of the most promising FHB control measures is host resistance. Inheritance of FHB resistance is complex as it involves multiple genes, whose effects may vary in different genetic backgrounds. Different types of resistance have been proposed: resistance to initial infection (incidence, INC), resistance to spread within the wheat head (severity, SEV), resistance to kernel damage, resistance to DON accumulation (Boutigny et al., 2008), and yield tolerance (Mesterhazy, 1995). Initially, many breeding programs focused on SEV-reducing resistance, but as DON testing has become more common and direct economic penalties for farmers have been implemented, the focus has shifted more toward resistance to accumulation of DON and *Fusarium*-damaged kernels (FDK) (Brown-Guedira et al., 2008).

Sources of genetic resistance have been identified in Asian spring wheats such as Sumai 3 and its derivatives, South American and European cultivars, and some U.S. soft red winter wheat cultivars including Ernie (PI 584525) (McKendry et al., 1995), the full-sibs Truman (PI 634824) (McKendry et al., 2005) and Bess (PI 642794) (McKendry et al., 2007), Coker 9511 (PI 643095), NC-Neuse (PI 633037) (Murphy et al., 2004), and Jamestown (PI 653731) (Griffey et al., 2010). These cultivars are known to be partially resistant, and some have been included in studies investigating the genetics governing their resistance. Quantitative trait loci (QTL) conferring FHB resistance were recently mapped in the cultivars NC-Neuse (Petersen et al., 2013) and Truman (Islam et al., 2011a; Islam et al., 2011b; Islam et al., 2011c; McKendry, 2012). While many studies have focused on the genetic basis of FHB resistance in Asian wheat sources, resistance in U.S. sources have not yet been characterized to the same degree.

Phenotypic evaluations of FHB resistance can be challenging and costly; thus marker-assisted selection (MAS) is typically utilized as a complement to phenotypic selection. Several cases of successful MAS for major-effect QTL, such as *Fhb1*, have been reported (Miedaner et al., 2006; Kang et al., 2011; Balut et al., 2013), while a limited number of reports of MAS for minor-effect QTL are available. Identification and mapping of QTL in U.S. sources of resistance, as well as validation of mapped QTL, remain important research objectives for the efficient use of MAS (Griffey, 2005; Brown-Guedira et al., 2008). Validation of QTL generally refers to the verification that a QTL is effective in genetic backgrounds different to the original mapping population (Collard and Mackill, 2008).

The objectives of the current study were to: 1) Validate genomic regions associated with FHB resistance in NC-Neuse and Truman by characterizing resistance QTL in a Bess / NC-Neuse DH population; 2) develop preliminary assays to use in MAS and allele enrichment in breeding programs based on validated QTL; and 3) evaluate these assays on lines from recent Uniform Southern Winter Wheat Scab Nurseries (USWWSN) to estimate resistance allele frequencies and single QTL effects.

Materials and methods

Plant Materials

An F_1-derived DH population was developed from a cross between the soft red winter wheat cultivars NC-Neuse and Bess, which both are moderately resistant to FHB. NC-Neuse (PI 633037) (*Rht-B1a*, *Rht-D1b*, *null* allele for *Ppd-B1*, 2BS:2GS:2GL:2BL translocation chromosome) was released in 2003 from North Carolina State University (Murphy et al.,

2004). Bess (PI 642794) (*Rht-B1b*, *Rht-D1a*, *Ppd-B1b*) was released from the University of Missouri in 2005 (McKendry et al., 2007). Bess has been used as a resistant check in the USWWSN since 2006-07. Bess is an early maturing, slightly shorter full-sib of the cultivar Truman (McKendry et al., 2005). Truman has been used as a resistant check in the Uniform Northern Winter Wheat Scab Nursery for several years. NC-Neuse and Bess differ at the *Ppd-B1* locus on chromosome 2B, where NC-Neuse carries a *null* allele and Bess the *Ppd-B1b* (sensitive) allele. Both cultivars have the *Ppd-A1a* (insensitivity) allele and the *Ppd-D1b* (sensitivity) allele. Most DH lines containing both *Rht-B1b* and *Rht-D1b* (double-dwarfs) and DH lines containing neither of the height-reducing alleles (tall) were discarded from the population during seed increase, and 98 DH lines were used in the current study.

Phenotypic Evaluations

The population parents and DHs were evaluated for FHB resistance traits in seven inoculated environments under mist-irrigation: Cunningham Research Station in Kinston, NC in field seasons 2011-12, 2012-13, and 2013-14; Lake Wheeler Field Laboratory, Raleigh, NC, in seasons 2012-13 and 2013-14; and Columbia, Missouri in seasons 2011-12 and 2012-13.The location/year combination will from here on be referred to as 'KIN', 'LW', and 'MO', followed by the last two digits of the season. The entries were planted in mid to late October in 1.2 meter plots, with 15 cm spacing between plots. A randomized complete block design with two or three replications was utilized in all environments. In Kinston, NC and Columbia, MO the plots were planted into corn debris to promote disease development. Fertilization, irrigation, and other agronomic treatments followed standard management procedures for North Carolina (Weisz, 2013) and Missouri (Conley et al., 2003).

Plots in Kinston and Raleigh, NC were inoculated with three applications of autoclaved corn kernels infected with *F. graminearum* isolates collected from the previous year's crop (four g $plot^{-1}$ $application^{-1}$). Applications were made at one-week intervals starting three weeks before the average heading date of entries in the nursery. In Columbia, MO, individual plots were spray-inoculated at 75% flowering using a CO_2 backpack sprayer. The inoculum was developed from a *F. graminearum* single isolate, previously evaluated for aggressiveness, concentrated to 70,000 macroconidia mL^{-1}. Overhead mist irrigation was applied to promote disease development, and was terminated once FHB symptoms started appearing on the wheat heads.

Data on INC and SEV were collected about 21 days after anthesis. Incidence was scored by counting the number of infected heads from a subsample of 20-35 primary heads from the middle of a plot (thereby evaluated as a percentage of infected heads in each plot). Disease severity was rated using a modified Horsfall-Barrett scale with ten categories developed by Stack and McMullen (2011). Plant height (HT) was measured on experimental entries in KIN13, LW13, KIN14, and LW14, and heading dates (HD) were recorded in all environments. The HD was recorded when 50% of primary heads in a plot were fully emerged from the flag leaf sheath.

At crop maturity, wheat heads were harvested from the center 60 to 90 cm of each plot, dried, and threshed using an Almaco stationary thresher (Almaco; Nevada, Iowa) without fan. Samples were cleaned using hand sieves in front of table-top fans set at low speed to ensure minimal loss of *Fusarium*-damaged seeds. Percent FDK was estimated by separating and counting the number of diseased kernels in the total number of kernels in a

random 10 g grain subsample. Grain subsamples (60-80 g sample^{-1}) underwent DON analysis at the University of Minnesota.

Data Analysis

Data were evaluated using SAS statistical software version 9.3 (SAS Institute Inc., 2012) (Appendix C). Initial evaluation included checking for violations of the assumptions of analysis of variance and linear mixed models through the use of PROC UNIVARIATE and QQPLOT. These analyses showed that a logarithmic transformation was necessary to achieve normality for the traits SEV, FDK, and DON. The traits INC, HD, and HT were not transformed.

Experimental factors included genotype, environment, and replication within environment. An environment was defined as a combination of location and year because some locations were not represented in all years. Analysis of variance was conducted using the SAS procedure PROC MIXED (Littell et al., 2006), with genotypes considered as fixed effect factors, while all other factors (environment, genotype x environment, and replication within environment) were treated as random effects.

Due to significant genotype x environment interactions, adjusted means for use in QTL analyses were estimated using PROC MIXED within and across all environments for INC, SEV, FDK, DON, HD, and HT. Genotype was treated as a fixed effect factor to obtain the phenotypic means of the genotype adjusted for all non-genetic effects. Heading date was included as a covariate by adding HD to the model as a fixed effect factor. In the models for across-environments analyses, HD was nested within environment.

Heritabilities of all FHB resistance traits, HD, and HT were estimated on a per-plot basis and on an entry-mean basis according to Holland et al. (2002). SAS code from the website http://www4.ncsu.edu/~jholland/heritability/Inbreds.html was modified for this purpose.

The PROC CORR procedure in SAS was used to calculate all phenotypic correlations.

Genotypic data collection

Leaf tissue from 5-8 seedlings of parents and each DH line was pooled and genomic DNA was extracted using a CTAB protocol. DNA concentrations were quantified and diluted to 50 ng μL^{-1}. The population was genotyped with 13 polymorphic SSR markers and 3 KASP markers for *Ppd-B1*, *Rht-B1*, and *Rht-D1*. Sequences for SSR primers were obtained from GrainGenes 2.0 (http://wheat.pw.usda.gov/GG2/index.shtml). Polymerase chain reactions (PCR) were conducted as described by Maloney et al. (2011). In PCR reactions for analysis on the ABI3730 Genetic Analyzer (Applied Biosystems, Foster City, CA), the forward primer was labeled directly with FAM, NED, PET, or VIC. In PCR reactions for analysis on the LI-COR 4300 DNA Analysis System (LI-COR Biosciences, Lincoln, NE), forward primers were modified to include the M13 sequence (5'-CACGACGTTGTAAAACGAC-3') on the 5' end for universal fluorescent labeling. For the KASP markers, assay mix preparation and PCR protocols were conducted according to LGC Genomics protocols (LGC Genomics, Hoddesdon, UK). Fluorescent endpoint genotyping was done using a LightCycler 480 (Roche Applied Sciences, Indianapolis, IN).

The population was genotyped with the Infinium iSelect 90K SNP chip array (Wang et al., 2014). SNP calls were made using Genome Studio data analysis software (Illumina, San Diego, CA). Markers with more than 20% missing data were excluded, and segregation distortion was analyzed for all markers using a Chi Square test (expected 1:1 segregation ratio).

Map Construction and QTL Analyses

A genetic linkage map was constructed using MapMaker/Exp version 3.0 (Lincoln et al., 1993) according to the software protocol. The threshold for log-likelihood (LOD) was initially set at 15.0 to develop tight, core linkage groups. Once core linkage groups were established, the LOD was set to 5.0 to add more markers to each group. Final maps and distances were calculated using the Kosambi function (Kosambi, 1944) and default LOD score of 3.0. Linkage groups were assigned to chromosomes using information from the 90K SNP consensus map (Wang et al., 2014), the 9K consensus map (Cavanagh et al., 2013), and the wheat SSR consensus map (Somers et al., 2004). SNP positions within linkage groups were also verified using these consensus maps.

QTL analyses for traits within environments and across environments were conducted using Composite Interval Mapping (CIM) and Multiple Interval Mapping (MIM) in Windows QTL Cartographer 2.5 (Wang et al., 2004). Result files from CIM were used for the MIM analysis. The critical LOD value to declare QTL significance was 3.2, based on 1000 permutations. Only additive effects were estimated.

Comparison of QTL Intervals

The linkage maps from the NC-Neuse / AGS 2000 RIL population, the Truman / MO 94-317 RIL population, and the Bess / NC-Neuse DH population were aligned to the 9K SNP consensus map and the 90K SNP consensus map with common *IWA* and *IWB* SNP markers in order to compare and validate QTL positions.

KASP Assay Development

A set of KASP assays was developed based on SNP markers in validated QTL regions on chromosomes 1A, 2B, 3B, 4A, and 6A. Primers were designed using Primer Picker software with default settings (LGC Genomics, Hoddesdon, UK). KASP assays included two allele-specific forward primers with tail sequences and one common reverse primer. Assay mix preparation and PCR protocols were conducted according to LGC Genomics protocols. Fluorescent endpoint genotyping was done using a LightCycler 480. The KASP assays were run on the population parents and a subset of DHs to test if the assays would give the same results as the original SNP calls. It was thus possible to determine which KASP assays created tight, differentiated clusters for easy interpretation.

To obtain an estimate of resistance allele frequencies and to test QTL effects in a set of lines other than our mapping population, representative KASP assays from each of the five validated QTL regions were run on 116 entries (including four check cultivars) from the 2013 and 2014 USWWSN. The USWWSN is an annual collaborative program to evaluate resistance to FHB in breeding lines submitted by public and private breeding programs. Nursery cooperators evaluated entries in mist-irrigated inoculated nurseries and recorded data on INC, SEV, FDK, DON, and HD. Annual reports including phenotypic data, genotypic

information about previously identified QTL (*Fhb1*, 2DL from Wuhan-1/W14, 5A from Ning7840, 5A from Ernie, and 3BL from Massey), and a list of the locations and cooperators involved are available at http://scabusa.org/pdfs_dbupload/suwwsn13_report.pdf and http://www.scabusa.org/pdfs_dbupload/suwwsn14_report.pdf.

Analysis of variance was conducted using PROC MIXED to test effects of the five validated QTL regions and previously identified QTL from other resistance sources among the 2013 and 2014 USWWSN entries. Only disease means for each entry were available from each test location, therefore locations were treated as replications. The model included QTL, genotype nested within QTL, year, replication nested within year, and residual error. Similar to the analysis method described by Balut et al. (2013), QTL and genotype nested within QTL were treated as fixed effect factors, while year and replication within year were treated as random effect factors. Adjusted means were generated and compared for each QTL and for each disease trait.

Results

Phenotypic Data Analysis

Data were analyzed from seven environments: KIN12, KIN13, KIN14, LW13, LW14, MO12, and MO13. The DH population displayed normal distributions for adjusted means of INC, SEV, FDK, and DON across environments after transformation of SEV, FDK, and DON (data not shown).

Significant genotypic effects were found for all resistance traits in the across-environments analysis (Table B.1) and in all individual environments (data not shown). The

variance components for all other random-effect factors (environment, replication within environment, and genotype x environment interaction) for all four disease traits were significantly different from zero.

Bess had significantly lower mean values for INC, SEV, FDK, and DON compared to NC-Neuse averaged over environments (Table B.2). Several transgressive segregants were identified; two DH lines had significantly lower DON levels than Bess, and between one and five DH lines had significantly higher disease means than NC-Neuse, depending on the disease trait. In all individual environments Bess had disease means significantly lower than or similar to NC-Neuse. The mean HD of NC-Neuse and Bess were significantly different averaged over environments. NC-Neuse had a mean HD two days later than Bess. The range between the earliest- and the latest-heading DHs was fifteen days averaged over all environments. The mean HT of NC-Neuse and Bess were not significantly different.

The entry mean heritabilities for INC, SEV, FDK, and DON were moderate to high; FDK had the lowest heritability (0.63), while SEV had the highest heritability (0.83) (Table 4.1). The entry mean heritability for HD (0.95) and HT (0.94) were high.

Pairwise Pearson correlations between adjusted means for the four disease traits were highly significant (r = 0.80 to 0.88, $P < 0.0001$); the highest correlation was observed between FDK and DON (r = 0.88), and the lowest was between SEV and DON (r=0.80) (Table 4.2). Pairwise correlations between the four disease traits and HD ranged from 0.09 to 0.40 (Table 4.2). Positive correlations were found between HD and the disease traits INC, FDK, and DON ($P < 0.01$). For these traits, a later heading date was associated with higher disease scores. The mean HT for NC-Neuse and Bess were not significantly different, but

there was significant genotypic variation within the population. Pairwise correlations between adjusted means for the disease traits and HT ranged from -0.16 to -0.33 (Table 4.2). Significant negative correlations ($P < 0.01$) were found between HT and the disease traits INC, SEV, and FDK. For these traits, a shorter plant height was associated with higher disease scores. A positive correlation (r = 0.32, $P = 0.0015$) between HD and HT was also observed, indicating that later lines generally were taller. Despite our attempt to reduce the effect of HD through multiple inoculation dates, HD had a significant effect on several disease traits in the across-environments analyses. Phenotypic means adjusted for effects of HD were generated for each line and used in the QTL analyses.

Linkage Map for Bess / NC-Neuse DH population

A total of 4014 markers were placed on the genetic linkage map consisting of 51 linkage groups, covering a total map length of 3337.7 cM (Table E.1). All chromosomes were represented. Of the 4014 markers, 3999 were SNPs, 12 SSRs, and three KASP assays for the major gene loci *Ppd-B1*, *Rht-B1*, and *Rht-D1*.

Significant distortions from the expected 1:1 marker segregation ratio and lack of recombination in the DH lines were observed on linkage group 2B.1 (position 0-103.8, chromosome 2BS). The NC-Neuse alleles in this area were significantly overrepresented (allele frequency of ~0.85) compared to the Bess alleles. These results were probably due to the presence in NC-Neuse of the 2BS:2GS·2GL:2BL translocation chromosome. Reduced recombination and preferential transmission has been associated with this translocation in other mapping populations (Nyquist 1962; Tsilo et al., 2008). The markers with distorted segregation ratios were included on the linkage map to represent the chromosomal region.

Some markers displaying distorted segregation ratios were also included on linkage groups 1D.1, 2B.1, 5B.1, 6A.1, 6A.3, and 7B.1 to help cover large unrepresented areas, as some of these groups had poor marker coverage.

QTL Mapping

Quantitative trait loci were considered reproducible if they were associated with resistance in multiple environments and/or with multiple disease resistance traits. A total of twelve reproducible resistance QTL were mapped to ten different chromosomes (Table 4.3); the NC-Neuse allele provided resistance at seven of these loci, and the Bess allele provided resistance at five loci. Due to the relatively small population size, QTL effects may have been overestimated (Beavis, 1998).

The NC-Neuse alleles at the QTL *Qfhb.nc-1A.2, Qfhb.nc-2A.3, Qfhb.nc-4A.2a, Qfhb.4A.2b, Qfhb.nc-5B.1, Qfhb.nc-6A.2* and *Qfhb.nc-6A.3* were associated with reduced disease. The Bess alleles at the QTL *Qfhb.nc-1B.4, Qfhb.nc-2B.1, Qfhb.nc-3B.2, Qfhb.nc-4D.1,* and *Qfhb-5D.1* were associated with reduced disease (Table 4.3).

The QTL interval for *Qfhb.nc-2B.1* included the *Ppd-B1* marker (position 50.9 cM). Bess possesses the *Ppd-B1b* (sensitive) allele, and NC-Neuse possesses a *null* allele at this locus on the 2BS:2GS:2GL:2BL translocation chromosome. The ratio of DH lines with the Bess allele to DH lines with the NC-Neuse allele at *Ppd-B1* was heavily distorted toward the NC-Neuse allele (frequency ~0.85).

Two QTL were mapped to chromosome 4A, these were designated *Qfhb.nc-4A.2a* and *Qfhb.4A.2b*. The QTL *Qfhb.nc-4A.2a* QTL reduced disease in the greatest number of environment compared to the other identified FHB QTL.

The *Qfhb.nc-4D.1* QTL region spanned the *Rht-D1* locus. Bess possesses the ***Rht-D1a*** (wild-type) allele at this locus, while NC-Neuse has the *Rht-D1b* (height reducing) allele. This result suggested that the height reducing allele at the *Rht-D1* locus is associated with susceptibility.

Two QTL were mapped to chromosome 6A, but to two different 6A linkage groups. These QTL were designated *Qfhb.nc-6A.2* and *Qfhb.nc-6A.3*.

Quantitative trait loci associated with HD were mapped to chromosomes 1A.2, 4B.1, 5A.2, 6A.3, and 7D.1 (Table 4.4). At four of these QTL, Bess alleles were associated with an earlier HD, and at one QTL the NC-Neuse allele was associated with earlier heading. The QTL *Qhd.nc-1A.2* mapped to interval 149.1 – 169.4 cM, and did not overlap with the resistance QTL *Qfhb.nc-1A.2* at position interval 195.5 – 220.1 cM. The *Qhd.nc-4B.1* QTL mapped to interval 99.7 – 143.8 cM on chromosome 4B, which did not include the *Rht-B1* marker (position 37.5 cM). The *Qhd.nc-5A.2* QTL, at which the Bess allele was associated with earlier heading, was the only QTL mapped to chromosome 5A in this study. The *Qhd.nc-6A.3* QTL mapped to interval 6.2 - 20.8 cM on the 6A.3 linkage group, and did not overlap with the *Qfbh.nc-6A.3* QTL in interval 52.2 – 57.9 cM. The NC-Neuse allele at the *Qhd.nc-7D.1* QTL was associated with a later HD compared to the Bess allele. This is an agreement with results from the NC-Neuse / AGS 2000 mapping population, where a HD QTL was mapped to the same region of chromosome 7D, and the NC-Neuse allele was associated with a later HD.

The QTL associated with plant height were mapped to chromosomes 3B, 4B, 7B, and 7D (Table 4.5). The *Qht.nc-4B.1* QTL interval (54.3 – 73.3 cM) did not include *Rht-B1*

(position 37.5 cM). The *Qht.nc-7D.1* QTL did not overlap with the *Qhd.nc-7D.1* QTL. None of the QTL associated with HT overlapped with identified resistance QTL.

Comparisons of FHB Resistance among Groups with Different Allelic Combinations of Rht Genes

The 98 DH lines with different allelic combinations at the *Rht-B1* and *Rht-D1* loci were analyzed using the adjusted group means of INC, SEV, FDK, DON, HD, and HT (Table 4.6). Eight lines had neither *Rht-B1b* nor *Rht-D1b* (tall), 37 lines had *Rht-B1b*, 50 lines had *Rht-D1b*, and two lines had both *Rht-B1b* and *Rht-D1b* (double dwarfs). The lines in the tall group (*Rht-B1a*, *Rht-D1a*) had significantly lower INC, SEV, FDK, and DON than lines in the other three groups. The mean plant height of lines in the tall group was higher than the means of the other groups. There were no significant differences among the group with *Rht-B1b* and the group with *Rht-D1b* for any of the disease traits, HD, or HT. The two lines in the double dwarf group (*Rht-B1b*, *Rht-D1b*) had significantly higher SEV, FDK, and DON than lines in the other three groups. The double dwarfs headed significantly earlier than the tall lines and lines with *Rht-D1b*, and were shorter than the tall lines.

Identification of Overlapping QTL

The QTL *Qfhb.nc-1A.2*, *Qfhb.nc-4A.2a*, and *Qfhb.nc-6A.3* identified in this study overlapped with resistance QTL identified in the NC-Neuse / AGS 2000 population on chromosomes 1A, 4A, and 6A (Figure 4.1) (Petersen et al., 2013). In both mapping studies, NC-Neuse alleles at these loci were associated with resistance. The resistance QTL region on chromosome 1A was associated with reduced INC, SEV, FDK, and DON in the NC-Neuse /

AGS 2000 population, and with reduced INC and DON in the Bess / NC-Neuse population in the present study. The overlapping resistance QTL region on chromosome 4A was associated with reduced FDK and DON in the NC-Neuse / AGS 2000 population, while it was associated with reduced INC, SEV, FDK, and DON in the present study. The overlapping resistance QTL region on chromosome 6A was associated with reduced INC and SEV in the NC-Neuse / AGS 2000 population, and with FDK and DON in the Bess / NC-Neuse population in the present study.

The QTL *Qfhb.nc-2B.1* and *Qfhb.nc-3B.2* overlapped with resistance QTL identified on chromosomes 2B and 3B in the Truman / MO 94-317 population. In the present study, Bess alleles were associated with resistance at both QTL, and Truman alleles were associated with resistance in the Truman / MO 94-317 mapping study (Islam et al., 2011a; Islam et al., 2011b; Islam et al., 2011c). The QTL region on chromosome 2B was associated with reduced SEV under greenhouse conditions in the Truman / MO 94-317 study, while it was associated with reduced SEV and FDK in this study. The QTL region on chromosome 3B was associated with reduced INC, SEV (under both greenhouse and field conditions), and DON in the Truman / MO 94-317 study. In the present study, the QTL on chromosome 3B was associated with reduced SEV.

Test of Selected KASP assays Using USWWSN Entries

Fourteen KASP assays were determined to be the most representative for the five validated QTL regions on chromosomes 1A, 2B, 3B, 4A, and 6A (Table 4.7). When genotyping the entries from the USWWSN, an entry was categorized as having the resistance allele at the *Qfhb.nc-1A.2* QTL if it had the NC-Neuse allele for markers *IWB29758*,

IWB41257, and *IWB54518*. For the other QTL, similar categorizations of lines into groups with and without resistance alleles at the QTL were conducted based on the selected KASP assays.

For the 116 entries from the 2013 and 2014 USWWSN, the frequencies of the resistance alleles at each of the five validated QTL regions were: 0.03 for *Qfhb.nc-1A.2*, 0.26 for *Qfhb.nc-2B.1*, 0.04 for the *Qfhb.nc-3B.2*, 0.35 for *Qfhb.nc-4A.2a*, and 0.40 for *Qfhb.nc-6A.3*. When comparing phenotypic means of USWWSN entries with and without resistance alleles at QTL validated in this study, the QTL *Qfhb.nc-1A.2*, *Qfhb.nc-3B.2*, and *Qfhb.nc-6A.3* were associated with no significant effect on any of the disease traits (data not shown). The *Qfhb.nc-2B.1* QTL was associated with a significant reduction of INC, SEV, FDK, and DON (Table 4.8). Of the 29 entries in the resistant allele group for *Qfhb.nc-2B.1*, four entries possessed *Fhb1*, the 5A QTL from Ning8740, and the 2DL QTL from Wuhan-1, while one entry possessed *Fhb1* and the 5A QTL from Ning8740, and six entries possessed *Fhb1*. The *Qfhb.nc-4A.2a* QTL was associated with increased INC, FDK, and DON. Among the non-NC-Neuse and Bess QTL evaluated in the USWWSN, the resistance alleles at the 5A QTL from Ning8740, 2DL QTL from Wuhan-1 or W14, and *Fhb1* were associated with significantly reduced SEV, FDK, and DON. The resistance allele at the 3BL QTL from Massey was associated with no significant effect on any of the resistance traits (data not shown), while the resistance allele at the 5A QTL from Ernie was associated with a significant increase in INC, SEV, FDK, and DON. In entries with *Fhb1* (n=20), the addition of *Qfhb.nc-2B.1* significantly reduced INC, FDK, and DON after accounting for the effect of *Fhb1* (data not shown).

Results from this preliminary test indicated that the *Qfhb.nc-2B.1* QTL markers could be good candidates for MAS. For the QTL *Qfhb.nc-1A.2* and *Qfhb.nc-3B.2*, the frequencies of entries with resistance alleles at the QTL were very low, and small sample sizes therefore likely affected the outcome of the test. The results also suggested that, although genomic regions associated with FHB resistance have been validated, not all markers from the present study may be appropriate for immediate use in MAS across a range of germplasm. For certain chromosomal regions, further marker validation is required. It should also be noted that the statistical model used for this preliminary test did not account for differences in heading date, or other segregating loci, such as *Ppd*, *Vrn*, and *Rht* loci, among the USWWSN entries. Genetic factors other than the FHB QTL specified in the test may therefore have influenced the resistance levels.

Discussion

Multiple QTL associated with resistance in the Bess / NC-Neuse DH population were identified in this study based on phenotypic data collected from seven inoculated, mist-irrigated FHB trials in North Carolina and Missouri. Twelve of these QTL were significant in more than one environment and/or for different resistance traits, and therefore were considered reproducible. Adjusted means generated within and across environments were used in the QTL analysis so that resistance QTL with significant effects in multiple environments could be identified. The reproducible QTL mapped to chromosomes 1A, 1B, 2A, 2B, 3B, 4A (2 QTL), 4D, 5B, 5D, and 6A (2 QTL), with NC-Neuse alleles providing resistance at the QTL on chromosomes 1A, 2A, 4A, 5B, and 6A, while Bess alleles provided resistance at the QTL on chromosomes 1B, 2B, 3B, 4D, and 5D.

The QTL identified in the present study were compared to previous QTL mapping results from studies involving NC-Neuse and Truman by alignment of linkage maps. Five overlapping QTL regions were identified. The QTL *Qfhb.nc-1A.2*, *Qfhb.nc-4A.2a*, and *Qfhb.nc-6A.3* from the present study overlapped with the QTL regions *Qfhb.nc-1A*, *Qfhb.nc-4A*, and *Qfhb.nc-6A* identified in the NC-Neuse / AGS 2000 population (Petersen et al., 2013). NC-Neuse alleles conferred resistance at all three QTL in both studies. The QTL *Qfhb.nc-2B.1* and *Qfhb.nc-3B.2* from the present study overlapped with QTL regions identified in the Truman / MO 94-317 study (Islam et al., 2011a; Islam et al., 2011b; Islam et al., 2011c; McKendry, 2012). In both studies, Truman or Bess alleles conferred resistance.

The *Qfhb.nc-1A.2* QTL mapped to the distal end of chromosome 1A. Several FHB resistance QTL have been mapped to chromosome 1A, including one on the distal end in the Norwegian line NK93604 (Semangn et al., 2007). A QTL associated with reduced SEV was identified in the Chinese line CJ 9306 (Jiang et al., 2007), and mapped proximal to the region identified in NC-Neuse. Another QTL was mapped to the centromeric area of chromosome 1A in the Chinese cultivar Wangshuibai (Yu et al., 2008). Eckard et al. (2015) mapped resistance from the hard winter wheat cultivars Lyman and Overland to chromosome 1AS. The *Qfhb.nc-1A.2* QTL appears to be the first FHB resistance QTL mapped to chromosome 1AL in a U.S. cultivar.

The *Qfhb.nc-2B.1* QTL overlapped with the 2B QTL region identified in Truman, which was associated with reduced SEV under greenhouse conditions (Islam et al., 2011a). The 2B QTL identified in Truman contained the SSR marker *Xgwm271*, which was also near a QTL on chromosome 2B in Ernie (Liu et al., 2007; Abate et al., 2008). The Ernie QTL

mapped between markers *Xgwm271* and *Xgwm276b*, and was associated with reduced SEV under greenhouse conditions and resistance to FDK and DON. A QTL has been reported on chromosome 2B in the Chinese cultivar Sumai 3 (Zhou et al., 2002) that significantly reduced SEV.

The *Qfhb.nc-3B.2* QTL from the present study overlapped with the QTL region on chromosome 3B identified in Truman by Islam et al. (2011a; 2011b; 2011c). The SSR marker *Xgwm77* was located within the QTL region in both populations. This QTL region on the proximal end of chromosome 3BL was also identified in Ernie (Liu et al., 2007; Abate et al., 2008; Liu et al., 2013), with markers *Xwmc653*, *Xwmc307*, and *Xwmc1* as the most closely linked, and in Massey (Liu et al., 2013), where *Xbarc164* was the most closely linked SSR marker. *Xbarc164* was also within the QTL region identified on chromosome 3B in Truman. The SSR markers *Xwmc1*, *Xgwm77*, *Xgwm264b*, *Xwmc762*, *Xbarc164*, *Xwmc418*, and *Xwmc827* all mapped within or very close to the QTL regions identified in Ernie, Truman, Massey, and Bess.

The *Qfhb.nc-4A.2a* QTL mapped to chromosome 4A, on which three other QTL were mapped. A QTL was mapped in the German cultivar Pirat (Holzapfel et al., 2008) and another in the Swiss winter wheat cultivar Arina (Paillard et al., 2004). Both QTL were located on the distal end of chromosome 4A. A QTL in the hard winter wheat cultivar Lyman was mapped on chromosome 4A with peak marker *IWA482* (Eckard et al., 2015), which mapped within the *Qfhb.nc-4A* QTL interval identified in the NC-Neuse / AGS 2000 population. *IWA482* mapped just outside the *Qfhb.nc-4A.2a* QTL interval identified in the present study.

The *Qfhb.nc-6A.3* QTL was mapped to chromosome 6AL. Wright et al. (2014) reported a resistance QTL in the cultivar Jamestown which mapped on chromosome 6A, close to markers *IWB73509* and *IWB53065* (S. Malla, personal communication, 2015). According to the 90K SNP consensus map, these markers are located within the *Qfhb.nc-6A.3* QTL region validated in the present study. Four other QTL have been mapped to chromosome 6A; these include resistance from the German cultivar Dream (Schmolke et al., 2005), the French cultivar Apache (Holzapfel et al., 2008), the U.K. cultivar Spark (Srinivasachary et al., 2008), and the hard winter wheat U.S. cultivar Overland (Eckard et al, 2015). The QTL in Overland was mapped between markers *IWA2812* and *IWA1282*, with peak markers *IWA3527* and *IWA2235*. The marker *IWA3527* mapped to position 123.3 cM on the NC-Neuse / AGS 2000 linkage map, which was about 10 cM from the *Qfhb.nc-6A* QTL interval identified in the NC-Neuse / AGS 2000 population. The marker *IWA2812* mapped about 20 cM from the *Qfhb.nc-6A.3* QTL interval identified in the present study. Since FHB resistance QTL from NC-Neuse, Jamestown, and Overland have been mapped to this region of chromosome 6A, it may be a good candidate for MAS in both soft and hard winter wheat.

The *Qfhb.nc-4D.1* QTL including the *Rht-D1* locus on chromosome 4D was found associated with variation for SEV in LW14 and FDK in KIN12. In both cases, the Bess allele (*Rht-D1a*) conferred resistance compared to the NC-Neuse (*Rht-D1b*) allele. Other studies have shown an increased susceptibility associated with the height-reducing alleles at the *Rht-D1* locus (Draeger et al., 2007; Srinivasachary et al., 2008; Liu et al., 2013). Interestingly, the group means of DH lines with *Rht-B1b* were not significantly different from the group means

of DH lines with *Rht-D1b* for any of the disease traits, HD, or HT in the across-environments analysis. Because the two groups had very similar mean HT, it may not have been plant height per se that reduced SEV and FDK in LW14 and KIN12, respectively, but pleiotropic effects of the *Rht-D1* locus. The *Qfhb.nc-4D.1* QTL did not have a significant effect across environments which could suggest either a relatively low effect of the QTL, or that the effect is more pronounced in certain environments. The disease levels in LW14 and KIN12 were moderate. This corresponded with results described by Srinivasachary et al. (2009), where *Rht-D1b* was associated with a very significant increase in disease levels in field environments with moderate disease pressure and a less significant increase in disease levels under heavy FHB pressure.

The validated QTL regions on chromosomes 1A, 2B, 3B, 4A, and 6A were targeted for KASP assay development, as KASP assays generally are fast to run and easy to interpret, making them useful in MAS (Chen et al., 2010). Within the overlapping QTL regions, there were some dissimilarities in markers associated with the QTL peaks between the mapping studies. Thus, a set of KASP assays was developed based on peak SNP markers in the present study, which did not necessarily include the KASP assays developed for QTL on chromosomes 1A, 4A, and 6A in the NC-Neuse / AGS 2000 population. The dissimilarities in QTL peak markers between populations were likely due to differences in polymorphic markers, population type and size, linkage disequilibrium (LD), and linkage maps. Even though the Bess / NC-Neuse population was genotyped with the 90K SNP chip array, there were gaps on the linkage map and regions with marker distortion were observed on several

chromosomes. This was probably due to the relatively small population size, which may have reduced the number of recombinants.

The results varied in our preliminary test of KASP assays using recent USWWSN entries. Based on the markers used to categorize entries as having the *Qfhb.nc-2B.1* QTL or not, the group of entries with the resistance alleles had significantly lower disease means than the group of entries without the resistance alleles. This could indicate that the KASP markers represented the *Qfhb.nc-2B.1* region well and could be used across a range of germplasm. For the *Qfhb.nc-1A.2*, *Qfhb.nc-3B.2*, and *Qfhb.nc-6A.3* QTL, categorizing the entries using our KASP assays did not results in any significant differences among group means. Based on the markers used to categorize entries as having the *Qfhb.nc-4A.2a* QTL or not, the group of entries with the resistance alleles was, surprisingly and unexpectedly, associated with greater disease means than the group of entries without the resistance alleles. The results suggested that several of the KASP markers developed in the present study may not represent the FHB QTL regions well when haplotyping a range of germplasm. One of the reasons could be different LD in a broad set of unrelated lines compared to the bi-parental mapping population. For the overlapping QTL regions identified on chromosomes 1A, 4A, and 6A, KASP assays developed based on the NC-Neuse / AGS 2000 population may be used instead of the KASP assays developed based on the present study. Additional marker development and validation is recommended.

In conclusion, several genomic regions associated with FHB resistance were verified and targeted for marker development. Markers developed for the *Qfhb.nc-2B.1* QTL were identified as the best candidates for use in MAS. Following further marker validation, marker

haplotypes for the other QTL regions may also be used to conduct MAS and resistance allele enrichment in breeding populations.

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Table 4.1. Per plot and entry mean heritability estimates, their standard errors (SE), and harmonic means of environments (ENV) and replications (REP) used to calculate entry mean heritability for incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), deoxynivalenol content (DON), heading date (HD), and plant height (HT).

	INC	SEV	FDK	DON	HD	HT
Per plot basis heritability	0.23	0.28	0.14	0.20	0.64	0.65
(SE)	(0.03)	(0.04)	(0.03)	(0.03)	(0.04)	(0.04)
Entry mean heritability	0.82	0.83	0.63	0.72	0.95	0.94
(SE)	(0.03)	(0.03)	(0.06)	(0.04)	(0.01)	(0.01)
Harmonic mean of ENV	6.92	6.92	5.96	5.96	6.98	4.00
Harmonic mean of REP	17.14	17.10	15.42	15.50	18.47	8.32

Table 4.2. Pearson correlation coefficients between across-environments adjusted means for the four disease traits: Incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON) content, and heading date (HD) and plant height (HT).

Trait	SEV	FDK	DON	HD	HT
INC	r = 0.85 *** †	r = 0.86 ***	r = 0.85 ***	r = 0.31 **	r = -0.33 ***
SEV	…	r = 0.82 ***	r = 0.80 ***	r = 0.09 NS	r = -0.30 **
FDK	…	…	r = 0.88 ***	r = 0.28 **	r = -0.30 **
DON	…	…	…	r = 0.40 ***	r = -0.16 NS
HD	…	…	…	…	r = 0.32 **

† Significance levels of correlation coefficients are indicated as: NS ($P > 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

Table 4.3. Reproducible quantitative trait loci (QTL) associated with reduced incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), and deoxynivalenol content (DON) in the doubled haploid (DH) population derived from a cross between Bess and NC-Neuse. QTL were determined based on adjusted phenotypic means calculated within and across seven environments.

QTL[†]	Chromosome; position; marker interval	Trait_Env[‡]	Position	Peak marker	Position interval	LOD[§]	$R^{2¶}$	Resistance source[#]
Qfhb.nc-1A.2	1A.2; 195.5 - 220.1; *IWB29758 - IWB73950*	INC_KIN13	207.8	*IWB36272*	195.5 - 210.0	3.8	13.3	NC-Neuse
		INC_LW13	215.1	*IWB54518*	205.9 - 220.1	3.2	10.8	NC-Neuse
		DON_MO12	206.8	*IWB36272*	205.6 - 216.7	5.8	18.0	NC-Neuse
Qfhb.nc-1B.4	1B.4; 24.6 - 40.1; *IWB31692 - IWB9040*	INC_LW14	35.9	*IWB38247*	26.9 - 37.2	3.3	7.0	Bess
		DON_all	35.9	*IWB38247*	24.6 - 40.1	5.9	11.0	Bess
Qfhb.nc-2A.3	2A.3; 56.0 - 61.4; *IWB42392 - IWB3746*	INC_KIN13	58.9	*IWB836*	56.0 - 61.2	3.8	13.0	NC-Neuse
		FDK_MO12	58.9	*IWB836*	58.2 - 61.4	3.2	10.8	NC-Neuse
Qfhb.nc-2B.1	2B.1; 46.5 - 68.2; *IWB31987 - IWB40514*	SEV_all	56.2	*IWA5830*	46.6 - 48.4	5.2	15.1	Bess
		SEV_MO13	56.2	*IWA5830*	47.3 - 49.5	3.2	12.8	Bess
		FDK_KIN13	64.8	*IWB1769*	59.1 - 68.2	5.4	15.1	Bess
		DON_KIN12	56.2	*IWA5830*	46.5 - 50.3	6.0	21.1	Bess

Table 4.3. Continued

Qfhb.nc-3B.2	3B.2; 48.5 - 73.8; *IWB35616 - IWA4267*	SEV_KIN12	50.8	*IWB24497*	48.5 - 51.6	3.2	6.2	Bess
		SEV_LW14	70.1	*IWB57595*	62.7 - 73.4	4.7	14.9	Bess
		SEV_MO12	71.1	*IWB57595*	68.4 - 73.8	3.5	16.1	Bess
Qfhb.nc-4A.2a	4A.2; 3.0 – 49.4; *IWA2106 - IWB26481*	INC_LW14	43.9	*IWB29438*	37.9 - 49.4	3.8	16.1	NC-Neuse
		SEV_LW14	24.7	*IWB52747*	19.3 - 31.2	3.7	13.5	NC-Neuse
		FDK_all	31.0	*IWB44184*	8.0 - 45.3	6.0	23.3	NC-Neuse
		FDK_KIN12	40.9	*IWB29438*	31.9 - 49.4	3.9	13.9	NC-Neuse
		FDK_KIN13	29.0	*IWB44184*	24.6 - 34.9	4.7	13.1	NC-Neuse
		FDK_KIN14	31.0	*IWB44184*	4.1 - 46.8	5.8	20.7	NC-Neuse
		DON_all	36.9	*IWB29438*	33.3 - 39.4	3.6	11.6	NC-Neuse
		DON_KIN14	32.0	*IWB44184*	3.0 - 48.6	6.8	18.7	NC-Neuse
Qfhb.nc-4A.2b	4A.2; 65.6 – 113.1; *IWB12132 - IWA4859*	SEV_KIN12	87.3	*IWB36777*	65.6 - 113.1	7.7	20.0	NC-Neuse
		DON_MO13	99.6	*IWB28864*	90.6 - 107.2	4.4	17.4	NC-Neuse

Table 4.3. Continued

Qfhb.nc-4D.1	4D.1; 18.0 – 56.2; *IWB49801 - IWB61486*	SEV_LW14	45.4	*IWB54349*	40.0 - 56.2	7.5	10.0	Bess
		FDK_KIN12	31.6	*IWB53820*	18.0 - 36.4	4.0	9.6	Bess
Qfhb.nc-5B.1	5B.1; 40.9 – 46.5; *IWB51347 - IWB8972*	FDK_all	44.4	*IWB14332*	41.8 - 45.9	4.9	15.1	NC-Neuse
		FDK_KIN14	43.9	*IWA7181*	40.9 - 46.5	4.3	13.3	NC-Neuse
Qfhb.nc-5D.1	5D.1; 69.9 – 124.0; *IWB50247 - IWB54292*	INC_KIN14	97.5	*IWB61072*	69.9 - 121.5	5.9	26.7	Bess
		SEV_MO13	108.5	*IWB61072*	99.3 - 121.0	3.5	15.3	Bess
		DON_all	111.5	*IWB61072*	94.5 - 124.0	4.8	12.9	Bess
Qfhb.nc-6A.2	6A.2; 0.0 – 23.3; *IWB12224 - IWB74194*	INC_KIN12	11.3	*IWB2543*	0.0 - 19.6	7.5	17.5	NC-Neuse
		INC_LW14	11.3	*IWB2543*	0.0 - 23.3	6.4	20.2	NC-Neuse
		SEV_LW13	11.3	*IWB2543*	8.7 - 17.4	5.7	17.6	NC-Neuse
		FDK_KIN12	2.0	*IWB12224*	0.0 - 8.6	7.1	21.2	NC-Neuse
		FDK_LW14	3.0	*IWB12224*	0.0 - 7.0	3.3	17.8	NC-Neuse
		DON_all	11.3	*IWB12224*	0.0 - 20.9	7.1	17.8	NC-Neuse
		DON_KIN13	11.3	*IWB2543*	10.5 - 14.1	3.6	15.8	NC-Neuse

Table 4.3. Continued

Qfhb.nc-6A.3	6A.3; 52.2 – 57.9; *IWA6517 - IWA3585*	FDK_MO13	53.3	*IWB39452*	53.0 - 57.9	4.0	17.1	NC-Neuse
		DON_MO13	53.5	*IWB33725*	52.2 - 55.1	3.7	14.0	NC-Neuse

† QTL, quantitative trait loci. The prefix *Qfhb.nc* is followed by the chromosomal location of the QTL.
‡ Trait_Env, Trait_Environment. The trait/environment combination with which the QTL was associated.
§ LOD, logarithm of odds.
¶ Percentage of phenotypic variation explained by the marker at the peak of the QTL.
# Parental source contributing the resistance allele.

Table 4.4. Reproducible quantitative trait loci (QTL) associated with heading date (HD) in a doubled haploid (DH) population derived from a cross between the cultivars Bess and NC-Neuse. QTL were determined based on adjusted phenotypic means calculated within and across environments.

QTL[†]	Chromosome; position; marker interval	Trait_Env[‡]	Position	Peak marker	Position interval	LOD[§]	$R^{2¶}$	Early HD allele[#]
Qhd.nc-1A.2	1A.2; 149.1 - 169.4; *IWB11161 - IWA6042*	HD_all	157.6	*IWB6517*	149.5 - 165.1	5.2	16.5	Bess
		HD_KIN12	157.6	*IWB6517*	149.7 - 169.1	6.4	19.6	Bess
		HD_KIN14	152.2	*IWB45538*	149.1 - 169.4	4.4	10.3	Bess
Qhd.nc-4B.1	4B.1; 99.7 - 143.8; *IWB75098 - IWB73485*	HD_all	123.9	*IWB38327*	99.7 - 138.1	6.3	20.1	Bess
		HD_KIN12	123.9	*IWB38327*	106.2 - 128.7	4.4	19.6	Bess
		HD_KIN14	118.9	*IWB38327*	111.8 - 124.6	3.9	18.6	Bess
		HD_LW14	124.9	*IWB38327*	114.4 - 143.8	10.5	25.8	Bess
		HD_MO12	138.8	*IWB66445*	114.1 - 143.3	4.8	14	Bess
Qhd.nc-5A.2	5A.2; 0.0 - 33.2; *IWA6606 - IWB69484*	HD_KIN12	17.2	*IWB72774*	0.0 - 33.2	4.8	18.5	Bess
		HD_KIN14	6.0	*IWB27297*	0.0 - 14.0	7.6	24.9	Bess
		HD_LW14	1.0	*IWB33924*	0.0 - 8.3	7.4	24.5	Bess

Table 4.4. Continued

Qhd.nc-6A.3	6A.3; 6.2 - 20.8; *IWB26966 - IWB47278*	HD_all	14.2	*IWA2685*	12.9 - 15.8	3.2	8.2	NC-Neuse
		HD_LW13	15.2	*IWA2685*	11.7 - 20.8	4.9	13.1	NC-Neuse
		HD_MO12	11.7	*IWA1962*	6.2 - 22.4	4.9	10.5	NC-Neuse
Qhd.nc-7D.1	7D.1; 28.3 - 57.9; *IWB517 - IWB58968*	HD_LW13	51.3	*IWB16634*	31.8 - 54.9	5.4	22.2	Bess
		HD_LW14	51.3	*IWB16634*	28.3 - 57.9	6.9	19.2	Bess

† QTL, quantitative trait loci. The prefix *Q* is followed by *hd* (heading date) and then the chromosomal location of the QTL.
‡ Trait_Env, Trait_Environment. The trait/environment combination that the QTL was associated with.
§ LOD, logarithm of odds.
¶ Percentage of phenotypic variation explained by the marker at the peak of the QTL.
# Parental source contributing the early heading date (HD) allele.

Table 4.5. Reproducible quantitative trait loci (QTL) associated with plant height (HT) in a doubled haploid (DH) population derived from a cross between the cultivars Bess and NC-Neuse. QTL were determined based on adjusted phenotypic means calculated within and across environments.

QTL[†]	Chromosome; position; marker interval	Trait_Env[‡]	Position	Peak marker	Position interval	LOD[§]	$R^{2¶}$	Short HT allele[#]
Qht.nc-3B.3	3B.3; 9.8 - 36.9; *IWB47235 - IWB50139*	HT_all	17.7	*IWB65329*	16.1 - 22.8	3.7	16	NC-Neuse
		HT_KIN14	30.1	*IWB33964*	17.7 - 36.9	5.4	17	NC-Neuse
		HT_LW14	16.3	*IWB1595*	9.8 - 23.6	6.0	18.2	NC-Neuse
Qht.nc-4B.1	4B.1; 54.3 - 73.3; *IWA3287 - IWA908*	HT_KIN14	59.7	*IWB39342*	54.3 - 64.4	6.2	18.8	Bess
		HT_LW14	68.9	*IWB7077*	66.0 - 73.3	4.0	14.2	Bess
Qht.nc-7B.1	7B.1; 87.7 - 105.0; *IWB14040 - IWB10070*	HT_KIN13	97.1	*IWB39616*	89.2 - 105.0	4.3	15.4	NC-Neuse
		HT_LW13	97.1	*IWB39616*	87.7 - 105.0	4.3	15.4	NC-Neuse
Qht.nc-7D.1	7D.1; 69.8 - 82.9; *IWB46253 - IWB26628*	HT_KIN13	75.1	*IWB46253*	71.0 - 77.1	3.9	13.7	Bess
		HT_LW13	75.1	*IWB46253*	71.3 - 77.5	3.9	13.7	Bess
		HT_LW14	75.1	*IWB46253*	69.8 - 82.9	4.6	18.3	Bess

[†] QTL, quantitative trait loci. The prefix *Q* is followed by *ht* (plant height) and then the chromosomal location of the QTL.
[‡] Trait_Env, Trait_Environment. The trait/environment combination that the QTL is associated with.
[§] LOD, logarithm of odds.
[¶] Percentage of phenotypic variation explained by the marker at the peak of the QTL.
[#] Parental source contributing the short plant height (HT) allele.

Table 4.6. Adjusted means and their standard errors (SE) across environments of *Fusarium* head blight incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON) content, heading date (HD), and plant height (HT) of doubled haploid (DH) groups having different allelic combinations at the *Rht-B1* and *Rht-D1* loci. The DH lines were derived from a cross between the cultivars Bess and NC-Neuse.

Allelic combinations[†]	**n**[‡]	**INC (SE)**	**SEV (SE)**	**FDK (SE)**	**DON (SE)**	**HD (SE)**	**HT (SE)**
Rht-B1a, *Rht-D1a* (tall)	8	45.1[a] (1.4)	13.0[a] (1.0)	8.3[a] (1.0)	6.0[a] (1.0)	115.4[a] (0.2)	97.8[a] (0.6)
Rht-B1b, *Rht-D1a* (semi-dwarf)	37	55.6[b] (0.7)	17.3[b] (1.0)	11.2[b] (1.0)	8.0[b] (1.0)	114.7[ab] (0.1)	89.5[b] (0.3)
Rht-B1a, *Rht-D1b* (semi-dwarf)	50	55.4[b] (0.6)	18.3[bc] (1.0)	11.2[b] (1.0)	8.5[bc] (1.0)	115.9[a] (0.1)	90.0[b] (0.3)
Rht-B1b, *Rht-D1b* (double dwarf)	2	54.7[b] (2.8)	19.1[c] (1.1)	14.8[c] (1.1)	9.4[c] (1.1)	113.8[b] (0.3)	89.8[b] (1.3)

[†] Allelic combinations at the *Rht-B1* and *Rht-D1* loci. The *a* alleles are wild-type alleles, and the *b* alleles are height-reducing alleles.

[‡] n indicates the number of DH lines in each group.

[§] Adjusted means followed by the same letter within a column are not significantly different at $P < 0.05$. Because genotypes with different allelic combinations were unevenly replicated, individual t-tests were used to compare each pair of group means.

Table 4.7. Primer sequences for KASP assays developed for validated *Fusarium* head blight (FHB) resistance QTL on chromosomes 1A, 2B, 3B, 4A, and 6A, used to genotype the 2013 and 2014 Southern Uniform Winter Wheat Scab Nurseries.

QTL	SNP[†] no.	SNP name	Primer A1[‡]	Primer A2[•]	Primer C1
Qfhb.nc-1A.2	*IWB29758*	Excalibur_rep_c101787_89	GATCAAGATAGCAAGATGGTTTCCG	CGATCAAGATAGCAAGATGGTTTCCA	GAAGGAGATGACATTGCCGACGAT
Qfhb.nc-1A.2	*IWB41257*	Kukri_c14733_486	ACAATGAGAAGACCCTTTCTG	CTGCTACAATGAGAAGACCCTTTCTA	CAGAAGAACCCAAAATCGAGACGCTA
Qfhb.nc-1A.2	*IWB54518*	RAC875_c17651_1174	GGGAACCACTAGTTTGAAGTCCG	GGGAACCACTAGTTTGAAGTCCA	TCGCAGGGGATCATGGCGACAA
Qfhb.nc-2B.1	*IWA4606*	wsnp_Ex_c7003_12065828	GAAATTTGATCACCGGTCCTGCATA	AAATTTGATCACCGGTCCTGCATG	TCCCACGTGCCATATACGGTTGTTA
Qfhb.nc-2B.1	*IWB1769*	BobWhite_c23950_145	GACGAGATTACCATCGACGACG	GACGAGATTACCATCGACGACA	GCCAAGTGGGTAGAAGCTGATACTA
Qfhb.nc-3B.2	*IWA4755*	wsnp_Ex_c7967_13522958	AATACTATGTCTGCGGCTTCAGTCA	ACTATGTCTGCGGCTTCAGTCG	AGTAGATCACTCCATTGCTGTTCATTCAT
Qfhb.nc-3B.2	*IWA6381*	wsnp_Ku_c10291_17065480	CGTGAAACGCTCATGCCCACTA	GTGAAACGCTCATGCCCACTG	TTGGCGAGGATGCAGATTCACTCAA
Qfhb.nc-3B.2	*IWB65344*	TA001128-1276	ATGAATGGGAATGACTACGCATCAAAA	GAATGGGAATGACTACGCATCAAAG	CTTTCCGAAGCTTCACTCTCAGCTT

Table 4.7. Continued

Qfhb.nc-4A.2a	*IWB56116*	RAC875_c278_2934	ACAAATTCTTGTG AGGTCAAAACTCA	ACAAATTCTTGTG AGGTCAAAACTCG	CATCTCCGAGTTCT AATCCTGGAGTT
Qfhb.nc-4A.2a	*IWB44184*	Kukri_c33315_159	CCCTTTAAGAGCTC ATTATGGCG	GCCCTTTAAGAGC TCATTATGGCA	CTTAGAGATCCTG AAGAACTAGTTTT ATAT
Qfhb.nc-4A.2a	*IWA6418*	wsnp_Ku_c11227_18 378980	GTATTTCCGACCAC CGCGGTG	GTATTTCCGACCAC CGCGGTA	TCTGCGAGATGCA TGCTAAGGCAAA
Qfhb.nc-6A.3	*IWB11269*	BS00082640_51	CATCTTATATTCAT GATAGCGAATTCT GG	ACATCTTATATTCA TGATAGCGAATTC TGA	CCGTACTTTTTGTG CTTGGCCTTCAT
Qfhb.nc-6A.3	*IWB35085*	IAAV5761	TTTGGCCAGTCAA GCGACCGA	GGCCAGTCAAGCG ACCGG	GGGCGCGGAGGAC CCCTTA
Qfhb.nc-6A.3	*IWB7113*	BS00022489_51	GTGCCATGGCTTCT GCTCCTTT	GCCATGGCTTCTGC TCCTTC	GCGGTGAACAGAG GCTACTTTGAAT

† SNP, single nucleotide polymorphism. The prefixes *IWA* and *IWB* are followed by an index number.
‡ Primer sequences are given without the FAM (A1) and VIC (A2) tails.

Table 4.8. Mean incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) values for groups of lines from the 2013 and 2014 Uniform Southern Winter Wheat Scab Nursery with and without resistance alleles at quantitative trait loci (QTL) associated with resistance to *Fusarium* head blight (FHB).

QTL[†]	Allele[‡]	n[§]	INC		SEV		FDK		DON	
Qfhb.nc-2B.1	S	81	60.2	***	34.7	***	30.6	***	9.8	***
	R	29	56.3		31.6		27.1		8.1	
Qfhb.nc-4A.2a	S	74	58.4	***	34.6	NS	29.2	*	8.9	*
	R	39	61.9		33.6		31.0		10.1	
Ning_5A	S	109	59.5	*	34.5	***	30.3	***	9.5	***
	R	6	54.7		28.0		19.9		5.1	
Ernie_5A	S	108	58.9	**	33.3	***	28.9	***	8.8	***
	R	8	64.9		44.4		40.5		14.4	
Wuhan-1_2DL	S	110	59.4	NS	34.5	***	30.3	***	9.5	***
	R	6	57.8		26.5		19.0		4.8	
Sumai 3_*Fhb1*	S	96	59.3	NS	35.1	***	30.6	***	9.8	***
	R	20	59.4		29.0		25.5		6.7	

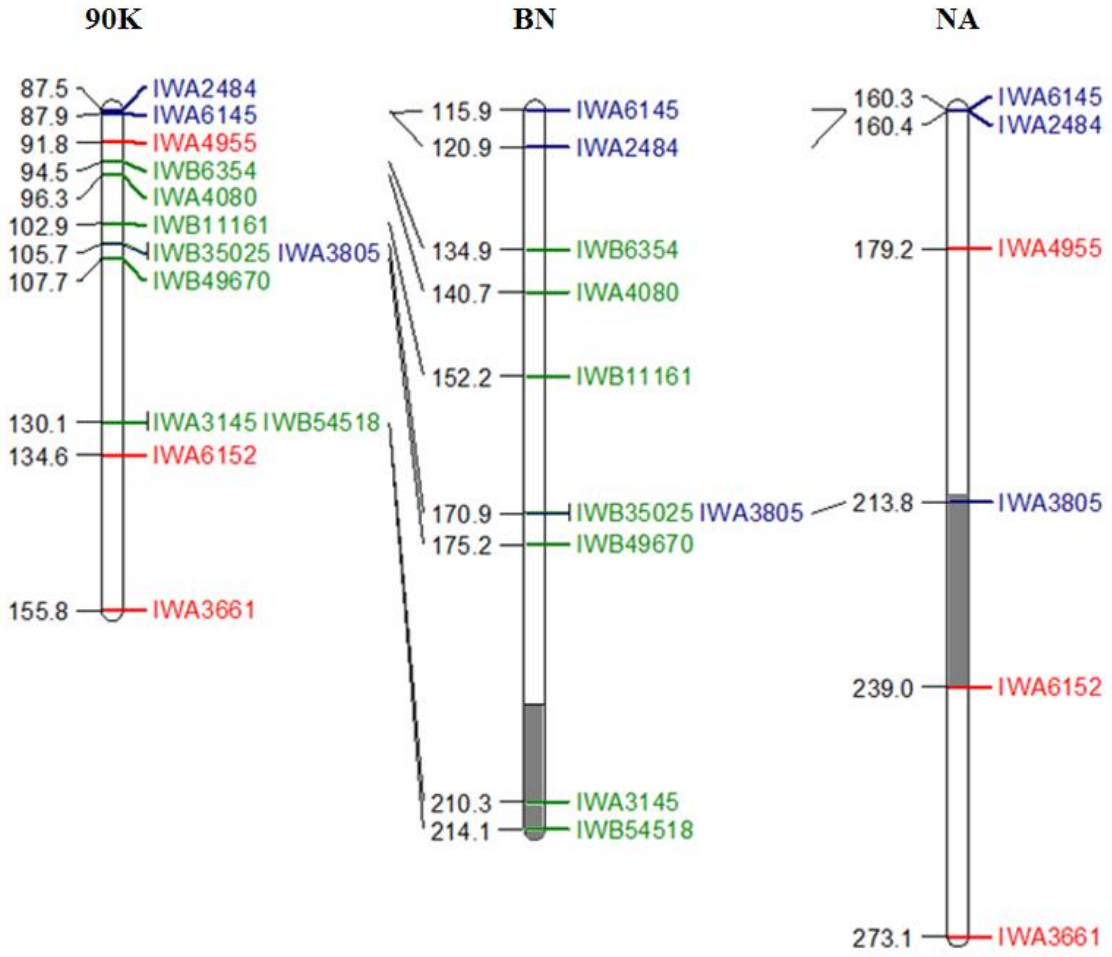
[†] QTL, quantitative trait loci. The two QTL presented with the prefix *Qfhb.nc-* followed by the chromosomal location were validated in the present study. The other four QTL are presented according to the source of resistance followed by chromosome number. The QTL *Fhb1* is located on chromosome 3BS.

[‡] Indicates presence of resistance allele (R) or susceptibility allele (S) at the respective QTL.

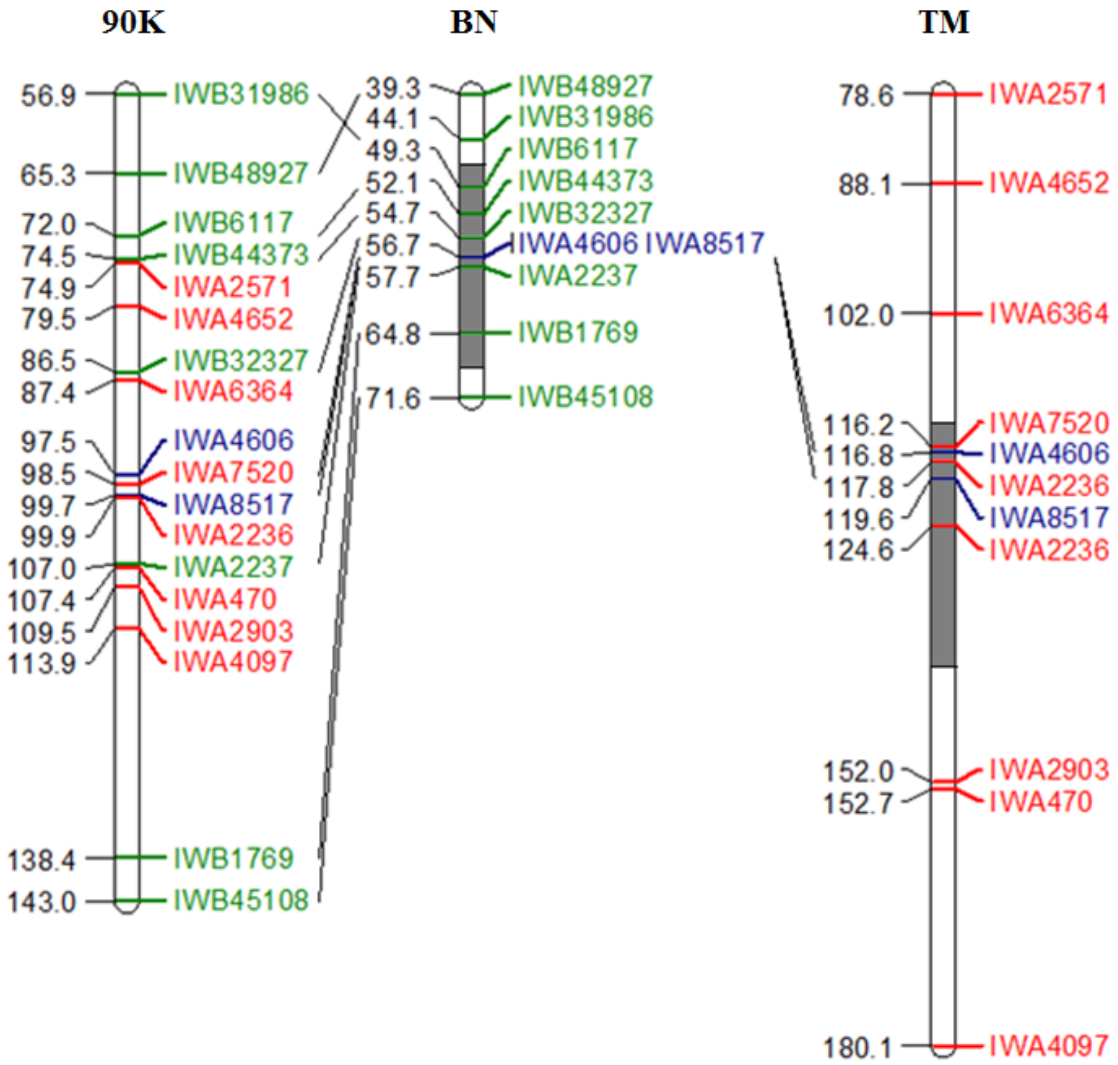
[§] n indicates the number of lines in the allele group.

[¶] Significance levels of mean comparisons are indicated as: NS ($P > 0.05$), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

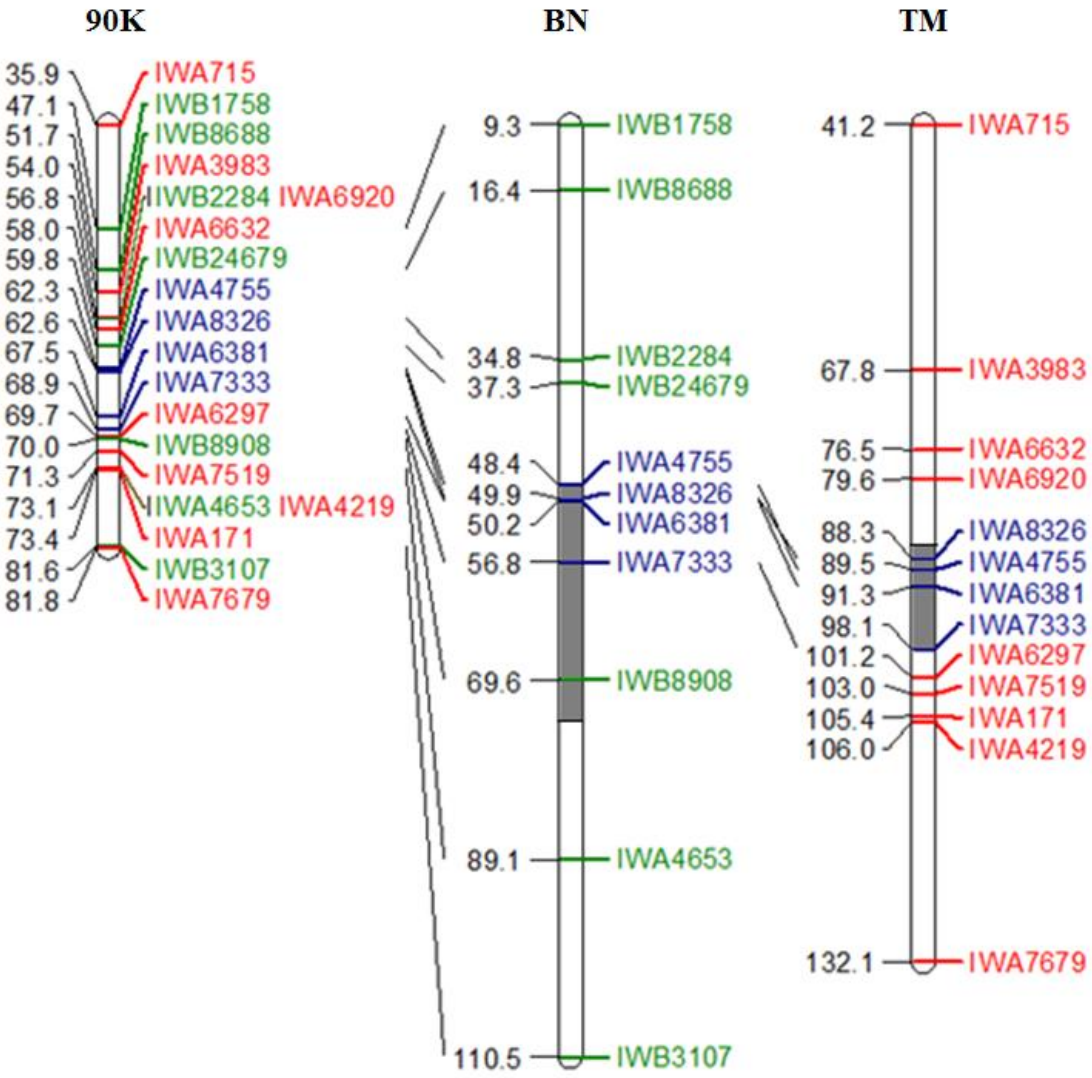
(a) Chromosome 1A



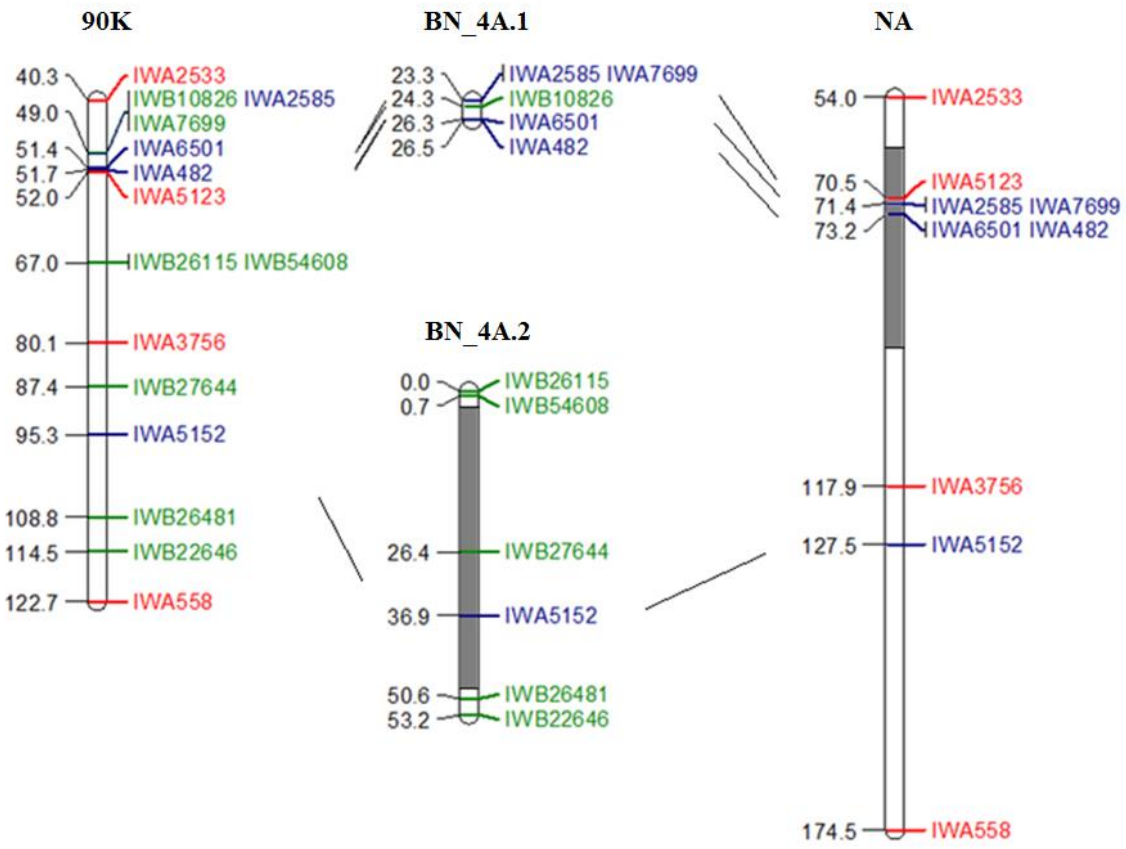
(b) Chromosome 2B



(c) Chromosome 3B



(d) Chromosome 4A



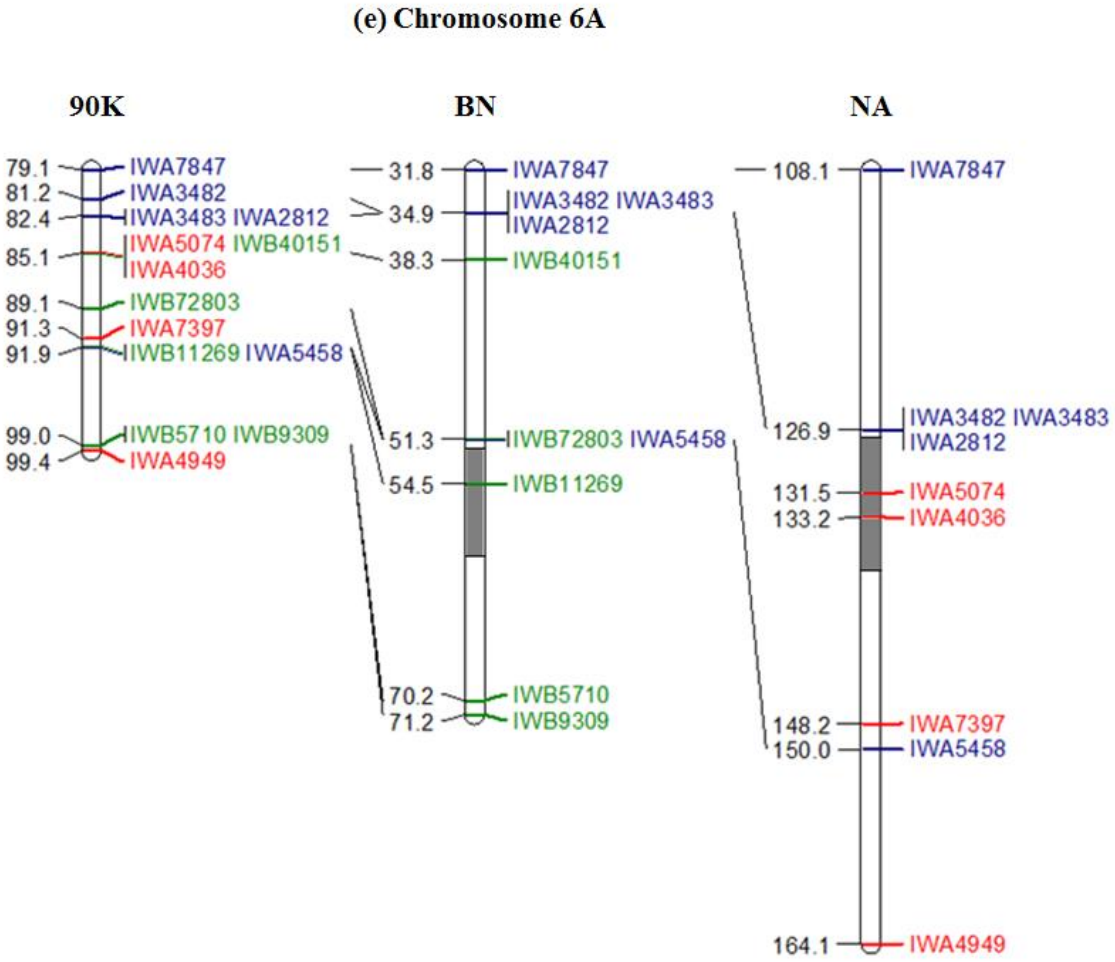


Figure 4.1. Comparative views of validated genomic regions associated with *Fusarium* head blight (FHB) resistance based on the Bess / NC-Neuse (BN) linkage map, the published 90K SNP consensus map (Wang et al., 2014), a linkage map developed for the NC-Neuse / AGS 2000 (NA) population, and a linkage map developed for the Truman / MO 94-317 (TM) population. Representative SNP markers from the validated regions on chromosomes 1A (a), 2B (b), 3B (c), 4A (d), and 6A (e) are shown. Marker names are at the right and map distances are on the left for each genetic map. Quantitative trait loci associated with resistance to FHB are indicated by blocks of grey on the linkage groups. Common markers between the 90K SNP map and more than one other linkage map are shown in blue and are connected with solid lines. Common markers between the 90K SNP map and the Bess / NC-Neuse map are shown in green, and common markers between the 90K SNP map and the NC-Neuse / AGS 2000 map or the Truman / MO 94-317 map are shown in red. The genetic linkage maps were drawn using MapChart 2.1 software (Voorrips, 2002).

APPENDICES

APPENDIX A - Supplemental Material for Chapter 3

Table A.1. Variance component estimates (Var.), their standard errors (SE), and significance levels (*P*) of the random effects in the mixed-model analysis of incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) concentration obtained across all environments for the NC-Neuse / AGS 2000 recombinant inbred line population.

	INC		**SEV**		**FDK**		**DON**	
Random factor	Var. (SE)	*P* †	Var. (SE)	*P*	Var. (SE)	*P*	Var. (SE)	*P*
Env	128.45 (98.51)	***	0.09 (0.06)	***	0.24 (0.16)	***	0.13 (0.10)	***
Rep (Env)	25.58 (12.99)	***	0.01 (0.01)	***	0.01 (0.01)	***	0.04 (0.02)	***
Genotype	50.05 (8.49)	***	0.03 (0.00)	***	0.11 (0.01)	***	0.13 (0.02)	***
Genotype x Env	40.69 (8.31)	***	0.02 (0.00)	***	0.07 (0.01)	***	0.07 (0.01)	***
Residual	246.91 (9.26)	….	0.12 (0.00)	….	0.19 (0.01)	….	0.17 (0.01)	….

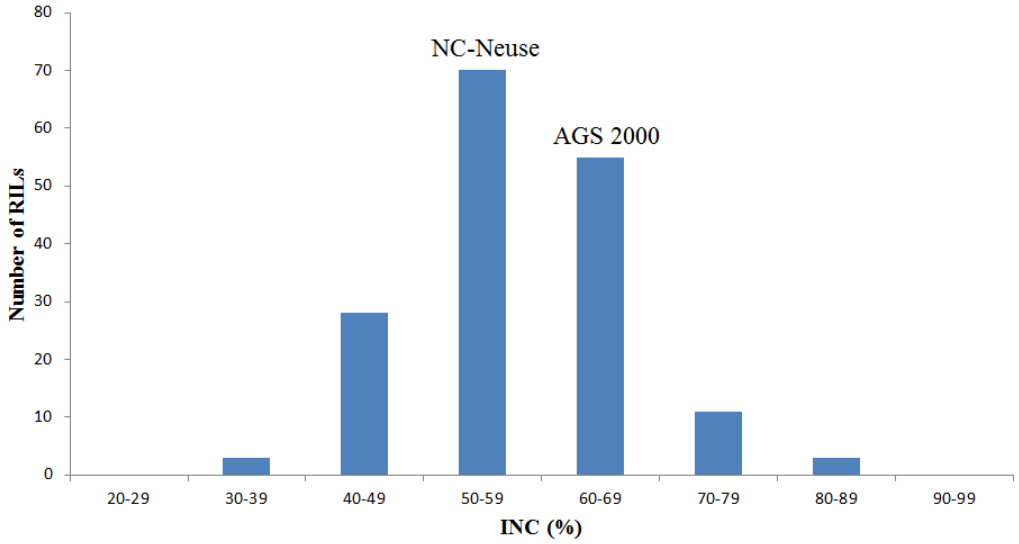
† Significance levels of effects are indicated as: *** ($P < 0.001$).

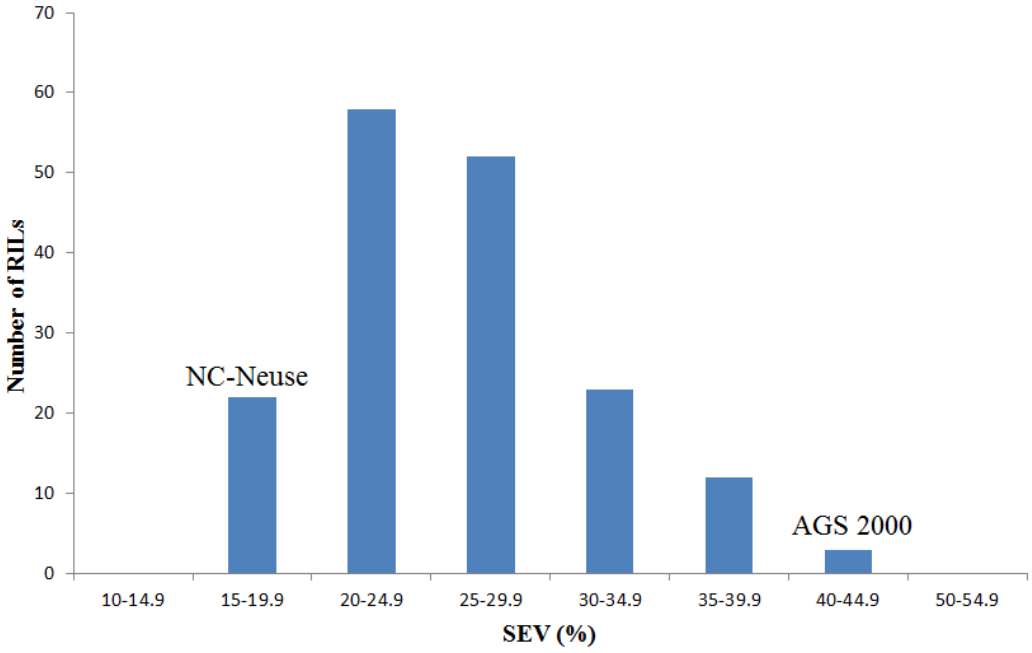
Table A.2. Adjusted means of population parents and recombinant inbred lines (RILs) for *Fusarium* head blight incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), deoxynivalenol content (DON), heading date (HD), and plant height (HT) calculated across and within environments. The range of mean values among RILs is listed for each trait. Means of AGS 2000 and NC-Neuse in the same column followed by different letters are significantly different at $P < 0.05$.

Trait	AGS 2000	NC-Neuse	RIL mean	RIL range
	Across environments			
INC	60.3[a]	52.9[a]	57.9	37.3 – 80.6
SEV	44.1[a]	18.9[b]	26.2	15.5 – 41.1
FDK	16.0[a]	10.8[b]	14.4	5.4 – 30.3
DON	20.4[a]	11.7[b]	15.3	5.8 – 40.7
HD	104[a]	109[b]	107	103 - 113
HT	90[a]	87[a]	88	76 – 103
	Kinston, NC 2011			
SEV	57.3[a]	32.2[b]	42.4	15.9 – 74.6
FDK	42.1[a]	20.3[b]	30.5	8.2 – 80.7
DON	48.5[a]	15.7[b]	25.8	5.4 – 65.9
HD	107[a]	109[b]	108	104 - 114
	Kinston, NC 2012			
FDK	11.4[a]	5.6[b]	10.7	1.1 – 34.8
DON	15.3[a]	14.4[a]	12.2	2.1 – 69.6
HD	90[a]	96[b]	94	87 - 101
	Salisbury, MD 2012			
INC	38.6[a]	56.1[b]	54.6	10.0 – 90.0
SEV	35.9[a]	18.6[b]	25.4	10.0 – 50.0
FDK	8.7[a]	11.4[a]	13.4	2.5 – 36.1
DON	11.1[a]	10.8[a]	14.1	3.1 – 35.5
HD	107[a]	112[b]	110	105 - 117
HT	82[a]	87[b]	84	70 - 100
	Kinston, NC 2013			
INC	56.8[a]	49.7[b]	55.6	19.7 – 86.0
SEV	45.4[a]	19.3[b]	29.7	11.7 – 72.9
FDK	26.6[a]	23.9[a]	21.8	6.1 – 53.0
DON	13.1[a]	12.5[a]	14.0	2.6 – 50.1
HD	107[a]	112[b]	110	105 - 115
HT	88[a]	85[a]	85	66 - 105

Table A.2. Continued

	Lake Wheeler, NC 2013			
INC	49.9[a]	30.2[b]	42.7	13.3 – 80.3
SEV	27.5[a]	10.5[b]	16.8	7.9 – 38.3
HD	106[a]	110[b]	109	105 - 117
HT	91[a]	89[a]	89	75 - 112
	Kinston, NC 2014			
INC	78.6[a]	69.6[a]	75.0	32.1 – 95.5
SEV	61.8[a]	21.8[b]	31.8	11.4 – 78.3
FDK	14.8[a]	9.1[b]	13.9	4.0 – 34.9
DON	32.3[a]	14.1[b]	23.3	7.6 – 63.3
HD	111[a]	118[b]	116	109 - 120
HT	88[a]	85[a]	86	65 - 100
	Lake Wheeler, NC 2014			
INC	77.4[a]	59.4[b]	61.4	36.2 – 89.6
SEV	48.0[a]	16.8[b]	22.3	10.6 – 43.1
FDK	9.7[a]	5.7[b]	9.1	1.2 – 29.9
DON	19.8[a]	6.3[b]	11.6	1.5 – 46.3
HD	114[a]	120[b]	118	113 - 123
HT	98[a]	95[a]	96	82 - 105


NC-Neuse
AGS 2000
Number of RILs
INC (%)
0
10
20
30
40
50
60
70
80
20-29
30-39
40-49
50-59
60-69
70-79
80-89
90-99


NC-Neuse
AGS 2000
Number of RILs
SEV (%)
0
10
20
30
40
50
60
70
10-14.9
15-19.9
20-24.9
25-29.9
30-34.9
35-39.9
40-44.9
50-54.9

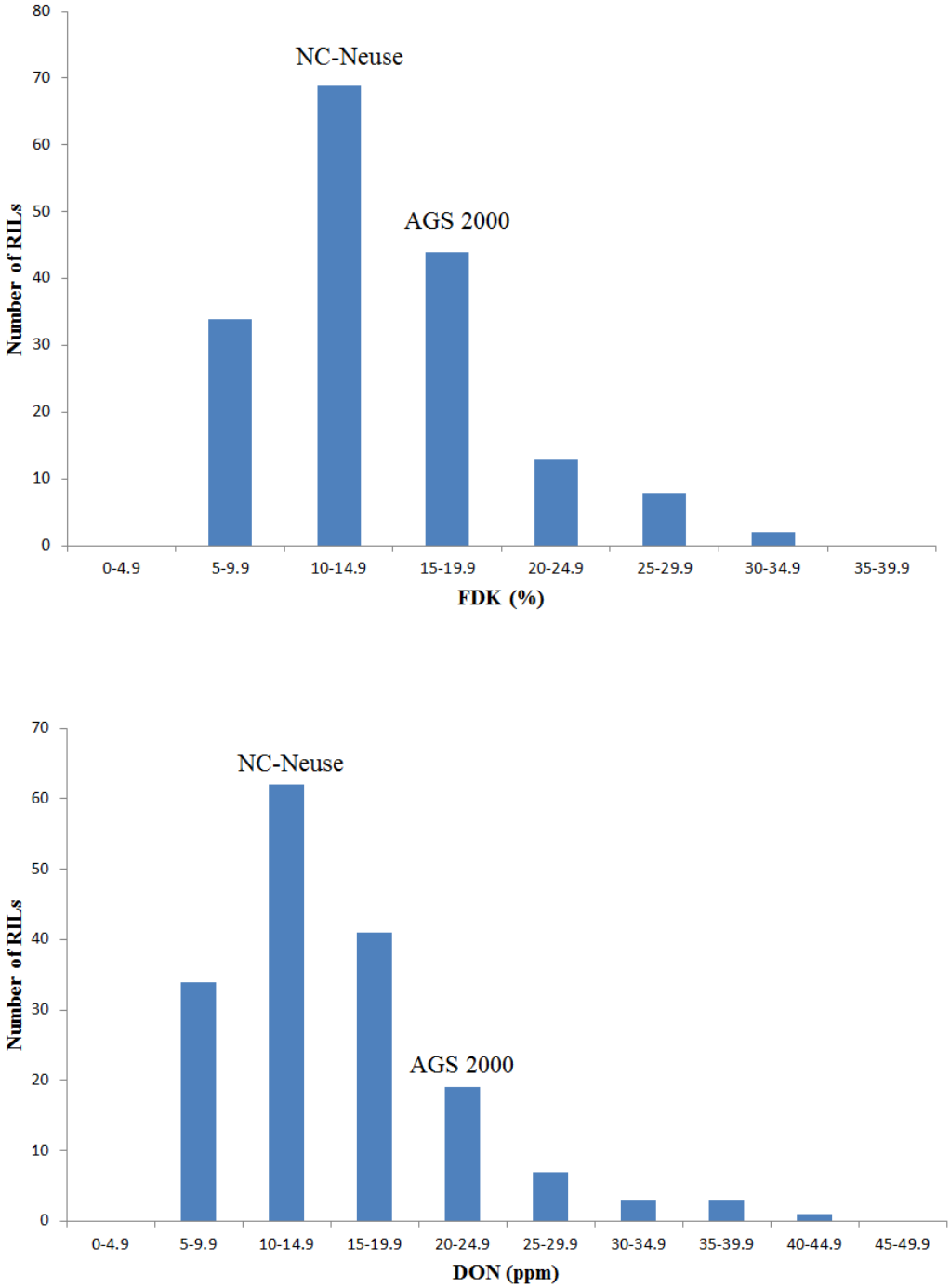


Figure A.1. Graphs showing frequency distributions of mean values for *Fusarium* head blight incidence (INC), disease severity (SEV), *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) content in the AGS 2000 / NC-Neuse recombinant inbred line (RIL) population. INC data was collected in a total of 5 environments, while SEV, FDK, and DON data was collected from 6 environments each.

APPENDIX B - Supplemental Material for Chapter 4

Table B.1. Variance component estimates (Var.), their standard errors (SE), and significance levels (*P*) of the random effects in the mixed-model analysis of *Fusarium* head blight incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) concentration obtained across seven environments for the Bess / NC-Neuse doubled haploid population.

	INC		**SEV**		**FDK**		**DON**	
Random factor	Var. (SE)	*P* †	Var. (SE)	*P*	Var. (SE)	*P*	Var. (SE)	*P*
Env	445.20 (265.46)	*	0.06 (0.04)	*	0.80 (0.51)	*	0.37 (0.24)	*
Rep (Env)	26.38 (12.38)	*	0.01 (0.00)	*	0.00 (0.00)	*	0.03 (0.01)	*
Genotype	83.93 (14.85)	***	0.06 (0.01)	***	0.09 (0.02)	***	0.11 (0.02)	***
Genotype x Env	32.99 (8.46)	***	0.03 (0.01)	***	0.10 (0.01)	***	0.09 (0.01)	***
Residual	240.48 (10.51)	….	0.12 (0.01)	….	0.14 (0.01)	….	0.15 (0.01)	….

† Significance levels of effects are indicated as: * ($P < 0.05$), *** ($P < 0.001$).

Table B.2. Adjusted means of Bess, NC-Neuse, and doubled haploid (DH) progeny for *Fusarium* head blight incidence (INC), severity (SEV), *Fusarium*-damaged kernels (FDK), deoxynivalenol content (DON), heading date (HD), and plant height (HT) calculated across and within seven environments. The range of mean values among DH lines is listed for each trait. Means of Bess and NC-Neuse in the same column followed by different letters are significantly different at $P < 0.05$.

Trait	Bess	NC-Neuse	DH mean	DH range
		Across environments		
INC	38.4[a]	60.4[b]	53.6	32.0 – 76.0
SEV	10.8[a]	20.9[b]	18.1	9.7 – 39.9
FDK	6.4[a]	13.0[b]	11.6	4.7 – 22.7
DON	5.4[a]	10.3[b]	8.6	3.4 – 19.1
HD	115[a]	117[b]	115	109 – 124
HT	94[a]	93[a]	93	75 – 109
		Kinston, NC 2012		
INC	20.6[a]	59.6[b]	49.3	15.0 – 85.4
SEV	8.0[a]	20.8[b]	16.7	8.0 – 43.9
FDK	5.4[a]	11.8[b]	8.9	3.2 – 29.7
DON	9.0[a]	17.2[b]	16.9	3.3 – 43.5
HD	93[a]	96[b]	94	85 – 104
		Kinston, NC 2013		
INC	28.4[a]	61.1[b]	45.0	6.7 – 86.7
SEV	14.1[a]	26.3[b]	23.3	8.0 – 81.0
FDK	54.7[a]	70.3[b]	65.1	42.7 – 80.5
DON	5.1[a]	21.1[b]	9.4	2.8 – 23.9
HD	108[a]	111[b]	109	101 – 121
HT	98[a]	94[b]	91	75 – 115
		Kinston, NC 2014		
INC	46.7[a]	49.2[b]	48.3	10.0 – 82.5
SEV	20.2[a]	26.6[b]	23.6	8.0 – 62.6
FDK	7.3[a]	11.2[b]	12.2	2.7 – 30.2
DON	9.3[a]	12.6[b]	15.5	2.5 – 42.8
HD	117[a]	118[a]	117	109 - 122
HT	88[a]	91[a]	87	70 - 105
		Lake Wheeler, NC 2013		
INC	8.1[a]	22.9[b]	18.6	2.8 – 52.2
SEV	8.3[a]	16.5[b]	12.0	6.3 – 35.0
HD	109[a]	111[b]	110	100 – 122

Table B.2. Continued

	Lake Wheeler, NC 2014			
INC	36.7[a]	68.0[b]	60.7	9.4 – 95.0
SEV	10.8[a]	27.2[b]	24.0	8.0 – 64.0
FDK	3.0[a]	8.0[b]	7.4	1.3 – 20.1
DON	6.7[a]	10.1[b]	11.9	2.7 – 57.6
HD	117[a]	120[b]	118	110 - 124
HT	98[a]	94[a]	92	76 – 114
	Columbia, MO 2012			
INC	56.7[a]	76.7[b]	77.2	50.0 – 95.0
SEV	9.5[a]	23.3[b]	15.4	5.9 – 34.6
FDK	1.8[a]	6.0[b]	6.6	1.6 – 17.5
DON	1.7[a]	3.2[b]	2.9	1.0 – 10.4
HD	119[a]	118[a]	119	111 - 127
	Columbia, MO 2013			
INC	73.3[a]	93.3[b]	82.5	45.0 – 100.0
SEV	8.8[a]	26.8[b]	20.3	6.5 – 53.9
FDK	4.9[a]	16.8[b]	10.2	12.3 – 29.0
DON	4.7[a]	9.1[b]	6.6	2.1 – 18.0
HD	143[a]	142[a]	143	140 – 153

APPENDIX C – Examples of SAS Code Used for Data Analyses

*Checking for violations of the assumptions for analysis of variance and linear mixed models: Example of test for normal distribution of non-transformed data, log-transformed data, and square root transformed data (with INC variable):

```
data NApop_allyrs; set NApop_allyrs;
logINC = log(INC);
sqINC = sqrt(INC);
run;
proc univariate data= NApop_allyrs normal plot;
var INC;
qqplot INC /normal(mu=est sigma=est color=red l=1);
run;
proc univariate data= NApop_allyrs normal plot;
var logINC;
qqplot INC /normal(mu=est sigma=est color=red l=1);
run;
proc univariate data= NApop_allyrs normal plot;
var sqINC;
qqplot INC /normal(mu=est sigma=est color=red l=1);
run;
```

*Checking for violations of the assumptions for analysis of variance and linear mixed models: Example of macro for making residuals vs. predicted plots (with INC variable):

```
%macro respred(trait);
proc glm data=NApop_allyrs;
        class line;
        model &trait = line;
        output out=&trait.plot predicted=pred residual=resid;
run;
proc sgplot data=&trait.plot;
        title "&trait resid vs. pred";
scatter x=pred y=resid;
xaxis label ='pred' interval=auto;
yaxis label='resid' interval=auto;
run;
%mend;
%respred(INC);
%respred(logINC);
%respred(sqINC);
run;
```

```
*Program for estimation of variance components and heritabilities for each FHB trait, HD,
and HT:
data one;
set one;
if line in (  'barley' )    then delete;
log_SEV=log(SEV);
log_FDK=log(FDK);
log_DON=log(DON);
run;
proc freq data=one noprint; where line not in (  'AGS2000'  'C9835'  'Ernie'  'Neuse'  'barley');
  tables line/list out=outlin;
  run;
*****;
data traitds  ;
 set  one;    where line not in (  'AGS2000'  'C9835'  'Ernie'  'Neuse'  'barley');
 geno= line;
 run;
proc freq data=traitds  noprint;
 tables geno/list out=outentry;
 run;
proc sort data=traitds;
  by env rep  geno ;
```

```
 run;
 proc sort data=traitds;
 by loc;
 run;
**************************;
ods graphics on;
*************************************************************************;
*** first, estimate variance components for each trait separately;
%macro varcomp(trait);
TITLE "varcomp &TRAIT";
proc mixed data = traitds;
class env rep geno;
model &trait = ;
random env rep(env) geno geno*env;
run;
TITLE;
QUIT;
%mend;

%macro heritabilityH (dataset, trait);
*** all effects are random;
TITLE "&TRAIT";
```

```
 proc freq data= &dataset noprint;  where &trait ne . ;
  tables env*geno*Rep/list out=outfq1 nocum nopercent;
   run;
 proc print data= outfq1;
 run;
** number of repetitions per gen and env;
proc freq data=outfq1  noprint;
  tables  geno*env /list out=outfq2 nocum nopercent;  **per env;
  tables  geno     /list out=outfq3 nocum nopercent;  **over all env;
  run;
 proc print data= outfq2(obs=10);
run;
 proc print data= outfq3(obs=10);
run;
** number of env per geno and env;
proc freq data=outfq2 noprint;
  tables  geno  /list out=outfq4 nocum nopercent;  **per env;
title "&trait - Number of env per gen ";
data env_num;
 set outfq4 (drop=percent );
  inv_e= 1/count;
  run;
```

```
 proc print data=  env_num(obs=10);
*run;
  proc means data = env_num  noprint;
  var inv_e;
  output out=out_e(drop=_TYPE_ _FREQ_) mean=;
  run;
  data out_e;
  set out_e;
  HM_e = 1/inv_e;
  run;
  title2 "Average number of env";
 proc print data= out_e;
 run;
  data _null_;
    set out_e;
          call symput ("Hme", Hm_e );
          run;
 ************************************************;
title "&trait total number of reps over all env per gen";
data rep_num;
 set outfq3 (drop=percent );
  inv_r= 1/count;
```

```
 run;
 proc print data= outfq3(obs=10) ;
run;
  proc means data = rep_num noprint;
  var inv_r;
  output out = out_r(drop=_TYPE_ _FREQ_) mean= ;
  run;
  data out_r;
    set out_r;
    Hm_r = 1/inv_r;
  run;
  title2 "Average number of reps";
 proc print data= out_r;
 run;
  data _null_;
    set out_r;
          call symput ("Hmr", Hm_r );
run;
*****************************************************************;
proc mixed asycov data = &dataset  ;  where &trait ne .;
 class  env rep geno  ;
 model &trait =  ;
```

```
 random env rep(env)  geno   geno*env  ;
 ods output covparms = estmat&trait; ods output asycov = covmat&trait;
run;
proc iml;
Start seh(V, C, LG, LP, H, SE);
   Vp = LP`*V;
   Vg = LG`*V;
    H = VG/Vp;
    d = (1/Vp)*(LG - (LP*H));
   VH = d`*C*d;
   SE = sqrt(VH);
 finish seh;
*****;
use estmat&trait; read all into V;
use covmat&trait; read all into cov;
C = cov(|1:nrow(cov), 2:ncol(cov)|);
*Note that SAS introduces an extra first column into the covariance matrix which must be
removed;
C = cov(|1:nrow(cov), 2:ncol(cov)|);
*order of variance components in v and c matrices is V(E), V(R), V(G), V(GE), V(error);
LG = {0, 0, 1, 0, 0};  *random effects rep(env)  geno   geno*env  plus residual  ;
LP = {0, 0, 1, 1, 1};
```

```
call seh(V, C, LG, LP, H, SE);
print "&trait - Heritability on a Plot Basis &trait", H, SE;
*inserting the harmonic mean of environments in which lines were tested, and the harmonic
mean of total replications across all environments;
e = &Hme;
r = &HMr;
LP = 0//0//1//(1/e)//(1/r);
call seh(V, C, LG, LP, H, SE);
print "&trait - Heritability on a Family Mean Basis ", e, r, H, SE;
quit; run;
*****;
%mend;
***** *BY ENV          ****;
proc sort data= traitds;
 by env;
 run;
 ods pdf file = "BY_ENV_analysis.pdf";

title 'by env log_DON';
proc mixed asycov data =traitds covtest plots=all;  by env; where DON ne .;
 class  env rep  geno  ;
 model  log_DON  =;
```

```
 random    rep   geno  ;
 ods output covparms = estmatdon ;
 ods output asycov = covmatdon;
run;

title 'by env log_FDK';
 proc mixed asycov data =traitds covtest  plots=all; by env; where FDK  ne .;
 class  env rep  geno  ;
 model  log_FDK =;
 random   rep  geno  ;
 ods output covparms = estmatFDK ;
 ods output asycov = covmatFDK;
run;

title 'by env log_SEV';
proc mixed asycov data =traitds covtest  plots=all; by env;where SEV  ne .;
 class  env rep  geno  ;
 model  log_SEV =;
 random    rep geno ;
  ods output covparms = estmatSEV;
 ods output asycov = covmatSEV;
run;
```

```
title 'by env INC';
proc mixed asycov data =traitds covtest  plots=all; by env;where INC  ne .;
 class  env rep  geno  ;
 model  INC =;
 random    rep geno ;
  ods output covparms = estmatINC;
 ods output asycov = covmatINC;
run;

title 'by env HT';
proc mixed asycov data =traitds covtest  plots=all; by env;where SEV  ne .;
 class  env rep  geno  ;
 model  HT =;
 random    rep geno ;
  ods output covparms = estmatSEV;
 ods output asycov = covmatSEV;
run;

title 'by env HD';
proc mixed asycov data =traitds covtest  plots=all; by env;where SEV  ne .;
 class  env rep  geno  ;
 model  HD =;
```

```
 random    rep geno ;
  ods output covparms = estmatSEV;
 ods output asycov = covmatSEV;
run;
TITLE;
quit;
ods pdf close;
ODS PDF FILE= "VARCOMP.PDF";
%varcomp(log_DON);
%varcomp(log_FDK);
%varcomp(log_SEV);
%varcomp(INC);
%varcomp(HT);
%varcomp(HD);
QUIT;
ODS PDF CLOSE;


ods pdf file="heritability_REML_DON.pdf"  ;
%let trait= DON;
TITLE "ANALYSIS OF VARIANCE &TRAIT";
proc mixed asycov data =traitds covtest plots=all  where DON ne .;
 class  env rep  geno  ;
```

```
 model  log_DON  =;
   random  env   rep( env)  geno  geno*env ;
  ods output covparms = estmatkghat;
 ods output asycov = covmatDON;
run;
%HeritabilityH (traitds , DON) ;
run;
TITLE;
quit;
ods pdf close;


 ods pdf file="heritability_REML_FDK.pdf"  ;
 %let trait= FDK;
TITLE "ANALYSIS OF VARIANCE &TRAIT";
proc mixed asycov data =traitds covtest  plots=all;
 class  env rep  geno  ;
 model  log_FDK  =;
   random  env   rep( env)  geno  geno*env ;
 ods output covparms = estmatkghat;
 ods output asycov = covmatkgha;
run;
%HeritabilityH (traitds , FDK);
```

```
run;
TITLE;
quit;
ods pdf close;

 ods pdf file="heritability_REML_SEV.pdf"  ;
%let trait= SEV;
TITLE "ANALYSIS OF VARIANCE &TRAIT";
proc mixed asycov data =traitds covtest  plots=all;
 class  env rep  geno  ;
 model  log_SEV =;
   random  env   rep( env)  geno  geno*env ;
 ods output covparms = estmatkghat;
 ods output asycov = covmatkgha;
run;
%HeritabilityH (traitds , SEV );
run;
TITLE;
quit;
ods pdf close;

 ods pdf file="heritability_REML_INC.pdf"  ;
```

```
 %let trait= INC;
TITLE "ANALYSIS OF VARIANCE &TRAIT";
proc mixed asycov data =traitds covtest  plots=all;
 class  env rep  geno  ;
 model INC  =;
   random  env   rep( env)  geno  geno*env ;
 ods output covparms = estmatkghat;
 ods output asycov = covmatkgha;
run;
%HeritabilityH (traitds , INC  );
run;
TITLE;
quit;
ods pdf close;

 ods pdf file="heritability_REML_HT.pdf"  ;
 %let trait= HT;
TITLE "ANALYSIS OF VARIANCE &TRAIT";
proc mixed asycov data =traitds covtest  plots=all;
 class  env rep  geno  ;
 model HT  =;
   random  env   rep( env)  geno  geno*env ;
```

```
 ods output covparms = estmatkghat;
 ods output asycov = covmatkgha;
run;
%HeritabilityH (traitds , HT  );
run;
TITLE;
quit;
ods pdf close;


 ods pdf file="heritability_REML_HD.pdf"  ;
 %let trait= HD;
TITLE "ANALYSIS OF VARIANCE &TRAIT";
proc mixed asycov data =traitds covtest  plots=all;
 class  env rep  geno  ;
 model HD  =;
   random  env   rep( env)  geno  geno*env ;
 ods output covparms = estmatkghat;
 ods output asycov = covmatkgha;
run;
%HeritabilityH (traitds , HD  );
run;
TITLE;
```

```
quit;
ods pdf close;

*Calculation of means adjusted for all non-genetic factors for all FHB variables, HD, and HT
(across environments);
%macro mixed1(trait);
proc mixed data = NA_allyrs_RILs covtest;
class geno env rep;
model &trait = geno;
random env rep(env) geno*env ;
lsmeans geno / pdiff;
run;
%mend;
%mixed1(INC);
%mixed1(logSEV);
%mixed1(logFDK);
%mixed1(logDON);
%mixed1(HD);
%mixed1(HT);
run;
```

```
* Calculation of means adjusted for all non-genetic factors for all FHB variables, HD, and
HT (across environments) with HD as a covariate:
%macro mixed2(trait);
proc mixed data = NA_allyrs_RILs covtest;
class geno env rep;
model &trait = geno HD(env);
random env rep(env) geno*env ;
lsmeans geno;
run;
%mend;
%mixed2(INC);
%mixed2(logSEV);
%mixed2(logFDK);
%mixed2(logDON);
run;



* Calculation of means adjusted for all non-genetic factors for all FHB variables, HD, and
HT (within environments);
%macro mixed3(trait);
proc mixed data = NA_allyrs_RILs covtest; by env;
class geno rep;
```

```
model &trait = geno;
random rep ;
lsmeans geno / pdiff;
run;
%mend;
%mixed3(INC);
%mixed3(logSEV);
%mixed3(logFDK);
%mixed3(logDON);
%mixed3(HD);
%mixed3(HT);
run;

* Calculation of means adjusted for all non-genetic factors for all FHB variables, HD, and
HT (within environments) with HD as a covariate;
%macro mixed4(trait);
proc mixed data = NA_allyrs_RILs covtest; by env;
class geno rep;
model &trait = geno HD;
random rep;
lsmeans geno;
run;
```

```
%mend;
%mixed4(INC);
%mixed4(logSEV);
%mixed4(logFDK);
%mixed4(logDON);
run;
```

```
*Comparison of groups means of genotypes from USWWSN with and without resistance
allele at QTL (example with QTL_6A);
%macro mixed5(trait);
proc mixed data = UFHBN_all_0403 covtest;
class year geno rep QTL_6A;
model &trait = QTL_6A geno(QTL_6A);
random year rep(year);
lsmeans QTL_6A /;
run;
%mend;
%mixed5(INC);
%mixed5(SEV);
%mixed5(FDK);
%mixed5(DON);
```

```
run;

*Comparison of groups means of genotypes from USWWSN with and without resistance
allele at two different QTL (example with QTL_6A and Fhb1 combined);
%macro mixed6(trait);
proc mixed data = UFHBN_all_21reps covtest;
class year geno rep Fhb1 QTL_6A;
model &trait = Fhb1 QTL_6A Fhb1*QTL_6A geno(Fhb1*QTL_6A);
random year rep(year);
lsmeans Fhb1 QTL_6A Fhb1*QTL_6A /pdiff;
run;
%mend;
%mixed6(INC);
%mixed6(SEV);
%mixed6(FDK);
%mixed6(DON);
run;

*Comparison of group means of genotypes with and without Rht-B1b (Rht1) and Rht-D1b
(Rht2) in the Bess / NC-Neuse population;
proc glm data = NBpop_allyrs;
class env rep line Rht1 Rht2;
```

```
model INC = env rep(env) Rht1 Rht2 Rht1*Rht2 line(Rht1 Rht2);
lsmeans Rht1 Rht2 Rht1*Rht2 / stderr pdiff;
run;
```

APPENDIX D – Genetic linkage map for the NC-Neuse / AGS 2000 recombinant inbred line (RIL) population

Table D.1. Genetic linkage map for the NC-Neuse / AGS 2000 recombinant inbred line population organized by linkage group number, chromosome label, marker, and marker position within each linkage group. The prefix 'IWA' designates SNP markers, 'wPt', 'rPt', and 'tPt' DArT markers, 'Xbarc', 'Xcfa', 'Xcfd', 'Xgwm', 'Xpsp', and 'Xwmc' SSR markers, and Ppd_B1 and Vrn_B1 are KASP markers.

Link. group number	Chromosome label	Marker	Position (cM)
1	1A	IWA6644	0
1	1A	wPt730618	3.6
1	1A	wPt666537	3.9
1	1A	wPt3198	3.9
1	1A	wPt6709	3.9
1	1A	wPt734000	3.9
1	1A	wPt732520	3.9
1	1A	wPt741357	6.8
1	1A	wPt666776	6.8
1	1A	wPt671790	6.8
1	1A	wPt664778	6.8
1	1A	wPt4676	6.8
1	1A	wPt669484	10.6
1	1A	wPt8455	11.7
1	1A	wPt1924	12.2
1	1A	Xpsp2999	14.8
1	1A	wPt2527	17.4
1	1A	wPt0196	18.4
1	1A	Xcfd15	34.3
1	1A	wPt6122	42.4
1	1A	wPt731412	42.7
1	1A	wPt664586	42.7
1	1A	wPt4666	43.1
1	1A	wPt734107	45.3
1	1A	IWA4644	49.5
1	1A	wPt734078	52.8
1	1A	wPt667172	56.9
1	1A	wPt668236	58
1	1A	IWA4506	64.8

1	1A	wPt665622	83.6
1	1A	wPt665985	87.6
1	1A	wPt665920	90.9
1	1A	IWA6431	97
1	1A	IWA5866	97.7
1	1A	IWA1583	99.4
1	1A	IWA1582	99.4
1	1A	IWA1142	100.1
1	1A	IWA164	100.1
1	1A	IWA3399	101.3
1	1A	IWA3347	101.3
1	1A	IWA1580	101.3
1	1A	IWA8615	101.7
1	1A	IWA5080	101.7
1	1A	IWA3346	101.7
1	1A	IWA492	101.7
1	1A	IWA1934	101.7
1	1A	IWA8255	105.5
1	1A	IWA1933	107.8
1	1A	IWA3073	107.8
1	1A	IWA4061	107.8
1	1A	IWA4646	107.8
1	1A	IWA4071	107.8
1	1A	IWA1615	113.1
1	1A	IWA8070	113.1
1	1A	IWA5692	113.1
1	1A	IWA6835	113.1
1	1A	IWA6378	113.4
1	1A	Xbarc83	117.3
1	1A	wPt733015	119.8
1	1A	wPt669299	126.4
1	1A	wPt668306	126.4
1	1A	wPt669297	126.4
1	1A	wPt732546	129.7
1	1A	wPt731806	129.7
1	1A	wPt734216	130.3
1	1A	wPt733007	130.4
1	1A	wPt733464	130.6
1	1A	wPt730148	131

1	1A	wPt7951	132.7
1	1A	Xwmc278	132.7
1	1A	Xwmc469	133.3
1	1A	IWA2584	145.2
1	1A	IWA5777	145.2
1	1A	IWA162	154.1
1	1A	IWA7869	154.1
1	1A	IWA7868	154.1
1	1A	IWA7871	158.3
1	1A	IWA6382	159.6
1	1A	IWA3496	159.6
1	1A	IWA6595	160
1	1A	IWA931	160
1	1A	IWA3019	160.3
1	1A	IWA3406	160.3
1	1A	IWA6145	160.3
1	1A	IWA6144	160.3
1	1A	IWA2485	160.3
1	1A	IWA2491	160.3
1	1A	IWA2489	160.4
1	1A	IWA2484	160.4
1	1A	wPt9757	165.2
1	1A	IWA4955	179.2
1	1A	IWA530	195.5
1	1A	IWA3805	213.8
1	1A	IWA1587	221.6
1	1A	IWA886	221.6
1	1A	wPt4408	227.6
1	1A	wPt9938	227.6
1	1A	wPt5660	227.8
1	1A	wPt730885	228
1	1A	wPt667252	228
1	1A	wPt733904	228
1	1A	wPt733811	228.4
1	1A	wPt7339	230.1
1	1A	Xwmc716	231
1	1A	IWA6152	239
1	1A	IWA3378	249.9
1	1A	wPt733820	261.8

1	1A	wPt730902	261.8
1	1A	wPt8644	261.8
1	1A	wPt732377	261.8
1	1A	wPt667288	261.8
1	1A	wPt732881	261.8
1	1A	tPt1419	261.8
1	1A	wPt5077	261.8
1	1A	wPt734288	261.8
1	1A	wPt0497	262.8
1	1A	Xbarc17	271.5
1	1A	IWA4123	273.1
1	1A	IWA3661	273.1
1	1A	IWA4120	273.1
2	1B	wPt7422	0
2	1B	wPt2261	5
2	1B	wPt5435	9.9
2	1B	rPt2869	15.3
2	1B	Xgwm273	19.4
2	1B	IWA4073	26.5
2	1B	IWA7331	26.5
2	1B	IWA5370	26.5
2	1B	IWA4715	26.5
2	1B	IWA2583	26.8
2	1B	IWA8557	26.8
2	1B	IWA2998	26.8
2	1B	IWA7703	27.1
2	1B	IWA7117	27.1
2	1B	IWA3092	27.1
2	1B	IWA2345	27.1
2	1B	IWA1566	27.1
2	1B	IWA4093	27.1
2	1B	IWA2881	27.1
2	1B	IWA2561	27.1
2	1B	IWA6611	27.1
2	1B	IWA6610	27.1
2	1B	IWA2150	27.1
2	1B	IWA8275	27.1
2	1B	IWA7504	27.1
2	1B	IWA7934	27.1

2	1B	IWA6290	27.1
2	1B	IWA2197	27.8
2	1B	IWA3620	28.1
2	1B	IWA8619	28.1
2	1B	IWA4389	28.1
2	1B	IWA6259	28.8
2	1B	IWA6890	28.8
2	1B	IWA3631	28.8
2	1B	IWA7977	28.8
2	1B	IWA6448	30.7
2	1B	IWA1109	36.4
2	1B	IWA3502	36.4
2	1B	IWA7375	36.4
2	1B	IWA3588	36.4
2	1B	IWA3587	36.4
2	1B	IWA7721	36.4
2	1B	IWA7723	36.4
2	1B	IWA7722	36.4
2	1B	IWA7720	36.4
2	1B	IWA5561	36.4
2	1B	IWA2517	36.4
2	1B	IWA5665	36.4
2	1B	IWA5664	36.4
2	1B	IWA4811	36.4
2	1B	IWA7594	37.5
2	1B	IWA4987	37.5
2	1B	IWA128	38.5
2	1B	IWA189	38.8
2	1B	IWA7017	40.2
2	1B	IWA4556	40.2
2	1B	IWA4557	40.2
2	1B	IWA3307	40.5
2	1B	IWA6107	42.2
2	1B	IWA515	44.8
2	1B	Xwmc419	51.7
2	1B	wPt0705	58.9
2	1B	wPt9857	65.3
2	1B	IWA7178	75.1
2	1B	IWA7179	75.1

2	1B	IWA8398	78.6
2	1B	IWA5769	80.9
2	1B	IWA8246	80.9
2	1B	IWA3017	81.4
2	1B	IWA4153	81.9
2	1B	IWA4154	81.9
2	1B	IWA4155	81.9
2	1B	IWA6646	83
2	1B	IWA5915	84
2	1B	IWA5749	84
2	1B	IWA5352	86.5
2	1B	IWA8507	86.5
2	1B	IWA7422	91
2	1B	IWA3097	91
2	1B	IWA5446	91
2	1B	IWA5447	91
2	1B	IWA695	93.9
2	1B	IWA8139	98.6
2	1B	IWA8542	98.6
2	1B	IWA1092	102
2	1B	IWA7992	115
2	1B	IWA8332	115
2	1B	IWA1791	127.3
2	1B	IWA2078	134.5
2	1B	IWA4022	141.9
3	1D.1	IWA3125	0
3	1D.1	IWA3124	1.3
4	1D.2	IWA1572	0
4	1D.2	IWA6675	1.7
4	1D.2	IWA5019	3.1
4	1D.2	IWA57	3.1
4	1D.2	IWA5020	3.1
4	1D.2	IWA5018	3.1
4	1D.2	IWA362	3.1
4	1D.2	IWA1193	5.7
4	1D.2	IWA165	5.7
4	1D.2	IWA5163	8.6
4	1D.2	IWA1464	8.6
4	1D.2	IWA2164	8.6

4	1D.2	wPt666414	15.5
4	1D.2	wPt9380	20
4	1D.2	wPt666832	20.6
4	1D.2	wPt668040	20.6
4	1D.2	wPt0413	25.7
4	1D.2	Xgwm337	31.7
4	1D.2	wPt731920	32.8
4	1D.2	wPt732845	32.8
4	1D.2	wPt666411	40.9
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7	2D.1	wPt667054	186.4
7	2D.1	wPt1301	187.9
7	2D.1	Xgwm382c	188.5
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7	2D.1	wPt665836	192.6
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11	3D.1	wPt7705	1.2
11	3D.1	wPt4991	1.5
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12	3D.2	wPt740619	83.5
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12	3D.2	wPt733640	92.1
12	3D.2	wPt741333	92.2
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13	4A	Xbarc78	14.5
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13	4A	wPt3349	22.4
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13	4A	IWA558	33
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13	4A	wPt800147	60.6
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16	5A.2	Xbarc117	90.3
16	5A.2	Xgwm304	90.6
16	5A.2	Xwmc705	90.9
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19	5D.2	IWA5012	0.6

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24	7A	wPt7113	12.2
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27	7D.2	Xwmc506	13.5
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27	7D.2	wPt744148	14.5
27	7D.2	wPt663918	14.7
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27	7D.2	wPt733104	14.7
27	7D.2	wPt744444	15.9
27	7D.2	wPt2565	18
27	7D.2	Xbarc184	22.4
27	7D.2	wPt731831	56.1
27	7D.2	wPt664727	56.1
27	7D.2	wPt731305	56.1
27	7D.2	wPt744644	57.3
27	7D.2	wPt743102	59
27	7D.2	wPt664320	67.1

APPENDIX E – Genetic linkage map for the Bess / NC-Neuse doubled haploid (DH) population

Table E.1. Genetic linkage map for the Bess / NC-Neuse doubled halploid population organized by linkage group number, chromosome label, marker, and marker position within each linkage group. The prefixes 'IWA' and 'IWB' designate SNP markers, ‘Xcfd’, ‘Xgwm’, and ‘Xwmc’ SSR markers, and Ppd_B1, Rht_B1, and Rht_D1 are KASP markers.

Link. group number	Chromosome label	Marker	Position (cM)
1	1A.1	IWB74213	0
1	1A.1	IWB27821	0
1	1A.1	IWA6644	0
1	1A.1	IWB74216	0.6
1	1A.1	IWB7628	0.6
1	1A.1	IWB71109	2.5
1	1A.1	IWB9222	2.5
1	1A.1	IWA1376	6.1
1	1A.1	IWB25590	6.6
1	1A.1	IWB21832	6.6
1	1A.1	IWB57448	14.7
1	1A.1	IWA8622	14.7
1	1A.1	IWB11098	14.7
1	1A.1	IWB17894	14.7
1	1A.1	IWB7470	14.7
1	1A.1	IWB8696	17
1	1A.1	IWB20403	18.8
1	1A.1	IWB12616	18.8
1	1A.1	IWB29206	22.7
2	1A.2	IWB71174	0
2	1A.2	IWA4033	0
2	1A.2	IWB45123	0
2	1A.2	IWB35649	0
2	1A.2	IWB71175	0
2	1A.2	IWA1481	0
2	1A.2	IWB46642	2.4
2	1A.2	IWB42452	2.9
2	1A.2	IWB54276	4.6

2	1A.2	IWB57005	4.6
2	1A.2	IWB4186	4.6
2	1A.2	IWB1201	4.6
2	1A.2	IWB7219	4.6
2	1A.2	IWA1479	7.2
2	1A.2	IWB12206	7.2
2	1A.2	IWA4754	7.2
2	1A.2	IWB36389	7.2
2	1A.2	IWB36835	8.1
2	1A.2	IWB71171	8.1
2	1A.2	IWB26930	10.5
2	1A.2	IWB74770	10.5
2	1A.2	IWA4644	11.1
2	1A.2	IWB36797	11.1
2	1A.2	IWA2452	11.1
2	1A.2	IWB55537	11.1
2	1A.2	IWB34835	11.1
2	1A.2	IWB45180	11.1
2	1A.2	IWB53884	12
2	1A.2	IWB11202	12
2	1A.2	IWB11053	12.9
2	1A.2	IWB11301	12.9
2	1A.2	IWA4506	20.4
2	1A.2	IWB31764	22.5
2	1A.2	IWB23185	22.5
2	1A.2	IWB38726	22.5
2	1A.2	IWB21788	22.5
2	1A.2	IWB31415	22.5
2	1A.2	IWB23832	22.5
2	1A.2	IWB31763	22.5
2	1A.2	IWB59888	22.5
2	1A.2	IWB53646	22.5
2	1A.2	IWB50401	24.6
2	1A.2	IWB34675	24.6
2	1A.2	IWB26042	25.6
2	1A.2	IWB20633	25.6
2	1A.2	IWB8188	26.9
2	1A.2	IWB31935	29.9

2	1A.2	IWB54031	29.9
2	1A.2	IWB48819	32
2	1A.2	IWB72800	34.1
2	1A.2	IWB66891	34.1
2	1A.2	IWB66889	34.1
2	1A.2	IWB72799	34.1
2	1A.2	IWB72065	39.7
2	1A.2	IWB69751	39.7
2	1A.2	IWB31602	39.9
2	1A.2	IWB72066	40.1
2	1A.2	IWB61685	40.1
2	1A.2	IWB47933	45.1
2	1A.2	IWB73417	45.1
2	1A.2	IWB45602	45.1
2	1A.2	IWA3373	45.1
2	1A.2	IWB9220	48.9
2	1A.2	IWB3515	48.9
2	1A.2	IWB28824	48.9
2	1A.2	IWB36084	48.9
2	1A.2	IWB26888	48.9
2	1A.2	IWB61401	52
2	1A.2	IWB58229	52
2	1A.2	IWB1215	52.5
2	1A.2	IWA3115	52.5
2	1A.2	IWA3398	52.5
2	1A.2	IWA2922	52.5
2	1A.2	IWA2655	53.7
2	1A.2	IWB52279	53.7
2	1A.2	IWA1583	53.9
2	1A.2	IWA2921	53.9
2	1A.2	IWA7421	53.9
2	1A.2	IWB34853	53.9
2	1A.2	IWA2656	53.9
2	1A.2	IWB57257	53.9
2	1A.2	IWA1582	53.9
2	1A.2	IWB66213	64.5
2	1A.2	IWB14317	65.3
2	1A.2	IWB414	65.8

2	1A.2	IWB40764	65.8
2	1A.2	IWA5631	65.8
2	1A.2	IWB16658	65.8
2	1A.2	IWB9465	65.8
2	1A.2	IWA3666	65.8
2	1A.2	IWB43760	65.8
2	1A.2	IWB35945	65.8
2	1A.2	IWB10807	65.8
2	1A.2	IWB46432	65.8
2	1A.2	IWB12529	65.8
2	1A.2	IWA2981	65.8
2	1A.2	IWA7945	65.8
2	1A.2	IWA4577	65.8
2	1A.2	IWA7065	65.8
2	1A.2	IWB55677	65.8
2	1A.2	IWB31887	65.8
2	1A.2	IWB35410	65.8
2	1A.2	IWB10918	65.8
2	1A.2	IWB12595	65.8
2	1A.2	IWA3532	65.8
2	1A.2	IWB53037	65.8
2	1A.2	IWA6971	65.8
2	1A.2	IWB65452	65.8
2	1A.2	IWB8273	65.8
2	1A.2	IWB30625	65.8
2	1A.2	IWB53598	65.8
2	1A.2	IWB66135	65.8
2	1A.2	IWA6972	66
2	1A.2	IWB11893	66.2
2	1A.2	IWA4265	66.4
2	1A.2	IWA3528	66.5
2	1A.2	IWA7779	66.6
2	1A.2	IWA7104	66.7
2	1A.2	IWA42	66.8
2	1A.2	IWA4576	66.9
2	1A.2	IWB56573	67
2	1A.2	IWB49698	67.1
2	1A.2	IWB53107	67.2

2	1A.2	IWB42121	67.3
2	1A.2	IWA3533	67.4
2	1A.2	IWB30251	67.4
2	1A.2	IWA2982	67.4
2	1A.2	IWB55848	69.4
2	1A.2	IWB65878	69.4
2	1A.2	IWA1279	69.4
2	1A.2	IWA3695	69.4
2	1A.2	IWB35883	69.4
2	1A.2	IWB10292	69.4
2	1A.2	IWA6044	69.4
2	1A.2	IWB31760	69.4
2	1A.2	IWB46963	69.4
2	1A.2	IWB26574	69.4
2	1A.2	IWB25621	69.4
2	1A.2	IWB49136	69.4
2	1A.2	IWA5268	69.4
2	1A.2	IWB2336	75.3
2	1A.2	IWB31877	78.1
2	1A.2	IWB22450	78.1
2	1A.2	IWB11087	98
2	1A.2	IWA3496	98.1
2	1A.2	IWB43647	98.1
2	1A.2	IWB46385	98.1
2	1A.2	IWB49095	98.1
2	1A.2	IWA6595	98.1
2	1A.2	IWB13718	98.1
2	1A.2	IWB34701	98.1
2	1A.2	IWA6382	98.1
2	1A.2	IWB25493	98.1
2	1A.2	IWB6944	98.1
2	1A.2	IWB9588	98.1
2	1A.2	IWB40889	98.1
2	1A.2	IWB40969	99.1
2	1A.2	IWB40044	99.1
2	1A.2	IWB46717	99.1
2	1A.2	IWA3637	99.4
2	1A.2	IWB11883	99.6

2	1A.2	IWB9335	99.6
2	1A.2	IWA7871	99.6
2	1A.2	IWA7573	99.6
2	1A.2	IWB63864	103.4
2	1A.2	IWA7868	103.4
2	1A.2	IWB6743	104.4
2	1A.2	IWB9513	105.2
2	1A.2	IWB2320	105.5
2	1A.2	IWB9512	105.8
2	1A.2	IWB63865	105.8
2	1A.2	IWB65808	106.4
2	1A.2	IWB49731	106.4
2	1A.2	IWB33406	114
2	1A.2	IWA2489	114
2	1A.2	IWB3489	114.8
2	1A.2	IWB35171	115.3
2	1A.2	IWA2485	115.5
2	1A.2	IWA6144	115.7
2	1A.2	IWA6145	115.9
2	1A.2	IWA6146	116.1
2	1A.2	IWB675	116.3
2	1A.2	IWB36631	116.5
2	1A.2	IWB49402	116.5
2	1A.2	IWB22509	116.5
2	1A.2	IWB38172	116.5
2	1A.2	IWB52379	116.5
2	1A.2	IWB59527	116.5
2	1A.2	IWA2484	120.9
2	1A.2	IWB60627	120.9
2	1A.2	IWB40942	120.9
2	1A.2	IWB4323	125.5
2	1A.2	IWB73012	125.5
2	1A.2	IWB6354	134.9
2	1A.2	IWB8167	134.9
2	1A.2	IWA6624	134.9
2	1A.2	IWB12650	134.9
2	1A.2	IWB31849	134.9
2	1A.2	IWB9545	134.9

2	1A.2	IWB62520	135.4
2	1A.2	IWA4080	140.7
2	1A.2	IWB4630	140.7
2	1A.2	IWB40338	143.2
2	1A.2	IWB9628	143.2
2	1A.2	IWB47990	143.2
2	1A.2	IWB11161	152.2
2	1A.2	IWB45538	152.2
2	1A.2	IWB54196	152.6
2	1A.2	IWB6029	152.6
2	1A.2	IWB6517	152.6
2	1A.2	IWA6042	170.9
2	1A.2	IWB35025	170.9
2	1A.2	IWB65422	170.9
2	1A.2	IWA3805	170.9
2	1A.2	IWB11406	172.5
2	1A.2	IWB40130	172.5
2	1A.2	IWA5046	174.9
2	1A.2	IWA5047	175
2	1A.2	IWB72493	175.1
2	1A.2	IWB49670	175.2
2	1A.2	IWB61497	175.2
2	1A.2	IWB29758	205.4
2	1A.2	IWB36272	206.8
2	1A.2	IWB41257	208.7
2	1A.2	IWA3145	210.3
2	1A.2	IWB9961	210.3
2	1A.2	IWB36329	213.2
2	1A.2	IWB54518	214.1
2	1A.2	IWB54358	217.1
2	1A.2	IWB30247	217.1
2	1A.2	IWB30243	218.1
2	1A.2	IWB73950	218.1
2	1A.2	IWB54870	223.7
2	1A.2	IWB596	223.7
2	1A.2	IWB66369	223.7
2	1A.2	IWB74025	226.9
2	1A.2	IWB75223	226.9

3	1B.1	IWB47800	0
3	1B.1	IWB7123	0
3	1B.1	IWB49209	0
3	1B.1	IWB47978	0
3	1B.1	IWB11290	0
3	1B.1	IWB65421	0
3	1B.1	IWB11262	0
3	1B.1	IWB44730	0
3	1B.1	IWB7122	0
3	1B.1	IWB69597	0
3	1B.1	IWB12632	0.4
3	1B.1	IWB44815	0.4
3	1B.1	IWB26242	2.3
3	1B.1	IWB26464	2.3
3	1B.1	IWB24983	2.3
3	1B.1	IWB45798	2.3
3	1B.1	IWB9761	2.3
3	1B.1	IWB47982	3.3
3	1B.1	IWB47981	3.3
3	1B.1	IWB24688	10
3	1B.1	IWB12157	12.3
3	1B.1	IWB7368	12.3
3	1B.1	IWB44529	14.3
3	1B.1	IWB39278	14.3
3	1B.1	IWB38627	14.3
3	1B.1	IWB48324	14.3
3	1B.1	IWB5922	14.3
3	1B.1	IWB6024	15.8
3	1B.1	IWB7731	15.8
3	1B.1	IWB22922	15.8
3	1B.1	IWB10589	15.8
3	1B.1	IWB24687	15.8
3	1B.1	IWB28223	15.8
3	1B.1	IWA1808	16.2
3	1B.1	IWB54787	16.8
3	1B.1	IWB64595	16.8
3	1B.1	IWB71754	16.8
3	1B.1	IWB17505	16.8

3	1B.1	IWB9830	16.8
3	1B.1	IWB10312	16.8
3	1B.1	IWB12602	16.8
3	1B.1	IWB10492	16.8
3	1B.1	IWB1453	16.8
3	1B.1	IWA4073	16.8
3	1B.1	IWA1396	16.8
3	1B.1	IWB66722	16.8
3	1B.1	IWB29508	16.9
3	1B.1	IWB73112	17.2
3	1B.1	IWB64596	17.3
3	1B.1	IWB28331	17.3
3	1B.1	IWB12603	17.3
3	1B.1	IWB29507	17.3
4	1B.2	IWA4504	0
4	1B.2	IWB41164	0
4	1B.2	IWA7703	1.3
4	1B.2	IWB8781	1.3
4	1B.2	IWB11695	1.3
4	1B.2	IWB40373	1.7
4	1B.2	IWB29515	1.7
4	1B.2	IWB9942	1.7
4	1B.2	IWB63847	1.7
4	1B.2	IWB33204	1.7
4	1B.2	IWB9645	2.7
4	1B.2	IWB60711	2.7
4	1B.2	IWB9236	2.7
4	1B.2	IWB41867	2.7
4	1B.2	IWB60993	2.7
4	1B.2	IWB60713	2.7
4	1B.2	IWB63352	2.7
4	1B.2	IWB69254	3.2
4	1B.2	IWB24659	3.2
4	1B.2	IWB51586	3.2
4	1B.2	IWB35982	3.2
4	1B.2	IWB12252	3.2
4	1B.2	IWB56393	3.2
4	1B.2	IWB28269	3.2

4	1B.2	IWB35375	3.2
4	1B.2	IWB23213	3.2
4	1B.2	IWB73550	3.2
4	1B.2	IWA6450	3.2
4	1B.2	IWB68665	3.2
4	1B.2	IWB25551	3.2
4	1B.2	IWB69962	4.5
4	1B.2	IWB11525	4.5
4	1B.2	IWB66085	4.5
4	1B.2	IWA1578	4.5
4	1B.2	IWB53510	4.6
4	1B.2	IWB16228	6.1
5	1B.3	IWB72563	0
5	1B.3	IWB72564	0
5	1B.3	IWB73370	0
5	1B.3	IWB31929	0
5	1B.3	IWB49946	0
5	1B.3	IWB69496	0.9
5	1B.3	IWB7992	0.9
5	1B.3	IWB5590	0.9
5	1B.3	IWB2337	1.4
5	1B.3	IWB31711	1.5
5	1B.3	IWB63690	1.6
5	1B.3	IWB69495	1.7
5	1B.3	IWB72533	1.8
5	1B.3	IWB7443	1.8
5	1B.3	IWA188	1.8
5	1B.3	IWB47330	1.8
5	1B.3	IWA2084	14.1
5	1B.3	IWB72962	14.1
5	1B.3	IWA6107	14.1
5	1B.3	IWB72078	14.1
5	1B.3	IWB72738	16
5	1B.3	IWB1336	16
5	1B.3	IWB1335	16
5	1B.3	IWB63365	16
5	1B.3	IWB31661	16
5	1B.3	IWB40993	16.1

5	1B.3	IWA790	16.4
5	1B.3	IWA7700	16.4
5	1B.3	IWB4238	16.4
5	1B.3	IWB12200	16.4
5	1B.3	IWA270	16.4
5	1B.3	IWA4316	16.4
5	1B.3	IWB63364	16.4
5	1B.3	IWB13774	17.9
5	1B.3	IWB42604	17.9
5	1B.3	IWA2254	17.9
5	1B.3	IWB60063	17.9
5	1B.3	IWA4203	17.9
5	1B.3	IWA7560	17.9
5	1B.3	IWB29900	17.9
5	1B.3	IWA7982	17.9
5	1B.3	IWB2041	17.9
5	1B.3	IWB13397	17.9
5	1B.3	IWB52413	17.9
5	1B.3	IWB10630	17.9
5	1B.3	IWB51604	17.9
5	1B.3	IWB24077	17.9
5	1B.3	IWA1959	17.9
5	1B.3	IWB10984	17.9
5	1B.3	IWA7787	17.9
5	1B.3	IWB7235	17.9
5	1B.3	IWB36034	17.9
5	1B.3	IWB12292	17.9
5	1B.3	IWA2861	18.4
5	1B.3	IWB48944	18.4
5	1B.3	IWB6543	18.4
5	1B.3	IWB35652	18.4
5	1B.3	IWB6215	18.4
5	1B.3	IWB43476	18.4
5	1B.3	IWB26949	18.4
5	1B.3	IWB26948	18.4
5	1B.3	IWA775	18.4
5	1B.3	IWB60433	18.4
5	1B.3	IWB13393	18.4

5	1B.3	IWB19579	18.4
5	1B.3	IWB9327	18.4
5	1B.3	IWB9457	18.4
5	1B.3	IWB52353	18.4
5	1B.3	IWA1231	18.4
5	1B.3	IWB35930	18.4
5	1B.3	IWB26950	18.4
5	1B.3	IWB43475	18.4
5	1B.3	IWB6504	18.4
5	1B.3	IWB47566	18.4
5	1B.3	IWB55419	18.4
5	1B.3	IWB23636	18.4
5	1B.3	IWB9693	18.4
5	1B.3	IWB26192	18.4
5	1B.3	IWB14729	18.4
5	1B.3	IWB8134	18.4
5	1B.3	IWB29159	18.4
5	1B.3	IWB31909	18.4
5	1B.3	IWA1889	19.2
5	1B.3	IWA1890	19.2
5	1B.3	IWB61131	19.2
5	1B.3	IWB36064	19.2
5	1B.3	IWB12813	19.2
5	1B.3	IWB30729	19.2
5	1B.3	IWA6945	19.2
5	1B.3	IWB10226	19.2
5	1B.3	IWB52480	19.2
5	1B.3	IWB31943	19.2
5	1B.3	IWB73651	19.2
5	1B.3	IWB25041	19.2
5	1B.3	IWB27292	19.2
5	1B.3	IWA6926	19.2
5	1B.3	IWB47303	19.2
5	1B.3	IWA6134	19.2
5	1B.3	IWA6133	19.2
5	1B.3	IWA4139	19.2
5	1B.3	IWB56695	19.2
5	1B.3	IWB69088	19.2

5	1B.3	IWA3712	19.2
5	1B.3	IWB71192	19.2
5	1B.3	IWB11968	19.2
5	1B.3	IWA5635	19.7
5	1B.3	IWB31681	19.7
5	1B.3	IWA2315	19.7
5	1B.3	IWA2626	19.7
5	1B.3	IWA6558	19.7
5	1B.3	IWB57715	19.7
5	1B.3	IWA5228	19.7
5	1B.3	IWB74900	19.7
5	1B.3	IWB45575	19.7
5	1B.3	IWB14950	19.7
5	1B.3	IWB55709	19.9
5	1B.3	IWB11230	20.2
5	1B.3	IWB9175	20.2
5	1B.3	IWB43583	20.2
5	1B.3	IWB71348	20.2
5	1B.3	IWB49534	20.2
5	1B.3	IWB3677	20.2
5	1B.3	IWB10143	20.2
5	1B.3	IWA4819	20.2
5	1B.3	IWA2788	20.7
5	1B.3	IWB44943	20.7
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21	3A.3	IWB8509	5.4
21	3A.3	IWB40771	8.6
21	3A.3	IWB55044	8.6
21	3A.3	IWB61269	10.3
21	3A.3	IWA3559	17.2
21	3A.3	IWA3560	17.2
22	3B.1	IWB62087	0
22	3B.1	IWB34431	4.5
22	3B.1	IWB23456	4.5
22	3B.1	IWB4561	4.5
22	3B.1	IWB40812	4.5
22	3B.1	IWB63525	4.5
22	3B.1	IWB58249	4.5
22	3B.1	IWB51900	5
22	3B.1	IWB71813	5
22	3B.1	IWB1428	5.3
22	3B.1	IWB58513	5.6
22	3B.1	IWB32711	5.6
22	3B.1	IWB57529	5.6
22	3B.1	IWA6092	9.3
22	3B.1	IWB56327	9.3
22	3B.1	IWB56326	9.7
22	3B.1	IWB53999	9.7
22	3B.1	IWB56857	11.4
22	3B.1	IWB8289	11.4

22	3B.1	IWB4759	16.1
23	3B.2	IWB9189	0
23	3B.2	IWB48086	3.2
23	3B.2	IWB20573	3.2
23	3B.2	IWB7760	5
23	3B.2	IWB67233	5
23	3B.2	IWB6207	9.3
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23	3B.2	IWB32646	9.3
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23	3B.2	IWB8688	16.4
23	3B.2	IWB8689	16.4
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23	3B.2	IWB28930	17.6
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23	3B.2	IWB25620	24.4
23	3B.2	IWB55771	27
23	3B.2	IWB55431	27.6
23	3B.2	IWB58218	28.1
23	3B.2	IWB56124	29.1
23	3B.2	IWA3725	29.1
23	3B.2	IWB35832	29.1
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23	3B.2	IWB41708	34.8
23	3B.2	IWB73993	34.8
23	3B.2	IWB2284	34.8
23	3B.2	IWB6373	35.1
23	3B.2	IWA3732	35.4
23	3B.2	IWB42771	37.3

23	3B.2	IWB1657	37.3
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23	3B.2	IWB52028	41.6
23	3B.2	IWB12062	41.6
23	3B.2	IWB580	41.6
23	3B.2	IWB26800	44.2
23	3B.2	IWB61879	44.2
23	3B.2	IWB26799	44.2
23	3B.2	IWB45903	45.2
23	3B.2	IWB45902	45.2
23	3B.2	IWA6482	45.2
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23	3B.2	IWA8326	49.9
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23	3B.2	IWB62638	50.1

23	3B.2	IWB25180	50.1
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23	3B.2	IWB75323	50.1
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23	3B.2	IWB56471	50.1
23	3B.2	IWB4823	50.1
23	3B.2	IWA2622	50.1
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23	3B.2	IWA6380	50.5
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23	3B.2	IWA628	50.7

23	3B.2	IWA629	50.7
23	3B.2	IWB24497	50.8
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23	3B.2	Xgwm264B	53.2
23	3B.2	IWB74433	53.8
23	3B.2	IWB21579	56.8
23	3B.2	IWA7333	56.8
23	3B.2	Xgwm077	62.5
23	3B.2	IWB65344	67.5
23	3B.2	IWB7901	67.7
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23	3B.2	IWB49738	68
23	3B.2	IWB8908	69.6
23	3B.2	IWB27904	70
23	3B.2	IWB27903	70.1
23	3B.2	IWB57595	70.1
23	3B.2	IWB58917	75
23	3B.2	IWA4267	75
23	3B.2	IWB35379	75
23	3B.2	IWB38030	75
23	3B.2	IWA4268	75
23	3B.2	IWB58916	75
23	3B.2	IWA4269	75
23	3B.2	IWB10634	75
23	3B.2	IWB58918	75
23	3B.2	IWB38942	76
23	3B.2	IWB38943	76
23	3B.2	IWB8347	78.9
23	3B.2	IWB43184	78.9
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23	3B.2	IWA5775	94.3
23	3B.2	IWA3018	96.6
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23	3B.2	IWB3107	110.5
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23	3B.2	IWB2621	120
23	3B.2	IWA3332	120.5
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24	3B.3	IWB62896	3.9
24	3B.3	IWB49437	3.9

24	3B.3	IWB46714	3.9
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24	3B.3	IWB55459	8.3
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24	3B.3	IWB23604	28.3
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24	3B.3	IWB50499	29.1
24	3B.3	IWB50423	29.1
24	3B.3	IWB33964	29.1
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25	3D.1	IWB49729	0
25	3D.1	IWB64430	0
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25	3D.1	IWB75016	0
25	3D.1	IWB35481	0
25	3D.1	IWB62158	0
25	3D.1	IWB11115	0
25	3D.1	IWB9543	0
25	3D.1	IWB56283	0
25	3D.1	IWB34431	0
25	3D.1	IWB63461	0
25	3D.1	IWB59180	0
25	3D.1	IWB55337	0

25	3D.1	IWB64174	0
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25	3D.1	IWB44215	0
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25	3D.1	IWB68188	0
25	3D.1	IWB4561	0
25	3D.1	IWB57908	0
25	3D.1	IWB59200	0
25	3D.1	IWB57989	0
25	3D.1	IWB57909	0
25	3D.1	IWB23510	0
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25	3D.1	IWB4058	0
25	3D.1	IWB5083	20.6
25	3D.1	IWB48230	20.6
25	3D.1	IWB11940	20.6
25	3D.1	IWB8145	20.6
25	3D.1	IWB11941	21
25	3D.1	IWB11833	21.4
25	3D.1	IWB18436	22
26	3D.2	IWB22531	0
26	3D.2	IWB22532	0
26	3D.2	IWB57983	0
26	3D.2	IWB9864	0
26	3D.2	IWB43605	0
26	3D.2	IWB10661	0
26	3D.2	IWB50461	0
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26	3D.2	IWB64548	0
26	3D.2	IWB54793	0
26	3D.2	IWB13275	0
26	3D.2	IWB58760	0.2
26	3D.2	IWB42149	0.2

26	3D.2	IWB7538	0.2
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26	3D.2	IWB12656	5.7
26	3D.2	IWB11519	5.7
26	3D.2	IWB27626	5.7
26	3D.2	IWA643	6.2
26	3D.2	IWB22530	6.8
26	3D.2	IWB27498	8.9
26	3D.2	IWB40719	8.9
26	3D.2	IWB50460	8.9
26	3D.2	IWA4081	8.9
26	3D.2	IWB7769	8.9
26	3D.2	IWB9185	12.6
26	3D.2	IWB15330	12.6
27	4A.1	IWB52625	0
27	4A.1	IWB21310	5.9
27	4A.1	IWB40004	5.9
27	4A.1	IWB42813	23.3
27	4A.1	IWA2585	23.3
27	4A.1	IWB12427	23.3
27	4A.1	IWB42814	23.3
27	4A.1	IWB10827	23.3
27	4A.1	IWA7699	23.3
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27	4A.1	IWA3191	23.3
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27	4A.1	IWB35273	23.3
27	4A.1	IWB8381	23.3
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27	4A.1	IWB45853	23.3
27	4A.1	IWB65412	23.3
27	4A.1	IWB65411	23.3
27	4A.1	IWB10826	24.3
27	4A.1	IWB25837	25.7
27	4A.1	IWA568	26.2
27	4A.1	IWA6501	26.3

27	4A.1	IWA482	26.5
28	4A.2	IWB68318	0
28	4A.2	IWB26115	0
28	4A.2	IWB34721	0.2
28	4A.2	IWB34669	0.4
28	4A.2	IWB42091	0.6
28	4A.2	IWB29177	0.7
28	4A.2	IWA2106	0.7
28	4A.2	IWB54608	0.7
28	4A.2	IWB27643	24.7
28	4A.2	IWB49186	24.7
28	4A.2	IWB56116	24.7
28	4A.2	IWB60773	24.7
28	4A.2	IWB52747	24.7
28	4A.2	IWB27644	26.4
28	4A.2	IWB74494	26.4
28	4A.2	IWB44327	26.4
28	4A.2	IWA7653	28
28	4A.2	IWA2505	28
28	4A.2	IWB44184	28
28	4A.2	IWA5152	36.9
28	4A.2	IWA3188	36.9
28	4A.2	IWA485	36.9
28	4A.2	IWB4577	36.9
28	4A.2	IWB11729	36.9
28	4A.2	IWA8168	36.9
28	4A.2	IWA6418	36.9
28	4A.2	IWB29438	36.9
28	4A.2	IWB26481	50.6
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28	4A.2	IWB22646	53.2
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28	4A.2	IWB23361	53.2
28	4A.2	IWB60092	53.2
28	4A.2	IWB23362	53.2
28	4A.2	IWA6563	53.2

28	4A.2	IWB23363	53.2
28	4A.2	IWB7057	53.2
28	4A.2	IWB23359	53.2
28	4A.2	IWB23882	53.2
28	4A.2	IWB41812	53.2
28	4A.2	IWB64766	53.2
28	4A.2	IWB60091	53.7
28	4A.2	IWB74685	58.7
28	4A.2	IWB9701	58.7
28	4A.2	IWB36288	58.7
28	4A.2	IWB11041	58.7
28	4A.2	IWB12104	58.7
28	4A.2	IWB30713	58.7
28	4A.2	IWB32055	58.7
28	4A.2	IWB12132	58.7
28	4A.2	IWB68352	84.3
28	4A.2	IWB68386	84.8
28	4A.2	IWB63979	84.8
28	4A.2	IWB48738	86.3
28	4A.2	IWB19550	86.5
28	4A.2	IWB19549	86.9
28	4A.2	IWB58087	87.3
28	4A.2	IWB36777	87.3
28	4A.2	IWB25284	93.1
28	4A.2	IWB42416	93.1
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28	4A.2	IWB71544	93.4
28	4A.2	IWB22422	93.4
28	4A.2	IWB11060	93.6
28	4A.2	IWB5324	93.6
28	4A.2	IWB2634	94.1
28	4A.2	IWB71810	94.1
28	4A.2	IWB71809	94.1
28	4A.2	IWB11988	95.1
28	4A.2	IWB72230	95.6
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28	4A.2	IWB22105	95.6
28	4A.2	IWB48468	95.6

28	4A.2	IWB48955	95.6
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28	4A.2	IWB64422	95.6
28	4A.2	IWB30310	95.6
28	4A.2	IWB42117	95.6
28	4A.2	IWB57948	95.7
28	4A.2	IWB53396	95.8
28	4A.2	IWB53675	95.8
28	4A.2	IWB44829	95.8
28	4A.2	IWB19810	95.8
28	4A.2	IWA7365	96.8
28	4A.2	IWA4084	96.8
28	4A.2	IWA4858	96.8
28	4A.2	IWB48829	96.8
28	4A.2	IWB28864	98.6
28	4A.2	IWB72383	100.4
28	4A.2	IWA1410	100.4
28	4A.2	IWA4859	100.7
28	4A.2	IWB46373	119.5
28	4A.2	IWB48591	119.5
28	4A.2	IWB35445	119.5
28	4A.2	IWB6643	119.5
28	4A.2	IWA2764	121.9
28	4A.2	IWB35921	122.5
29	4B.1	IWB71989	0
29	4B.1	IWB27808	0.2
29	4B.1	IWB71725	0.4
29	4B.1	IWB932	0.9
29	4B.1	IWB71727	0.9

29	4B.1	IWB60203	0.9
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29	4B.1	IWB71988	0.9
29	4B.1	IWB12274	0.9
29	4B.1	IWA8108	7.1
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29	4B.1	IWB23112	8.8
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29	4B.1	IWB34767	22
29	4B.1	IWB30977	22
29	4B.1	IWB60105	22
29	4B.1	Rht_B1	37.5
29	4B.1	IWB44094	52.9
29	4B.1	IWA3287	56.8
29	4B.1	IWB35570	57.8
29	4B.1	IWB38540	57.8

29	4B.1	IWB49052	59.7
29	4B.1	IWB52053	59.7
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38	6A.1	IWB67414	6.8
38	6A.1	IWB67416	6.8
38	6A.1	IWA7007	6.8
38	6A.1	IWB66015	6.8
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49	7B.1	IWB3244	130.8
49	7B.1	IWB10979	130.8
49	7B.1	IWB65549	130.8
49	7B.1	IWB63121	143
49	7B.1	IWA7907	143
49	7B.1	IWB63122	143.1
49	7B.1	IWB34912	143.4
49	7B.1	IWA7717	143.4
49	7B.1	IWB69965	143.4
49	7B.1	IWB50231	143.4
49	7B.1	IWB25774	145
49	7B.1	IWB2670	145
49	7B.1	IWB48348	146.1
49	7B.1	IWB9100	146.1
49	7B.1	IWB13061	146.1
49	7B.1	IWB73054	149.4
49	7B.1	IWB60676	151.4
49	7B.1	IWB56648	151.4
49	7B.1	IWB9002	151.4

49	7B.1	IWB21594	151.4
49	7B.1	IWB8667	151.4
49	7B.1	IWB11254	151.4
49	7B.1	IWB56649	151.4
50	7B.2	IWB37096	0
50	7B.2	IWB48219	0
50	7B.2	IWB36362	0
50	7B.2	IWB857	0
50	7B.2	IWB62475	0
50	7B.2	IWB73261	3.5
50	7B.2	IWB26655	3.5
50	7B.2	IWB21716	3.5
50	7B.2	IWB24039	7.2
50	7B.2	IWB25100	7.2
50	7B.2	IWB45629	19.8
50	7B.2	IWB48669	24.9
50	7B.2	IWB73641	25.5
50	7B.2	IWB6469	25.5
50	7B.2	IWB9064	25.5
50	7B.2	IWB10676	25.5
50	7B.2	IWB29106	25.5
50	7B.2	IWB24509	25.5
50	7B.2	IWB24200	27.1
50	7B.2	IWB10815	27.1
50	7B.2	IWB64169	32.2
50	7B.2	IWB75239	32.6
50	7B.2	IWB64015	32.6
50	7B.2	IWB24202	32.6
50	7B.2	IWB57443	32.6
50	7B.2	IWB57016	32.6
50	7B.2	IWB8762	35.2
50	7B.2	IWA6801	35.2
50	7B.2	IWB55488	39.3
50	7B.2	IWB56656	39.3
50	7B.2	IWB25320	39.3
50	7B.2	IWB57013	41.1
50	7B.2	IWB3312	41.1
50	7B.2	IWB75238	41.4

50	7B.2	IWB65255	41.7
50	7B.2	IWB13461	41.7
50	7B.2	IWB8824	42.7
50	7B.2	IWB48670	42.7
50	7B.2	IWB5969	42.7
50	7B.2	IWB61786	44.5
50	7B.2	IWB8571	44.5
50	7B.2	IWB74819	44.6
50	7B.2	IWB29026	45.1
50	7B.2	IWB64172	45.5
50	7B.2	IWB64173	45.7
50	7B.2	IWB47863	45.7
50	7B.2	IWB60653	45.7
51	7D.1	IWB22038	0
51	7D.1	IWB45329	0
51	7D.1	IWB11990	0
51	7D.1	IWB10503	0
51	7D.1	IWB22037	0
51	7D.1	IWB12634	0
51	7D.1	IWB9595	0
51	7D.1	IWB23865	0
51	7D.1	IWB38527	3.1
51	7D.1	IWB46586	3.1
51	7D.1	IWB52796	4.7
51	7D.1	IWB18737	4.7
51	7D.1	IWB3933	4.7
51	7D.1	IWB26752	4.7
51	7D.1	IWB50978	5.7
51	7D.1	IWA3745	5.7
51	7D.1	IWA3746	5.7
51	7D.1	IWA4131	5.7
51	7D.1	IWB24931	5.7
51	7D.1	IWA5972	6.2
51	7D.1	IWB22849	7
51	7D.1	IWA3749	7
51	7D.1	IWB17211	7.7
51	7D.1	IWB63607	7.7
51	7D.1	IWB61302	7.7

51	7D.1	IWB65448	8.6
51	7D.1	IWB24689	8.6
51	7D.1	IWB338	8.6
51	7D.1	IWB47867	8.6
51	7D.1	IWB517	8.6
51	7D.1	IWB13273	51.3
51	7D.1	IWB39125	51.3
51	7D.1	IWB29912	51.3
51	7D.1	IWB58045	51.3
51	7D.1	IWB16634	51.3
51	7D.1	IWB34569	54.4
51	7D.1	IWB58968	58.4
51	7D.1	IWB58471	60.5
51	7D.1	IWB44202	60.5
51	7D.1	IWB45751	61.9
51	7D.1	IWB65929	62.3
51	7D.1	IWA5249	63.9
51	7D.1	IWB49398	63.9
51	7D.1	IWA5557	63.9
51	7D.1	IWA604	63.9
51	7D.1	IWB6064	63.9
51	7D.1	IWB36226	64.4
51	7D.1	IWA1257	64.4
51	7D.1	IWB18199	66.2
51	7D.1	IWB13301	68.1
51	7D.1	IWB46253	68.1
51	7D.1	IWB48773	76.4
51	7D.1	IWB26629	76.4
51	7D.1	IWB53451	77.5
51	7D.1	IWA2273	79.6
51	7D.1	IWB45846	80.2
51	7D.1	IWB34689	80.4
51	7D.1	IWB19310	80.6
51	7D.1	IWB65357	80.6
51	7D.1	IWB8024	80.8
51	7D.1	IWB26628	81.1
51	7D.1	IWB23951	84.5
51	7D.1	IWB9724	85

51	7D.1	IWB8604	85.1