

ABSTRACT

BENSON, JARED HOWELL. Improving Marker Assisted Selection in Soft Winter Wheat for Fusarium Head Blight Resistance with QTL Validation, Genome-Wide Association, and Genomic Selection. (Under the direction of Dr. Gina Brown-Guedira).

Fusarium head blight (FHB) is a devastating disease of wheat with worldwide distribution. An increase in the frequency and intensity of outbreaks of the disease revealed that current North American cultivars lack sufficient levels of resistance. The development and release of resistant varieties has been complicated by the quantitative nature of resistance and high levels of genotype by environment interaction. In order to increase the resistance levels in elite U.S. cultivars, regional uniform scab screening nurseries have been established to evaluate adapted cultivars and breeding lines. In addition, mapping in bi-parental populations has been conducted to identify resistance QTL that may be deployed by marker assisted selection. The first objective of our research was to evaluate the effect of QTL introgressed from exotic sources into an elite soft red winter wheat cultivar. The resistance genes/QTL *Fhb1* and *Qfhs.umc-2DL* were introgressed from the Chinese Spring wheat germplasm W14 by accelerated backcrossing to NC-Neuse that has moderate FHB resistance. Selected BC₂F_{2:3} lines having *Fhb1* and/or *Qfhs.umc-2DL* had lower severity of FHB than was observed on backcross-derived lines without either resistance gene. Although lower levels of FHB were observed on backcross lines with the QTL, they were not significantly more resistant than the recurrent parent NC-Neuse. In addition, lines having both QTL were not significantly more resistant than lines having *Fhb1*, indicating that the effects of the two resistance genes may not be additive.

Experiments using backcrossing to evaluate QTL in different backgrounds are useful for validation of QTL effects but can be expensive and time-consuming to conduct. The second objective of our research was to use genome wide association analysis to validate QTL effects in a population of diverse lines and at the same time identify new QTL. A set of a set of 258 lines were selected from entries in the regional scab screening nurseries and genotyped with DArT markers distributed throughout the genome, as well as SSR and STS markers previously reported as linked to QTL for FHB resistance. Since no major sub-groups were identified in these germplasm using the program STRUCTURE, a combination of the kinship matrix and principal components was used to correct for population structure when performing association mapping. Data collected on FHB incidence, FHB severity, and Fusarium Damaged Kernels (FDK) from evaluation of the 2008, 2009 and 2010 regional scab screening nurseries were standardized and used as phenotypes in the analysis. Markers located in 23 regions on 14 chromosomes were significantly associated with at least one scab resistance trait. Multiple QTL were identified on chromosomes 1A, 1B, 2D, 3B, and 6A. The effect of the *Fhb1* resistance gene that was known to segregate in these germplasm was confirmed. In addition, 16 of the QTL co-occurred with previously mapped QTL for FHB resistance.

The higher accuracy of genomic selection over conventional marker assisted selection suggests genomic selection would be an efficient method to breed for scab resistance. The genomic selection model ridge regression was found to produce higher accuracies than Bayes C π .

Improving Marker Assisted Selection in Soft Winter Wheat for Fusarium Head Blight
Resistance with QTL Validation, Genome-Wide Association, and Genomic Selection

by
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DEDICATION

I would like to dedicate this thesis to my wife Jennifer Benson in recognition of her support,
love, and encouragement which she so generously gives to me.

BIOGRAPHY

Jared Howell Benson is the fifth child of Jerry Lee and Calleen Howell Benson. Though born in Lexington, Kentucky, he spent his formative years in New Milford, Connecticut. While attending Brigham Young University, Jared began coursework in the Genetics and Biotechnology department. As part of the program curriculum, he worked in the plant genetics research lab on quinoa and was mentored by senior students. After two semesters, and with the trust of professors, Jared was given his own research project to work on endophytes in forage grass. After completing a Bachelor of Science degree, he continued his studies at BYU and to pursue a Master of Science degree under the supervision of Dr. Brad Geary. His Masters research was focused on how amending soil through pH and Ca would reduce pink rot in potato. During his graduate work at BYU, Jared cycled with Dr. Josh Udall, who became a friend and important mentor in his education and influence in his decision to pursue a Ph.D. Jared started a Ph.D at North Carolina State University as a Monsanto Fellow and a Provost Scholar. After his first year, on June 6, 2009, he married Jennifer Nichole Luther in the Dallas, Texas temple of the Church of Jesus Christ of Latter-day Saints. During his third year, he and his wife gave birth to their first child, Libby Claire Benson.

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CHAPTER 1. LITERATURE REVIEW

I. Molecular Breeding

A. Marker Assisted Breeding

Traditional breeding conducts selection based on evaluation of lines in greenhouse studies and field nurseries. Marker assisted breeding (MAB) uses genotypic data to drive selection. Markers may directly or indirectly improve a trait by selecting alleles that have been statistically associated with phenotype 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 linkage disequilibrium (LD) or associated with a functional variant, the trait is improved indirectly by selection at the marker locus (Hospital, 2003).

The term marker assisted selection (MAS) was first used by Beckmann & Soller (1986), although the technique was previously mentioned by Neimann-Sorenson and Robertson (1961), Smith (1967), Tanksley and Rick (1980), Tanksley (1983) and Soller and Beckmann (1983). Markers can complement traditional selection for traits which are: costly or hard to phenotype, not selected for in early generations due to low heritability, or recessive.

Methods of MAB include: allele enrichment, pyramiding, foreground and background selection, marker assisted recurrent selection, and genomic selection. Marker assisted breeding methods are intended to increase the genetic gain per cycle, reduce cost, or give greater control over donor germplasm. The implementation and success of MAS depends on a plethora of factors: heritability of the trait, relative cost of genotyping and phenotyping,

population structure generating false positive associations, LD between markers and functional variants, type of molecular marker, or statistical analysis.

B. Population Structure

A broad description of population structure given by Wright (1951), is the “entire pattern of relatedness among individuals.” A population which can be partitioned into subgroups is often referred to as stratification (Sneller et al. 2009). A population which has structure will be composed of lines which are genetically differentiated. However, population structure is not necessarily present if a set of lines are unequally related (Zhang et al., 2009). Events such as selection, drift, bottlenecks, domestication, adaptation, breeding history, and geographic origin will lead to deviations from lines being independent from one another (Sneller et al., 2009; Sorrells and Yu, 2009; Stich et al., 2008).

Measures of a population are relative due to the ascertainment bias of individuals for a population (Hedrick, 2005). Comparisons between individuals of a population, such as distance between any two entries, will depend on all pair-wise evaluations. Dendograms based upon similarity or diversity measures can be employed to visualize relationships, but lack the ability to quantify such structure.

Principal component analysis (PCA) is an approach to identify co-ancestry and population structure. Random markers from the genome are used to summarize variation through a covariance matrix, from which eigenvectors are determined. The eigenvectors represent subsets of the data which explain a proportion of the total variation. The “top” eigenvectors are used as axes of co-ancestry (Price et al., 2006; Sneller et al., 2009) and may represent sup-populations based upon genetic ancestry or long range LD.

Structured association (SA) is an approach that assigns membership of a sample to one or more subgroups. Programs such as STRUCTURE (Pritchard, 2000) use a Bayesian Monte Carlo Chain analysis to calculate probabilities for group membership. The number of subgroups must be defined prior to analysis and then inferred from the log probability of the data $\ln P(d)$ of multiple levels of subgroups. Hardy-Weinberg equilibrium is a key assumption of the analysis (Hubisz et al., 2009). The effect of admixture can be added to a model so that membership of an entry can be among multiple subgroups.

The SA and PCA methods capture some of the many levels of relatedness (Kang et al., 2008), while a kinship matrix allows for a pair wise evaluation of relatedness between all samples (Myles et al., 2009). An array of kinship matrices can be calculated based upon pedigree, Kotterman K_s , similarity indices, or estimates of alleles identical by descent (IBD) and identical by state (IBS) (Yu, 2006). Depending upon the method to calculate kinship, estimates of relation can be negative, suggesting that the individuals are more diverse than under random mating conditions (Stich et al., 2008). A kinship measure based upon markers has been found to give more accurate estimates of relatedness than pedigrees (Myles et al., 2009; Zhang et al., 2009).

C. Linkage Disequilibrium

Linkage disequilibrium, also known as gametic phase disequilibrium, arises when alleles at different loci occur together in a non-random association (Hendricks, 1987). The likelihood of alleles co-occurring should follow the probability based upon Mendel's law of independent assortment. Forces that generate LD are admixture, selection, domestication, mating system, population structure, genetic drift, and mutation.

The pattern and strength of LD will vary within a genome and across populations. Linkage disequilibrium will be produced in specific genomic locations by selection and gene conversion, while factors like geography or mating system will influence genome wide LD (Somers et al., 2007; Zhang et al., 2010b). Epistatic interactions and selection can cause LD to persist between distant loci even while linkage equilibrium (LE) exists for adjacent genes (Gupta et al., 2005). Independent assortment will decay inter-chromosome LD, while recombination is the main process which breaks down intra-chromosome LD (Caldwell et al., 2006). Larger effective population sizes will also help contribute to faster rates of LD decay (Gupta, 2010). Among autogamous species, recombination is less effective than in allogamous species in reducing LD because of cross-over events between homozygous chromatids (Caldwell et al., 2006) even though recombination might be more frequent (Flint-Garcia, 2003).

Lewontin (1964) proposed a disequilibrium coefficient, D , to measure the degree of LD. The D coefficient is based upon the difference between the frequencies of expected and observed genotypes in a population, $D = Pr_{A_1B_1} - Pr_{A_1} Pr_{B_1}$, where $Pr_{A_1B_1}$ is the observed occurrence of the genotype A_1B_1 and Pr_{A_1} and Pr_{B_1} are the frequencies of each allele. The measure is bounded by 0 and 1. When D is greater than zero, LD is present. The standard D is highly influenced by allele frequencies. Attempts to normalize D have led to alternate measures such as: D' , r^2 , d , Q , and δ . These additional LD measures are calculated by dividing the standard D by a unique quantity representing allele frequencies in the population (Oraguzie et al., 2007).

The measures δ and d are generally applied in epidemiological settings while D' and r^2 are

commonly used in association studies. The D' ,

$$D' = \begin{cases} \frac{D}{\min(p_1q_2p_2q_1)} & D > 0 \\ \frac{D}{\min(p_1q_1p_2q_2)} & D < 0 \end{cases}$$

coefficient is robust and better suited for loci with greater differences in allele frequencies than D or r^2 (Flint-Garcia, 2003; Gupta et al., 2005). The effect of recombination history and physical distance are better measured by D' . For multi-allelic loci, it is also the standard method. Heterozygous loci and loci with high rates of mutation can cause problems in estimating D' .

If mutation is a substantial factor, the r^2 , ($D^2/[P_A P_a P_B P_b]$), correlation coefficient is an alternative measure which takes into consideration both recombination and mutation. As a correlation between alleles, r^2 is better for bi-allelic markers, when fewer haplotypes are present, and for low frequency alleles (Oraguzie et al., 2007). The correlation of QTL and markers is better indicated by r^2 , and thus preferred for association studies over D' .

Both D' and r^2 are bounded by 0 and 1. Depending on the number of haplotypes present, values of D' and r^2 may be very similar. When $r^2 = 1$, allele frequencies are equal while D' can give values of 1 with missing data and slight differences in allele frequency. The type of molecular marker used in estimating LD must be considered because as the number of alleles increases, so do the number of haplotypes, driving down r^2 estimates (Sorrells and Yu, 2009).

D. Molecular Markers

Molecular DNA markers are assays designed to detect differences in the DNA sequence between two or more samples. Restriction Fragment Length Polymorphisms (RFLPs) (Botstein, 1980) were among the first molecular DNA markers. A need for faster, easier, and cheaper markers gave way to development of PCR based markers such as Random Amplified Polymorphic DNA (RAPD) (Welsh and McClelland, 1990; Williams et al., 1990), Short Sequence Repeats (SSR), Amplified Fragment Length Polymorphism (AFLP) (Vos, 1995), and Single Nucleotide Polymorphism SNPs, among others. Currently over 40 different markers have been characterized (Gupta et al., 2002). Each marker has attributes that influence its application (Table 1.1). The type of marker used can be based on considerations such as: type of DNA polymorphism targeted, detection, visualization, ability to be automated, inheritance pattern, ability to detect heterozygosity (H), reliability, cost, technical skill, multiplex level, a priori information required for development, and the quality or quantity of DNA needed for assays. To date, the most amenable markers for MAS in plant breeding are microsatellite or SSR markers and SNPs.

E. Microsatellites

Microsatellite markers have several alternate names and close variants including SSR (Hearne et al., 1992), Short Tandem Repeat (STR) (Edwards et al., 1991), or Sequence Tagged Microsatellite Site (STMS) (Beckmann and Soller, 1990) that will be considered jointly. These markers are designed to anneal primers to conserved sequences flanking repeated motifs.

A repeated motif of 1-8 bp in length is known as a microsatellite. Larger motifs of 10-30 bp are referred to as minisatellites and >30 bp motifs are known as satellites (Ellegren, 2004). Microsatellites are preferred over their larger counterparts because they are ubiquitous throughout the genome (Tautz and Renz, 1984), while larger complements are more likely to occur in telomeric regions. The number of repeats is assumed to be effected by polymerase slippage or unequal crossing over. Simple microsatellites have repeats of one motif; multiple motifs are referred to as compound (Weber, 1990). If sequence interruptions occur within or between motifs then it is classified as an imperfect SSR.

These highly reproducible co-dominant markers can be found within genes and in untranslated regions of coding sequences, but are mainly in non-coding regions. The degree of polymorphism is affected by the source from which the microsatellite was derived. Markers that originate from expressed sequence tags (EST) have less polymorphism because of sequence conservation (Scott, 2001). 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.

Multi-plexing of SSRs into single reactions for PCR or fragment analysis enables this marker assay amenable to automation. The high level of polymorphism per locus, speed of genotyping, and cost has made SSRs a common marker in breeding applications. Use of SSRs between related species, such as barley and wheat, has not been particularly successful (Roder et al., 1995). Issues with homoplasmy, in which alleles are identical by state not descent, can interfere with markers being diagnostic in MAB.

F. SNPs

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. Insertion or deletion (indels) are generally not classified as SNPs, although they may often be detected with a number of technologies used to detect SNP variants. There is the potential for four alleles, but SNPs are generally bi-allelic because mutation rates are nominal. They may occur in either non-coding or coding sequences and are considered evolutionarily stable due to a low mutation rate. The frequency of SNPs has ranged from 1/104bp for Maize to 1/1032 bp in Cassava (Edwards, 2007). The frequency of SNPs for an elite world-wide collection of bread wheat is 1/335 bp (Ravel et al., 2006b).

A SNP marker is a misnomer since many markers detect single base variants. For example, an RFLP can produce polymorphic bands if a SNP resides within the restriction recognition site. Generally, SNP marker analysis refers to a high throughput method of detection such as: MALDI-TOF-MS, genotyping by sequencing (GBS), oligonucleotide ligation assays (OSA), or dynamic allele specific hybridization (DASH) among many others.

Development of SNPs has been expensive and timely due to a need for sequence information but new technology is making the process easier for orphan crops such as amaranth or oats (Maughan et al., 2009). Using restriction enzymes to reduce the complexity of the genome, fragments are joined with unique barcode adapters and then pooled with other ligated fragments of multiple individuals for sequencing via Roche 454-pyrosequencing (Maughan et al., 2009) or Illumina goldengate/infinium assays (Elshire et al., 2011). This process was described as genotyping by sequencing (GBS) by Elshire et al. (2011).

G. DArT

Diversity array technology (DArT) markers are assays designed to screen for thousands of markers in one reaction on a microarray platform. This proprietary process is based upon reducing the complexity of the genome to a reproducible portion with restriction enzymes (RE) and size selection. The resulting genome fraction represents 10 to 20 % of the genome (Akbari, 2006). The digested DNA fragments are added to a microarray slide for hybridization to complementary oligonucleotide probes. The spotted oligonucleotides of the microarray slide represent complementary polymorphic fragments from a diversity panel that underwent the same complexity reduction process. A hybridization signal identifies compatible sequences and the presence of a marker (Jaccoud et al., 2001; Wenzl et al., 2004). An absence signal would indicate polymorphism in the restriction site or along the hybridization sequence (Newell et al., 2010).

The bi-allelic DArT marker is classified as dominant, although it could be possible to identify co-dominance if other alleles are represented and known. The oligonucleotides could be sequenced and turned into a PCR based marker for single marker analysis, though the assay is designed to generate thousands of markers independent of sequence information. Mapping studies have suggested that DArTs are more largely represented in clusters in gene rich areas. Mapping of DArTs has met some complications due to single markers mapping to multiple loci (Newell et al., 2010).

H. Linkage Mapping

The goal of linkage analysis is to identify associations between functional variants and marker loci (Sneller et al., 2009). The key difference between bi-parental and association

mapping is centered around recombination (Myles et al., 2009). The degree and extent of recombination is reflected in differences of experimental design between the two methods.

Bi-Parental Mapping

Bi-parental mapping utilizes progeny of a single cross in segregating F_2 , backcross, recombinant inbred (RIL), or double haploid (DH) population. Each population has attributes that need to be considered in relation to mapping. The RIL and DH populations are commonly used because the population can be phenotyped for multiple traits in multiple environments (Lehmensiek, 2009).

These populations are highly structured, family based, and have very high LD, requiring relatively few markers to span the genome. A majority of bi-parental studies are often composed of 100-250 individuals limiting the power to detect QTL and the resolution to intervals of 10-20 cM. Generally only the two alleles in the parents are evaluated for a gene or QTL. Several different linkage analysis models can be used to detect significant associations in bi-parental populations. In these models markers are considered fixed.

A QTL can be mapped to a single marker using regression or ANOVA. This method, known as single marker analysis (SMA), does not require a genetic map, but is greatly influenced by recombination. Quantifying the magnitude of a QTL effect with SMA can be misleading. A given QTL effect may be equivalent if a large effect QTL is associated with a distant marker or a small effect QTL and a nearby marker.

Interval mapping (IM) (Lander and Botstein, 1989) helps to balance the effect of recombination between marker and QTL by determining the likelihood of a QTL at every interval between two markers. If marker density is high then results will be similar to SMA.

The optimal setting for IM is to have markers evenly spaced with large intervals between, allowing for many recombination events (Tanksley, 1993). When multiple QTL are being mapped, their effects can interact with the analysis and alter estimates (Jansen, 1993).

Composite interval mapping (CIM) and multiple interval mapping (MIM) (Jansen 1993) use the same approach of evaluating a QTL at each position within the genome but control for additional QTL by using random markers as cofactors in multiple regression for a more accurate analysis.

Many QTL have been mapped with these methods, although the implementation of QTL by MAS has been limited (Bernardo, 2008). The LD in such populations is maximized. 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.

New analytical methods may increase the precision of analysis, but power resides in the experimental design. Considerations need to be given to population size, heritability of the trait, number of QTL, epistasis, marker saturation and order (Asins, 2002). Tanksley (1993) stated that many QTL studies are composed of populations with 100-250 individuals, limiting the detection to major effect QTL. The Beavis effect is another attribute of small mapping populations, leading to overestimation of QTL effects. If QTL are overestimated, then the actual number of QTL determining a trait will be underestimated (Beavis, 1994; Beavis, 1998). Though population size and heritability have been determined to be the most important factors (Lynch and Walsh, 1998), epistasis (Carlborg and Haley, 2004; Holland,

2004) and accuracy of marker order (Lehmensiek et al., 2005) also influence power and accuracy of QTL mapping.

I. Association Analysis

Like bi-parental mapping, association analysis (AA) relies on the correlation between marker loci and functional variants. Association mapping can be conducted in populations without crossing (Iwata et al., 2009). A broader base of experimental units also allows for more QTL and alleles to be evaluated (Myles et al., 2009). The net effect is that historical recombination allows a greater chance to reduce LD through accumulated meiosis giving greater mapping resolution.

Several additional advantages are gained by using an association study such as: more efficient use of resources, evaluation of multiple traits, and a greater probability of identifying polymorphic markers (Brescaglio and Sorrells, 2006a). Association studies can be divided into two different experimental designs, family or population based. The intent of the analysis can also be separated into two categories, candidate gene analysis (CGA) or genome wide association studies (GWAS). The main difference between the two is the portion of the genome being targeted and the resolution. A CGA can be used to fine map QTL or genes while a GWA study can be applied to improve MAS by aiding in identification and confirmation of effective markers for selection (Brescaglio and Sorrells, 2006b).

Even though AA has the potential for greater power than bi-parental mapping, the chances of Type I and II errors are also greater. Type I errors, known as false positives, occur from population structure creating covariance among sub-groups and neutral SNPs associated with structure.

The method for accounting of structure depends on the design and goal of the experiment. Genomic control, which uses an Armitage statistic based on the median value of null unlinked markers to adjust linked markers (Price et al., 2006), would be better for CGA (Sneller et al., 2009) and when there is not significant confounding (Devlin and Roeder, 1999; Kang et al., 2008). Genomic control does not determine any aspect of genetic ancestry or relatedness, and would not be the best option for GWAS studies where complex structure can exist.

Inferring genetic ancestry is an alternative method to detect and adjust for population structure (Price et al., 2010). The two most common approaches for population based analyses are structured association (SA) and principle component analysis (PCA). The SA and PCA approaches are treated as fixed effect covariates that adjust for population structure in linear models. The probability of sub-group membership as defined by STRUCTURE (Q) or eigenvectors from PCA (P) serve as the fixed covariates. Although SA and PCA can calculate an estimate of ancestry, they lack the ability to detect familial patterns or cryptic relatedness. The use of SA is not ideal for when there are large numbers of subgroups, deviations from HWE are great (Balding, 2006; Sneller et al., 2009) or complex patterns of relatedness exist (Zhao et al., 2007).

The PCA correction can be better when the number of subgroups is high due to each axis being orthogonal, therefore the value of a sample in an axis does not change depending on how many axes are used (Price et al., 2006). Some complications of PCA are identifying what are the top eigenvectors to include, the biological significance of groups, or that the axes may reveal long range LD but not population structure (Price et al., 2010).

The SA and PCA only capture some of the many levels of relatedness (Kang et al., 2008) while a kinship matrix allows for a pair wise evaluation of relatedness between all samples (Myles et al., 2009). The inclusion of a kinship matrix as a random effect with SA or PCA into a mixed linear model has proven to reduce false positives and increase power (Yu, 2006; Zhao et al., 2007).

When kinship matrices have negative values, problems occur with the matrix not being positive definite. A common fix is to change negative relationships to zero, although this disregards information of the relationship between unrelated individuals, and requires inclusion of covariates like P or Q in the model (Stich et al., 2008). Simple kinship matrices have been found effective to reduce false positives and avoid the issue of not being positive semi-definite (Kang et al., 2008; Zhao et al., 2007).

While Mixed Linear Models (MLM) have been popular and efficient models to account for structure, they are not without issues. Problems with MLM include: not resolving peaks finely, inability to handle complex peaks, and not controlling LD due to selection (Stich et al., 2008).

A large sample size will help increase power and reduce type I errors in populations with large and complex structure although, cryptic relatedness may prevent the complete elimination of stratification (Sneller et al., 2009). A reduction of structure may be better than elimination so that some power remains to detect and discriminate functional variants between true and false positives (Zhao et al., 2007).

Type II errors, or false negatives, can arise from over-correction of multiple hypothesis testing, low LD between markers and functional variants, an unbalanced design

(Brescaglio and Sorrells, 2006a) or MLM controlling away functional variants. False negatives may reduce the ability to detect rare or low frequency variants (Brescaglio and Sorrells, 2006a), but controlling against false positives is paramount to identifying markers and QTL to be used in marker assisted breeding.

Multiple hypothesis testing (MHT) corrections are essential in GWAS studies where thousands of tests are performed and multiple tests are positive due to linkage or LD to a causal locus. Corrections for MHT such as the Bonferroni are often too conservative, and the probability for a type I error is set too low (Oraguzie et al., 2007). The false discovery rate (FDR), which is the expected proportion of false discoveries within the group of tests that reject the null hypothesis, may also be too conservative if tests are positively correlated, as when markers are collinear or in strong LD with one another linked (Storey and Tibshirani, 2003).

The q value is an alternative approach to define significance associated with FDR. Instead of measuring the false positive rate as a p value does, q measures the false discovery rate. Storey and Tibshirani (2003) clarify the difference between the two measures, “the false positive rate is the rate that truly null features are called significant. The FDR is the rate that significant features are truly null.” The q value has been applied to microarray studies and is suited well for genome-wide studies.

With the increase in power, ability to screen diverse populations, and more genomic resources, association studies have burgeoned. The analysis can be performed in a myriad of platforms such as PLINK (Purcell et al., 2007), Powermarker (Liu and Muse, 2005), ASREML (Gilmour et al., 2002), R (Ihaka and Gentleman, R: A language for data analysis

and graphics), SAS (SAS Institute), and TASSEL (Bradbury, 2006). The analytical models have been refined with methods such as the efficient mixed model association (EMMA) (Kang et al., 2008; Zhao et al., 2007), population parameters previously defined (P3D) and compression (Zhang et al., 2010b).

One drawback to association studies is difficulty with estimating and interpreting gene and allele effects. Extent of LD, population structure, QTL x environment interaction, and sampling error are a number of the reasons why gene effects are biased and can give false inferences (Breseghello and Sorrells, 2006a; Breseghello and Sorrells, 2006b). A lack of genomic resources combined with the size and complexity of the genome has limited the number of association studies in wheat to

studies on high-molecular weight glutenins (Ravel et al., 2006a), milling quality (Breseghello and Sorrells, 2006a), baking quality (Crossa et al., 2007), and *Staganospora nodorum* resistance (Tommasini et al., 2007).

J. Genomic Selection

Many MAS techniques, like MABC and allele enrichment, rely on accurate detection of QTL and the magnitude of their effects. The numerous QTL with small effects of polygenic traits are often undetected and not used due to: allelic variation, genetic background, inaccurate mapping, inadequate statistical models, low heritability, small populations, population structure, or significance thresholds (Heffner 2009, Schon et al. 2004, Xu 2003). The result is that only a few markers associated with few QTL are used that explain a small proportion of the genetic variance. Many important and disease traits are

governed by a large number of QTL with small effects. Thus, MABC and allele enrichment leave a major portion of genetic gain untapped.

Genomic selection (GS) is an approach that attempts to capture all genetic variation with a high density of genome wide markers. The GS method put forth by Meuwissen et al. (2001) uses the joint effects of all marker loci to calculate a genomic estimated breeding value (GEBV). A genome that is saturated with markers should have all QTL in LD with at least one marker.

A GS model can be applied to predict the GEBV of individuals who lack phenotypic data but have been genotyped. In order to predict GEBV, a model must be developed or “trained” by estimating marker effects in an initial reference population which has been both genotyped and phenotyped. The prediction of GEBV for selection candidates without phenotyping is proposed to save cost (Heffner et al., 2009) and speed up selection with the use of off-season nurseries, increasing genetic gain per unit time (Heffner et al., 2010). Complex, low heritability, or negatively correlated traits can have greater genetic gains with GS over phenotypic methods if multiple selection cycles per year are used (Dekkers, 2007; Heffner et al., 2009). Additional advantages that have been put forth include maintaining genetic diversity (Heffner et al., 2010) and lower levels of inbreeding through a reduction of sib co-selection (Daetwyler et al., 2007).

Cross validation of lines within a prediction set allows for estimates of accuracy (r) of GS models based upon the correlation between the true breeding value (TBV) and GEBV of lines. The Pearson correlation coefficient, r , as a measure of accuracy can be related to the gain from selection with the formula $R = ir\sqrt{\sigma_A}$, where R is the response to selection, i is

selection intensity, $\sqrt{\sigma_A}$ is the additive variance, and r is accuracy (Heffner et al., 2009; Rutkoski et al., 2010). Accuracy can be increased in several manners such as: larger training sets, higher trait heritabilities, or greater genetic relatedness among lines in the training and prediction populations.

It has been shown that larger training, or estimation, sets are more accurate than smaller ones, and more influential in improving model accuracy than an increase in marker number (Heffner et al., 2011; Lorenzana and Bernardo, 2009). The composition of the training set is a balancing act between representing all the genetic diversity and increasing relatedness to selection candidates. Accurate calculation of breeding values will depend on: the estimation set representing the entire diversity of alleles, the degree of relatedness to individuals in the prediction set (Heffner et al., 2009; Rutkoski et al., 2010), and the statistical model chosen to estimate marker effects.

Several models have been proposed to reduce the dimensionality of GS that is caused by having more marker predictors than phenotypes, termed “large p , small n ” (Jannink et al., 2010). Two common model types involve regression or Bayesian estimation.

Stepwise regression (SR) was an early approach to estimate significant marker effects. With markers as fixed effects, combinations of markers are fit to the phenotypic data to determine significance. Non-significant markers are treated as having no effect, and are not included in the final model. Models with larger p value thresholds have been shown to have greater accuracy than more strict models (Hospital et al., 1997). The use of SR was short lived as Goddard & Hayes (2007) and Meuwissen (2001) pointed out that SR overestimates QTL with large effects and lacks the ability to capture small effect QTL.

The ridge regression (RR) model is based on a normal distribution of random marker effects which have identical variances. Marker effects are estimated simultaneously and all loci are retained in the model. Each marker effect is shrunk toward zero by the same factor due to the assumption of equal variance. Heffner et al. (2009) notes that equal variance does not mean that QTL effects are also equal, implying that large QTL effects will be underestimated by RR. While LD between functional variants and marker loci is still the underlying factor driving GS, Habier et al. (2007), found that RR was able to estimate GEBV in the absence of LD because the inclusion of all markers allowed superior estimation of genetic relationships. It has been proposed that RR will be best suited for GS designs for which there are few large effects with many small effect QTLs.

Meuwissen (2001) developed two Bayesian methods, A and B, to alleviate the assumption of equal variance. Based upon inverse χ^2 distributions and random markers, the models differ in premise of whether marker loci can have no effect. The Bayes A model requires that all marker effects are greater than zero and thus retains all markers in the model. The Bayes B analyses allow some markers' effects to be equal to zero, which are not included in calculating GEBV. The probability (π) of loci with no effect is defined a priori. When $\pi = 0$, the Bayes B equals the Bayes A model.

A third Bayesian model termed Bayes C_π , returns to the supposition of common variance, but only for markers with effects greater than zero. The proportion of markers with effects greater than zero can be estimated by Bayes C_π , generally with an initial value of $\pi = 0.5$. The Bayesian methods can be computationally intensive and need to be run with 1,000s of iterations to allow for convergence.

Many simulation studies have reported the superiority of the Bayes B model, yet no clear dominant model has been accepted in plant breeding. The attributes of the experimental design and trait greatly influence the accuracy of each model. Both Bayes A and B models performed better when there were fewer QTL (Habier et al., 2007). When levels of LD are lower and greater amounts of marker in equilibrium with traits, RR generated greater accuracies than Bayes B (Habier et al., 2007). While Bayes B does not appear to be effected by marker density and can handle collinear markers, RR does perform better when marker spacing and LD extended over larger regions (Heffner et al., 2009). The composition of the training set might also dictate model choice. If many individuals of the prediction set are related or are progeny of the estimation set, than RR could generate greater accuracies than Bayes B because of genetic relationships which can be captured (Habier et al., 2007).

II. WHEAT

A. Taxonomy

Wheat is in a taxonomical lineage that contains many important cereals, grasses, and small grain crops. As a member of the *Poaceae* family, genomic similarity at this taxonomic level can be found in syntenic blocks throughout the genomes of wheat, rice, maize, and forage grasses (Gustafson et al., 2009).

The degree of similarity increases between the members of the supertribe, *Triticoideae*. Members of the supertribe share the same base chromosome number ($n=7$) and

higher levels of synteny. Within *Triticoideae*, the *Triticeae* tribe includes several important small grain genera such as rye (*Secale*), barley (*Hordeum*), and wheat (*Triticum*) in addition to many weedy grass genera like *Aegilops*, *Agropyron*, *Elmyus*, and *Leymus* (Devos et al., 2009).

Many of the *Triticeae* genera will hybridize readily and produce fertile progeny as can be seen in the case of Triticale (*Triticum aestivum* x *Secale cerealis*) and polyploid wheat (*Triticum* x *Aegilops*). The ability to intermate has been capitalized on in cultivated wheat to introgress novel genetic material. Examples of the incorporation of disease resistance genes from related genera has included *Sr26* from *Agropyrum elongatum*, *Yr8* *Aegilops comosa*, and the 1RS translocation from rye to the long arm of chromosomes 1B or 1A in wheat (Worland and Snape, 2001).

The genera *Triticum* and *Aegilops* are the most closely related within the *Triticeae*. Morphological and molecular studies both demonstrate the close relationship between the genera and have been considered to be monophyletic (Barkworth and von Bothmer, 2009).

Wheat exists in wild and domesticated forms of diploid, tetraploid and hexaploid species. Events relating to domestication were the culminating factors leading to subspeciation, speciation, and polyploidization. Subspecies exist for several wheat's, although some taxonomists contend that many subspecies should be individual species (Barkworth and von Bothmer, 2009). The disparity of taxonomic classification can be exemplified in diploid wheat. In extreme situations, five diploid wheat's have been classified as separate species contrasted with five subspecies of a single species.

There are two accepted wild diploid wheat species, *T. boeoticum* ($2n=2x=14$, A^bA^b) and *T. urartu* ($2n=2x=14$, A^uA^u). Differences phenotypically and genetically separate the wild species (Kilian et al. 2007b), in addition to a hybridization barrier (Johnson and Dhaliwal, 1976). An alternate name for *T. boeoticum* is wild einkorn wheat, which gave rise to cultivated einkorn wheat or *T. monococcum* ($2n=2x=14$, $A^m A^m$). Production of einkorn wheat can still be found in regions of Italy and Turkey; the grain is used as feed for swine and poultry (Feldman, 2001; Worland and Snape, 2001). The species *T. urartu* remained wild and through hybridization with *Ae. speltoides* ($2n=2x=7$, SS), gave rise to tetraploid wheat.

Due to different cytotypes of *Ae. speltoides* (BB , GG), two different species resulted from the hybridization with *T. urartu*, namely *T. turgidum* subsp *dicoccoides* ($2n=4x=21$, A^uA^uBB) and *T. timopheevii* subsp *armenicum* ($2n=4x=14$, A^uA^uGG).

The specie *T. turgidum* subsp *dicoccoides* is also known as wild emmer wheat. Domesticated emmer wheat (*T. turgidum* subsp *dicoccum*) has thicker grain and a less fragile rachis (Table 1.2) than its wild derivative. Sup-species of *T.turgidum* subsp *diccicum*, include durum, rivet, Persian, and Khoasan (Dvorak, 2009; Gustafson et al., 2009).

Like *T. turgidum* subsp *dicoccum*, the wheat specie *T. timopheevii* subsp *timopheevii* is the domesticated tetraploid of subsp *armenicum*. Both the BB and GG genomes of tetraploid wheat derive from an ancestor of *Ae. speltoides*, but from different hybridization events. Translocation events have differentiated the BB and GG genomes and are likely the basis for intermating issues between the tetraploid species (Feldman, 2001). A second

hybridization event occurred for both domesticated tetraploids giving rise to hexaploid wheat.

The hexaploid wheat, *T. zhucovskyi* ($2n=6x=42$, $A^uA^uA^mA^mGG$), is formed from the cross of *T. timopheevii* and *T. monococcum* subsp *monococcum*. Common wheat (*Triticum aestivum* L. $2n=6x=42$, AABBDD), derives from a different interspecific hybridization event of *T. turgidum* subsp *dicoccum* and *Ae. tauschii* ($2n=2x=7$, DD). Cytoplasm is maintained through the female tetraploid emmer wheat. Unlike diploid and tetraploid wheat progenitors, no wild hexaploid wheat is known (Feldman, 2001). Several subspecies of common bread wheat have been identified based upon four traits causing dramatic morphological changes (Table 1.2).

B. Flowering & Reproductive Behavior

Wheat is a determinate annual. The transition from a vegetative state to induction of flowering depends on photoperiod (*PPD*) and vernalization (*VRN*) genes. The loci associated with major photoperiod effects are three homoeologous genes on group 2 chromosomes.

The vernalization genes can have the greatest effect on flowering and determines growth habit as a summer or winter annual. A complex interaction between three homoallelic genes on group 5 chromosomes (*Vrn-A1*, *Vrn-B1* and *Vrn-D1*) and photoperiod dictates when an undifferentiated apex will flower (Yan et al., 2003; Yan et al., 2006; Yan et al., 2004). Multiple alleles at these loci and additional vernalization genes have been found,

though the strength of effects in establishing vernalization requirements are minor in comparison (Yan, 2009).

The *Vrn-1* and *Vrn-3* genes are dominant for spring type, and *Vrn-2* is dominant for winter type. Spring type is dominant over winter. The *Vrn1* gene commences the change from a vegetative to reproductive stage of the terminal apex. When *vrn2a* or *vrn2b* is present, flowering proceeds as spring type. In the presence of transcribed *Vrn2*, the genes *Vrn1* and *Vrn3* are down-regulated and the photoperiod effect is stymied. The transcription of *Vrn2* is reduced by vernalization and short days, leading to flowering.

The flowering wheat head is composed of spikelets that are positioned opposite one another along the rachis. The terminal spikelet is perpendicular to the others. Each spikelet has multiple florets. Florets are perfect flowers. The palea and lemma are inner glumes that surround each floret. A floret contains three stamens, one pistil, and two lodicules. The lodicules help to separate the palea and lemma during anthesis (Bewley et al., 2006) although some wheat varieties are cleistogamous.

Reproductive traits, particularly naked or free threshing kernels, aided in the diversification and domestication of wheat species. Several genes influence the free threshing nature of kernels. The *Q* gene on 5AL confers free threshing through square headed ears, softer glumes, and rigidity of the rachis. This gene is partially dominant with intermediate phenotypes. Expression of *Q* is suppressed by the dominant *Tg* allele on 2D, resulting in hulled grains (Worland and Snape, 2001).

Other traits which influence threshing include a non brittle rachis and spike compactness. A brittle rachis is ideal for dispersal and self sowing, but not for cultivation and

harvesting. A homoeologous series *Br-A1*, *Br2*, *Br3* on 3DS, 3A, and 3B respectively regulate rachis fragility. A compact spike which is particular to club wheat can be controlled by two genes, *C* on 2D, and *S-D1* on 3D (Worland and Snape, 2001).

C. Production

Wheat production has extended far from the Fertile Crescent, into the arctic circle in Finland and Norway to 45° South in Argentina and Chile (Kilian, 2009). The ability to grow wheat in severe climates is possible because of selection for traits that alter heading date to protect the plant from environmental extremes. In addition to PPD and VRN genes, earliness per se (*EPS*) QTL allow for flowering to be altered by a few days. An estimated 76 *EPS* QTL involved with heading and flowering via photoperiod and temperature sensitivity have been recognized (Worland and Snape, 2001).

Wheat is consistently one of the largest crops in global annual acres planted and tonnage harvested. According to 2008 Food and Agricultural Organization (FAO) estimates, 689,945,712 metric tons were harvested from 223,564,097 Ha (552,438,915 acres) (<http://Faostat.fao.org>). The average consumption per person in the United States was 137 lbs/year for 2008 (USDA Economic Research Service, www.ers.usda.gov).

The six U.S. wheat economic market classes generally reflect specific growing regions and end use. The classes are presented in order of most acres planted.

Hard red winter (HRW) is grown primarily in the Great-Plains of the mid west, east of the Rocky Mountains and west of the Mississippi river. The Economic Research Service (ERS) of the United States Department of Agriculture (USDA) estimated 40% of the total

U.S. annual harvest is from this market class, with Kansas producing the largest amount of wheat in the U.S.

Hard red spring (HRS) is grown in Minnesota, Wisconsin, both Dakotas, and Montana. The cold, harsh winters necessitate spring type planting because of winter kill of autumnal sown seed. This region is well-known for high protein and bread making attributes.

Soft red winter (SRW) is produced east of the Mississippi, in breeding programs from Illinois to Louisiana. The softer seed coat and lower protein content has different milling and baking qualities, and is used for cracker, cookie, cakes, pretzels, and batters.

Soft 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 is often used for bakery products and crackers.

Durum wheat is a hard grained tetraploid wheat with high protein. It is raised in North Dakota and Montana on roughly 3.2 million acres specifically for pastas.

Hard white wheat, which is grown in eastern Kansas, Nebraska, and Colorado, is a relatively new market class in the U.S. The white bran has less tannins and phenolics than red wheat, resulting in a less bitter taste in whole wheat breads. Asian noodles are also made from this class. The environments where it can be grown are limited because of issues with pre-harvesting sprouting (PHS). Wet conditions during ripening and harvesting are ideal for PHS. A close association exists between QTL for PHS and the genes controlling seed coat color. Breeders generally limit breeding material to within market class unless there is a need to acquire diversity for a trait such as disease resistance. It is time consuming to

reconstitute the linkage blocks that confer agronomics for particular environments and market class characteristics (Anderson, 2003).

In addition to a marker class being regionally adapted and grown, they also reflect gross phenotypes. These characteristics include growth habit (Spring/Winter), bran coat color (Red/White), and milling properties (Hard/Soft). Color is regulated by three homoeologous genes: *R1*, *R2*, and *R3* on the group 3 chromosomes (Gustafson et al., 2009; Kilian, 2009). Red kernels carry at least one dominant allele. The *R* genes can act additively, creating a dosage effect (Bewley et al., 2006). It has been observed that red wheat has greater dormancy effects and resists pre-harvest sprouting better than white wheat, and is more suitable for more humid environments.

The hardness of the endosperm is controlled by linked genes *Pina*, *Pinb*, and *Gsp-1* on the short arm of chromosome 5D. The dominant alleles for these genes confer the soft wheat phenotype. Lower grinding pressure is used for milling soft wheat. The higher the grinding pressure the more damage starch granules experience, allowing the granules to absorb more water, which is ideal for bread making (Kilian, 2009).

D. Reduced Height Genes

Semi-dwarfed wheat has contributed to an increase in production. Genes that reduce height (*RHT*) are heavily represented by few alleles, though more than 21 different alleles of several loci have been identified in shortening the stature of wheat (McIntosh et al., 1998). A reduction in yield is generally expected with dwarfing genes; although several alleles increase yield significantly. The incorporation of two homoeologous alleles, *Rht-B1b* and *Rht-D1b* on chromosomes 4B and 4D respectively, increased straw strength, spikelet fertility,

and yield compared to tall genotypes (Worland and Snape, 2001). Commonly referred to as the green revolution genes, *Rht-B1* and *Rht-D1*, formerly *Rht1* and *Rht2*, are from an induced mutation in the Japanese landrace 'Daruma' (Konzak, 1987; Worland and Snape, 2001). The new alleles were brought to North America by S.C. Salmon who released the alleles in the variety 'Gaines', although the genes were popularized by Norman Borlaug (Gustafson et al., 2009; Worland and Snape, 2001). A large proportion of the U.S. wheat varieties possess one of these two alleles (Guedira et al., 2010), which reduce height to around 80-90 cm depending upon the genetic background. At present, the *Rht-B1* locus has five alleles and *Rht-D1* has four (McIntosh et al., 1998). Alternate alleles at these loci are used infrequently due to extreme dwarfing. The alleles that produce dwarfing at the 4B and 4D loci are gibberellic acid (GA) insensitive and have short coleoptiles. The use of GA insensitive alleles is not recommended for environments with low initial water availability. Smaller coleoptiles can affect seedling germination and stand establishment under arid conditions (Konzak, 1987). Use of GA sensitive *RHT* genes provides an alternative in water limited environments.

The most widely used GA sensitive dwarfing gene is *Rht8*. Originally from Akakomugi, another Japanese landrace, the allele has been in use in Italy and surrounding areas since 1930. The gene is closely linked to *Ppd-D1*, giving an advantage at lower latitudes due to photoperiod insensitivity. Allelic variants of *Rht8* and other GA sensitive dwarfing genes are not commonly used due to severe dwarfing or a decrease in yield.

E. Genetics

The common bread wheat (*Triticum aestivum* subsp *aestivum*) genome is an estimated 16 Gb in size and 2,500 cM in length (Somers, 2004). Each of the ancestral diploid parents of bread wheat contributed seven chromosomes. The individual genomes are: A- 4.93 pg, B- 5.15 pg, and D- 5.1 pg. The three genomes are highly collinear except for retrotransposons (Devos et al., 2009). Homologous pairing and disomic inheritance is maintained by a major gene *Ph1* on 5B. Smaller effect pairing genes like *Ph2* on 3DS or those on 5AL, 5DL, 5AS, 3AL, 3BL and 3DL (Feldman, 1993) have nominal contribution and cannot entirely compensate for the loss of *Ph1* (Devos et al., 2009). Homoeologous loci could be heterozygous causing intra-genomic hybrid vigor.

Coding DNA is thought to be 4-8% of the total genome. The euchromatic DNA is isolated in a few gene islands between abundant amounts of repetitive DNA. An estimated 80-90% of the DNA is repetitive (Smith and Flavell, 1975). A large quantity of retrotransposons and transposons are incorporated in the genome (Devos et al., 2009). Wheat is considered to have a low degree of polymorphism.

F. Genomic Resources

The hexaploid constitution of wheat allows for many cytogenetic stocks, including monosomic, nulli-tetrasomic, telosomic stocks, and deletion lines. Conversely the large homoeologous genome has made many molecular techniques performed in *Arabidopsis thaliana* and rice very difficult. Transposon tagging or chromosome walking are not

common techniques in wheat. Extensive EST and BAC libraries are being established to advance sequencing, cloning, and marker development.

The development of markers is a constant process. An international endeavor in wheat has produced thousands of SSRs. Microsatellite markers were designed from low copy DNA (Roder, 1998), ESTs (Eujayl et al., 2002), and DNA specifically enriched for SSR motifs (Gupta et al., 2002). Microsatellite acronyms often designate lab origin and genome location. The homoeologous genomes of polyploid wheat require special attention for locus specificity. Conserved loci can amplify multiple fragments across ancestral genomes. Implementation of SSRs from related species such as barley has not been successful (Roder et al., 1995).

Akbari et al, (2006) developed DArT markers for hexaploid wheat. Thirteen cultivars from Australian and European germplasm were used to represent genomic diversity. The combination of restriction enzymes, *PstI/TaqI*, were used to give the greatest amount of polymorphic fragments. Currently over 7,000 probes have been identified as potential DArT markers. It was estimated that around 10% of the probes were redundant as opposed to linked loci. An extensive genetic map is being constructed with chromosome and location assignment, although not all markers have been mapped to a single locus (Akbari, 2006, Diversity Arrays, www.diversityarrays.com). Markers have been mapped to all 21 chromosomes with telomeres containing a larger percentage. The D genome had significantly fewer DArT markers than the A or B genomes.

As markers are developed to represent and saturate the entire genome, target and allele specific markers have been designed for key genes and QTL. The *Rht-B1* and *Rht-D1*

markers have been designed to detect the polymorphism causing reduced height (Ellis et al., 2002). Development of perfect markers is preferred, although closely linked markers like the UMN10 marker closely linked to the *Fhb1* gene also provide efficient means of selection (Liu et al., 2008).

The first genetic map of wheat began in 1989 by the International *Triticeae* Mapping Initiative (ITMI) utilizing RFLP's. A RIL population of 115 lines was developed from the cross of CIMMYT cultivar 'Opata' by synthetic wheat W7984. The ITMI population has been re-created with 1,500 RILs and 200 DH (Somers and Humphreys, 2009) and redistributed to create more saturated and precise genetic maps as new marker platforms are developed. Currently at least 13 different linkage maps are available on GrainGenes2.0 (<http://wheat.pw.usda.gov/GG2/index.shtml>). Consensus maps combine multiple bi-parental genetic maps, which are limited in the number of segregating markers. A SSR consensus map of four mapping populations was created by Somers et al. (2004) and a composite map of eleven different mapping studies by Appels et al. (2003). The new combined maps have over 1,200 SSR and 4,000 SSR, RFLP, and AFLP markers, respectively.

G. LD in Wheat

In wheat, LD has been found to extend > 40 cM (Crossa et al., 2007; Somers et al., 2007) to less than <1 cM on some regions of 2D and 3B (Breseghello and Sorrells, 2006a; Tommasini et al., 2007). Estimates of genome wide LD ($r^2 > .2$) were <10 cM (Chao et al., 2007; Crossa et al., 2007). Elite varieties which have undergone intense selection have higher LD than landraces (Steffenson et al., 2007). Comparison of LD in an international

collection of durum wheat to that of a Mediterranean subgroup showed greater levels of LD, 20cM compared to 10cM (Maccaferri et al., 2006).

III. FUSARIUM HEAD BLIGHT

A. Background

In 1884 in England, W.G. Smith described a disease of wheat which he coined as Scab. The disease was reported at farms in Ohio and Delaware in 1890, followed by Indiana in 1891, Pennsylvania and Iowa in 1892 (Stack, 2003). This floral disease was labeled Fusarium head blight (FHB), though scab is still frequently used because of lesion development on glumes. The disease spread globally reports of scab in Siberia, Germany, Netherlands, and Denmark (Stack, 2003) and China (Rudd et al., 2001).

B. Economic Loss

In 1999, the United States Department of Agriculture (USDA) made the pronouncement that FHB caused the worst epidemic for wheat or barley since stem rust, (*Puccinia graminis* Pers.) (Wood et al., 1999). Windels et al.(2000) estimates yield loss since 1990 has been 500 million bushels, while Johnson et al.(2003) determined the financial loss from 1991 to 1997 to be \$1.3 billion in direct loss and escalating to \$4.8 billion through indirect impacts. The epidemics of the 1990s resulted in less wheat acreage and fewer rotations with corn and or soybean (Johnson et al., 2003).

Wheat fields with FHB infection can have reduced yields through several different mechanisms. Wheat heads may have fewer kernels due to the pathogen sterilizing florets or clogging vascular tissue which prematurely ripens the spikelet (Dill-Macky, 2003). Tainted kernels may have higher ash content causing lower levels of starch, volume, or weight (Dexter et al., 1997; Dill-Macky, 2003; Steffenson et al., 2007). Lighter kernels can be blown away during combining (Bai and Shaner, 2004).

In addition to a yield reduction, the quality of the grain is affected. Diseased grain that is harvested will have weaker gluten protein strength (McMullen et al., 1997) and toxic secondary metabolites, such as deoxy-nivalenol (DON), nivalenol, 3ADON, and 15ADON, all which affect milling and baking quality (Dexter et al., 1996). Grain may be docked or rejected at elevators if DON levels are above established thresholds. The European Union allows 1.25 ppm in unprocessed bread flour, and 0.5 ppm in breads or 0.2 ppm in baby food (Buerstmayr et al., 2009b). Other nations such as the U.S. and Canada have limits around 1.0 ppm for wheat products.

When these nivalenol based toxins are ingested by humans they may suffer from: Akakabi toxicosis, toxic aleukia, convulsions, anorexia and vomiting (Goswami and Kistler, 2004; Stack, 2003). Ingestion as feed by cattle will lead to: feed refusal, diarrhea, emesis, and hemorrhaging (Goswami and Kistler, 2004; Snijders, 1990). The name vomitoxin was ascribed to DON by swine growers who fed their animals infected seed (Mirocha et al., 2003).

C. Symptoms

Wheat plants will generally show disease symptoms three to four days after natural infection or inoculation (Pugh et al., 1933). Early symptoms consist of lesions at the base of florets or a water soaked appearance (Bushnell et al., 2003). Pugh et al.(1933) reported progressive symptoms to be: purple-brown lesions on bracts, bleaching of florets, and pink discoloration due to perithecia formation. If wheat is bearded, the awns may twist or curl (Goswami and Kistler, 2004). The top fraction of an infected wheat head may die prematurely due to clogged vascular tissue (Dill-Macky, 2003). Infected kernels will frequently be white, pink, shriveled, or scabby; damaged kernels are called tombstones (Bushnell et al., 2003).

D. Modes of Infection

Ascospores and mycelium are the primary inoculum, although chlamyospores, macroconidia, and bits of hyphae have been observed to infect wheat (Sutton, 1982). Infection is limited to floral tissue and during a few developmental stages, namely flowering and early kernel development (Pugh et al., 1933). Susceptible growth stages would correspond to 10.1 to 11.3 on the Feekes scale and 50 to 83 on the Zadoks rating system. Fungal material is transported to the head by splashing water or wind (Gilbert and Fernando, 2004). The thick epidermal tissue of the palea, lemma, or glume stymies penetration (Seong et al., 2008). Tu et al. (1950) cited that infection may happen at the base of the glume where tissue is thinner. Hyphae may enter through wounds, circumventing morphological barriers and infecting plants (Kang and Buchenauer, 2000).

During dehiscence the lemma and palea separate, creating an opportunity for spores and other fungal material to enter (Kang and Buchenauer, 2000). If anthers protrude, they may become trapped between the palea and lemma (Trail, 2009). Exserted stamens provide ideal conditions for entrance and colonization of the pathogen since betaine and choline on anthers promote fungal growth (Strange et al., 1974) and prevent closure of the tissues. Pritsch et al. (2000) used scanning electron microscopy to report that entrance may occur through stomates, although appressorium were not observed by Seong et al. (2008) who argues stomata are not a likely route. After establishment, the pathogen spreads from floret to floret then apically to new spikelets through vascular tissue and the rachis (Bushnell et al., 2003; Goswami and Kistler, 2004).

The tricothene toxin DON is not required for infection, but is recognized as a virulence factor (Trail, 2009). The current theory is that DON assists in the spread of the fungus by being secreted in advance of fungal material (Bai and Shaner, 2004) and causing necrosis of cells (Trail, 2009) or cell wall degradation (Jansen et al., 2005).

E. Life Cycle

Most of the monocyclic life cycle of *Fusarium graminearum* (*Fg*) is spent in the haploid condition. The necrotroph is a filamentous ascomycete with a dikaryotic phase. Both mating genes, *Mat1-1* and *Mat1-2*, can be found beside one another in a haploid cell, allowing the pathogen to be homothallic (Trail, 2009). Outcrossing can occur but is infrequent (Goswami and Kistler, 2004).

A powerful discharge propels ascospores from asci jutting out of perithecia, into the wind for dispersion (Hallen and Trail, 2008). Ascospores are the primary inoculum and mode

of long distance movement. Spores germinate into vegetative mycelium, continuing with asexual conidia and fusiform development on the surface of a host plant and migrate via splashing of water. The pathogen may infect via sexually derived ascospores or asexual conidia. Overwintering structures can be saprophytic mycelia (Goswami and Kistler, 2004), perithecia, or ascospores (Trail, 2009). Bread wheat is a host of *Fg* and has been isolated from durum wheat (*T. turgidum* L. *durum*), barley (*Hordeum vulgare* L.), maize (*Zea mays*), rice (*Oryza sativa*), and *Agropyron*, *Lolium*, *Glycine*, *Medicago* species (Goswami and Kistler, 2004).

F. Environmental Influences on Infection

The warm, wet weather of spring is ideal for the concurrent events of ascospore release and flowering (Trail, 2009). Sutton (1982) determined that optimal infection of wheat occurs at 25 to 28°C, with infection still occurring between 15-32°C. Moisture is associated with perithecia development (Shaner, 2003). Wind and rain appear to be the most significant factors relating to dispersal (Parry and Parry, 1995; Shaner, 2003).

More controllable factors such as field management have been found to affect disease outbreaks. Disease severity was worse when wheat followed a corn rotation that used conservation or reduced till (Bai and Shaner, 2004; Dill-Macky, 2008). Maiorano et al.(2008) found a strong correlation ($R^2= 0.848$) between the DON levels in kernels and amount of corn stubble in a field (Maiorano et al., 2008). Amendment of soil with nitrogen was found to raise disease severity and DON levels (Lemmens et al., 2005). Chemical control of outbreaks is difficult due to limited effective fungicides. Triazole is one of the more

successful fungicides available to control scab although timing of application and spraying method will influence efficacy (Mesterhazy, 2003).

G. Population Genetics and Characterization

F. graminearum Schwabe (teleomorph *Gibberella zeae*) (*Fg*) is internationally distributed and the prevailing specie causing scab in North America (Liddell, 2003). Two other species have been identified as sources of scab, *F. culmorum*, and *F. avenaceum* (teleomorph *Gibberella avenaceae*) (Dill-Macky, 2003). Characterization of different species has generally been based upon morphological attributes. When grown on Potato dextrose agar (PDA) the mycelium of *Fg* is dark red and no microconidia are produced. Growth attributes on PDA are: no growth below 4°C or above 32°C, slow growth around 8°C and 32°C, fast growth between 12-28°C with optimal growth occurring at 25-28°C (Bai and Shaner, 1996).

Two isolates of *Fg* causing scab symptoms were similar morphologically but distinct reproductively. The variants were subdivided into two groups. Group 1 is heterothallic, lacks perithecia, and causes crown rot in addition to FHB symptoms. Group 2 is homothallic and solely produces scab symptoms (Bushnell et al., 2003; Dill-Macky, 2003). Group 1 has been reclassified and is now referred to as *F. pseudograminearum*, teleomorph *G. coronicola* (Aoki and O'Donnell, 1999). O'Donnell et al.(2004) states that based upon the analysis of eleven genes, the group 2 specie *Fg*, is nine morphologically identical, but genetically different species. The nine lineages locate to major global territories, except for lineage 7 which is internationally dispersed and the causative lineage in the U.S. The lineage 7 specie has maintained the name *Fg*. Inter-mating can and does occur between the lineages, but it

seldom outcrosses (Goswami and Kistler, 2004). No *Fg* races have been identified that have specific pathogen-host recognition (Buerstmayr et al., 2009b). The lack of host specific interaction has been a key factor for the success and wide implementation of the resistance gene *Fhb1*.

H. Genetics of *Fusarium Graminearum*

The genome of *Fg* is an estimated 36.1 Mb with 14,000 genes on four chromosomes (Guldener et al., 2006). Comparisons with related *Fusarium* species *F. verticillioides* (*Fv*), *F. oxysporum* (*Fo*), and *F. solani* (*Fs*), indicate similar genome sizes although chromosome fusion is likely the reason for fewer chromosomes occurring in *Fg* than *Fv*, *Fo*, or *Fs*.

Further comparison reveals that approximately 2,000 genes, which are unique to *Fg*, are up-regulated during infection. Genes that are conserved across other related *Fusarium* species and ascomycetes such as *Saccharomyces cerevisiae* are down-regulated during infection (Cuomo et al., 2007). Publication of the sequenced *Fg* genome has helped to elucidate potential key virulence, pathogenicity, and aggressiveness factors. There are 15 steps required for pathogens to synthesize DON (Desjardins, 2006). The trichothecene (TRI) cluster on chromosome 2 is composed of ten genes (Kimura et al., 2003). An interesting feature of *Fg* is the “repeat induced point mutation” (RIP) mechanism which causes copious point mutations to occur in duplicated sequence during meiosis (Cuomo et al., 2007). The end result is few paralogous genes and many non-functional sequences.

I. Characterizing Resistance

Fusarium head blight resistance is a quantitative trait. A spectrum of variation can be found in the resistance response to *Fg*, although complete resistance or immunity against scab has not been identified in wild or cultivated wheat. Resistance against scab is not a single trait, and can be evaluated under different phenotypes. The classifications of resistance were devised by Schroeder and Christiansen (1963) and Mesterhazy (1995). These particular classes are Type I - rate of incidence, type II - disease severity and spread of pathogen within the head, type III - accumulation of DON, and type IV - resistance to Fusarium damaged kernels or yield tolerance.

Spraying a conidial suspension over wheat plants and summing the number of infected spikes over total spikes will give a score of type I resistance. High disease pressure does not allow for discrimination between resistant and susceptible varieties for type I resistance (Bai and Shaner, 2004). Type II resistance can be evaluated by point inoculation and assessing spread in a green house.

Bai & Shaner (2004) address the difficulties of evaluating type I and II under field conditions, particularly when the lines being evaluated vary in their composition of resistances and high disease pressure. Lines that contain only type I resistance could appear more susceptible from disease spread. Contrasted with lines with mainly type II, they would appear more susceptible from multiple initial infection points.

Resistance against DON accumulation, type III, is necessary because even without complete type I or II resistance, infected grain can have DON present (Bai and Shaner, 2004). Prevention of DON accumulation in the grain can occur by various means such as DON degradation, failure of DON to relocate from other tissues, or reduced ability of the fungus to produce the mycotoxins (Jansen et al., 2005). Individual classes of resistance are not mutually exclusive.

J. Genetics of Resistance

The genetic and biochemical architecture of resistance is not well understood (Bai and Shaner, 2004). Wiese (1987), proposed that physiological resistance would be preferred over morphological resistance. Physiological resistance, based upon biochemical pathways, stops the pathogen after infection and is more complete, while morphological barriers can be thought of as disease escape and a reduction in the incidence of disease. Currently, no traditional R genes have been found which confer complete resistance. This is perhaps due to the lack of specific host virulence and the necrotrophic nature of scab.

A reduction in incidence was seen in cultivars that are awnless (Mesterházy, 1995), completely cleistogamous (Gilsinger, 2005), or taller (Srinivasachary et al., 2009; Srinivasachary et al., 2008; Voss, 2008). Severity and disease spread was faster in wheat plants that had small peduncles, compact spikes, or awns (Rudd et al., 2001). Susceptible cultivars were found to have double the amount of choline on anthers than more resistant varieties (Li and Wu, 1994). In an extensive review of scab resistance QTL by Buerstmayr et al. (2009b), 22 QTL regions were determined. Of the 22 regions, ten were associated with plant height and four with flowering date.

K. Breeding for Host Resistance

Breeding for resistance is complicated by resistance QTL in association with undesirable agronomic traits, extensive genotype x environment interaction, and difficulty in phenotyping (Bai and Shaner, 2004; Rudd et al., 2001). In order to direct efforts, breeders often attempt to enhance wheat by either improving the agronomic traits of resistant plants, increasing the resistance in advanced lines, or introduce new genes into the germplasm (Bai and Shaner, 2004)

A concerted effort to breed more resistant lines has produced several cultivars and advanced breeding lines like Sumai 3, Ernie, Freedom, Truman, W14, Frontana, Wangshuibai, Ning 7840, and CJ 9306. The Chinese cultivar Sumai 3 is from a cross between two moderately resistant parents ‘Funu’ x ‘Taiwan Xiaomai’ (Rudd et al., 2001; Waldron et al., 1999). The line has been used for more than 30 years in Chinese breeding programs (Bai and Shaner, 2004). Three QTL have been identified with resistance and one for susceptibility. The QTL on 3BS originally denoted as *Qfhs.ndsu-3Bs*, has been fine mapped (Cuthbert, 2006) and renamed *Fhb1* (Liu et al., 2005). The gene for *Fhb1* has not been characterized, although it has been observed that lines containing the gene convert DON into a less toxic substance (Lemmens et al., 2005). This particular gene has had R^2 values for phenotypic variation of 24.8% to 41.6% depending on the background (Anderson et al. 2001). A second QTL, which was on 6BS was also fine mapped (Cuthbert, 2007) and renamed *Fhb2*. Garvin and Anderson (2002) in their historical analysis of uniform regional scab nurseries for spring wheat, reported that 60% of advanced breeding lines with resistance had Sumai-3 in their pedigree. The popular line Sumai-3 is not ideal for SRW in the Eastern

U.S. due to being a tall, late maturing spring wheat that is susceptible to several diseases that are common in North America (Liu et al., 2005).

The cultivar ‘Ernie’ (Liu, 2007; Liu et al., 2005) is another transgressive segregant that contains QTL thought to be derived from North American winter wheat germplasm. Biparental mapping identified four QTL in Ernie, *Qfhs.umc-2B*, *Qfhs.umc-3BSc*, *Qfhs.umc-4B*, and *Qfhs.umc-5A*. The use of native QTL will avoid complications of bad agronomics in non-adapted traits. The idea of transgressive segregants is supported by the idea of multiple additive resistance QTL on different chromosomes coming together (Bai and Shaner 2004, Rudd 2001).

Currently, more than 50 mapping studies have reported 250 QTL associated with FHB (Buerstmayr et al., 2009b; Liu et al., 2009; Loffler et al., 2009). Every chromosome has been reported with multiple QTL (Liu et al., 2009). The actual number of QTL is fewer since several studies share parents or validated previous QTL in new genetic backgrounds. Loffler et al. (2009) and Liu et al. (2009) conducted meta-analyses on mapping studies, while a visual consensus map was compiled by Buerstmayr (2009b). The meta-analysis of Liu was the most extensive and reported 43 genomic regions associated with resistance. The Meta-QTL clusters were composed of 2-13 initial QTL from the mapping studies (Liu et al., 2009; Loffler et al., 2009). Clusters could co-localize in a meta-analysis when they correspond to different resistance types. A limitation with meta-analysis is that determination of allelic QTLs is not possible. The cultivars Sumai 3 and Wangshuibai share no common parents in their pedigrees, and have resistance mapping to the same locus associated with *Fhb1* (Yu et al., 2008).

Meta-QTL clusters associated with type II resistance were the most common followed by type I. A preponderance of type I and II resistance studies is likely due to the difficulty and cost of phenotyping for FDK and DON. Asian germplasm accounts for a majority of mapped resistance QTL, followed by European and then the Americas. Many QTL from Asia, Europe, and the Americas were found to map to similar regions, although some QTL remained unique to one Continental source (Liu et al., 2009).

It is possible that several of the reported QTL may be false positives (Buerstmayr et al., 2009b) because QTL analysis is affected by the number of lines, magnitude of a QTL effect, marker density, genetic background, genotype by environment interaction, and accuracy of phenotyping (Zhang et al., 2009). Bi-parental mapping requires QTL to be validated in additional crosses with different susceptible backgrounds or with NILs before application to MAS.

The effect of *Fhb1* has been assessed in 13 crosses with different susceptible parents (Pumphrey et al., 2007). In one background Pumphrey et al. (2007) found *Fhb1* to increase susceptibility. Relatively few other QTL have been validated; these include *Qfhs.nau-2DL*, *Qfhs.nau-3A*, *Qfhs.ifa-5A*, 4B-Wuhan1, *Qfhs.ifl-6AL*, *Qfhs.ifl-7BS* (Buerstmayr et al., 2009a; Haberle et al., 2007). Validation is an expensive and time consuming step and a limiting factor in MAS implementation.

The prospect of combining multiple QTL together to raise the level of resistance and joining of multiple types of resistance is promising. Many lines like Sumai 3 and Wangshuibai already contain multiple QTL. The additive effects are greater than non-additive forces, implying that pyramiding would be effective (Bai and Shaner, 1996).

The use of markers to assist in the breeding for resistance to FHB is promising. Scab is a low heritability trait for which traits can be difficult and costly to phenotype. The implementation of markers will assist in reducing the size of donor genomic fragments, pyramiding QTL, reducing the size of populations through allele enrichment, and selection in the absence of the pathogen.

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Table 1.1 Characteristics of RFLP, RAPD, STS, SSR, AFLP, SNP, and DArT markers as it applies to marker assisted breeding

Attribute	RFLP	RAPD	STS(CAPS)	SSRs	AFLPs	SNPS	DArT
Polymorphism type	Indel/subst	Indel/subst	Indel/subst	Motif Repeat	Indel/subst	substitution	Indel/Subst
Information needed a priori	None	None	Yes	Yes	None	Yes	None
Skill	Medium	Low	Low	low	medium/high	medium/high	high
Inheritance	co-dominant	dominant	co-dominant	co-dominant	dominant	Depends on detection	Pres/Abs
Automation	None	None	Yes	Yes	low	Yes	Yes
Development cost	med	low		high	low	high	medium
Running cost	Medium/high	low	Medium/high	low/medium	med	low	low
Reproducibility	high	low	high	high	high	high	high
No of loci detected	1-5	1-10	1-4	1-3	>70	1	many
Abundance	low	medium	high	medium	medium	high	Medium
PIC	Medium	Low	high	high	medium	Low	medium
Fingerprinting	good	better	good	Best	Best	better	Best
Gene tagging	better	better	better	better	Best	good	-
QTL Mapping	better	No	good	better	better	good	Best
MAS	good	No/good	better	better	good	better	-
Comparative mapping	better	good	better	better	better	better	-

Table 1.2 Latin Binomial, common name, genomic constitution, cultivation and threshing attributes of wild and cultivated diploid, tetraploid, and hexaploid wheat with defining domestication traits.

Ploidy	Genus	Specie	Subspecie	Genome	Cultivation	Threshing ¹	Common name	Characteristics ²
2x	<i>Triticum</i>	<i>urartu</i>		AA	wild			
	<i>Triticum</i>	<i>monococcum</i>	<i>aegilopoides</i>		wild		Wild einkorn	
	<i>Triticum</i>	<i>monococcum</i>		A ^m A ^m	cultivated	hulled	Einkorn	Non-brittle rachis from two recessive genes
	<i>Aegilops</i>	<i>speltoides</i>		SS	wild			
	<i>Aegilops</i>	<i>tauschii</i>		DD	wild			Tg
4x	<i>Triticum</i>	<i>timopheevii</i>	<i>timopheevii</i>	GGA ^m A ^m	cultivated	Hulled		Two grained, non-brittle
	<i>Triticum</i>	<i>militane</i>		GGA ^m A ^m	cultivated	Free		Mutant of timopheevii
	<i>Triticum</i>	<i>timopheevii</i>	<i>armenicum</i>	GGA ^m A ^m	wild			
	<i>Triticum</i>	<i>turgidum</i>	<i>dicoccooides</i>	AABB	Wild		Wild Emmer	Fragility of the spike, narrow grain
	<i>Triticum</i>	<i>turgidum</i>	<i>dicoccum</i>	AABB	cultivated	Hulled	Emmer	Less fragile rachis
	<i>Triticum</i>	<i>turgidum</i>	<i>durum</i>	AABB	cultivated	Free	Durum	Large grain, hard texture low gluten ideal for semolina and pasta
	<i>Triticum</i>	<i>turgidum</i>	<i>polonicum</i>	AABB	cultivated	Free	Polish	Long, papery, loose glumes, P gene on 7A1
	<i>Triticum</i>	<i>turgidum</i>	<i>turanicum</i>	AABB	cultivated	Free	Khorassan	Large glumes, large grains
	<i>Triticum</i>	<i>turgidum</i>	<i>carthlicum</i>	AABB	cultivated	Free	Persian	Round, non-keeled glumes
	<i>Triticum</i>	<i>turgidum</i>	<i>turgidum</i>	AABB	cultivated	Free	Rivet or cone	Softer grain than durum
6x	<i>Triticum</i>	<i>turgidum</i>	<i>parvicocum</i>	AABB		Free		Naked, small grain, compact head
	<i>Triticum</i>	<i>aestivum</i>	<i>aestivum</i>	AABBDD	cultivated	free	Common	tgtgQQccSS - normal spike and grains
	<i>Triticum</i>	<i>aestivum</i>	<i>compactum</i>	AABBDD	cultivated	free	Club	tgtgQQCCSS - compact spike normal grain
	<i>Triticum</i>	<i>aestivum</i>	<i>spelta</i>	AABBDD	cultivated	hulled	Spelt	TgTgqqccSS - normal spike and grains
	<i>Triticum</i>	<i>aestivum</i>	<i>vavilovii</i>	AABBDD	cultivated	hulled		TgTgQQccSS - normal spike and grains
	<i>Triticum</i>	<i>aestivum</i>	<i>macha</i>	AABBDD	cultivated	hulled	macha	tgtgqqccSS - normal spike and grains
	<i>Triticum</i>	<i>aestivum</i>	<i>sphacrocum</i>	AABBDD	cultivated	free	shot	tgtgQQccss - normal spike, spherical grain
	<i>Triticum</i>	<i>aestivum</i>	<i>petrapavlovski</i>	AABBDD	cultivated			
	<i>Triticum</i>	<i>zhucovskyi</i>		GGAAA ^m A ^m	cultivated	hulled		

¹ Free threshing wheat's have softer glumes and stiffer rachis,

² Tg-tenacious glumes, Q-Free threshing, C-compact spike, S-spherical grain, naked-free threshing.

CHAPTER 2. Validation of *Fhb1* and *Qfhs.nau-2DL*, for *Fusarium* Head Blight Resistance
in the Soft Red Winter Wheat, NC-Neuse

Abstract

Severe outbreaks of *Fusarium* head blight in wheat spurred the identification and development of varieties with higher levels of resistance. The majority of resistance QTL identified thus far have originated in Asian and European germplasm that is not adapted to the soft winter wheat growing region of the eastern United States. There are a limited number of validation studies conducted on the mapped QTL, particularly in U.S. market classes outside of hard red spring wheat. Validation is crucial to ensure markers are diagnostic and QTL effects are transferable to alternate backgrounds. We report the evaluation of *Fhb1* and *Qfhs.nau-2DL*, which have been identified to reduce the damage of infection caused by *Fusarium* head blight, in the background of the soft red winter wheat NC-Neuse. An accelerated backcross population from the cross NC-Neuse (moderately resistant) x VA01W-476 (*Fhb1* and *Qfhs.nau-2DL*) was used to create sets of BC₂F_{2,3} lines that were composed of lines with no QTL, one QTL or both QTL together. Backcrossed lines were assessed in inoculated FHB nurseries over two years. The backcross derived lines with no QTL, had significantly more disease severity and *Fusarium* damaged kernels than derived lines having either *Fhb1*, *Qfhs.nau-2DL* or both. Although lower levels of FHB were observed on backcross lines with the QTL, they were not significantly more resistant than the recurrent parent NC-Neuse. In addition, lines having both QTL were not significantly more

resistant than lines having *Fhb1*, indicating that the effects of the two resistance genes may not be additive.

Introduction

In North America, the primary pathogen that elicits the disease Fusarium head blight (FHB) in wheat (*Triticum aestivum* L.) is *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* Schwein)(Nganje, 2004; O'Donnell et al., 2004). Resistance to FHB, referred to as scab, is quantitatively inherited with no known race specific resistance factors. Biparental populations have been a standard method to identify and map genes or QTL. To date, greater than 50 peer reviewed publications have presented results on more than 250 QTL associated with several resistance traits (reviewed by Buerstmayr et al. (2009a), Liu et al. (2009), and Loffler et al. (2009).

The largest number of mapped QTL and sources of resistance originate from Asia followed by Europe (Liu et al., 2009). The Chinese cultivar Sumai 3 has been a popular parent and stable source of resistance for more than 30 years (Bai and Shaner, 2004). Of the scab QTL mapped in Sumai 3, the *Fhb1* gene on 3BS has the largest effect at reducing disease severity and has been used globally (Anderson et al., 2001; Waldron et al., 1999). The effect of *Fhb1* has been validated in multiple backgrounds with positive reductions in resistance with r^2 values ranging from 24.8 to 41.6% (Anderson, 2003). However, Pumphrey et al. (2007) conducted a validation of *Fhb1* in 13 populations and found an increase in susceptibility associated with *Fhb1* for scabby kernels in one hard red spring wheat background, indicating that *Fhb1* may not be effective in every background. Despite

successful efforts to increase the level of scab resistance in U.S. soft winter wheat breeding material, the deployment of elite varieties with exotic resistance QTL has been slow due to lack of QTL validation, strong QTL x environment interaction, and linkage with undesirable agronomic traits (Bai and Shaner, 2004; Rudd et al., 2001). Although highly resistant, Sumai 3 is not an ideal parent for soft red winter (SRW) wheat in the Eastern U.S. due to a spring growth habit, being tall, late maturing, and susceptible to several diseases that are common in North America (Liu et al., 2005).

The development of cultivars such as W14 and CJ 9306 were steps toward bringing popular and effective QTL from Asian sources such as Sumai 3, Wangshuibai, and Ning 7840 into other germplasm (Jiang et al., 2006). These cultivars were developed to have greater levels of FHB resistance in backgrounds with better agronomics than previous sources of resistance. The cultivar W14 has the QTL combination of *Fhb1* on chromosome 3B and *Qfhs.nau-2DL* on chromosome 2D (Jiang et al., 2007a). Thought to originate from Wangshuibai, *Qfhs.nau-2DL* (Jiang et al., 2007a; Mardi, 2005), has been found to account for substantial reductions in susceptibility.

The additive effects of scab QTL are reported to be substantial, implying that pyramiding would be effective at reducing disease (Bai and Shaner, 1996). The effect of multiple QTL can be seen in original sources of resistance like Wangshuibai and Ning7840, but few studies have validated multiple QTL of different origins in alternate backgrounds (Haberle et al., 2007), particularly in SRW (Brown-Guedira et al., 2008).

This study evaluated the effects of *Fhb1* and *Qfhs.nau-2DL*, alone and in combination, in the background of moderately resistant soft red winter wheat cultivar NC-Neuse, which is adapted to the Mid-Atlantic region of the United States.

Materials and Methods

Plant material

A BC₂F_{2.3} population with lines, that differ in composition for *Fhb1* and *Qfhs.nau-2DL*, was derived from the cross NC-Neuse*3/ VA01W-476 (Roane /W14). The cultivar NC-Neuse (PI 633037) (Murphy et al. 2004) is a soft red winter wheat with moderate scab resistance. . The advanced breeding line VA01W-476, is a double haploid line having *Fhb1* and *Qfhs.nau-2DL*, selected from a cross of the Chinese spring wheat germplasm W14 (PI 641164) and the soft red winter wheat cultivar Roane (PI 612958) (Griffey et al. 2001) The selected lines had either no QTL (NC-Neuse alleles at markers flanking the resistance QTL), *Fhb1*, *Qfhs.nau-2DL*, or *Fhb1* and *Qfhs.nau-2DL*.

Marker assisted selection was started in the BC₁ generation by retaining plants which contained *Fhb1* and *Qfhs.nau-2DL* alleles. Plants were then selected based upon the highest percentage of the recurrent parent NC-Neuse genotype at background SSR loci. The chosen plants were backcrossed again to NC-Neuse and marker selection repeated. Plants which were not culled after the second round of selection were allowed to self-pollinate.

At the BC₂F₂, plants homozygous for *Rht-D1b* were selected for to avoid height segregation. Plants which were homozygous for the presence or absence at *Fhb1* or *Qfhs.nau-2DL* were allowed to self, producing 34 BC₂F_{2.3} lines with no QTL, *Fhb1*,

Qfhs.nau-2DL, and *Fhb1* with *Qfhs.nau-2DL*. The no QTL lines represent NC-Neuse per se and the effects of backcrossing. The recurrent parent NC-Neuse and susceptible variety Coker 9835 were included as checks.

Genotyping

Plant tissue was collected from the leaf of a single seedling from BC₁, BC₂, and BC₂F₂ plants and stored at -80°C. A plate grinding machine was used to pulverize the tissue. Isolation of DNA was followed according to the CTAB method of Pallotta et al. (2003). Thirty-five background SSRs distributed throughout the genome were used to select for the recurrent parent genotype for accelerated backcrossing. The presence of *Fhb1* and *QFhs.nau-2DL* were based on flanking markers UMN10, GWM533, GWM493, and CFD233, GWM608, GWM539 respectively. Primer sequences were obtained from Graingenes 2.0 (<http://wheat.pw.usda.gov/GG2/index.shtml>).

Amplification of microsatellite markers was carried out in 12 µl reactions that constituted 1.2 µl 10X PCR buffer with 15 mM MgCl₂, 0.96 µl dNTPs (2.5 mM), 0.24 µl each fluorescent-dye-labeled primer (10 µM), 0.18 µl *taq* polymerase (5 units/ µl), 7.18 µl H₂O, and 2 µl of template DNA. The conditions for thermal cyclers started with a two minute denaturation step at 95°C followed by 35 cycles of 94 °C for 30 secs, 52-61 °C for 30 secs, 72 °C for 45, and ended with an extension step at 72 °C for 10 minutes. A 0.6 µl aliquot of PCR reaction was added to a 9.5 µl solution of formamide and LIZ dye standard which was then denatured for 5 min at 95 °C. Electrophoresis was carried out on an ABI 3130 (Applied Biosystems, Foster City, CA). The software Genemarker v1.85 (Softgenetics, State

College, PA) was used to analyze and call SSR alleles which were manually checked for accuracy.

FHB Evaluation

The BC₂F_{2:3} lines and the recurrent parent NC-Neuse were grown at Kinston, NC (2009) and BC₂F_{2:4} lines were grown at Kinston, NC (2010) and Blacksburg, VA (2010) in randomized complete block designs with two replications. The experimental units were single row plots in 2009 and two row plots in 2010. All plots were one meter in length.

Multiple virulent strains of *Fusarium graminearum* were isolated from local infected wheat using mung bean agar and cultured on Joffees medium. Spores were gathered from all of the strains and aggregated for inoculum. Two different inoculation methods were used to establish high disease pressure for Kinston, NC. The first inoculation occurred three weeks prior to anthesis of the earliest lines, by dispersing corn kernels infected with *F. graminearum* on the ground around all plots. The second inoculation involved spraying wheat heads at 50% anthesis with a conidial suspension (3×10^4 spores/ml). The inoculation of corn kernels and the macroconidia suspension were prepared according to the protocols of Gilbert and Woods (2006). The Blacksburg, VA environment was inoculated with a conidial suspension (5×10^4 spores/ml) that was sprayed on each line at 50% anthesis.

The moisture level was kept high beginning at anthesis and until two weeks post anthesis of the latest maturing lines by an overhead misting system that operated for eight hours daily, at intervals of two minutes every half-hour. Phenotypic data for incidence and severity were gathered 21 days after spray inoculation. Incidence was recorded as the number of heads with symptoms divided by the total number of heads. Severity was a visual rating of

the percentage of diseased spikelets across diseased heads. Fusarium damaged kernels (FDK) was assessed after harvest and hand threshing as the number of seeds out of 100 that were diseased.

Statistical analysis

All analyses were conducted using SAS/STAT software SAS 9.2 (SAS Institute, Cary, NC). A mixed model was used to evaluate the effects of QTL in a NC-Neuse background. Environments and QTL were treated as fixed factors. The random effect of a line was nested within QTL. Multiple comparisons between QTL classes were adjusted according to the Tukey-Kramer method. Inclusion of F₂ or F₃ pedigree information as covariates in the analysis had no effect on the statistical significance of QTL line mean comparison with Tukey-Kramer. Entry mean heritability estimates were calculated from variance components of random lines according to the protocol of Holland et al. (2003).

Results

The effects of *Fhb1* and *Qfhs.nau-2DL* were evaluated individually and in combination in the SRW wheat background of NC-Neuse, a popular cultivar in the Mid-Atlantic and Southern United States. A total of 34 BC₂F₂-derived lines tracing to 20 BC₂F₁ individuals were developed with varying QTL composition. The number of lines for the combined, *Fhb1*, *Qfhs.nau-2DL*, and no QTL classes were 10, 12, 8, and 4 respectively.

The range of observed phenotypes was not equal among the three environments (Table 2.1). The 2010 Blacksburg, VA environment had the smallest range and mean for SEV and FDK. Kinston, NC in 2010 had the greatest range for SEV and FDK. The least

square mean of the 2009 Kinston, NC environment was significantly different from the other environments for SEV and INC, while the 2010 Blacksburg, VA was the only significantly different environment for FDK (Table 2.1). The entry mean heritability was greatest for severity, $H^2 = .89$, followed by FDK and incidence at $H^2 = 0.69$, and $H^2 = 0.63$ respectively.

Significant differences were observed between groups of lines having different QTL composition (Table 2.2). Mean SEV and FDK of lines with no QTL were significantly greater than that observed for backcross-derived lines having either *Fhb1*, *Qfhs.nau-2DL* or both. In addition, lines having both *Fhb1* and *Qfhs.nau-2DL* had lower mean SEV and FDK than lines with only *Qfhs.nau-2DL*. However, significant differences were not observed for SEV, INC or FDK between the recurrent parent NC-Neuse and backcross-derived lines having either or both QTL. The mean SEV and FDK observed for lines having *Fhb1* was lower than that of lines with *Qfhs.nau-2DL*, though the difference was not significant.

Discussion

The validation of QTL effects in alternate backgrounds is key to ensuring that marker assisted selection will be effective across a breeding program or market class germplasm. Few scab resistance QTL have been validated in soft red winter wheat backgrounds (Brown-Guedira et al., 2008) leaving breeders to speculate about which QTL they should invest resources for deployment. The effects of *Fhb1* and *Qfhs.nau-2DL* from the experimental line VA01W-476 were assessed in the background of an elite cultivar, NC-Neuse, to validate each QTL effect alone and in combination. Backcross-derived lines with neither QTL were selected at the BC₂F₂ to represent the NC-Neuse parent per se and the effects of introgressing

the QTL. Although an accelerated backcrossing approach was implemented, a relatively small number of markers was used to recover the genome of the recurrent parent. Significantly lower levels of disease were observed on the recurrent parent than the no QTL class of lines, suggesting that alleles with negative effect on FHB resistance were also introgressed in these lines. Although lower levels of disease were observed for the backcross-derived lines having *Fhb1* and *Qfhs.nau-2DL* or both than on NC-Neuse, the differences were not significant. NC-Neuse was included only once in each experiment, leading to large standard errors associated with the mean phenotypes. The poor performance of the no QTL lines and lack of significance between the derived lines and NC-Neuse complicated interpretation of the QTL effects. Significant effects of the *Fhb1* gene and *Qfhs.nau-2DL* on SEV and FDK were observed in comparison with backcross-derived lines with neither QTL. Only when comparing lines having both *Fhb1* and *Qfhs.nau-2DL* with the no QTL lines were significant differences observed for INC. This is consistent with previous reports that the effects of *Fhb1* are primarily on reducing the spread of FHB rather than preventing initial infection (Cuthbert et al. 2006). The differences observed in this study between lines having both QTL and those with no QTL could be due to loss of alleles conferring resistance to infection in NC-Neuse in the no QTL lines. Alternately, there may be an interaction of the two QTL leading to reduced INC as well as SEV and FDK.

As in other validation studies (Cuthbert, 2006; Pumphrey et al., 2007), *Fhb1* was effective at decreasing severity and FDK in novel germplasm. The *Fhb1* gene had a greater effect than *Qfhs.nau-2DL* in this study, although the differences were not significant. In another SRW background of KY93C-1238-17-2, the effect of *Qfhs.nau-2DL* was found to be

greater than *Fhb1* (Agostinelli, unpublished data). Lines having the combination of both *Fhb1* and *Qfhs.nau-2DL* had the lowest mean levels of disease in both studies.

The effect of *Fhb1* and *Qfhs.nau-2DL* appear to be stable and effective in SRW material. Disease pressure was low in the screening nurseries, particularly for the 2010 environments due to high temperatures effecting inoculum viability and buildup. The spraying of the conidial suspension was an attempt to mitigate this effect, but development of infection was slower than expected. Higher disease pressure may have elucidated a greater effect from *Qfhs.nau-2DL* and *Fhb1*. Our data suggest that breeding programs would benefit most by utilizing the broad adaptability of *Fhb1* for severity type resistances in conjunction with other QTL for incidence or severity. Studies have suggested that scab QTL are mainly additive, and cultivars would benefit from multiple QTL to increase resistance (Somers et al., 2005). Small gains from combining both QTL together were observed in this study, suggesting a benefit to combining multiple QTL although the effects may not be entirely additive.

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Table 2.1. Minimum and maximum observed entry mean and least square mean estimate of severity, incidence, and percent scabby seed for each environment measured as percentages.

Environment	SEV			INC			FDK		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
2009 Kinston, NC	14	75	34 a ¹	0	68	59 a	6	41	20 a
2010 Kinston, NC	3	70	20 b	12	64	32 b	3	51	19 a
2010 Blacksburg, VA	3	50	17 b	8	67	26 b	1	23	6 b

¹Least square mean estimates for environments with different letters were significantly different at $p < 0.05$.

Table 2.2 Estimates of means and standard errors for severity (SEV), incidence (INC), and Fusarium damaged kernels (FDK) of backcross-derived lines having no QTL, *Fhb1*, *Qfhs.nau.2DL*, or both QTL.

Trait	Line QTL Composition	QTL Effect	Standard
		Estimate	Error
SEV	NC-Neuse	27.9ab ¹	6.0
	NC-Neuse + No QTL	39.9c	3.1
	NC-Neuse + <i>Qfhs.nau.2DL</i>	24.2b	2.5
	NC-Neuse + <i>Fhb1</i>	18.7ab	2.2
	NC-Neuse + both	13.2a	2.1
INC	NC-Neuse	38.0ab	7.2
	NC-Neuse + No QTL	47.5b	3.8
	NC-Neuse + <i>Qfhs.nau.2DL</i>	40.9ab	3.1
	NC-Neuse + <i>Fhb1</i>	36.9ab	2.7
	NC-Neuse + both	32.4a	2.5
PSS	NC-Neuse	15.7ab	2.6
	NC-Neuse + No QTL	24.3c	1.4
	NC-Neuse + <i>Qfhs.nau.2DL</i>	15.2b	1.2
	NC-Neuse + <i>Fhb1</i>	11.5ab	1.0
	NC-Neuse + both	10.4a	1.0

¹Means with different letters are significantly different at $p < 0.05$

CHAPTER 3. Population Structure and Linkage Disequilibrium of Winter Wheat in Regional Fusarium Head Blight Screening Nurseries

Abstract

Efforts to develop U.S. winter wheat (*Triticum aestivum* L.) cultivars with resistance to Fusarium head blight (FHB) has led to frequent use as parents of a limited number of resistant exotic lines and adapted cultivars with moderate FHB resistance. Understanding the effect of focused breeding efforts for FHB resistance on the genetic diversity, population structure (PS) and linkage disequilibrium (LD) in soft winter wheat germplasm in the eastern United States is important for marker-assisted breeding and use of association analysis to map resistance QTL. A set of 251 lines selected from three collaborative FHB screening nurseries was genotyped with SSR, STS and DArT markers to assess LD, genetic diversity, and PS. The genome-wide average of LD decay to $r^2 < 0.2$ was 9.9 cM. Moderate levels of LD ($r^2 > 0.2$) were generally constrained to markers less than 5 cM apart. Although the lines evaluated were targeted to distinct production zones (northern vs. southern) of the eastern winter wheat region, cluster and principal component analyses detected only a weak separation of lines based on location of the developing breeding program. The lack of major sub-groups in this population may be the result of a short, intense breeding history for scab resistance and wide-spread use of common resistant parents. Our data indicate that there is frequent admixture and sharing of germplasm among U.S. winter wheat programs focused on development of FHB resistant cultivars, which has increased the level of genetic diversity.

Introduction

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe is a disease of wheat (*Triticum aestivum* L.) of international importance. Although the most economical method of mitigating disease is the planting of resistant cultivars, developing FHB resistant cultivars is complicated by the quantitative inheritance of resistance and large genotype by environment interactions. Until relatively recently, breeding for FHB resistance in the U.S. relied on natural epidemics for testing and lacked a concerted effort to identify sources of resistance. Griffey (2005) reported that few U.S. winter wheat breeding programs actively bred for FHB resistance before 1990. This was due to infrequent outbreaks of FHB in North America and poor agronomics associated with the few identified exotic sources of resistance (Stack, 2003).

Several FHB outbreaks occurred in the 1990's in North America which resulted in an estimated \$4.8 billion in losses (Johnson et al., 2003; Windels, 2000). As a result, research efforts on FHB intensified and since 1997, breeding programs in the spring wheat region of the northern Great Plains and the eastern winter wheat region of North America have collaboratively evaluated experimental lines in regional uniform scab screening tests. These lines are grown in FHB inoculated and mist-irrigated disease nurseries in a myriad of environments across their respective production regions. The experimental lines entered in to the FHB screening nurseries may be released as cultivars and the most resistant selections are frequently shared across breeding programs for use as parents for crossing. Thus, the lines represent an important germplasm base for improving the FHB resistance of cultivars in their respective regions.

The relatively recent focus on breeding for FHB resistance and a lack of a large numbers of adapted cultivars with resistance has led to the frequent use as parents of unadapted sources of resistance. One such parent is the Chinese cultivar Sumai 3 which was found in 60% of the pedigrees of entries in the Uniform Regional Spring Wheat Scab Nursery (Garvin and Anderson, 2002). Additional Asian and European cultivars used as sources of resistance are reviewed in Liu et al. (2009) North American soft winter wheat lines such as Ernie, Freedom, Roane, and Goldfield were identified as moderately resistant to scab and were also used as parents by winter wheat breeders (Griffey, 2005).

Selection for FHB resistance, the introgression of resistance genes/QTL from unadapted sources, and use of limited number of adapted resistant parents may affect the genetic diversity, population structure and extent of linkage disequilibrium in breeding lines. These attribute of populations are of interest to plant breeders, especially in relation to association studies (Chao et al., 2010; Comadran et al., 2010; Newell et al., 2010) and marker-assisted breeding (Habier et al., 2007; Zhong et al., 2009). Comprehension of LD, the non-random association of alleles at different loci, and PS, the entire pattern of relatedness among lines, for a collection of individuals is key to maximize power, reduce false positives, correctly infer marker trait association, and to predict the variation explained by markers (Bressegello and Sorrells, 2006b; Newell et al., 2010). Quantifying these parameters depends on the type and density of markers (Sorrells and Yu, 2009) as well as the size and composition of a dataset (Price et al., 2006). A single study on LD or PS cannot be projected to represent an entire species (Sorrells and Yu, 2009).

In wheat, a lack of abundant genomic resources, particularly large numbers of SNP markers, has made it difficult to evaluate the genome with high marker density. Diversity Array Technology (DArT) markers provides a cost effective approach to generate thousands of genome-wide genetic markers. The detection of the presence or absence of polymorphic regions by DArT markers is accomplished through a complexity reduction of the genome with a pair of restriction endonucleases. Microarray analysis allows detection of hybridized genomic fragments and complementary probes in parallel for thousands of markers in a single hybridization (Akbari et al. 2006).

The objective of this research was to characterize the population structure, LD, and genetic diversity in a population of North American winter wheat selected from programs developing cultivars for FHB prone regions. Knowledge of these population attributes is essential to understand how to direct breeding activities and how marker-assisted breeding can be improved through association analysis.

Materials and Methods

Plant Material

A collection of 251 winter wheat lines (Appendix A) were selected out of collaborative FHB screening nurseries: the 2008, 2009 and 2010 Northern Uniform Winter Wheat Scab Nursery (NUWWSN, n=114), the 2008 and 2009 Preliminary Northern Uniform Winter Wheat Scab Nursery (PNUWWSN, n=64), and the 2008 and 2009 Uniform Southern Fusarium Head Blight Nursery (USFHBN, n=73). The selected lines included the check cultivars (Ernie, Tribute, Freedom, Bess, Truman, Pioneer brand 2545 and Coker 9835) and advanced experimental lines submitted by breeders at 15 public and three private winter

wheat breeding programs located in the eastern United States and Canada. The NUWWSN and PNUWWSN are used to evaluate winter wheat material adapted to Northern part of the growing region and the USFHBN includes entries from southern programs. Nursery entries frequently have a known source of FHB resistance in the pedigree and most, but not all, have been evaluated previously for FHB resistance. Thus, the material represented a wide range of resistance levels.

In order to assess if there was an effect of geography on population structure, entries were assigned to a Northern or Southern group based on location of the developing breeding program. The Northern group was further partitioned into a Midwest (MW) group that included entries from programs in Illinois, Indiana, Ohio, Missouri, and Kentucky and a Northeastern (NE) group from programs in New York, Michigan, and Ontario. The Southern group was divided into the Mid-Atlantic (MA) region (Virginia and Maryland) and Southeast (SE) region (Louisiana, Georgia, Arkansas and North Carolina).

Genotypic evaluation

DNA was isolated from a single plant of each entry to avoid problems with heterogeneity. Tissue from the primary leaf that was placed in a 96-well plate filled with silica gel in preparation for DNA extraction. Genomic DNA was isolated from dried leaf tissue following a CTAB method described by Diversity Arrays P/L, (<http://www.diversityarrays.com/>, Canberra, Australia). The quality of DNA was evaluated on a 0.8% agarose gel and normalized to 50 ng/ μ l. For each sample a 20 μ l aliquot of DNA was sent for DArT analysis.

Analyses were also performed with sequence tagged site (STS) markers diagnostic for the presence of the 1RS·1AL and 1RS·1BL wheat-rye translocation chromosomes (marker *Xscm09*; Saal and Wricke, 1999) and the *Sr36* introgression from *T. timopheevii* (marker *Xwmc477*; Tsilo et al. 2008). STS markers diagnostic for the *Rht-B1* and *Rht-D1* reduced height genes (Ellis et al. 2002), *Ppd-D1* photoperiod response locus (Beales et al. 2007), and the *Fhb1* scab resistance gene (markers *X3BS-SNP8*, *Xumn10*) were also evaluated (Liu et al. 2008; Bernardo et al. 2011). In addition, 73 simple sequence repeat (SSR) markers targeted to regions reported to harbor FHB resistance QTL were evaluated (Somers, 2004).

Amplification of SSR and STS markers was carried out in 12 µl reactions containing 1.2 µl 10X PCR buffer with 15 mM MgCl₂, 0.96 µl dNTPs (2.5 mM), 0.24 µl each fluorescent-dye-labeled primer (10 µM), 0.18 µl *taq* polymerase (5 units/ µl), 7.18 µl H₂O, and 2 µl of template DNA. The conditions for thermal cycling started with a two minute denaturation step at 95°C followed by 35 cycles of 94 °C for 30 secs, 52-61 °C for 30 secs, 72 °C for 45 secs, and ended with an extension step at 72 °C for 10 minutes. A 0.6 µl aliquot of PCR reaction was added to a 9.5 µl solution of formamide and LIZ dye standard which was then denatured for 5 min at 95 °C. Electrophoresis was carried out on an ABI 3130 (Applied Biosystems, Foster City, CA). The software Genemarker v1.85 (Softgenetics, State College, PA) was used to analyze and call SSR alleles which were manually checked for accuracy.

All samples were assayed by Diversity Arrays P/L (<http://www.diversityarrays.com/>, Canberra, Australia) with a high density wheat array that has 7,000 probes. Genotyping by DArT analysis was chosen as a cost effective approach to obtain genome-wide coverage of

markers (Akbari et al. 2006). All samples were analyzed concurrently to ensure accurate detection of polymorphism.

Linkage Disequilibrium

Of the 2,402 polymorphic loci in the DArT dataset, a position within a chromosome was available for 513 markers based upon a DArT consensus map constructed from 79 mapping populations (<http://www.diversityarrays.com/>). Of the 513 mapped DArT loci, 31% of the markers co-located to positions with other markers, leaving 352 unique loci.

Calculation of linkage disequilibrium for each chromosome was performed in JMP Genomics 5.0 (SAS Institute, Cary, NC) using the 351 DArT markers which have been assigned a unique location. The r^2 coefficient according to Hill and Robertson (1968) was used to calculate pair-wise LD estimates for the 19 chromosomes which had multiple DArT markers. The average extent of LD was calculated for linked and unlinked markers. Using the assumptions set forth by Newell et al. (2010), markers more than 40cM apart were considered unlinked.

An estimate of the effective population size (N_e) was calculated from the average extent of LD (r^2) among unlinked loci according to the formula, $N_e = \frac{1}{(2r^2)}$, proposed by Hedrick (2005). The rate of LD decay was determined with non-linear regression by plotting the genetic distance over which LD decayed in SAS 9.2 (SAS Institute, Cary, NC).

Population Structure

For analyses of population structure and cluster analysis, markers with a minor allele frequency (MAF) < 10% were excluded, leaving 2,064 DArT markers. Since DArT markers

are known to cluster in the wheat genome (Akbari, 2006; Tinker et al., 2009) and the selected germplasm varied for the presence of inter-specific translocations, the 2,064 DArT markers were further curated to reduce biasing measures of genetic relationship with highly redundant markers. The LD tagSNP feature in JMP genomics 5.0 (SAS Institute, Cary, NC) was used to identify groups of DArT markers with LD correlation coefficient of $r^2 > 0.75$ and a single marker from each group of markers was retained, reducing the 2,064 DArT markers to 900 non-redundant loci. The largest groups of redundant marker loci were associated with alien introgressions on chromosomes 1B and 2B. Two bins on chromosome 1B contained 83 polymorphic DArT markers that were reduced to two unique loci and one bin on chromosome 2B contained 47 collinear DArTs. After accounting for 2.3% missing data, there were a total of 220,704 marker data points in the data set used to assess population structure.

Cluster analysis was done using Nei's genetic distance 1972 (Nei, 1972) in JMP Genomics v 5.0 (SAS Institute, Cary, NC). Principal component analysis was performed in SAS 9.2 (SAS Institute, Cary, N.C.). The first ten eigenvectors of PCA were calculated from the correlation matrix derived from marker genotypes and eigenvectors were graphed to visualize relatedness of lines. Model-based analysis of population structure was done with the program STRUCTURE (Pritchard, 2000) with the conditions of admixture and non-correlated allele frequencies. The burn-in and simulation stages were both set at 50,000 iterations. The number of sub-groups, K, was evaluated from one to 15 and each analysis was replicated 8 times. The ad hoc ΔK method of Evanno et al. (2005) was used to determine the appropriate number of subgroups.

Population Diversity

The software Powermarker v. 3.25 (Liu and Muse, 2005) was used to determine allele frequencies, polymorphism information content (PIC), and gene diversity of each marker. The calculation of PIC and gene diversity for each marker was according to the formulas: $(PIC) = 1 - \sum_{i=1}^m P_i^2 - \sum_{i < j} 2P_i^2 P_j^2$ and gene diversity = $1 - \sum_{i=1}^m P_i^2$, where P_i and P_j are the proportion of the population carrying the *i*th and *j*th alleles, respectively. Estimates were determined for all lines and subsets of entries based on geographic divisions. PIC and gene diversity were calculated separately for bi-allelic DArT markers and multi-allelic SSR markers. The relative importance of a parental line was assessed by counting the occurrence of that line in the pedigree of other entries (Mikel and Dudley, 2005).

Results

The 251 experimental lines and cultivars included in these analyses included entries from all collaborating wheat breeding programs in the eastern production region. The number of lines evaluated from a single breeding program ranged from seven to 30 (Appendix B). Seventy percent of germplasm lines in the study were developed by breeding programs contributing to the two northern FHB screening nurseries (NUWWSSN and PNWWSSN) which included lines developed by programs in the MW region (128 lines) and a smaller number of lines (35) from the soft red and white winter wheat NE region of New York, Michigan and Ontario (Table 3.1). Thirty-seven and 50 of the selected entries were developed by breeding programs located in the MA and SE, respectively.

Genetic Diversity and Allele Frequencies

Average gene diversity and PIC values for DArT markers were 0.39 and 0.31, respectively. The observed values were similar across geographic divisions (Table 3.1). The mean number of alleles per SSR locus was 5.5. Marker *Xgwm349* had the largest number of alleles (10) and only two alleles were observed for seven SSR markers. The gene diversity and PIC means of SSR markers were 0.65 and 0.60, respectively.

While none of the 900 DArT markers were monomorphic within the Northern or Southern groups, 35 markers showed differences in allele frequency greater than 0.3 between these groups (Table 3.2). Chromosomes 2D and 7A had the greatest number of marker loci with differences in allele frequency between the Northern and Southern groups greater than 0.3 with 25 and 12 markers, respectively. Three of the five markers with allele frequency differences greater than 0.4, were located on 7A.

Differences in allele frequency of the DArT markers were observed between the MW, NE, MA, and SE germplasm (Table 3.2). No alleles were fixed in the MW group, although 43 markers were monomorphic in the NE group. Comparatively fewer loci, 2 and 14, were fixed for the SE and MA groups, respectively. The proportion of loci with differences in allele frequency greater than 0.5, was greatest between the NE and the southern lines, ranging from 5% to 9% of loci. Large differences in allele frequency were observed for few loci among the MW, SE, and MA lines (Table 3.2).

Major gene differences among regions

Evaluation with gene specific markers identified differences in the frequencies of major dwarfing genes, *Rht-B1b* and *Rht-D1b*, in this set of germplasm (Table 3.3). Although

both the *Rht-B1b* and *Rht-D1b* dwarfing genes were present in lines originating in each region, *Rht-B1b* was three times more common in entries from the MW than those from programs in the SE, MA or NE. *Rht-D1b* was most the common dwarfing gene in all other regions. A small number of lines (14) possessed neither *Rht-B1b* nor *Rht-D1b*.

The SSR marker *Xwmc477* was used to determine the presence of the *Sr36* gene, located on a segment of chromosome 2B introgressed into *T. aestivum* from the tetraploid wheat relative, *T. timopheevii* (McIntosh et al., 1998; Tsilo et al., 2008). This alien introgression was detected in a total of 57 lines that originated from the MA, SE, and MW and was more common in southern entries (Table 3.3). The rye-specific SSR marker *Xscm09* amplified a 208 bp or 224 bp fragments in lines carrying either the 1RS·1BL and 1RS·1AL translocation chromosomes, respectively, having the short arm of rye (*Secale cereale* L.) chromosome 1R translocated onto the long arm of either wheat chromosome 1B or 1A. Lines possessing the 1RS·1AL translocation were rare in the northern entries but more common in the southern material (Table 3.3). The 1RS·1BL translocation was detected in approximately a fifth (56) of all the germplasm assayed and was more frequent in entries developed by Northern programs. The frequency of the *Ppd-D1a* allele that confers photoperiod insensitivity at the *Ppd-D1* locus was similar among entries in all regions, ranging from 44% in MW to 54% in NE.

Population Structure

A dendrogram from hierarchical clustering based on genetic distance allowed us to visualize the relationship among lines. Two large, clusters were observed, each having both northern and southern lines (Figure 3.1). Ten smaller clusters of more closely related lines

were observed, some of which were composed primarily of entries originating from individual breeding programs (Appendix B). In particular, clusters 1, 6 and 7 were composed primarily of lines from the Purdue University, University of Missouri and Cornell University breeding programs, respectively. Fifteen of the 17 entries from the University of Illinois were placed into a large group (cluster 2) that included the cultivars Ernie (released by the University of Missouri) and Tribute (released by Virginia Polytechnic Institute). Cluster 10 included seven of the nine entries from the breeding program at North Carolina State University. Cluster nine included eight entries from the Virginia Polytechnic Institute, most of which had the cultivar Roane in their pedigrees (Appendix B). Clusters 2, 4, 5 and 8 were composed of entries from various programs across the region.

The first ten eigenvectors from PCA explained 63.1% of the genetic variation; the first two components accounted for 15.9% and 10.4% of the variation, respectively. A weak grouping of lines based on Southern or Northern origin was observed in the scatter plot of the first two principal components (Figure 3.2). Although more closely related individuals that clustered together in the dendrogram were in close proximity on the graph of the first two principal components, clusters were overlapping without distinct separation (data not shown).

Analysis in the program STRUCTURE indicated that these soft winter wheat germplasm represent a single population. Graphing the average $\ln P(D)$ of $K=1$ to $K=15$, as proposed by Pritchard (2000), did not identify a change in slope that would corresponded to the number of underlying subgroups (data not shown). The method of Evanno et al. (2005) which graphs the second order rate of change of $\ln P(D)$, denoted ΔK , also did not identify multiple subgroups.

Evaluation of the pedigrees of the 251 entries indicated that some lines appeared frequently in pedigrees. A number of full-sib lines selected from the same pedigree and lines having two or more crosses to a common parent were entered into the nursery and were included in the set of entries analyzed. The most common parents in pedigrees of nursery entries were the moderately FHB resistant SRWW cultivars Roane, Ernie, Hopewell and Freedom, which appeared in the pedigrees of 64 entries originating from both Northern and Southern breeding programs. Among the 20 most commonly used parents were the Chinese lines W14, And Ning 7840 as well as Pioneer brand 25R18 that has Sumai 3 as a parent. Each of these lines is known to possess the *Fhb1* resistance gene located on the short arm of chromosome 3B. Based on marker evaluation, 35 entries were determined to carry *Fhb1* introgressed from these and other Asian sources.

Linkage Disequilibrium

The 352 loci polymorphic in our study and located on the DArT consensus map were used to estimate LD across chromosomes having multiple markers. The average marker density was a marker per 7.8 cM. Chromosome 2D has the highest saturation of markers with an average density of 2.5 cM, although other D-genome chromosomes had poor coverage. Seventy-one markers were located on D-genome chromosomes, with few DArT markers on 3D, 5D, and 7D and no polymorphic DArTs located on chromosome 4D. In addition, only one polymorphic marker was located on 5A. The largest number of unique marker loci were assigned to the B-genome (148) followed by the A-genome (133). Across

the 19 chromosomes with multiple markers, a total map distance of 2,765 cM was represented.

Gaps in the spacing of markers were frequent. Of the 69 gaps greater than 10 cM, 43% (30) were less than 15 cM. Among the 18,344 pair-wise combinations of markers on the same chromosome, 10,252 (56%) were spaced less than 40 cM apart and were considered linked.

The level of LD observed for different chromosomes varied in this study. The distance over which LD decayed to r^2 below 0.2 ranged from 3.5 cM to 14.1 cM on chromosomes 2D and 7A, respectively (Table 3.4). The average genetic distance at which LD on all chromosomes decayed to $r^2 < 0.2$ was 9.9 cM (Figure 3.3). LD ranging from $r^2 = 0.13$ to 0.50, was present for markers less than 5 cM apart on each chromosome and was greatest for closely linked DArT on chromosomes 1D, 3A and 3B (Table 3.4). LD generally declined for markers between five and 10 cM apart. Using the genome-wide estimate of LD observed for unlinked markers ($r^2=0.011$), the effective population size for our collection of lines is 45 individuals.

Discussion

The recent focus on breeding for FHB resistance in soft winter wheat in Eastern North America and investment of resources to obtain disease data from collaborative, inoculated screening nurseries raises the possibility of identifying QTL in these germplasm by association analysis. Also, these data may be suitable for development of genomic selection models for improving levels of resistance by marker-assisted breeding. However,

the effects of a relatively short history of concentrated breeding for increased FHB resistance on LD, PS, and genetic diversity in this market class and growing region are not known. By genotyping a subset of entries from regional scab screening nurseries submitted over three years with genome-wide markers, we were able to examine the influence of selection for scab resistance on the structure and LD in a population of eastern winter wheat.

In our study, major sub-groups among the entries in the FHB screening nurseries were not observed. This is in contrast to a number of studies in wheat where population stratification has been reported. A collection of Chinese soft winter wheat composed of landraces and elite cultivars were found to segregate into two corresponding sub-populations (Hao et al., 2011). Within the subgroup of landraces, greater diversity was present, which can lead to more diffuse relationship among lines. Additionally, LD differed between the two clusters, with elite cultivars having greater LD over longer distances. Landraces have lower LD due to more meiotic events and recombination, in addition to different selection pressures (Sneller et al., 2009).

In a collection of U.S. hard wheat germplasm, two sub-populations were identified that corresponded to lines having winter or spring growth habits (Chao et al., 2010). Zhang et al. (2010) observed two major groups of contemporary winter wheat breeding lines: hard wheats from the U.S. Great Plains and soft wheats from the eastern growing region. Collections assayed in the before mentioned studies included germplasm from distinct genetic pools with limited admixture. In other studies, population stratification within a region and/or market class has been observed. Tommasini et al. (2007) reported two sub-groups of European winter wheat from multiple national programs. Four sub-groups were

reported in a collection of soft winter wheat cultivars from the U.S. with release dates that spanned approximately 30 years (Brescaglio and Sorrells, 2006a).

When entries in the current study were divided into four geographic regions of origin, it was clear that differences existed in allele frequencies for numerous loci, particularly for soft white winter wheat lines adapted to the most northern portion of the production region. Overall, the lower PIC values and number of alleles observed for the 35 entries from the NE, indicate that this region was less diverse in general. However, the NE lines did not form a distinctive sub-population, likely due to crossing to soft red winter germplasm by Northeastern breeding programs. We had supposed that the differences in latitude among the programs and the inclusion of both soft red and soft white winter wheat would result in distinct groups of lines, but did not find evidence for this. Zhang et al. (2010) also failed to identify subgroups within soft winter wheat from the eastern U.S.. In contrast, they identified three subgroups that corresponded to geography of developing programs within hard winter wheat from the Great Plains.

The absence of subgroups in our study could be explained by extensive exchange of germplasm among winter wheat breeders in the eastern U.S. It may also reflect the restriction of the germplasm evaluated to current advanced breeding lines and cultivars targeted for improving resistance to FHB. A similar result of a lack of subgroups in a European winter wheat population was reported in an AA study for FHB resistance (Miedaner et al., 2010).

Diversity of SSR and DArT markers was comparable for the regional subsets, with the exception of the NE region that was somewhat less diverse.. The mean gene diversity of SSR markers in this germplasm (0.65) was greater than the mean reported for a diverse

collection of elite winter wheat breeding lines by Zhang et al. (2010). This may be due to due to widespread crossing to exotic parents with scab resistance and targeting to SSR markers to regions with reported FHB resistance QTL.

An examination of pedigrees of the entries confirmed the frequent use of parents from different breeding programs and sharing of germplasm among programs. The varied group membership of lines observed in the STRUCTURE analysis in this study indicated that admixture has maintained genetic diversity within programs. Chao et al. (2010) found that diversity was greater within breeding programs than between, particularly for U.S. winter wheat. However, their population consisted of primarily hard red winter wheat from the Great Plains and included only a limited number of eastern soft wheat lines.

Genome wide estimates of LD give an indication of the variation that could be explained by genome wide association studies (GWAS). Estimates of LD observed in this study are within the range of reported estimates. Moderate levels of LD, $r^2 > 0.2$, were generally confined to markers less than 5 cM apart, a smaller distance than reported by Crossa et al. (2007). In contrast, the distance over which LD decayed in our study was greater than reported by Chao et al. (2010) and Zhang et al. (2010). Differences between studies may be attributed to the populations under examination and markers used. The historical population of international wheat germplasm for which Crossa et al. (2007) determined LD spanned 25 years of breeding, while the population of focus in this study represents material with a short and current breeding history that centers on FHB resistance. While time can increase the amount of recombination events, it can also create subgroups based upon current trends in breeding programs, which can cause LD to be greater. The

spacing and type of marker used to analyze LD is known to affect measurements and likely contributed to the greater LD reported in our study than that reported by Zhang et al. (2010) that included hard and soft winter wheat and which used SSRs as opposed to SNPs or DArTs by Chao et al. (2010) and Crossa et al. (2007), respectively. Multiple alleles of SSRs drive down r^2 estimates.

The current density of polymorphic DArT markers in our population limits the ability to map QTL with fine resolution, though it should be applicable to population based genome wide association studies. The average genome-wide density of the 352 mapped DArT markers was 7.8 cM. However overall genome coverage may have been greater since there were many more polymorphic DArT markers that were not present on the consensus map and we were able to identify 900 DArT loci with $r^2 < 0.75$. The lack of mapped positions for DArTs limits the application of the markers for some genetic studies. In addition, based on the markers having map location, it is evident that some chromosomes are under represented on the DArT array, particularly D-genome chromosomes (Akbari, 2006). With the DArT technology, one cannot easily go back to fill in gaps of low marker coverage.

This study did not identify subgroups within the population of 258 soft winter wheat lines enriched for scab resistance. Thus, the use of covariates from STRUCTURE or PCA is not strongly supported for use in correcting for population structure in association mapping.

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Table 3.1 Comparison of mean polymorphic information content (PIC), gene diversity, and number of fixed alleles of 73 SSR and 900 DArT markers for lines based on geographic location of the developing breeding program.

Region	No. of lines	Mean Gene Diversity SSR	Mean PIC SSR	Ave. No. of Alleles	Mean Gene Diversity DArT [†]	Mean PIC DArT [‡]	No. of Monomorphic DArT
Northern	168	0.64	0.59	4.5	0.39	0.31	0
Midwest	131	0.64	0.59	4.5	0.39	0.31	0
Northeast	35	0.56	0.50	3.7	0.34	0.27	43
Southern	84	0.64	0.59	4.5	0.39	0.30	0
Mid-Atlantic	38	0.61	0.55	4.1	0.35	0.28	14
Southeast	46	0.64	0.58	4.4	0.38	0.30	2
Total	252	0.65	0.60	5.5	0.39	0.31	0

$$^{\dagger}\text{Gene Diversity} = 1 - \sum_{i=1}^m P_{i=1}^2$$

$$^{\ddagger}\text{PIC} = 1 - \sum_{i=1}^m P_i^2 - \sum_{i<j} 2P_i^2 P_j^2$$

Table 3.2 Comparison of Northern (N), Southern (S), Midwest (MW), Northeast (NE), Mid-Atlantic (MA), and Southeast (SE) germplasm by the number of loci with differences in allele frequency >0.2 based upon 900 DArTs.

Geographic Comparison	Allele Frequency Difference			
	>0.2	>0.3	>0.4	>0.5
N vs. S	193	35	5	0
MW vs. NE	301	160	99	44
MW vs. SE	220	55	11	0
MW vs. MA	279	71	19	3
NE vs. SE	248	205	116	51
NE vs. MA	220	191	139	80
SE vs. MA	255	75	34	1

Table 3.3 Percentage of lines (n) in each region having major genes for photoperiod insensitivity (*Ppd-D1a*), reduced height (*Rht-B1b* and *Rht-D1b*), the *T. timopheevii*-derived translocation having *Sr36* resistance gene and the 1RS·1AL and 1RS·1BL translocations.

Locus	Region				Latitude	
	SE [†] (n=46)	MA [‡] (n=37)	NE [§] (n=35)	MW [¶] (n=133)	South (n=83)	North (n=168)
<i>Sr36</i>	37.0	43.2	0.0	18.0	39.8	14.3
<i>Ppd-D1a</i>	45.7	45.9	54.3	43.6	45.8	45.8
<i>Rht-B1b</i>	28.3	24.3	11.4	78.9	26.5	64.9
<i>Rht-D1b</i>	63.0	78.4	71.4	21.8	69.9	32.1
1RS·1AL	13.0	18.9	2.9	4.5	15.7	4.2
1RS·1BL	10.9	13.5	25.7	27.8	12.0	27.4

[†]SE= Southeast, [‡]MA = Mid-Atlantic, [§]NE = Northeast, [¶]MW = Midwest

Table 3.4 Mean estimates of the LD coefficient, r^2 , among linked and unlinked marker pairs, mean LD between markers less than 5 and 5 to 10cM apart and the distance over which LD decays to an $r^2 < 0.2$

Chromosome	Mean linked loci distance (cM)	Linked loci LD (r^2)	Unlinked loci LD (r^2)	Persistence of LD		LD Decay ($r^2 < 0.2$)
				<5 cM	5-10 cM	
1A	16.3	0.08	0.01	0.24	0.02	9.6
1B	17.6	0.07	0.01	0.23	0.04	7.0
1D	16.0	0.16	0.02	0.50	0.35	4.3
2A	10.3	0.16	0.01	0.29	0.01	7.9
2B	19.1	0.09	0.02	0.28	0.25	8.5
2D	15.6	0.10	0.03	0.24	0.09	3.5
3A	10.4	0.19	0.01	0.40	0.02	11.7
3B	16.4	0.10	0.01	0.35	0.04	8.4
4A	11.8	0.08	0.02	0.15	0.13	5.0
4B	17.5	0.07	0.01	0.24	0.02	6.7
5B	14.6	0.08	0.01	0.24	0.03	11.0
6A	12.5	0.06	0.01	0.15	0.05	7.9
6B	16.2	0.05	0.01	0.13	0.07	7.5
6D	11.7	0.15	0.01	0.30	0.35	12.2
7A	6.0	0.18	0.01	0.21	0.04	14.1
7B	11.6	0.07	0.01	0.18	0.03	11.4
Genome mean	13.7	0.10	0.01	0.25	0.08	9.9

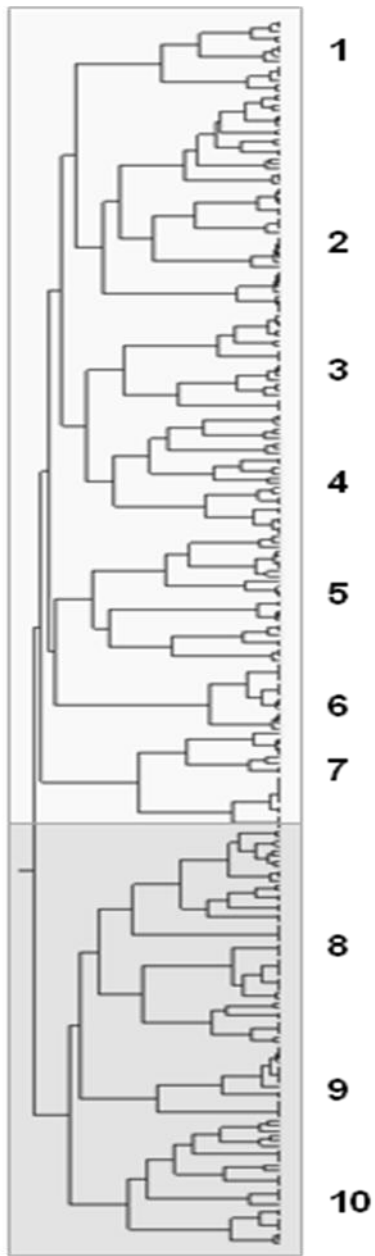


Figure 3.1 Dendrogram of 251 entries from the Northern Uniform Winter Wheat Scab Nursery, Preliminary Northern Uniform Winter Wheat Scab Nursery, and Uniform Southern Fusarium Head Blight Nursery based upon Neis 1972 genetic distance calculated from 900 non-redundant DArT markers

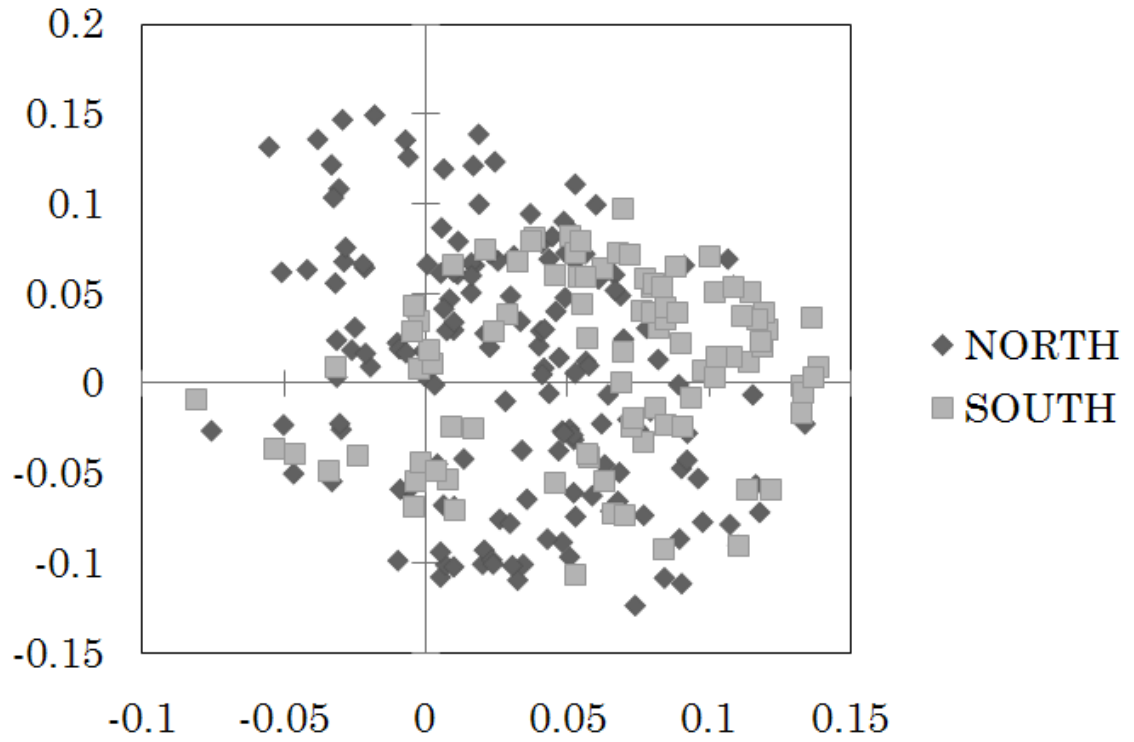


Figure 3.2 Distribution of entries, classified by nursery evaluated, according to the first two eigenvectors from principal component analysis

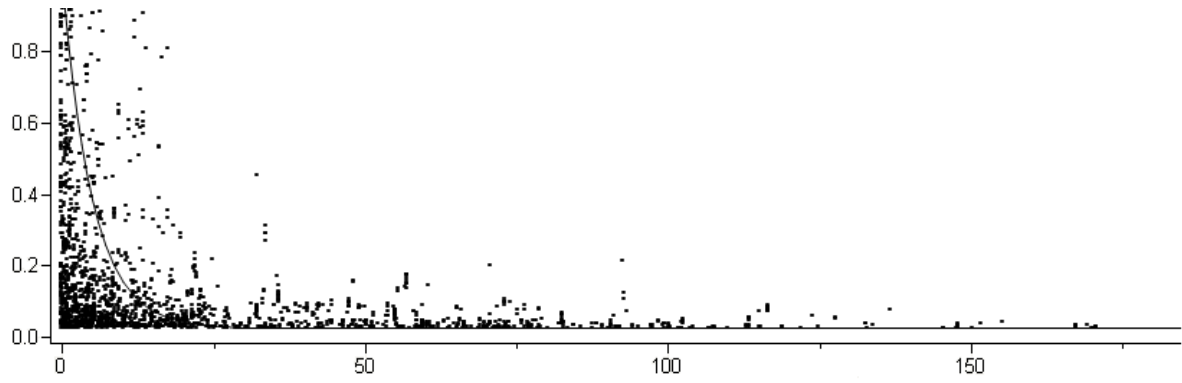


Figure 3.3 Genome-wide decay of the strength of LD (r^2) over distance (cM) across 19 chromosomes with multiple DArT loci.

CHAPTER 4. Association Analysis of Fusarium Head Blight Resistance in North American Soft Winter Wheat

Abstract

Genome wide association in population based studies can evaluate numerous alleles at different loci for multiple traits. The merits of association studies relative to bi-parental mapping include the potential for greater power and resolution to detect QTL associated with quantitative traits such as Fusarium head blight. We applied genome-wide association analysis to a population of Eastern North America soft winter wheat in relation to Fusarium head blight resistance to validate mapped QTL, evaluate the transferability and diagnostic capacity of marker trait associations, and identify new QTL in the germplasm. A subset of advanced breeding lines enriched for scab resistance was selected from three regional scab screening nurseries evaluated during 2008, 2009, and 2010. Entries were genotyped with SSR and STS markers located near reported FHB resistance QTL as well as DArTs distributed throughout the genome. We analyzed two mixed-linear models, one based upon DArTs to identify QTL regions and the second based upon the SSR and STS markers to validate the efficacy and diagnostic capability of these markers in this germplasm. Our analysis determined that markers linked to *Fhb1*, *Qfhs.umc-3BSc*, *Qfhs.umc-5A*, *Qfhs.nau-2D*, and *Rht-D1b* had significant effects for SEV, INC and FDK in these germplasm. In addition, significant SSR and STS markers were identified on chromosomes 1A, 1B, 2B, 3A, 6B and 6D. Twenty-three putative QTL on thirteen different chromosomes associated with resistance were identified based upon genome wide analysis of DArT markers. All but seven

of the QTL regions corresponded to previously identified QTL. While robust marker association were identified for some reported QTL, our results indicate that MAS for FHB in this market class should include many markers and resistance sources in order to capture variation at the large number of loci influencing resistance.

Introduction

Association analysis (AA) is an alternative approach to linkage based mapping (LM) for identifying, mapping, or validating genes and QTLs linked to phenotypes. Both methods are centered on identifying significant correlations between marker loci and functional variants which explain phenotypic variation (Sneller et al., 2009). The non-random association of alleles between two loci, such as markers and functional variants, is termed linkage disequilibrium (LD), and is the principle that allows for markers to be used as agents of selection for phenotypic improvement (Flint-Garcia, 2003).

The nature and extent of LD is the key feature which distinguishes AA and LM analysis and can be summarized in the nature of the experimental design. A bi-parental LM study is a highly structured family based design that maximizes LD. Population based AA, also known as linkage disequilibrium mapping, can be conducted without crossing (Iwata et al., 2009), incorporating a breadth of germplasm. Advantages can be gained by using a population based association study such as: more efficient use of resources, evaluation of multiple traits, a greater probability of identifying polymorphic markers (Bresghele and Sorrells, 2006a), and an increased relevance of results in an active breeding program (Sneller et al., 2009). There are two main benefits of using a population versus family based

association test. First, a broad base of experimental units allows for more QTL and alleles to be evaluated (Myles et al., 2009). A diverse genetic base also forms an ideal platform to evaluate the effect of a QTL when in many different genetic backgrounds. The validation of markers being diagnostic across germplasm is also simultaneously evaluated, facilitating the identification of robust markers and QTL for use in molecular breeding.

Second, resolution and power to detect QTL can be greater with population based analysis. Relying solely upon the LD that persists throughout breeding history allows recombination to reduce LD (Flint-Garcia, 2003; Sorrells and Yu, 2009). The smaller distance over which LD persists increases the amount of phenotypic variation which can be explained by markers, increasing the ability and resolution to detect and map QTL with small effects.

The wheat disease Fusarium head blight, caused by *Fusarium graminearum* Schwabe, is an international concern with severe periodic epidemics worldwide. Outbreaks are often acute due to a lack of complete resistance in cultivated or ancestral wheat. Resistance is complex and studies to date have reported greater than 250 QTL for FHB resistance from biparental mapping studies (Buerstmayr et al., 2009a; Liu et al., 2009; Loffler et al., 2009), of which 22 have been detected in more than one population. Successful application of marker assisted selection (MAS) for improving FHB resistance has been reported, particularly for the introgression of *Fhb1* (Waldron et al., 1999), *Qfhs.nau-2DL* (Jiang et al., 2007a), and *Qfhs.ifa-5A* (Buerstmayr et al., 2002; Jiang et al., 2007a; Jiang et al., 2007b). An increase in the level of resistance among advanced breeding lines has resulted from use of MAS to pyramid scab QTL (Anderson, 2003; Wilde, 2007). These resistance sources and the mapped

QTL have been targeted in efforts to increase resistance in eastern U.S.A. winter wheat breeding programs (Kang et al., 2011). However, the mapped resistance QTL from exotic sources are often linked with undesirable agronomic traits (Liu, 2007) and few of the many mapped QTL have been validated in alternate backgrounds (Buerstmayr et al., 2009a).

Investment in collaborative scab research through the United States Wheat and Barley Scab Initiative (USWBSI, <http://www.scabusa.org>) has been ongoing to increase the resistance levels in elite cultivars. With establishment of protocols for evaluation in inoculated mist-irrigated nurseries and extensive field screening of germplasm, moderate levels of FHB resistance have been identified in U.S. winter wheat germplasm (Bai and Shaner, 2004; Gilbert and Woods, 2006). The ability to phenotype experimental lines in breeder's nurseries and as part of a regional collaborative scab screening nursery program has contributed to development and release of soft winter wheat cultivars with improved FHB resistance, including the cultivars Truman, Bess, Jamestown and others (Griffey, 2005). Utilization of resistance QTL present in germplasm adapted to the eastern winter wheat growing region, termed native resistance, is desired to maintain diversity and to ensure that cultivars are developed that are protected against other common pathogens and insects, meet end-use quality specifications and have adequate yield potential in the target environments. While North American soft winter wheat lines have been identified with resistance, few such as Goldfield (Gilsinger, 2005), Ernie (Liu, 2007), and IL-1653 (Bonin and Kolb, 2009) have been the subject of published QTL mapping studies.

The cultivar Ernie, which has four mapped scab QTL, *Qfhs.umc-2B*, *Qfhs.umc-3Bs*, *Qfhs.umc-4B*, and *Qfhs.umc-5A*, is a common parent in the eastern regional scab screening

nurseries (Chapter 3). Three and two QTL associated with FHB resistances were mapped in Goldfield (2BS, 2B, 7B) and IL-1653 (2B, 4B). Both lines, Goldfield and IL-1653 occur directly as parents in pedigrees of some lines in our study, although the frequency is low. Mapping resistance in bi-parental populations developed from diverse U.S. germplasm has been delayed due to the time required for development of RIL populations in winter wheat and lack of a dedicated regional facility for development of double haploid populations. Several studies are currently underway to map resistance in other bi-parental mapping populations developed from crosses with eastern winter wheat germplasm. Association analyses can complement these efforts by confirming the effect of previously reported QTL in adapted lines and identifying new regions of the genome associated with resistance.

The objective of this study was to employ AA to identify putative QTL in soft winter wheat using genome-wide DArT markers and to validate SSR and STS markers linked to previously reported QTL in this germplasm. By selecting lines from regional scab screening nurseries, we made use of an available dataset of disease ratings on advanced breeding lines adapted to the eastern winter wheat region that were enriched for resistance to Fusarium head blight.

Materials and Method

Phenotypic data

A subset of 258 soft winter wheat lines was chosen from entries in the Northern Uniform Winter Wheat Scab Nursery (NUWWSN), Preliminary Northern Uniform Winter Wheat Scab Nursery (PNUWWSN), and the Uniform Southern Fusarium Head Blight

Nursery (USFHBN) that were evaluated in 2008, 2009, and 2010 (Table 4.1; Appendix A). The germplasm was composed of advanced breeding lines enriched for resistance and represents a range of the underlying genetic diversity.

The nurseries are divided such that breeding lines adapted to states in the Southeast are evaluated in the USFHBN. Breeding lines developed by programs in the Northeast and the Midwest are evaluated in the NUWWSN and PNUWWSN. Lines from the breeding programs in the Mid-Atlantic (VA and MD) are evaluated in all nurseries. Participating programs evaluated each nursery to which they contributed entries at a location within their state (Table 4.2). With the exception of the check cultivars and 37 repeated entries, lines were submitted to one nursery for one year.

A randomized complete block design was used at all locations, with either two or three replications, depending on the site. The sizes of the plots were one, two or four rows of approximately one meter in length. Two methods for inoculation were used, spreading of diseased corn kernels prior to flowering or a conidial suspension that is sprayed on a plot when it reaches 50% anthesis (Gilbert and Woods, 2006). Nursery collaborators adapted the methods of introduction of the pathogen to suit the environment where the nursery is grown. For example, at the Urbana, Illinois testing site, multiple scatterings of corn residue are done to ensure constant production of spores across maturity dates. The Kinston, North Carolina collaborator combined both methods of inoculation to combat high temperatures and a large range in heading date.

Three disease resistance phenotypes were evaluated. Incidence (INC) was measured as a percentage of infected heads in a sample. Severity (SEV) was a visual rating of the

percent of infected spikelets on infected heads. Fusarium damaged kernels (FDK) was a visual rating of the percent of scabby seed in a threshed sample.

Phenotypic data was also collected for heading date and plant height. Data was reported as the mean of an entry at the location. An analysis of variance was conducted on environment and line effects to test for significant differences.

Genotypes were not constant in all locations or years, and an environment consisted of a location for a single year. The correlation between the resistance phenotypes and heading date at each location were calculated using SAS 9.2 (SAS Institute, Cary, NC). The data from locations with significant ($p < 0.05$) positive correlation ($r^2 > 0.6$) between heading and disease ratings were removed to reduce confounding FHB resistance and heading date. The number of locations for which data for INC, SEV and FDK was included in the analyses varied for each nursery and each year (Table 4.1).

To account for differences in disease pressure across locations and years, data were standardized as follows: $(X_i - \mu_i) / SE_i$, where X_i is the entry mean for an environment, μ_i is the environment mean and SE_i is the standard error for an environment. Heritability was calculated on an entry mean basis according to the method described by Holland et al. (2003).

Genotypic Data

Seeds were submitted by nursery coordinators to the Eastern Regional Small Grains Genotyping Center, USDA-ARS Raleigh, NC. A single seed for each entry was germinated and tissue collected for isolation of DNA according to the CTAB protocol of Pallotta et al. (2003).

The population was genotyped with DArT markers (Diversity Arrays P/L, <http://www.triticarte.com.au/>, Canberra, Australia) as previously described (Chapter 3). DArTs with a minor allele frequency (MAF) less than 10% were removed from the association analysis. A total of 2062 DArT markers were tested for association with each disease phenotype. A consensus map of DArT markers (DiversityArrays P/L www.diversityarrays.com) and the ITMI mapping population (Sorrells et al. 2011) was used to assign markers to chromosomal locations.

Twenty chromosomes with a reported 25 different scab QTL were targeted for genotyping with sequence tagged site (STS), SSR, and KASPar markers (Appendix C). A larger number of markers were selected on chromosomes with multiple QTL at different loci (1B, 2D, 3B, 5A) and for QTL thought to be present in the selected germplasm (*Fhb1*, *Qfhs.umc-2B*, *Qfhs.umc-3B*, *Qfhs.umc-4BL*, *Qfhs.ifa-5A*, *Qfhs.umc-5A*). Additional SSR markers near repeatable QTL regions were selected from a review of the literature (Buerstmayr et al. 2009), and meta-analyses (Loffler et al. 2009, Liu et al. 2009). Markers flanking potential QTL were used to replace markers not commonly used by the USDA Eastern Regional Small Grains Genotyping Center, Raleigh, NC according to the SSR consensus map (Somers, 2004). KASPar markers specific for the dwarfing alleles of the major plant height loci *Rht-B1* and *Rht-D1* were evaluated since the dwarfing alleles at these loci have been implicated in susceptibility to FHB (Srinivasachary et al., 2009; Srinivasachary et al., 2008; Voss, 2008). Standard PCR conditions were used as described previously, with specific annealing temperatures for each marker as described on GrainGenes2.0 (<http://wheat.pw.usda.gov/GG2/>). Amplified fragments were separated on an ABI 3730xl

DNA analyzer (Applied Biosystems, Foster City, CA) and data were analyzed using GeneMarker v1.85 (Soft Genetics, State College, PA).

A MAF threshold < 0.05 was applied to markers with multiple alleles; if several alleles were below the MAF, they were pooled and treated as a single allele. Multi-allelic markers were coded as separate variables according to the presence or absence of an allele.

Linkage Disequilibrium

Heat maps and the degree of LD among all pair-wise markers targeted to QTL regions were calculated for each chromosome using JMP Genomics 5.0. The strength of LD was according to the measure D' to account for multiple alleles. Marker order and distance were according to the SSR consensus map (Somers, 2004) or the original QTL mapping report. Markers with a distance greater than 40 cM between loci were considered unlinked.

Association Analysis

To account for all pair-wise levels of relatedness among lines, a simple kinship matrix was calculated from a Spearman correlation matrix based upon the subset of 900 DArTs that were identified as being non-redundant ($r^2 < 0.75$) as previously described (Chapter 3). The same set of 900 markers was used for principal components analysis (PCA) in SAS 9.2 (SAS Institute, Cary, NC) with PROC PRINCOMP. The first ten eigenvectors were regressed using PROC REG onto the normalized disease ratings to determine if inclusion of eigenvectors as fixed covariates would be justified in the absence of sub-populations (Price et al., 2010).

Two mixed linear models were analyzed based upon different marker data sets: (1) a set composed of SSR, KASPar, and STS in regions flanking reported QTL and (2) an exploratory set composed solely of 2062 DArT markers distributed throughout the genome.

The model $y = X\beta + Zu + e$ was used for each of the marker association analyses. The column vector y is composed of phenotypic observations while the vectors β and u are columns of fixed and random effects. The X and Z are design matrices. Random residuals are contained within the vector e . The PCA eigenvectors were treated as fixed effects, while the kinship matrix was random.

A naïve model with only phenotypic and marker data was analyzed for each trait according to a general linear model. The naïve models serve as a reference to determine how well the kinship and PCA covariates help to correct for structure.

The analysis was run in the program TASSEL 3.0 (Bradbury, 2006) with compression set at 1 and population parameters previously defined (P3D). Allele effects were calculated by TASSEL. Multiple hypothesis testing correction was applied to the marker p values from TASSEL by calculating a false discovery rate (FDR) using PROC MULTTEST in SAS 9.2. A FDR threshold $p < 0.05$ was set to determine markers in significant association with a trait.

Results

Phenotypic data was collected from 42 environments over three years and all nurseries (Table 4.1). Four locations were in common between all three nurseries: Lexington, KY, Urbana, IL, Colombia, MO, and Blacksburg, VA. Phenotypic data from the Lexington, KY, Crowley, LA, Bay, AR and Fayetteville, AR locations were excluded from the analysis since in all years significant ($p < 0.05$) Pearson correlation coefficients of r^2 were

greater than 0.6 were observed between SEV, INC and FDK with heading date. Phenotypic data for SEV, INC and FDK were included from 29, 26, and 14 environments, respectively. Environments were significantly different for each trait ($p < 0.001$) in the analysis of variance.

The mean of all entries for days to heading across all locations within a nursery was seven to 12 days (Table 4.3). The lowest mean levels of disease was observed in the 2009-NUWWSN, but all nurseries had entries that ranged from moderately resistant to very susceptible (Table 4.3). The correlations between traits were all significant ($p < 0.001$) with the lowest Pearson correlation coefficients between FDK and both INC ($r = 0.43$) and SEV ($r = 0.45$). A higher correlation, $r = 0.71$, occurred between SEV and INC. The mean phenotypic score for severity was similar across all nursery by year combinations ranging from the low of 29% in 2009 NUWWSN and PNUWWSN to 36% in the 2008 USFHBN (Table 4.3). A similar trend was also present for FDK scores. A wider range of mean scores occurred for incidence.

Marker analyses

A total of 421 alleles were identified by 81 SSR and STS markers. The number of markers analyzed per chromosome was greatest for chromosome 3B, with 15 markers targeted to two QTL regions, followed by chromosomes 4B (ten markers), 5A (nine markers) and 2D (eight markers) (Appendix D). The number of markers assayed for the remaining 13 chromosomes ranged from one to four. After exclusion of alleles present in fewer than 5% of entries, an average of 4.3 alleles were analyzed per marker and the data for 353 alleles were converted into bi-allelic variables for association analyses.

The number of DArT markers with a MAF greater than 10% was 2062. All but 222 DArTs could be located to a chromosome, although not all were positioned on the consensus map. Only 887 markers were assigned a position within the consensus map. A total of 142 DArTs were mapped to multiple chromosomes. The chromosomes with the most markers were 1B, 3B, and 2B which had 191, 189, and 172 markers, respectively. Only four and six DArT markers were located on chromosomes 4D and 5D. Chromosome 5A had 20 markers, but only eight could be mapped, which located to a region between 45 and 50 cM on the DArT consensus map.

Linkage Disequilibrium

The level of genome-wide LD based on the analysis of DArT markers in this set of germplasm was previously reported (Chapter 3). Pair-wise calculation of LD among SSR and STS markers on the same chromosome identified combinations of markers with strong LD ($D' > 0.4$) persisting at distances greater than 30 cM between loci on chromosomes 1A (*Xgwm357/Xbarc17* and *Xwmc716/Xscm09*), 2B (*Xwmc770/Xgwm319*), and 2D (*Xgwm261/Xgwm539* and *Xgwm484*) (Figure 4.1). High LD ($D' > 0.4$) was present among neighboring markers on regions of chromosomes 2D, 3B, 4B, and 5A, which spanned from 4.7 to 14.5 cM (Figure 4.1). Two regions with D' greater than 0.4 on chromosome 3B were not in LD with each other (Figure 4.1).

Association Analyses

The first three eigenvectors from PCA had significant p values (< 0.005) for at least one trait when regressed onto mean phenotypic values for each entry. The second principal component was significant for SEV, INC and FDK. As a result, only the second principal

component was selected for inclusion with the kinship matrix as a covariate for association mapping. The addition of the second eigenvector and kinship matrix as fixed and random covariates, respectively, reduced the number of significant markers to less than a third of that observed with the naive models which included only marker and phenotypic data for each trait (Table 4.4).

SSR and STS markers: Using a threshold of $p < 0.05$ after correction for FDR, 30 SSR and STS marker-alleles were determined to be significantly associated with one or more disease ratings. Eighteen, seven, and twelve alleles were significant for SEV, FDK, and INC respectively (Table 4.5). Two loci, *Xbarc137* and *Xwmc705*, were significantly associated with all three traits. Six markers were significantly associated with both SEV and INC of FHB.

Two alleles of SSR marker *Xbarc137* on chromosome 1B were significantly associated with SEV (Table 4.6). The *Xbarc137-238* had the effect of significantly reducing SEV, INC and FDK and *Xbarc137-225* was associated with increased disease SEV (Figure 4.2). Marker *Xbarc137-238* was present in 39% of entries.

Two alleles of marker locus *Xwmc705* located on chromosome 5A were significantly associated with all three traits (Table 4.6). The 135 bp allele of *Xwmc705* significantly decreased both scab SEV and INC and the 133 bp allele was associated with increased FDK (Figure 4.3). Two additional markers in this region of 5A were associated with reduced SEV (*Xgwm156-283*) and INC (*Xgwm304-217*). Marker loci on chromosomes 2D and 3B were

also associated with multiple resistance ratings, although different markers and alleles within each region were significant for each trait (Table 4.6).

Significant effects on SEV were detected for numerous markers on the short arm of chromosome 3B linked to the *Fhb1* locus (Table 4.6). In each case, the alleles derived from Asian donor parents of *Fhb1* (*Xsnp3BS-8-1*, *UMN10-239*, *Xgwm533-145*, *Xgwm493-195* and *Xcfd79-280*) were associated with reduced disease SEV (Figure 4.4). Marker *Xsnp3BS-8-1* was also significantly associated with decreased INC. In contrast, marker alleles *Xcfd79-283* and *UMN10-236* were associated with increased disease severity (Figure 4.4).

A second region on chromosome 3B, near the centromere, had multiple significant marker loci associated with increased disease SEV: *Xgwm285-226*, *Xwmc1-149*, and *Xwmc787-162*. Two alleles, *Xgwm285-226* and *Xwmc1-149*, were also significantly associated with increased incidence while alternate alleles of *Xwmc1* (*Xwmc1-162*) and *Xwmc787* (*Xwmc787-158*) decreased incidence (Figure 4.5).

Three markers located on the long arm of chromosome 2D were associated with decreased INC and SEV while one SSR marker on the short arm of 2D (*Xgwm261-175*) was associated with decreased FDK (Table 4.6). A single locus on each chromosome: 1A, 2B, 3A, and 6D were associated with FDK. The *Xscm9-208* allele that is indicative of the presence of the 1RS·1AL translocation was associated with reduced disease INC. The *Rht-D1b* dwarfing allele was significantly associated with increased disease INC.

DArT markers: For the traits SEV, INC, and FDK a total of 71, 67, and 25 DArTs, respectively, were significant after multiple hypotheses testing (MHT) correction (Table 4.5).

Twelve markers were significant for all traits, with INC and SEV sharing an additional 29 DArTs in common. Six and three mutual markers were found for INC-FDK and SEV-FDK respectively, leaving 55 DArTs with effects on a single trait. For all markers significant for multiple traits, the effect of the presence alleles (coded as the “1” allele) were in the same direction (Table 4.7). Chromosome 2D had the greatest number of significant loci (55), followed by 1A (25), and 3B (19). An putative 23 QTL on 13 chromosomes were identified by DArT based AA. DArT loci were grouped into two QTL regions on chromosome 1B and 2B, and three regions on 1A, 2D, 3B, and 6A (Table 4.7). Only one DArT associated with INC was significant on each chromosome 4B and 5D.

The first cluster on chromosome 1A (1A.1) spanned 0.2cM and was composed of nine significant markers (Table 4.7). The presence allele of each locus was associated with an increase in SEV or INC (Table 4.7). Markers in the 1A.2 cluster, at 25.3 cM to 35.7 cM, were associated with SEV, and INC or FDK. The 1A.3 region was associated with FDK.

Seven loci from 49.0-56.7 cM on chromosome 2D were identified as increasing at least two traits, while two loci positioned at 72 cM resulted in a decrease of SEV levels (Table 4.7). The “1” allele at the 2D.3 QTL had significant effects on reducing all three resistance phenotypes.

There were four significant DArT loci in region containing the gene *Fhb1* (3B.1) for SEV, two of which were also significant for INC. Two other DArT loci on the short arm of 3B, near the centromere at position 58.4 cM, produced an increase for SEV. The 3B.3 QTL was identified by five loci at 131.5 to 136.9 cM with significant decreasing effects on SEV and INC.

Two and one DArT loci defined the 2B.1 and 2B.2 clusters (Table 4.7). The DArT loci wPt-6627 and wPt-8998 in the 2B.1 group were associated with a decrease in FDK and SEV respectively. The presence of the wPt-665645 locus at 85.5 cM was correlated with a decline in both SEV and INC.

The 6A.1 group spanned a 4.9 cM interval. A decrease in all three traits occurred for the “1” allele (Table 4.7). The second aggregate of significant DArTs at QTL 6A.2 extended from 21.3 to 26.9 cM and included five marker loci. The two outermost loci were associated with severity, while the inner three DArTs, all positioned at 24.2 cM had an effect of a decrease in incidence for the presence of the locus. The set of significant DArTs, 6A.3, covered a region spanning 22.5 cM and was represented by six loci that conferred a decrease in INC and FDK.

Seventeen DArT significant DArT markers could not be assigned to a relative position on a chromosome while two DArTs mapped to both chromosomes 2B and 5B. A chromosomal location could not be identified for an additional six significant DArT loci. The highest r^2 for among significant markers was 2.61, 1.69, and 1.41 for FDK, INC, and SEV respectively. The phenotypic variation captured for each trait was the sum of the most significant marker r^2 for each significant QTL region. The highest range of phenotypic variance captured was 18.5% for INC, while SEV had the lowest at 16.9%.

Discussion

Association analysis has become an important tool to help improve marker assisted breeding for quantitative traits such as FHB resistance in soft winter wheat.

Using a population that was enriched for scab resistance, we used DArTs to conduct a genome-wide scan for QTL. The same population was subjected to a GWAS to validate and ensure diagnostic capability of site specific markers such as SSRs and STS which have been reported in bi-parental mapping studies and used in MAS.

Differences in the range of heading days between locations were expected due to nurseries being evaluated across a large North to South geography. The time to anthesis is a paramount concern since flowering is the time at which plants are most susceptible and often relates to the timing of spore production and release from the pathogen (Trail, 2009). We are unable to determine how much an impact days to anthesis had on scab QTL detection, although no DArT, SSR, or STS markers that were significant for heading date were also significant for any of the resistance phenotypes (data not shown). The absence of markers being significant for heading date and scab resistance in this study is contrary to several reports (Buerstmayr et al. 2009). Our results did confirm previous results that lines possessing the *Rht-D1b* allele have higher levels of scab incidence (Srinivasachary et al., 2009; Voss, 2008). A single DArT marker, wPt-730427, on 2D was significant for both height and severity in our study (data not shown).

Validation of SSR and STS marker effects: The estimates of local LD among site specific markers often reflect the selection pressure for QTL/genes. In this study, the region with the highest LD, $D' = 0.59$, was found among markers associated with the gene *Fhb1* on 3B, which is likely the most prominent resistance factor that is a target of selection in this germplasm. Conversely, the lowest LD was found among markers on 7D, which has very few mapped

scab QTL. With the exception of chromosome 2D that had LD extending to 14 cM, the blocks of high LD, $D' > 0.4$, in two regions on 3B, as well as on 4B and 5A, were confined to markers within 5 cM. The influence of high levels of allelic diversity is seen with *Xgwm484*, which had nine alleles and maintained high LD, $D' = 0.62$, with marker loci up to 47 cM distant.

While strong LD between markers and functional variants is paramount to detection of QTL effects and selection of targets, it is also imperative that marker alleles be able to discriminate the presence or absence of the QTL in different backgrounds. Given the introduction of new alleles and the resulting high LD, five of six SSR and STS markers evaluated near *Fhb1* were significant in our study. In addition, four DArT markers in the *Fhb1* region were significant for SEV and two for INC. The one SSR marker for *Fhb1* that was not significant in the AA, *Xbarc147*, is not used for MAS in soft winter wheat germplasm due a high frequency of alleles identical in state with the *Fhb1* donors. False positives are also known to occur for even the “best” markers (*Xumn10* or *Xsnp3BS-8*) used for *Fhb1* selection; thus, use of multiple flanking markers currently offers the best solution, but can restrict diversity in the regions flanking the resistance gene.

This study resulted in identification of marker alleles that would be ideal to select for or against to increase the level of scab resistance in soft winter wheat germplasm. Selection for the 238 bp allele of *Xbarc137* on chromosome 1B would effectively reduce SEV, INC, and FDK, whereas avoiding the inclusion of the 226 bp allele of *Xgwm285* would prevent an increase in disease SEV and INC. Marker loci on 2DL are known to recombine frequently within the region containing *Qfhs.nau-2DL* or *Qfhs.crc-2DL*. Until more tightly linked

markers are available, our results suggest that the use of GWM157, GWM608, and CFD233 would be the most appropriate in soft winter wheat. An array of mapping studies from various cultivars have reported a QTL on the short arm of 5A. Out of ten marker loci targeted to this region of low recombination and high LD, only three were identified as significant for at least one trait. The locus *Xwmc705* identified the 135 bp allele as decreasing SEV and INC, while the 133 bp allele increased INC. Use of the *Xgwm156* and *Xgwm304* would also facilitate a decrease in SEV and INC respectively.

Validation of markers through association analysis allows identification of multiple robust markers to be used in a breadth of soft winter wheat. Except for chromosome 4B where no significant effects were detected, the high LD blocks on 2D, 3B, and 5A were associated with multiple significant site specific markers. These regions also represent common QTL that are likely selected in this germplasm. Genomic regions in which no markers were significant does not definitively mean that QTL were absent in that locale. Two possible explanations are that the QTL effects were too small to capture or the markers were not in strong enough LD with a functional variant.

Identification of FHB resistance QTL with DArTs: The use of DArT markers was advantageous for genome wide coverage to identify putative QTL. However, the lack of assignment to a chromosome position for many markers made interpretation of the number and position of QTL difficult. An additional problem is the uneven distribution of DArTs across chromosomes, particularly for 3D, 4D, 5A, and 5D, which had poor marker coverage (Chapter 3).

This study identified 23 regions on 13 chromosomes that were significantly associated with one of three FHB resistance traits. Three QTL were detected on each of four chromosomes (1A, 2D, 3B, and 6A) and two QTL each on 1B and 2B. Locations of significant DArT markers in ten regions correspond well to positions of meta-QTL reported by Loffler et al. (2009) (Table 4.8). In addition, other researcher have reported FHB resistance QTL near the 1A.3, 2B.1, 4A.1, 5B.1, and 7A.1 regions (Liu et al., 2009; Loffler et al., 2009). The 1A.1, 6A.1, 6A.3 and 6B.1 QTL in our study were not reported previously. Significant DArTs on 2A and 5D were not assigned on the consensus map and could not be compared with previously reported QTL on these chromosomes.

Some regions that were significant in our analysis corresponded to similar QTL regions reported for different resistant phenotypes. A majority of the mapped QTL in the literature are in association with severity or spread of Fusarium head blight. Liu et al. (2009) reported several QTL from meta-analysis which located to the same region, but in relation to different traits. This is not unexpected since the disease resistance ratings are highly correlated with one another, as seen in this study. We also found that many markers were significant for more than one disease measure.

Concordance between significant DArT and site specific makers was found for seven QTL: 1A.2, 1B.1, 1B.2, 2D.1, 2D.2, 3B.1 and 3B.2. The greater coverage of DArTs led to detection of QTL not identified with SSRs. Conversely, five QTL significant with the site specific markers were not detected by DArTs where coverage was sparse. This included significant SSR markers on chromosome 5A for which there were very few polymorphic DArT loci, as well as SSR markers on 6B and 6D. Significant DArT markers were also not

found associated with the QTL identified by the *Xscm09* rye-specific SSR marker (indicative of the 1RS·1AL translocation) and the STS marker for the *Rht-B1b* dwarfing gene.

Cultivars with mapped resistance QTL on multiple chromosomes such as CJ9306 (1AS, 2DL, *Fhb1*), Goldfield (2B, 2BS, 7B), Frontana (2B, 3A, 6B), and Ernie (2B, 3Bc, 4B, 5A), were present in the pedigrees of multiple lines in our study and could be sources for the resistant alleles of these QTL. A QTL mapped in Truman on chromosome 1B (A. McKendry, unpublished data), was identified in this study. The 238 bp for *Xbarc137* and five DArT markers at the 1B.1 QTL were found to significantly increase the level of resistance for each phenotype and together form a “Truman haplotype” at the QTL. The *Xwmc705-135* allele is found in Ernie, which may be the source of the significant effect to decrease SEV and INC. Given the bi-allelic nature of the DArT markers and high allele frequency observed for significant markers, we could not identify particular resistance sources for QTL identified only with DArTs in this study.

Resistance QTL on chromosomes 4A, 6D and 5D have previously only been reported in European and Asian material and from sources not commonly used in U.S. breeding programs. In contrast to the results with the SSR markers in the *Fhb1* region, AA with DArT markers did not allow us to determine if these QTL have been introgressed via European or Asian germplasm or are of native origin and co-localize with the exotic QTL.

Screening the germplasm with DArTs was useful for obtaining genome wide coverage at low cost, although direct application to MAS is limited since the marker is evaluated on a microarray and does not allow targeting to a single region by only a few DArT loci. However, the DArT assay can be applied to genomic selection, which could

complement the polygenic nature of scab resistance, avoids the arbitrary nature of identifying QTL by significance thresholds, and can be applied simultaneously to multiple traits using selection indices (Heffner et al., 2011).

The application of AA to a population derived from regional scab screening nurseries in our study was effective at identifying multiple QTL in diverse germplasm, and identifying robust markers for specific target QTL. A limitation to AA was that absolute allele effects could not be determined with this study. Some QTL specific markers, such as those on chromosome 1B, 2D, 3B, and 5A, were significant and robust in our analyses, supporting their continued use in MAS. However, we identified 23 different QTL by AA in these germplasm, even with limited genome-wide marker coverage, application of a conservative interpretation of QTL numbers and use of FDR for declaring significance. These results are consistent with those of bi-parental mapping studies that, with the exception of few genes like *Fhb1*, resistance to scab is characterized by numerous QTL of small effect. These data suggest that using information from all significant markers affecting FHB in populations derived from many resistance sources may be more effective at increasing FHB resistance than MAS for single or few QTL in bi-parental crosses. Alternatively, all marker data could be included to perform genomic selection for FHB resistance, which would have the advantage of allowing for simultaneous selection for other important traits and more rapid cycle time.

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Table 4.1 Number of breeding programs submitting entries and number of entries evaluated in to the Uniform Southern FHB Nursery (USFHBN), the Preliminary Northern Uniform Winter Wheat Scab Screening Nursery (PNUWSSN) and the Northern Uniform Winter Wheat Scab Screening Nursery (NUWSSN) in different years, number of locations reporting data for disease severity (SEV), disease incidence (INC), and Fusarium damaged kernels (FDK).

Nursery	Year	No. of Programs	No. of Entries	No. of Locations		
				SEV	INC	FDK
USFHBN	2008	9	37	4	3	3
	2009	9	54	4	4	5
PNUWSSN	2008	8	44	6	6	2
	2009	8	42	6	5	3
NUWSSN	2008	10	44	8	7	3
	2009	11	56	9	8	5
	2010	12	51	9	8	4

Table 4.2 Breeding programs that contribute entries to the USFHBN, NUWWSN, or the PNUWWSN and respective locations where nurseries are evaluated. Locations in bold were not included in the analysis

Program	Location	Nursery
Purdue U.	Lafayette, IN	PNUWWSN, NUWWSN, USFHBN
Sunbeam Extract	Not evaluated	PNUWWSN, NUWWSN
U. Nebraska	Mead, NE	NUWWSN
Cornell U.	Ithaca, NY	NUWWSN
U. of Guelph	Ridgetown, Ontario	NUWWSN
Syngenta	Brookston, IN, Bay, AR	PNUWWSN, NUWWSN, USFHBN
Michigan State U.	East Lansing, MI	PNUWWSN, NUWWSN
Ohio State U.	Wooster, OH	PNUWWSN, NUWWSN
U. of Maryland	Salisbury, MD	NUWWSN, USFHBN
U. of Illinois	Urbana, IL	PNUWWSN, NUWWSN
U. of Kentucky	Lexington, KY	PNUWWSN, NUWWSN, USFHBN
Virginia Polytechnic Institute	Blacksburg, VA	PNUWWSN, NUWWSN, USFHBN
U. of Missouri	Colombia, MO	PNUWWSN, NUWWSN, USFHBN
North Carolina State U.	Kinston, NC	USFHBN
Louisiana State U.	Crowley, LA	USFHBN
U. of Georgia	Griffin, GA	USFHBN
USDA-ARS Raleigh, NC	Not evaluated	USFHBN
U. of Arkansas	Kibler, AR, Fayetteville, AR	USFHBN

Table 4.3 Mean, minimum, and maximum observed phenotype across locations within a nursery by year combination for days to heading, severity, incidence, and Fusarium damaged kernels

Year	Nursery	Julian Days to Heading			Severity (%)			Incidence (%)			FDK (%)		
		Min. ¹	Max. ²	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
2010	NUWWSN	133	143	138	15	56	33	44	71	57	18	58	34
2009	NUWWSN	133	144	137	10	44	29	35	74	57	8	44	25
	PNUWWSN	132	142	136	16	59	29	35	82	60	9	65	31
	USFHBN	128	135	131	19	56	34	37	65	48	14	46	30
2008	NUWWSN	138	150	143	15	61	34	42	79	59	12	43	25
	PNUWWSN	140	152	145	16	52	33	46	86	68	14	62	37
	USFHBN	116	128	120	17	70	36	40	83	62	15	64	30

¹Minimum average phenotype of an entry across locations within a nursery

²Maximum average phenotype of an entry across locations within a nursery

Table 4.4 Number of markers after FDR correction that were significantly associated with scab severity (SEV), Fusarium damaged kernels (FDK), and disease incidence (INC) from linear model analysis with covariates K+P2 (Kinship +PC2) and without (Naïve)

Marker	Model	Trait		
		SEV	FDK	INC
DArT	Naïve	210	248	246
DArT	Kinship+P2	71	25	67
SSR	Naïve	54	43	53
SSR	Kinship+P2	18	7	12

Table 4.5 Number of DArT and SSR markers significantly associated with scab severity (SEV), Fusarium damaged kernels (FDK) and disease incidence (INC) by chromosome

Chromosome	SEV		FDK		INC	
	DArT	SSR	DArT	SSR	DArT	SSR
1A	16	-	2	1	7	1
1B	4	2	3	1	3	1
2A	2	-	-	-	1	-
2B	3	1	1	-	3	-
2D	26	2	11	1	18	2
3A	-	-	-	1	-	-
3B	10	10	-	-	9	5
4A	2	-	-	-	1	-
4B	-	-	-	-	1	-
4D	-	-	-	-	-	1
5A	-	2	-	1	-	2
5B	1	-	-	-	1	-
5D	-	-	-	-	1	-
6A	4	-	4	-	13	-
6B	-	1	2	-	5	-
6D	-	-	-	2	-	-
7A	1	-	-	-	1	-
Unknown*	2	-	2	-	3	-

*Markers which did not have a map location or mapped to multiple locations

Table 4.6 Location, frequency, p value of significant, $p < 0.05$, markers after FDR correction and allele effect of significant marker-alleles for severity (SEV), Fusarium damaged kernels (FDK), and Incidence (INC).

Marker-allele ¹	Chrom.	Frequency	SEV		FDK		INC		QTL ²
			p	Effect	p	Effect	p	Effect	
scm09-208	1A	7.5	-	-	-	-	0.006	-4.48	
cfa2219-250	1B	17.6	-	-	0.011	-3.77	-	-	1B.2
barc137-238	1B	39.5	0.015	-4.59	0.036	-5.47	0.013	-3.54	1B.1
barc137-225	1B	26.6	0.005	2.84	-	-	-	-	1B.1
wmc770-120	2B	#N/A	0.007	4.25	-	-	-	-	
gwm261-175	2D	46.9	-	-	0.015	-3.68	-	-	2D.1
gwm157-110	2D	6.3	0.005	-5.48	-	-	0.008	-5.33	2D.2
gwm608-150	2D	40.2	0.005	-2.57	-	-	-	-	2D.2
cf233-280	2D	13.3	-	-	-	-	0.006	-3.26	2D.2
wmc11-164	3A	61.3	-	-	0.010	-2.75	-	-	
Xsnp3BS-8	3B	14.1	0.008	-4.72	-	-	0.007	-3.62	3B.1
umn10-236	3B	64.8	0.006	3.04	-	-	-	-	3B.1
umn10-239	3B	12.9	0.006	-4.14	-	-	-	-	3B.1
gwm493-195	3B	25.4	0.006	-3.15	-	-	-	-	3B.1
gwm533-145	3B	11.3	0.005	-4.04	-	-	-	-	3B.1
cf279-280	3B	6.6	0.007	-5.97	-	-	-	-	3B.1
cf279-283	3B	23.8	0.007	3.46	-	-	-	-	3B.1
gwm285-226	3B	19.5	0.009	4.51	-	-	0.010	3.94	3B.2
wmc1-149	3B	23.8	0.005	3.03	-	-	0.007	3.01	3B.2
wmc1-162	3B	12.5	-	-	-	-	0.006	-3.58	3B.2
wmc787-162	3B	26.0	0.005	3.52	-	-	-	-	3B.2
wmc787-158	3B	16.0	-	-	-	-	0.007	-4.14	3B.2
Rht-D1b	4D	43.8	-	-	-	-	0.007	2.65	
wmc705-135	5A	19.5	0.007	-3.56	-	-	0.006	-2.82	
gwm156F-283	5A	26.2	0.005	-2.94	-	-	-	-	
wmc705-133	5A	16.0	-	-	0.010	3.53	-	-	
gwm304-217	5A	7.4	-	-	-	-	0.006	-4.35	
gwm219-163	6B	8.6	0.005	4.64	-	-	-	-	
barc23-243	6D	25.0	-	-	0.013	-3.6	-	-	
barc23-237	6D	25.8	-	-	0.011	3.29	-	-	

¹Number after marker name indicates size in base pairs

²Putative QTL identified with DArT markers

Table 4.7 Location, effect, and p value of significant, $p < 0.05$, markers after FDR correction in relation to resistance traits. Allele effects are in average standard error units. Chromosome locations are from Syn x Opatá and DArT consensus maps.

Putative		Chrom. ¹	cM	Freq. ¹²	SEV		INC		FDK	
QTL	Marker				p	Effect	p	Effect	p	Effect
1A.1	wPt-6280	1A	0.0	63.7	0.04	3.2	-	-	-	-
1A.1	wPt-744290	1A	0.2	61.3	0.02	2.7	0.04	3.5	-	-
1A.1	wPt-665724	1A	0.2	62.5	0.04	3.4	-	-	-	-
1A.1	wPt-743417	1A	0.2	27.0	0.04	3.7	0.04	3.0	-	-
1A.1	wPt-732520	1A	0.2	45.7	0.04	3.1	-	-	-	-
1A.1	wPt-666537	1A	0.2	46.5	0.04	3.0	-	-	-	-
1A.1	wPt-730618	1A	0.2	46.1	0.04	3.0	-	-	-	-
1A.1	wPt-734000	1A	0.2	45.7	0.05	2.9	-	-	-	-
1A.1	wPt-6709	1A	2.7	46.9	0.05	2.9	-	-	-	-
1A.2	wPt-667260	1A	23.3	81.3	0.04	4.3	0.02	4.3	-	-
1A.2	wPt-5776	1A	35.7	40.2	0.02	3.7	-	-	0.02	3.8
1A.2	wPt-1862	1A	35.7	49.6	0.04	3.1	-	-	-	-
1A.3	wPt-4801	1A	87.9	60.5	-	-	-	-	0.01	-2.4
ua ³	wPt-4712	1A	ua	85.9	-	-	0.03	4.2	-	-
ua	wPt-729787	1A	ua	87.1	-	-	0.04	4.5	-	-
ua	wPt-1167	1A	ua	80.1	0.02	4.6	-	-	-	-
ua	wPt-669418	1A	ua	80.9	0.04	4.7	-	-	0.02	4.1
ua	wPt-730929	1A	ua	85.9	0.04	4.1	-	-	0.02	3.5
ua	wPt-3198	1A	ua	46.1	0.04	2.9	-	-	-	-
1B.1	wPt-4325	1B	30.6	72.7	0.01	-4.0	0.01	-3.5	-	-
1B.1	wPt-1573	1B	35.6	41.0	-	-	-	-	0.01	3.5
1B.1	wPt-6425	1B	38.5	66.8	0.01	4.2	0.02	3.5	0.03	5.1
1B.1	wPt-3579	1B	48.9	80.1	0.05	-3.5	0.04	-3.8	-	-
1B.1	wPt-0705	1B	57.2	75.0	0.04	-3.3	-	-	-	-
1B.2	wPt-669831	1B	89.2	49.6	-	-	-	-	0.02	3.8
2A.1	wPt-0568	2A	ua	85.9	0.02	-5.0	-	-	-	-
2A.1	wPt-8826	2A	ua	67.6	0.03	-3.8	0.02	-3.1	-	-
2B.1	wPt-6627	2B	4.2	50.8	-	-	-	-	0.01	-3.1
2B.1	wPt-8918	2B	6.2	77.7	0.04	-3.8	-	-	-	-
2B.2	wPt-665645	2B	85.5	74.2	0.04	-2.2	0.02	-2.1	-	-
ua	wPt-3569	2B 5B	ua	63.7	-	-	0.02	-2.8	-	-

Table 4.7 Cont.

Putative		Chrom. ¹	cM	Freq. ¹²	SEV		INC		FDK	
QTL	Marker				p	Effect	p	Effect	p	Effect
ua	wPt-3995	2B 5B	ua	64.1	0.04	2.9	0.02	2.7	-	-
2D.1	wPt-734280	2D	49.0	42.2	0.00	4.4	0.00	4.0	0.03	4.0
2D.1	wPt-730889	2D	49.0	49.6	0.01	3.3	0.01	2.9	0.02	3.6
2D.1	wPt-665644	2D	49.0	49.6	0.02	3.0	0.03	2.6	0.02	3.4
2D.1	wPt-731409	2D	52.9	44.5	0.02	3.4	0.03	2.9	0.01	3.4
2D.1	wPt-733567	2D	52.9	48.8	0.03	3.2	-	-	-	-
2D.1	wPt-667312	2D	53.6	47.7	0.02	3.0	0.01	2.4	0.02	3.1
2D.1	wPt-740836	2D	56.7	57.0	-	-	0.01	2.9	0.01	2.5
2D.2	wPt-731941	2D	71.6	82.8	0.04	-4.4	-	-	-	-
2D.2	wPt-671737	2D	73.0	69.1	0.04	-1.9	-	-	-	-
2D.3	wPt-7466	2D	90.5	17.2	0.02	4.3	0.02	3.4	-	-
2D.3	wPt-3728	2D	90.5	15.6	0.01	4.9	0.02	3.9	-	-
2D.3	wPt-733725	2D	95.8	53.5	0.02	-3.1	-	-	-	-
2D.3	wPt-732052	2D	99.1	68.4	0.04	-3.4	-	-	-	-
2D.3	wPt-665836	2D	99.1	67.6	0.04	-3.5	-	-	-	-
2D.3	wPt-667765	2D	100.4	61.7	0.05	-3.0	-	-	-	-
2D.3	wPt-730677	2D	101.0	67.6	0.04	-3.3	-	-	-	-
2D.3	wPt-731220	2D	101.4	66.0	0.04	-3.2	0.03	-2.6	-	-
2D.3	wPt-671742	2D	102.8	73.8	0.01	-4.2	0.02	-3.5	0.02	-4.3
2D.3	wPt-730080	2D	102.8	73.8	0.02	-3.9	0.02	-3.3	0.01	-4.0
2D.3	wPt-730757	2D	102.8	73.0	0.02	-4.0	0.02	-3.5	0.02	-4.2
2D.3	wPt-668261	2D	102.8	70.7	0.02	-3.9	0.04	-3.1	0.02	-4.0
2D.3	wPt-665102	2D	102.8	71.5	0.04	-3.5	0.04	-3.1	0.01	-3.8
2D.3	wPt-1301	2D	103.6	68.8	0.04	-3.3	0.02	-2.6	-	-
2D.3	wPt-0153	2D	103.6	62.5	0.04	-3.1	-	-	-	-
2D.3	wPt-730427	2D	103.7	63.3	0.04	-3.3	-	-	-	-
ua	wPt-4329	2D	ua	66.0	0.01	-3.6	0.00	-3.5	-	-
ua	wPt-4144	2D	ua	57.0	0.05	2.7	0.01	2.9	-	-
ua	wPt-7636	2D	ua	85.2	-	-	0.02	-4.6	-	-
3B.1	wPt-741750	3B	25.1	48.8	0.01	-3.5	-	-	-	-
3B.1	wPt-1081	3B	25.4	52.3	0.05	-2.1	-	-	-	-
3B.1	wPt-3536	3B	25.7	63.7	0.01	-4.1	0.04	-2.8	-	-
3B.1	wPt-741331	3B	26.5	30.5	0.02	-3.6	0.02	-3.2	-	-
3B.2	wPt-4608	3B	58.4	22.3	0.04	3.5	-	-	-	-
3B.2	wPt-2091	3B	58.4	21.9	0.04	3.6	-	-	-	-
3B.3	wPt-5072	3B	131.5	12.5	0.04	-3.8	0.01	-4.6	-	-

Table 4.7 Cont.

Putative		Chrom. ¹	cM	Freq. ¹²	SEV		INC		FDK	
QTL	Marker				p	Effect	p	Effect	p	Effect
3B.3	wPt-7614	3B	131.5	12.5	0.05	-4.4	0.01	-4.8	-	-
3B.3	wPt-2559	3B	131.5	13.3	-	-	0.02	-4.3	-	-
ua	wPt-0324	3B	ua	12.1	0.04	-3.6	0.02	-4.1	-	-
ua	wPt-7786	3B	ua	12.9	-	-	0.04	2.7	-	-
4A.1	wPt-5003	4A	87.9	56.3	0.03	1.7	-	-	-	-
ua	wPt-6176	4A	ua	24.2	0.05	3.4	0.02	3.3	-	-
4B.1	wPt-1708	4B	56.2	62.9	-	-	0.04	2.7	-	-
5B.1	wPt-6880	5B	128.9	73.4	0.05	-3.3	-	-	-	-
5B.1	wPt-1348	5B	140.8	63.7	-	-	0.05	-2.7	-	-
5D.1	wPt-5870	5D	ua	72.3	-	-	0.02	-2.7	-	-
6A.1	wPt-667662	6A	-2.6	40.6	0.02	3.5	0.02	3.1	-	-
6A.1	wPt-734331	6A	2.3	38.7	0.00	4.2	0.00	3.5	0.02	3.3
6A.2	tPt-6278	6A	21.3	15.2	0.02	4.1	-	-	-	-
6A.2	wPt-667780	6A	24.2	75.8	-	-	0.04	-3.3	-	-
6A.2	wPt-664733	6A	24.2	77.0	-	-	0.04	-3.3	-	-
6A.2	wPt-733764	6A	24.2	75.0	-	-	0.04	-3.1	-	-
6A.2	wPt-7127	6A	26.9	35.2	0.05	-2.2	-	-	-	-
6A.3	wPt-732951	6A	86.5	71.9	-	-	0.03	-3.0	-	-
6A.3	wPt-666156	6A	87.9	78.9	-	-	0.05	3.0	-	-
6A.3	wPt-731854	6A	104.6	17.2	-	-	0.01	4.0	0.01	4.2
6A.3	wPt-732328	6A	104.6	17.2	-	-	0.01	4.0	0.01	4.2
6A.3	wPt-9474	6A	107.8	17.2	-	-	0.01	4.0	0.01	4.2
6A.3	wPt-6696	6A	109.0	70.3	-	-	0.04	-2.8	-	-
ua	wPt-2822	6A	ua	77.3	-	-	0.03	-3.3	-	-
ua	wPt-667624	6A	ua	88.3	-	-	0.04	4.3	-	-
6B.1	wPt-6116	6B	113.3	82.8	-	-	0.02	-4.0	-	-
6B.1	wPt-3045	6B	114.6	9.8	-	-	0.00	5.2	0.01	5.1
6B.1	wPt-1264	6B	114.6	9.8	-	-	0.00	5.2	0.01	5.1
6B.1	wPt-742453	6B	ua	87.1	-	-	0.01	-5.1	-	-
6B.1	wPt-9952	6B	ua	55.9	-	-	0.04	-2.7	-	-
7A.1	wPt-0275	7A	90.2	53.1	0.04	2.8	-	-	-	-
7A.1	wPt-6495	7A	162.3	50.8	-	-	0.02	1.6	-	-
ua	wPt-742401	NA	ua	30.9	0.04	3.0	-	-	-	-
ua	wPt-667198	NA	ua	84.8	0.02	5.2	0.02	4.6	-	-

Table 4.7 Cont.

Putative		Chrom. ¹	cM	Freq. ¹ ²	SEV		INC		FDK	
QTL	Marker				p	Effect	p	Effect	p	Effect
ua	wPt-667162	NA	ua	67.6	-	-	-	-	0.02	-2.1
ua	wPt-665037	NA	ua	84.8	-	-	-	-	0.01	-5.0
ua	wPt-732617	NA	ua	63.3	-	-	0.03	-2.4	-	-
ua	wPt-4791	NA	ua	50.0	-	-	0.03	-2.3	-	-

¹Chromosome

² Frequency of the “1” allele

³ua – unassigned chromosome

Table 4.8 Comparison of 23 genomic regions, which contain significant DArT loci associated with resistance phenotypes for scab, to meta-analysis QTL and review.

QTL	Location (cM)	Associated Phenotype	MetaQTL ¹	Source	References
1A.1	0-0.2	SEV, INC	na	na	Liu et al. 2009
1A.2	23.3-35.7	SEV, FDK	MQTL1	Wheaton, Pirat	Liu et al. 2009
1A.3	87.9	FDK	na	NK93604	Liu et al. 2009
1B.1	30.6-57.2	SEV, INC, FDK	MQTL2	Wangshuibai, Seri82, CM-82036, Truman	Loffler et al. 2009; Buerstmayr et al. 2009; McKendry unpublished
1B.2	89.2	FDK	MQTL3	Pirat, Biscay, History	Loffler et al. 2009; Liu et al. 2009
2A	Unknown	SEV, INC	na	na	na
2B.1	4.2-6.2	SEV, FDK	na	Goldfield	Liu et al. 2009; Buerstmayr et al. 2009
2B.2	85.5	SEV, INC	MQTL6	Ernie, Ning7840, Renan, Strongfield	Loffler et al. 2009; Buerstmayr et al. 2009; Liu et al. 2009;
2D.1	49-56.7	SEV, INC, FDK	MQTL7	Alondra, Wangshuibai, NyuBai, Gamenya	Loffler et al. 2009; Buerstmayr et al. 2009; Liu et al. 2009;
2D.2	71.6-73	SEV	MQTL8	Wuhan1, CS, CJ9306, Wangshuibai, DH181, Apache	Loffler et al. 2009; Buerstmayr et al. 2009; Liu et al. 2009;
2D.3	90.5-103.7	SEV, INC, FDK	(may be MQTL8)		

Table 4.8 Cont

QTL	Location (cM)	Associated Phenotype	MetaQTL ¹	Source	References
3B.1	25.1-26.5	SEV, INC	MQTL9 (<i>Fhb1</i>)	Sumai3, Ning7840, CJ9306, W14 and others	Loffler et al. 2009; Buerstmayr et al. 2009; Liu et al. 2009;
3B.2	58.4	SEV	MQTL10	Wangshuibai, Ernie, Apache, Massey, Truman	Loffler et al. 2009; Buerstmayr et al. 2009; Liu et al. 2009; McKendry unpublished
3B.3	131.5-136.9	SEV, INC	na	Hupei 57	Buerstmayr et al. 2009
4A	87.9	SEV, INC	na	Pirat, Arina	Buerstmayr et al. 2009; Liu et al. 2009;
4B	56.2	INC	MQTL11	IL94-1628, Ernie, Wangshuibai, Becker, Wuhan 1	Loffler et al. 2009; Buerstmayr et al. 2009; Liu et al. 2009;
5B	128.9-140.8	SEV, INC	na	Wangshuibai	Buerstmayr et al. 2009; Liu et al. 2009
5D	Unknown	INC	na	na	na
6A.1	-2.6-2.3	SEV, INC, FDK	na	na	na
6A.2	21.3-26.9	SEV, INC	MQTL16	Dream, Apache, Spark	Loffler et al. 2009; Buerstmayr et al. 2009; Liu et al. 2009;
6A.3	86.5-109	INC, FDK	na	na	na
6B.1	113.3-114.6	INC, FDK	na	na	na
7A.1	90.2	SEV, INC	na	Wangshuibai, Ritmo, NK93604, Romanus, Spark	Buerstmayr et al. 2009; Liu et al. 2009;

¹Meta-QTL number from Loffler et al. 2009

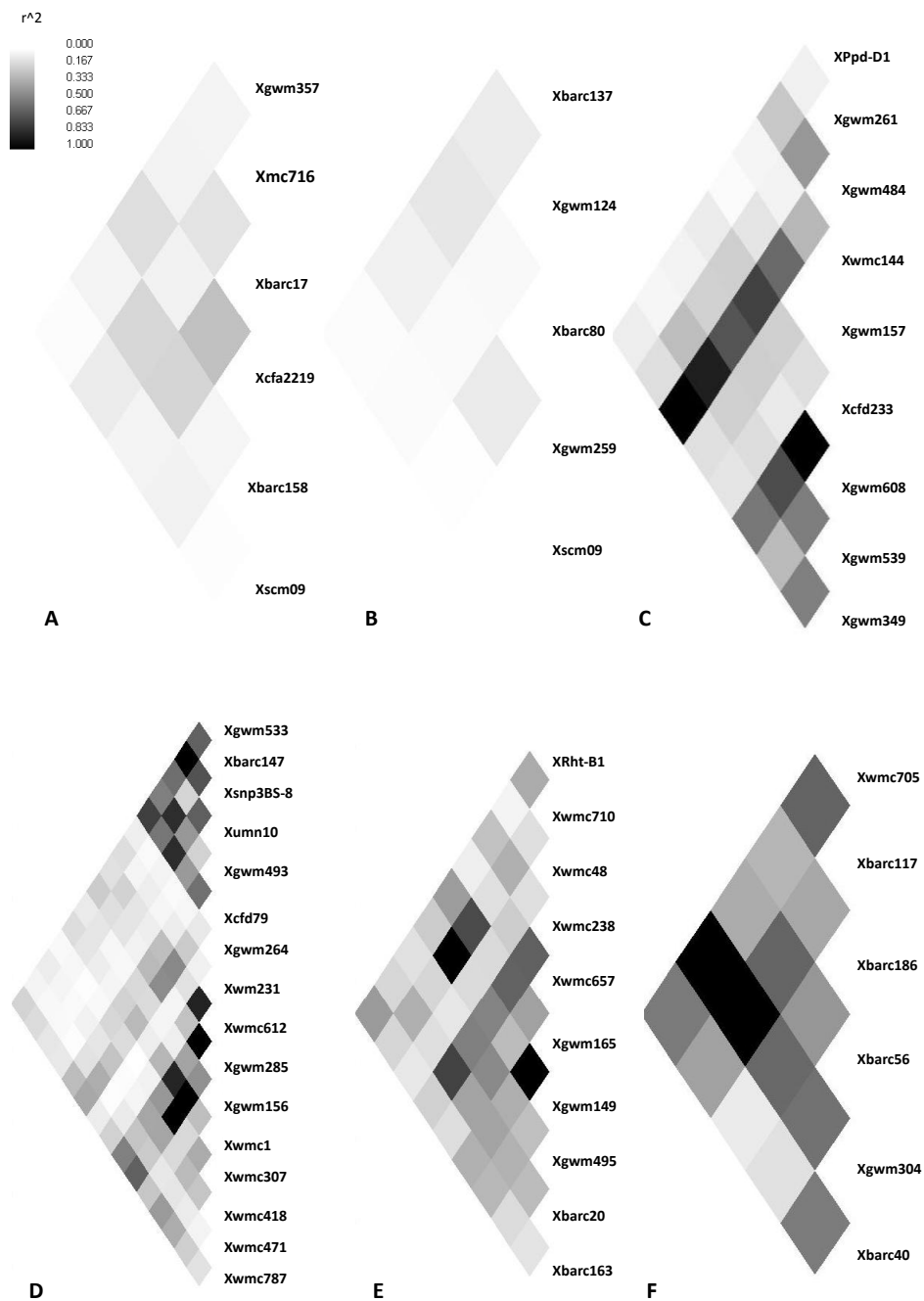


Figure 4.1. Estimation of linkage disequilibrium, D' , among all pair-wise site specific markers on chromosomes A.1A, B. 1B, C. 2D, D, 3B, E. 4B, and F. 5A. Markers are ordered according to the SSR consensus map (Somers et al. 2004).

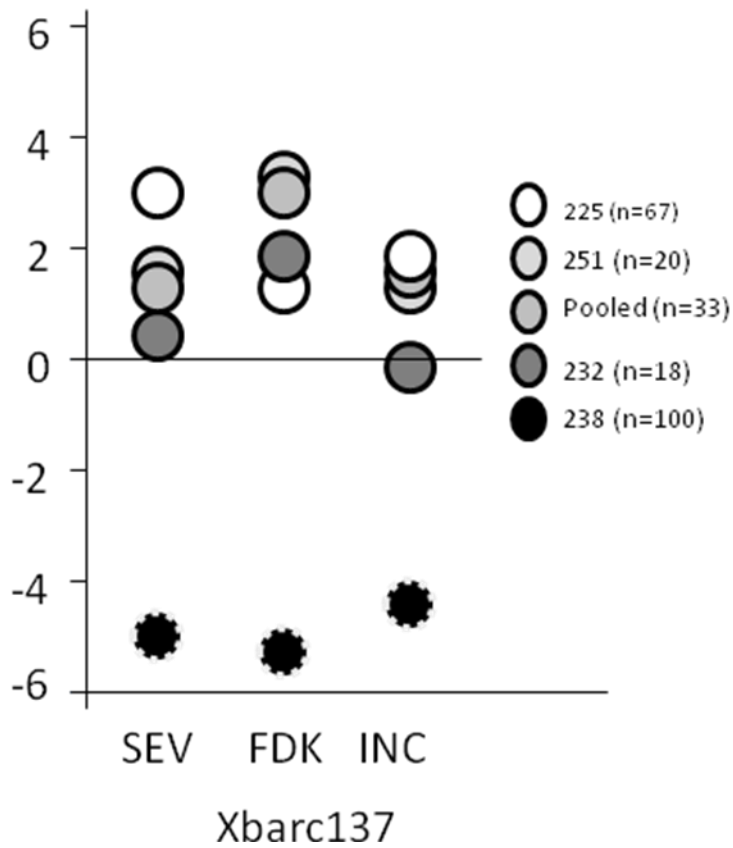


Figure 4.2. The relative allele effects of *Xbarc137* in averaged standardized error units for severity, Fusarium damaged kernels, and incidence to scab. Bubbles are colored coded according to allele size in base pairs and n indicates the numbers of entries possessing that allele. Dotted lines on outside of bubble indicate a significant allele effect.

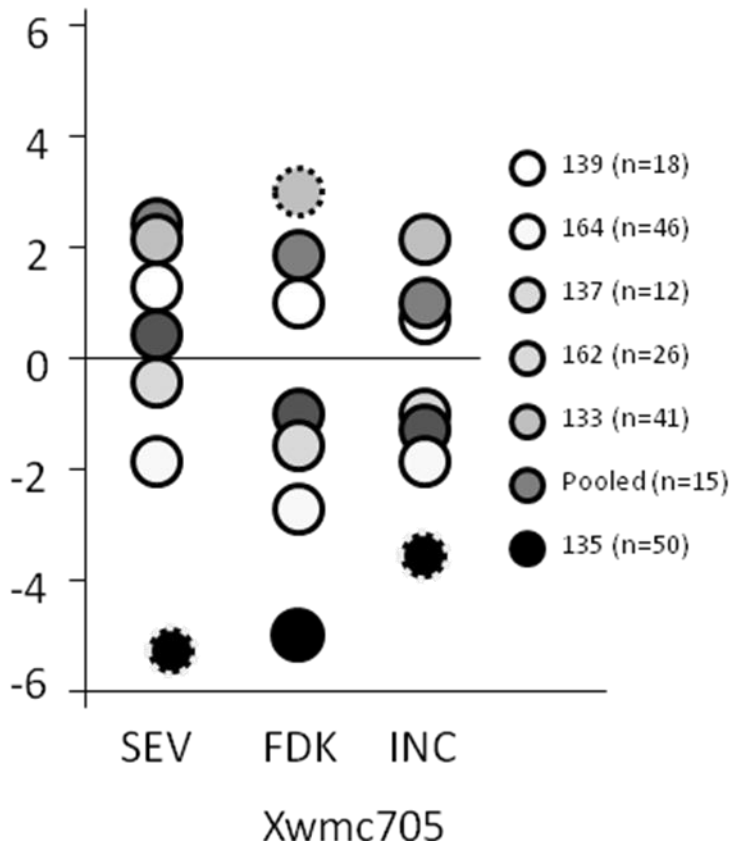


Figure 4.3. The relative allele effects of *Xwmc705* in averaged standardized error units for severity (SEV), Fusarium damaged kernels (FDK), and incidence (INC) of scab. Bubbles are colored according to allele size in base pairs and n indicates the numbers of entries possessing that allele. Dotted lines on outside of bubble indicate a significant allele effect.

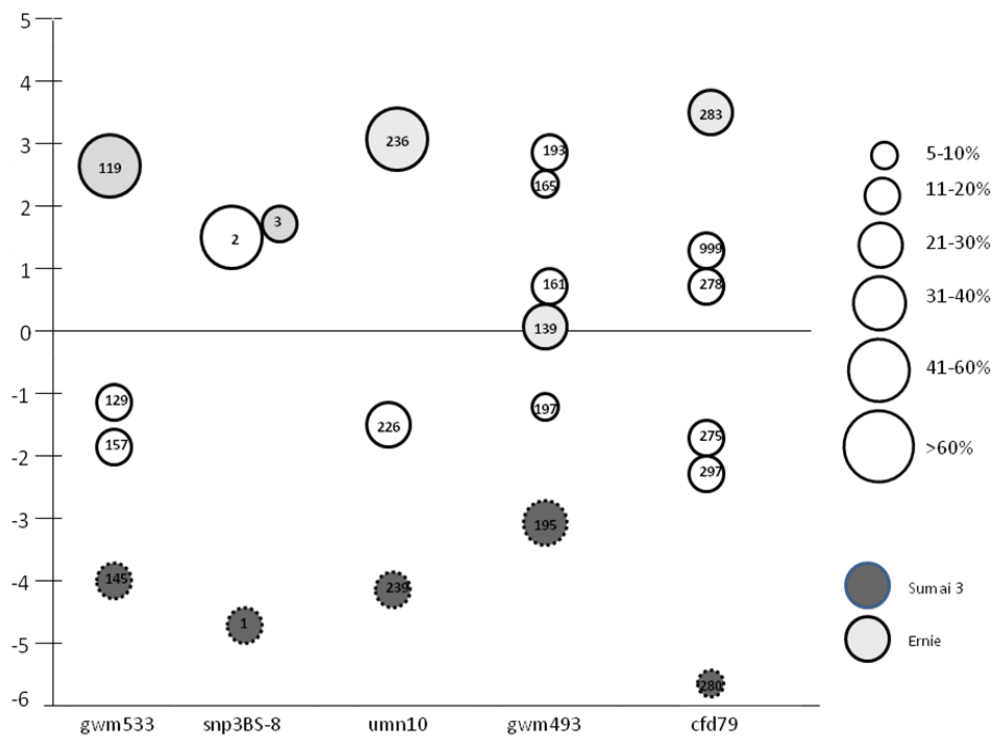


Figure 4.4. The relative allele effects of significant, $p < 0.05$, *Fhb1* markers after FDR correction in averaged standardized error units for severity to scab. Size of bubbles indicates relative allele frequency. Dotted lines on outside of bubble indicate a significant allele effect.

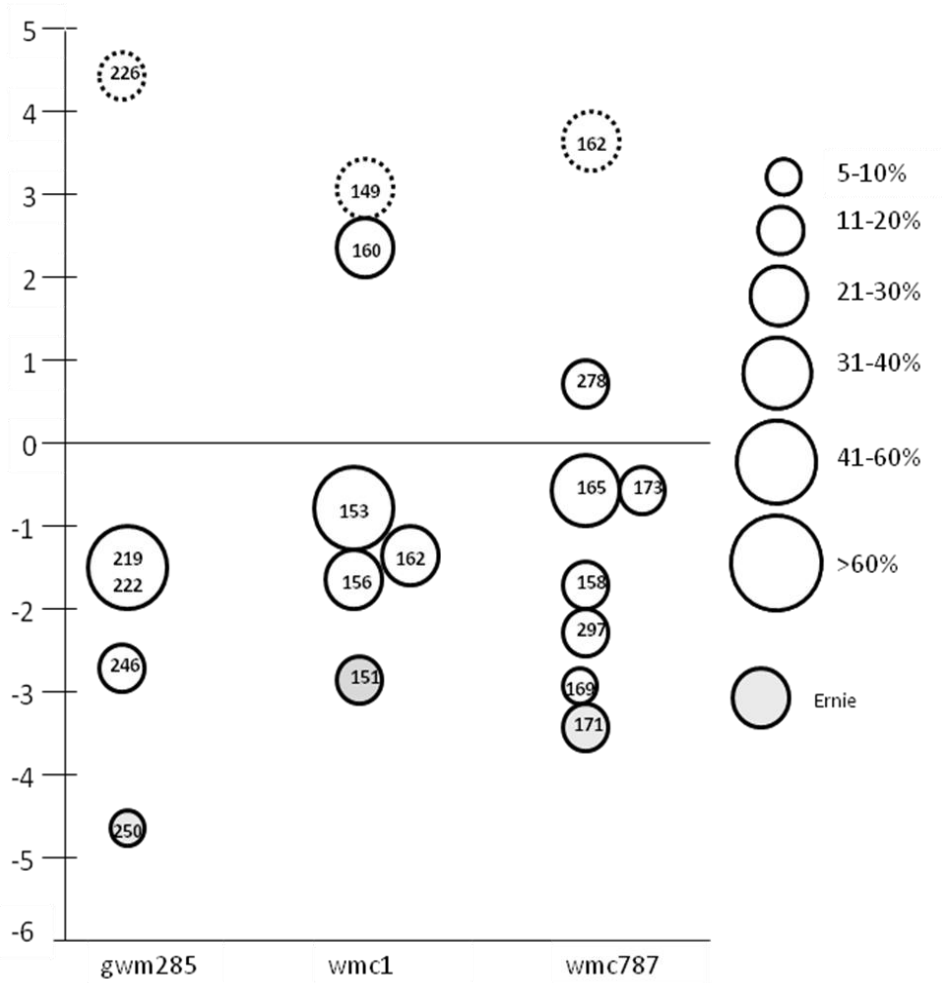


Figure 4.5. The relative allele effects of significant, $p < 0.05$, *Qfhs.umc.3BSc* markers after FDR correction in averaged standardized error units for severisty of scab. Size of bubbles indicates relative allele frequency. Dotted lines on outside of bubble indicate a significant allele effect.

CHAPTER 5. Evaluation of Genomic Selection Models for FHB Resistance in Multi-family Populations of Soft Winter Wheat

Abstract

Genomic selection (GS) is an approach to use the joint effects of all markers across a genome to predict the genomic estimated breeding value (GEBV) of an individual. Without the determination of significance levels, a greater portion of the phenotypic variation due to small effect quantitative trait loci (QTL) can be captured. The use of genomic selection is attractive for polygenic traits in addition to reducing cost and speeding up the deployment of cultivars. The accuracy of genomic selection is affected by trait heritability, marker number, training population size, relationship of lines in estimation to prediction sets, and choice of model. The application of genomic selection to plants has been limited in comparison to animal systems, where the method was originally developed. The intent of this study was to compare the accuracy of phenotypic selection, multiple genomic selection models, and conventional marker assisted selection for Fusarium head blight resistance in an empirical dataset of soft winter wheat. The mean accuracy based on phenotypic selection was higher than genomic selection accuracies in all but one case. Ridge regression and Bayes $C\pi$ produced the highest accuracies for severity among the multiple models tested, including traditional marker assisted selection. Negligible differences in accuracies were found when the number of markers was reduced to 900 from 261. When time and cost are taken into account, the efficiency of GS becomes favorable in comparison with phenotypic selection.

Introduction

Genomic selection (GS) is a marker assisted breeding method that utilizes all markers across the genome and does not determine significance between marker loci and traits (Piepho, 2009). The joint effect of all alleles in relation to trait phenotypes are estimated in an initial population, often termed a training or estimation set. Genomic estimated breeding values (GEBV) can then be determined for selection candidates within a prediction population based upon their genotype and the allele effects calculated in the estimation set (Meuwissen et al., 2001).

Implementation of GS has the potential to reduce the cost of developing cultivars. The cost of densely genotyping lines can be substantially less than the resources that would have been allocated to phenotyping lines in the field (Heffner et al., 2010). An increase in the gain per unit time and a reduction in cost and resources was demonstrated in simulations for corn and wheat crops which incorporate GS over alternative marker assisted selection methods by reducing breeding cycle length (Bernardo and Yu, 2007; Heffner et al., 2010). Conducting GS would mitigate the need to conduct multiple mapping populations for diversity, validate significant markers, evaluating QTL effects in relevant germplasm, and replicated trials over many environments (Heffner et al., 2009). Genetic gains from GS would be most beneficial under circumstances in which phenotypic selection is difficult and costly (Goddard and Hayes, 2007).

The recent focus on association analysis has been to address the aforementioned issues in relation to bi-parental mapping (Brescaglio and Sorrells, 2006b) though issues with population structure (Price et al., 2006) and correcting for multiple hypothesis testing

(Storey and Tibshirani, 2003) limit the capability of association studies to detect QTL. Prediction of line performance would be better with GS than association studies due to a greater capacity to capture small effect QTL (Meuwissen et al., 2001) and avoidance of biased marker effect estimates (Jannink et al., 2010; Lande and Thompson, 1990). Reported estimates of selection accuracy have been greater for GS than for marker assisted selection based on QTL estimates (Bernardo and Yu, 2007; Heffner et al., 2011).

The accuracy (r) of a genomic selection model can indicate the selection response and therefore the rate at which genetic gain can be achieved (Heffner et al., 2009). Accuracy of GS models is measured as the correlation between genomic estimated breeding values (GEBV) and the true breeding value (TBV). While TBV cannot be directly calculated, the estimated breeding value (EBV) allows for a translated estimate of model accuracy. Lorenz et al. (2011) describes the relationship as $r(\text{GEBV:EBV}) = r(\text{GEBV:TBV}) \times r(\text{EBV:TBV})$.

The number and type of marker in conjunction with training population size have been shown to influence GS accuracy (Lorenzana and Bernardo, 2009; Solberg et al., 2008). An increase in the size of an estimation set has been shown to augment accuracy more than an increase in the number of markers. The number of markers needed will be in relation to the size of the training population and number of QTL (Lorenz et al., 2011). A two fold increase in the number of trait loci from 10 to 1000, required a fivefold increase in the number of markers to give an equivalent accuracy (Hayes et al., 2009). The greater number of markers allows for more QTL to be in LD with at least one marker. An r^2 of 0.20 between neighboring markers was sufficient for traits of low heritability, $H^2 \sim 0.1$, and decreased to $r^2 = 0.15$ for high heritability, $H^2 \sim 0.5$ (Calus and Veerkamp, 2007). Increasing marker

saturation will not always improve accuracy; if training populations are not large enough markers can become collinear (Muir, 2007). The choice of GS model will also affect accuracy, and influenced by the number of markers. Ridge regression (RR) and Bayes A (BA) models incorporate all markers whereas Bayes B (BB) and Bayes C π (BC) allow for some markers to have no effect. The BB model will capitalize on greater marker density and the stronger LD between markers and QTL (Meuwissen et al., 2001; Rutkoski et al., 2010). Models that can take greater advantage of LD, such as Bayes, will have accuracies that persist over more generations of selection, since recombination will decay accuracy. Ridge regression is equivalent to a mixed model and can capture genetic relationships (Habier et al., 2007). Because of the ability to simulate a relationship matrix, RR can give greater accuracies than BB when LD is weaker and the density of markers is lower. The performance of RR relative to BB, generated lower accuracies according to Meuwissen et al. (2001) and Habier et al. (2007), although Jannink et al. (2010) suggests that large variances of few QTL was likely the reason why BB outperformed RR in these studies. Several reports have found that RR accuracy is similar or better than Bayesian models (Heffner et al., 2011; Lorenzana and Bernardo, 2009; Zhong et al., 2009). An accuracy of $\frac{1}{2}$ x the square root of the trait heritability has been proposed as the threshold for GS to generate gains exceeding phenotypic selection per unit time, since multiple cycles can be conducted with GS in the same time as phenotypic evaluation (Heffner et al. 2010).

The intent of this study was to determine if GS was a viable option for breeding for scab resistance in wheat. Abundant QTL identified by bi-parental mapping and GWAS indicate that the trait is polygenic. In addition to the quantitative nature of resistance,

phenotyping is complicated by extensive genotype by environment interaction. These criteria suggest that GS would be a viable alternative to traditional marker assisted breeding methods. In this research, a set of 258 lines phenotyped for severity and incidence of scab, were selected from three regional scab screening nurseries and genotyped with DArT markers. Data were used to evaluate the accuracy of MAS using significant markers, and GS accuracy using Bayes A, Bayes B, Bayes $C\pi$ and ridge regression. The effect of different size marker datasets was also tested.

Materials and Method

Phenotypic data

A set of 258 soft winter wheat lines was chosen from entries in the Northern Uniform Winter Wheat Scab Nursery (NUWWSN), Preliminary Northern United Winter Wheat Scab Nursery (PNUWWSN), and the Uniform Southern Fusarium Head Blight Nursery (USFHBN) that were evaluated in 2008, 2009, and 2010. The germplasm was composed of advanced breeding lines enriched for resistance and represents a range of the underlying genetic diversity (Chapter 4). The nurseries are divided such that breeding lines adapted to states in the Southeast are evaluated in the USFHBN. Breeding lines developed by programs in the Northeast and the Midwest are evaluated in the NUWWSN and PNUWWSN. Participating programs evaluated each inoculated nursery to which they contributed entries, at a location within their state, and therefore genotypes were not constant in all locations

Lines from the breeding programs in the Mid-Atlantic (VA and MD) are evaluated in all nurseries. With the exception of the check cultivars and 37 repeated entries, lines were

submitted to one nursery for only one year. Phenotypic data for single nursery grown and year was used for the repeated lines that did occur in multiple years or nurseries. A randomized complete block design was used at all locations, with either two or three replications, depending on the site. The sizes of the plots were one, two, or four rows of approximately one meter in length. Phenotypic data for incidence (INC) and severity (SEV) resistance phenotypes were evaluated (Chapter 4). Incidence (INC) was measured as a percentage of infected heads in a sample. Severity (SEV) was a visual rating of the percent of infected spikelets on infected heads. Best linear unbiased predictors were calculated in SAS 9.2 (SAS Institute, Cary, NC) using PROC MIXED, with entries grouped by environment.

Genotypic Data

Seeds were submitted by nursery coordinators to the Eastern Regional Small Grains Genotyping Center, USDA-ARS Raleigh, NC. A single seed for each entry was germinated and tissue collected for isolation of DNA according to the CTAB protocol of Pallotta et al (2003). Sample preparation for DArT assays was previously described (Chapter 3).

The population was genotyped with ~7,300 DArT markers (DiversityArrays P/L, <http://www.triticarte.com.au/>, Canberra, Australia) as previously described (Chapter 3). A consensus map of DArT markers (DiversityArrays P/L www.diversityarrays.com) was used to position markers to chromosomal locations. Polymorphic DArTs were assessed for redundancy with LD tagSNP at $r^2 > 0.75$ in JMP Genomics 5.0 (SAS Institute, Cary, NC). Sets of loci with an $r^2 > 0.75$ were grouped into bins. A single marker, with the least amount of missing data, was used to represent the other redundant markers in a bin. A further

reduced marker set was constructed from the non-redundant DArTs by selecting only mapped loci that were spaced at least two cM from the next adjoining marker.

Training and Validation set construction

The average of three cross validation datasets were used for determining genomic selection accuracy of a model. The three different datasets were based upon the 2008, 2009, and 2010 regional scab screening nurseries. A cross validation set was composed of all entries evaluated for one given year, thus giving three possible cross validation sets. The remaining two years of data was used to compose the training population (Table 5.1). Missing genotypic data was substituted by the mean frequency for that marker for a given dataset.

Statistical analysis

Two genomic selection models, ridge regression (RR) and Bayes C_{π} , were compared for accuracy in relation to severity and incidence. The two genotypic datasets were evaluated for each trait and statistical model to assess if a reduced marker panel would be feasible to conduct genomic selection via regional USDA-ARS genotyping centers. The panels consisted of 900 non-redundant DArTs, as determined by $r^2 < 0.75$. The second genotypic data set was a further reduction into 261 loci spread across the genome that were spaced at least two cM apart. A comparison of accuracies for P, RR, BA, BB, BC, and association analysis (AA) models was carried out only for severity due to computational demand.

Each model was analyzed on all three training/validation set combinations. The analysis was performed in the statistical software package R 2.12.1 (Hornik, 2011). Ridge regression BLUPs were determined with the package EMMA

(<http://mouse.cs.ucla.edu/emma/>). The BA, BB, and BC code were adapted from Dekkers et al. (2009), with BC modifications provided by Dr. Mark Sorrells. Each Bayes model was set to 2500 iterations with the probability of marker effects being equal to zero (π) of 0, 0.95, and 0.5 respectively.

Prediction Accuracy

The accuracy of phenotypic selection was assessed by randomly splitting the environments for each year into two groups and correlating the observed phenotypes of lines between groups PROC CORR in SAS 9.2 (SAS Institute, Cary, NC). The accuracy of GS models was determined by the correlation of predicted GEBV of lines in the prediction set by the observed phenotypes of the prediction set. The GEBV from AA were determined from regression coefficients of multiple linear regression models, which selected the best significant ($p < 0.15$) fitting markers from significant results of association analyses (Chapter 4).

Results

The accuracy for phenotypic selection ranged from 0.37 for INC in 2008 to 0.72 for SEV in 2010. Except for the 2008 validation of INC, phenotypic accuracies exceeded genomic selection accuracies for RR and BC regardless of the number of markers in the genotypic dataset (Table 5.2). In all but one instance, accuracies for RR were slightly better than BC for predicting SEV, particularly with larger numbers of markers. Accuracies of BC and RR were similar for INC, except for the 2010 validation set where BC had higher accuracies with both marker sets. Generally, the inclusion of more markers resulted in small increases in accuracy over the smaller genotypic dataset (Table 5.2). However, in three cases

(2009-RR, 2010-BC, and 2008 BC) a slight increase in accuracy was observed with the smaller number of marker loci. Identical accuracies occurred for both datasets in two models (2008 & 2010-RR).

The mean phenotypic accuracy for SEV across all three validation sets was also higher than that observed with the other Bayes models, BA and BB (Figure 5.1). The selection model including only markers from the AA was the least accurate ($r = 0.38$), followed by BB and BA. Overall, the GS models with the highest mean accuracy was RR and BC.

Discussion

Genomic selection is a marker assisted breeding technique that does not focus on identifying underlying genetic architecture of traits nor significant markers. The lack of assigned p values allows for greater phenotypic variation to be captured and explained by marker loci. This feature is ideal for quantitative traits such as Fusarium head blight which have moderate heritability and are costly and difficult to phenotype. While our results indicate that selection based on phenotype remains the most accurate approach to improve scab resistance, other factors such as time and costs come into play when evaluating efficiency. In addition, r_P in this study is inflated since data from different locations in the same year are correlated to determine r_P , where as the cross validation sets for determining r_{GS} are for lines evaluated in different years from the estimation sets. While a corrected r_{GS}

can be determined by rGS/h (Heffner et al. 2011), the unbalanced nature of our dataset makes comparisons of heritability unreliable.

The performance of RR and BC were equivalent for several scenarios or had slight differences of one or two percentage points in all but three comparisons. While RR produced greater accuracies over all other methods, the performance of RR over BC was marginal. The BC model would prove superior to RR if selection was being conducted in several generations removed from the training population since BC exploits LD between marker and QTLs better.

Number of marker used for GS had little effect on accuracy. The small training populations and effective population size are two possible reasons why more markers are not capitalized upon to capture a larger portion of the phenotypic variance (Fernando et al., 2007; Muir, 2007). Our results suggest that genomic selection can be done with a minimal number of bi-allelic markers such as DArTs for improving scab resistance.

The accuracies observed for GS for SEV using RR and BC ranged from 0.48 to 0.60 and were greater than 0.50 in all but one validation set. When factors such as heritability, relative costs of genotyping versus evaluation of FHB resistance in the field, and the number of cycles of selection per year are taken into account, the efficiency of GS becomes favorable in comparison with phenotypic selection.

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Table 5.1 The year(s) and number of lines in training and validation sets.

Training Set Composition	No. of lines	Validation set	No. of lines
2008/2009	211	2010	47
2008/2010	135	2009	123
2009/2010	174	2008	84

Table 5.2 Accuracy (r) of phenotypic evaluation (P), ridge regression (RR), and Bayes C π (BC) for severity and incidence.

Cross Validation Set	Severity					Incidence				
	rP	900 DArT		261-DArT		rP	900 DArT		261-DArT	
		rRR	rBC	rRR	rBC		rRR	rBC	rRR	rBC
2008	0.55	0.53	0.48	0.52	0.50	0.37	0.40	0.41	0.40	0.40
2009	0.63	0.54	0.51	0.56	0.55	0.67	0.47	0.47	0.46	0.46
2010	0.72	0.57	0.55	0.52	0.60	0.58	0.47	0.55	0.47	0.49
Mean	0.64	0.55	0.51	0.53	0.55	0.54	0.45	0.48	0.44	0.45

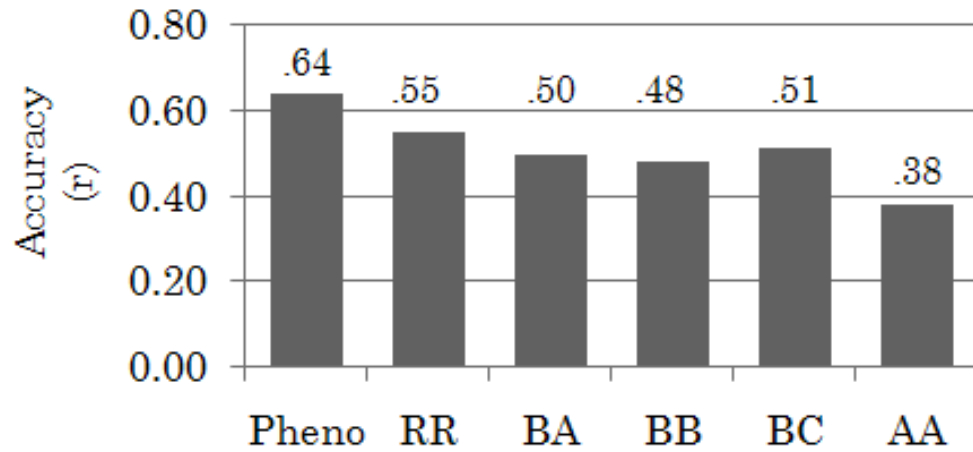


Figure 5.1 Average SEV prediction accuracy for phenotypic (Pheno), ridge regression (RR), Bayes A (BA), Bayes B (BB), and Bayes $C\pi$ (BC), and marker assisted selection (AA) across validation sets. GS accuracies were based upon 900 DArT loci, AA was determined from markers identified by multiple linear regression on significant results from association analysis

APPENDIX

Appendix A. Name, pedigree and developing program of 258 lines from the Uniform Southern Fusarium Head Blight Nursery (USFHBN), Northern Uniform Winter Wheat Scab Nursery (NUWWSN), and Preliminary Northern Uniform Winter Wheat Scab Nursery (PNUWWSN) grown over the 2008, 2009, and 2010 seasons.

Entry	Pedigree	Developing Program	Year Evaluated	Nursery Evaluated
AR97002-10-2	AR 369-4-2 / NING 8026	U. of Arkansas	2008	USFHB
AR97002-2-1	AR 369-4-2 / NING 8026	U. of Arkansas	2008, 2009	USFHB
AR97124-4-2	P88288C1-6-1-2 / TERRA SR204	U. of Arkansas	2008	USFHB
AR99028-1-1	Sabbe / P89204A8-1-59	U. of Arkansas	2008	USFHB
AR99039-5-2	ARR664-21-1 / NC97BGTD7	U. of Arkansas	2009	USFHB
AR99054-4-1	AR679-9-1-2 / Roane	U. of Arkansas	2009	USFHB
AR99071-7-2	AR 682-11-1-1 / FFR522W	U. of Arkansas	2009	USFHB
AR99254-7-1	P86300RB1-4-3-2-104 / NC97BGTAB9	U. of Arkansas	2009	USFHB
AR99263-7-1	P2118b4 / Jaypee	U. of Arkansas	2009, 2008	USFHB
ARGE97-1048-6	Mason//Sha 3/Catbird	U. of Arkansas	2009	USFHB
GA031307-DH14	AGS 2000 / VA01W-461	U. of Georgia	2008	USFHB
GA031354-DH30	VA01W-461 / USG 3592	U. of Georgia	2008	USFHB
GA031454-DH31	VA01W-461 / USG 3592	U. of Georgia	2008	USFHB
GA031454-DH38-7	VA01W-461 / USG 3592	U. of Georgia	2009	USFHB
GA031454-DH7	VA01W-461 / USG 3592	U. of Georgia	2008	USFHB
GA981621-5E34	AGS 2485 / P26R61	U. of Georgia	2008	USFHB
GA991109-1-G1	ERNIE / PION 2684 // 901146-4E-15	U. of Georgia	2009	USFHB
GA991109-6E8	Ernie / Pio 2684 // 901146	U. of Georgia	2008	USFHB
GA991209-6E33	901146 / 96004 // AGS2000	U. of Georgia	2009	USFHB
GA991371-6E12	GA931521 / 2*AGS 2000	U. of Georgia	2008	USFHB
LA01141D-98-6-2	LA841/PI225160/LA841	Louisiana State U.	2009	USFHB
LA01162D-131-8	LA422/CIM1FHBN#5//PIONEER 26R61	Louisiana State U.	2009	USFHB
LA01162D-136-8	LA422/CIM1FHBN#5//PIONEER 26R61	Louisiana State U.	2009	USFHB
LA01164D-43-7-B	LA422/FUTAI8944//PIONEER 26R61	Louisiana State U.	2009	USFHB
LA01164D-94-2	LA422/FUTAI8944//PIONEER 26R61	Louisiana State U.	2009	USFHB
LA03187C-2	LA97447D-9-2/LA95181BUB6-1	Louisiana State U.	2009	USFHB
NC05-19896	BURR / NC96BGTA6 SIB // NATCHEZ	North Carolina State U.	2008	USFHB
NC05-20671	P92188(SN) / NC95-22365 // ROANE	North Carolina State U.	2009	USFHB
NC05-21937	SHAAN 85-15 (FHB) / SS 520 // NC-NEUSE	North Carolina State U.	2009	USFHB
NC05-23015	BURR / NC96BGTA6 SIB // NATCHEZ	North Carolina State U.	2009	USFHB

Appendix A Cont.

Entry	Pedigree	Developing Program	Year Evaluated	Nursery Evaluated
NC05-25066	SHAAN85-15 / SS 520 // NC-NEUSE(SCAB)	North Carolina State U.	2008	USFHB
NC06-20288	JACKSON / NC95-25305 // NC96-13965	North Carolina State U.	2009	USFHB
NC07-22927	P2580 / 20 // NC-Neuse	North Carolina State U.	2009	USFHB
NC07-23170	NC-Neuse // PI 531193 (JG1) / ROANE	North Carolina State U.	2009	USFHB
B030543	VA93-54-429/LA85422	Syngenta	2009, 2008	USFHB
D05*6437	PIO2552/L96*9266	Syngenta	2008	USFHB
ARS03-3806	X94-748-2-2/TAM 301	USDA-NC	2009	USFHB
ARS03-4736	KS00U755/TX98D1170	USDA-NC	2009	USFHB
ARS03-5358	Pat/TX98D2106	USDA-NC	2009	USFHB
ARS04-1249	Lakin/KS2023-U18	USDA-NC	2009	USFHB
ARS05-0242	Coker 9835/RL6042//TX99D4657	USDA-NC	2009	USFHB
ARS05-0443	Neuse/TX98D2334	USDA-NC	2009	USFHB
ARS05-1044	KS2132-U138/Trego	USDA-NC	2009	USFHB
ARS05-1234	KS2016-U2/Lakin	USDA-NC	2009	USFHB
MD01W233-06-16	McCormick/Choptank	U. of Maryland	2008	USFHB, NUWWSN,
MD01W255-08-1	Roane/MD71-19	U. of Maryland	2009	USFHB
MD02W81-08-2	Freedom/Ning7840//VA97W533	U. of Maryland	2009	NUWWSN
MD02W81-08-6	Ning7840/Freedom//VA97W533	U. of Maryland	2009	USFHB
MD03W61-09-1	25R42/CHESAPEAKE	U. of Maryland	2010	NUWWSN
MD03W91-09-8	25R42/TRIBUTE	U. of Maryland	2010	NUWWSN, NUWWSN,
MD99W483-06-11	VA97W358/RENWOOD3260	U. of Maryland	2008	USFHB
TRIBUTE	VA98W-593=VA92-51-39(IN71761A4-31-5-48//71-54-147/MCN1813)/AL870365 (CK747*2/Amigo)	Virginia Polytechnic U.	2008	USFHB
VA04W-360	French Line VR95B717/Roane//VA96W-391 (IN81401A1-32-2/ CK9803)	Virginia Polytechnic U.	2008	USFHB
VA04W-90	SS 520(96-54-158=FFR555W/ GORE) /PION2552//ROANE (VA93-54-429),F11	Virginia Polytechnic U.	2009	PNUWWSN, USFHB
VA05W-425	Roane/3/Ning7840/Coker9904//Pioneer2552	Virginia Polytechnic U.	2008	NUWWSN
VA05W-510	Roane / Pion 2684 // OH 552	Virginia Polytechnic U.	2009	USFHB
VA05W-534	Goldfield/Tribute//Gibson	Virginia Polytechnic U.	2009	USFHB
VA05W-640	RENWOOD 3260*2//W14/RENWOOD 3260/3/RENWOOD 3260,BC3F7	Virginia Polytechnic U.	2009	USFHB
VA05W-641	Renwood 3260*2//Futai8944/Renwood 3260/3/Renwood 3260	Virginia Polytechnic U.	2008	USFHB
VA05W-777	3007-8-12-2 (Roane*2//W14/Roane /3/Roane), BC3F6	Virginia Polytechnic U.	2008	PNUWWSN
VA06W-553	Roane/3/Ning7840/Coker9904//Pioneer2552	Virginia Polytechnic U.	2008	PNUWWSN
VA06W-558	VA96W-348/P92823A1-1-4-4-5(Clark*4/ Ning7840)// McCormick	Virginia Polytechnic U.	2008, 2009	PNUWWSN, NUWWSN
VA06W-561	OH618//Roane/Sisson"S" (VA96W234)	Virginia Polytechnic U.	2008	PNUWWSN
VA06W-578	Roane / Pion 2684//OH 552 (P71761A4-31-5-33/MD55-286-21: FHB-RES),F9	Virginia Polytechnic U.	2009	PNUWWSN

Appendix A Cont.

Entry	Pedigree	Developing Program	Year Evaluated	Nursery Evaluated
VA06W-587	ROANE//OH 552(P71761A4-31-5-33/MD55-286-21: FHB-RES)/AGS 2000 (UGA89482E7=P2555/ PF94301//FL302),F8	Virginia Polytechnic U.	2009	USFHB
VA06W-608	Freedom/Neuse"S" (NC96-13374)//RC-Strategy (VA98W-586)	Virginia Polytechnic U.	2008	USFHB
VA06W-612	FREEDOM/ NEUSE"S" (NC96-13374)// VA98W- 688[ROANE"S" (91-54-219) //FFR555W/GORE],F9	Virginia Polytechnic U.	2010	NUWWSN
VA06W-615	Roane/OH 552//RC-Strategy (VA98W-586)	Virginia Polytechnic U.	2008, 2009	PNUWWSN, NUWWSN
VA06W-622	IL89-6489/Sisson"S" (VA97W-375)// Ernie	Virginia Polytechnic U.	2008	PNUWWSN
VA07W-568	Roane / Ernie//McCORMICK,F9	Virginia Polytechnic U.	2009	USFHB
VA07W-580	Goldfield (P89118RC1-X-9-3-3-1= INW9241/P79410D1-3//CLARK: FHB- RES)/TRIBUTE//IL4162,F8	Virginia Polytechnic U.	2009	NUWWSN
VA07W-591	FREEDOM/NC96-13374 // RC-STRATEGY,F8	Virginia Polytechnic U.	2009	PNUWWSN
VA07W-594	FREEDOM/ NEUSE"S" (NC96-13374) // RC- STRATEGY,F9	Virginia Polytechnic U.	2010	NUWWSN
VA07W-600	OH 552/SS550//RC-STRATEGY,F9	Virginia Polytechnic U.	2009	NUWWSN
VA07W-601	OH 552/ SISSON"S" (SS550)// RC-STRATEGY,F9	Virginia Polytechnic U.	2010	NUWWSN
VA07W-607	IL89-6489(PIONEER 9021L// ROLAND/IL77-2656: FHB-RES)/ Sisson"S" (VA97W-375= CK9803/FREEDOM)// ERNIE,F8	Virginia Polytechnic U.	2009	USFHB
VA07W-643	COKER 9474(SCAB RES)/ McCormick"S" (VA98W- 590),F7	Virginia Polytechnic U.	2009	PNUWWSN
VA07W-672	RENWOOD 3260*2//W14/ RENWOOD 3260 (VA96W-326)/3/ RENWOOD 3260,BC3F7	Virginia Polytechnic U.	2009	NUWWSN
VA08W-734	COKER 9474(FHB-RES)/ IL97-2945 (FHB-RES) //TRIBUTE,F7	Virginia Polytechnic U.	2010	NUWWSN
NW07505	Trego/Thunderbolt F3	Cornell U.	2010	NUWWSN
NY03179FHB-10	NY7387/Caledonia//Caledonia-2///Caledonia	Cornell U.	2009	NUWWSN
NY03179FHB-12	NY7387/Caledonia//Caledonia-2///Caledonia	Cornell U.	2009	NUWWSN
NY03180FHB-10	NY7387/Caledonia//Caledonia-2///Caledonia	Cornell U.	2009	NUWWSN
NY88046-7088	MD286-21/Harus	Cornell U.	2010	NUWWSN
NY93246SP-9070	Harus/3/92145:91009(Geneva/U1273-5-18- 8)/NY73116-4W	Cornell U.	2008	NUWWSN
NY94052-9340	Pio2737w/Harus	Cornell U.	2008, 2010	NUWWSN
NY99045-3110	Geneva/P2737W	Cornell U.	2010	NUWWSN
NY99068-3251	NY87048W-7387/P25W33	Cornell U.	2010	NUWWSN
NYCalresel-L	Reselection from Caledonia	Cornell U.	2008	NUWWSN
NYW103-102-9103	Cayuga/ Caledonia	Cornell U.	2009	NUWWSN
NYW103-21-9183	Cayuga/ Caledonia	Cornell U.	2009	NUWWSN
NYW103-70-9232	Cayuga/Caledonia	Cornell U.	2008	NUWWSN
ACF12004	No pedigree	U. of Guelph	2009	NUWWSN
ACF126103	Movokrimka/Arina	U. of Guelph	2010, 2009	NUWWSN
ACF213003B	Harding/TF174	U. of Guelph	2010, 2009	NUWWSN
DH22/24	Ruby/Frontana #1 x AC RON/ Ruby/Frontana #1	U. of Guelph	2008	NUWWSN
DHF/SF,23	Frontana x Sumai 3	U. of Guelph	2008	NUWWSN

Appendix A Cont.

Entry	Pedigree	Developing Program	Year Evaluated	Nursery Evaluated
GS-0-EM0681	Radiant/FE9	U. of Guelph	2010	NUWWSN
GS-1-EM0362	GS-0-EM0672/Radiant = FE9/2* <i>Radiant</i>	U. of Guelph	2010	NUWWSN
RCUOGtr35	No pedigree	U. of Guelph	2009	NUWWSN
MSULineE3024	980775,(Caledonia, Geneva / Geneva)//NY85020-395	Michigan State U.	2010	NUWWSN
MSULineE5011	Caledonia / NY88024-117	Michigan State U.	2008	NUWWSN
MSULineE5011B	Caledonia / NY88024-117	Michigan State U.	2010	NUWWSN
MSULineE5024	D6234 / Pioneer 25W33	Michigan State U.	2009	PNUWWSN
MSULineE6001	Pioneer 25W60 / CJ 9306	Michigan State U.	2008	NUWWSN
MSULineE6002	VA96W-403-WS / CJ9403	Michigan State U.	2008	NUWWSN
MSULineE6003	VA96W-403-WS / W14	Michigan State U.	2008, 2009	NUWWSN
MSULineE6012	Caledonia / Pioneer Brand 25W33	Michigan State U.	2010	NUWWSN
MSULineE6038	VA96W-403-WS / CJ9403	Michigan State U.	2008	PNUWWSN
MSULineE6059	D9070 / Pioneer 2552	Michigan State U.	2008	PNUWWSN
MSULineE7035R	MSU Line D6234 / W14	Michigan State U.	2009	NUWWSN
MSULineE8052	P2552 / D8006	Michigan State U.	2010	NUWWSN
IL01-34159	IL84-2191 / IL87-2834 // IL90-6364 / IL96-24851 (= IL90-6364 // IL90-9464 / Ning 7840)	U. of Illinois	2008	PNUWWSN
IL02-19463	Patton / Cardinal // IL96-2550	U. of Illinois	2008	NUWWSN
IL04-10118	IL95-2516/ IL98-12212	U. of Illinois	2008	NUWWSN
IL04-10721	IL95-4162/ IL97-7010	U. of Illinois	2009, 2008	NUWWSN
IL04-10741	IL95-4162/ IL97-7010	U. of Illinois	2009, 2008	NUWWSN
IL04-11003	IL96-3073/ Roane	U. of Illinois	2009	PNUWWSN
IL04-17204	IL97-3578/ Ernie	U. of Illinois	2008	PNUWWSN
IL04-17762	IL97-3578/ IL97-7010	U. of Illinois	2009	PNUWWSN
IL04-24668	IL98-13404/ IL97-3578	U. of Illinois	2010	NUWWSN PNUWWSN,
IL04-7874	G65201/ IL98-12212	U. of Illinois	2008, 2009	NUWWSN
IL04-7942	G65201/ IL98-12212	U. of Illinois	2009	NUWWSN
IL04-8445	IL94-1653/ IL97-3578	U. of Illinois	2008	PNUWWSN
IL05-15079	NEL-1538/ KY93C-38-17-1	U. of Illinois	2009	PNUWWSN
IL05-27333	IL96-24851-1/ IL97-3574// IL97-3950	U. of Illinois	2009	PNUWWSN
IL05-27522	IL96-24851-1/ IL97-3574// IL99-2536	U. of Illinois	2009	PNUWWSN
IL06-14262	IL00-8530/ IL97-1828	U. of Illinois	2010	NUWWSN
IL06-7550	IL97-3632/ IL98-4632	U. of Illinois	2010	NUWWSN
IL79-002T-B-B	IL94-6727 / IL96-6472	U. of Illinois	2008	PNUWWSN
KY00C-2059-16	91C-170-3/2552	U. of Kentucky	2008	NUWWSN
KY00C-2059-19	KY91C-170-3/2552	U. of Kentucky	2009	NUWWSN
KY00C-2143-08	90C-048-59/90C-160-14	U. of Kentucky	2008, 2009	NUWWSN
KY00C-2567-01	SS 520/25W33	U. of Kentucky	2009	NUWWSN
KY00C-2755-03	2552/Allegiance	U. of Kentucky	2008	NUWWSN

Appendix A Cont.

Entry	Pedigree	Developing Program	Year Evaluated	Nursery Evaluated
KY02C-3005-25	25R18/MCCORMICK	U. of Kentucky	2009	PNUWWSN
KY02C-3005-44	25R18/MCCORMICK	U. of Kentucky	2008	PNUWWSN
KY02C-3006-46	25R18/VA97W-375WS	U. of Kentucky	2010	NUWWSN
KY02C-3007-45	25R18/Allegiance	U. of Kentucky	2009	PNUWWSN
KY02C-3008-01	25R18/92C-0010-17	U. of Kentucky	2010	NUWWSN
KY03C-1192-34	KY93C-0876-66//KY96C-0059-21	U. of Kentucky	2010	NUWWSN
KY03C-2170-24	VA01W-476/Roane	U. of Kentucky	2009	PNUWWSN
KY04C-2151	25R18/VA01W-476	U. of Kentucky	2010	NUWWSN
KY97C-0321-05-2	Kristy/VA94-52-25//2540	U. of Kentucky	2008	NUWWSN
KY99C-1205-06-1	25R26/ USG 3209//2540	U. of Kentucky	2008	PNUWWSN
BESS	MO 11769/Madison	U. of Missouri	2008, 2009	USFHB
ERNIE	Pike/3/Stoddard/Blueboy//Stoddard/D1707	U. of Missouri	All	All PNUWWSN, NUWWSN
MO041020	960429/960112	U. of Missouri	2008, 2009	NUWWSN
MO041687	MO 960304/MO 960815	U. of Missouri	2009	PNUWWSN
MO050101	MO 11769/Madison	U. of Missouri	2008, 2009	NUWWSN
MO050143	MO 11769/Madison	U. of Missouri	2008	NUWWSN
MO050144	MO 010708 RS	U. of Missouri	2009	NUWWSN
MO050219	MO 010708 RS	U. of Missouri	2009	NUWWSN
MO050261	MO 94-182/VA 91-54-219	U. of Missouri	2008	PNUWWSN
MO050617	960815/IL 91-14163	U. of Missouri	2008	PNUWWSN
MO050699	950016/3/950016//90X54-1-1/MO 91-1009	U. of Missouri	2008	NUWWSN
MO050771	MO 960120/MO 960304	U. of Missouri	2009	NUWWSN
MO050917	Truman 'S'/960815	U. of Missouri	2008	PNUWWSN
MO050921	Ernie/Truman "S"	U. of Missouri	2008, 2009	NUWWSN
MO051150	960815/IL 91-14163	U. of Missouri	2008	PNUWWSN
MO071411	MO 980429/P86958RC4-2-1-1-10	U. of Missouri	2009	NUWWSN
MO071722	MO 980429/Ernie	U. of Missouri	2009	PNUWWSN
MO080104	L910097/MO-92-599	U. of Missouri	2010	NUWWSN
MO080864	981020//P92201D5-2/980725	U. of Missouri	2010	NUWWSN
MO081652	PL-2552/980829	U. of Missouri	2010	NUWWSN
TRUMAN	MO11769/Madison	U. of Missouri	All	PNUWWSN
FREEDOM	GR876/4/Logan*3/3/Va63-52-12/Logan/Blueboy	Ohio State U.	All	PNUWWSN
OH02-12678	FOSTER/HOPEWELL//OH581/OH569	Ohio State U.	2008	NUWWSN
OH02-12686	FOSTER/HOPEWELL//OH581/OH569 OH581/IN83127E1-24-5-2-1-31//5088B-D-32- 1/OH601	Ohio State U.	2009	PNUWWSN
OH02-13567	OH552/HOPEWELL	Ohio State U.	2008	NUWWSN
OH03-235-2	IL91-14167/OH599	Ohio State U.	2008	NUWWSN
OH03-41-45	P.92227C5-1-1/BL930390	Ohio State U.	2008	PNUWWSN

Appendix A Cont.

Entry	Pedigree	Developing Program	Year Evaluated	Nursery Evaluated
OH04-264-58	OH645/HOPEWELL	Ohio State U.	20092010	NUWWSN
OH04-268-39	HOPEWELL/VA96-54-372	Ohio State U.	2008, 2009	PNUWWSN, NUWWSN
OH05-101-1	HOPEWELL/PIONEER 25R26	Ohio State U.	2009	NUWWSN
OH05-152-68	OH685/PATTON	Ohio State U.	2009	PNUWWSN
OH05-164-76	PIONEER 25R18/OH686	Ohio State U.	2009	PNUWWSN
OH05-200-74	OH629/HOPEWELL	Ohio State U.	2009	PNUWWSN
OH05-248-38	OH685/OH686	Ohio State U.	2009	NUWWSN
OH05-249-32	OH685/OH686	Ohio State U.	2009	PNUWWSN
OH05-72-6	PIONEER 25R18/VA97W-375 (Vahart / Frondoso /5/ Vahart /4/(KY4097-37 , Citr12658 , Frondoso /3/ Trumbull // Hope / Hussar) /6/ Asosan /7/ Norin 10 / Brevor /8/(VA55-16-23 , Citr13351 , Supreza / Fultz /5/ Kawvale /4/ Fultz / Hungarian // Illinois No. 1/ Wabash /3/ TrumBull *3// Hope / Hussar) , VA66-54-10) /9/ Arthur , IL71-5662) / 10 /(W9018A , Pioneer Line W521 / Pioneer S76)/ 11 /(W689D-2 , Coker 68-15 /5/(MO7510 , Etoile de Choisy // Thorne / Clarkan /4/ Pawnee /3/(Pd3848A5- 5-1 - 26 , Citr12454 , Trumbull / W38 // Fultz / Hungarian))	Ohio State U.	2009	PNUWWSN
PIONEER2545		Pioneer	All	PNUWWSN, NUWWSN
01946A1-16-48-5	981477/981128//INW0304/981250	Purdue U.	2010	NUWWSN
059A1-2-4-3	Truman/INW0316/4/9819/3/Freedom//Ernie/INW9824	Purdue U.	2010	NUWWSN
99691A2-5-4-16-1	NC97BGTD8/Patterson//92212/3/9560	Purdue U.	2010	NUWWSN
P.011010A1-15	97395/981129//INW0316	Purdue U.	2008	NUWWSN
P.0128A1-22-22	L4/Foster/4/Gfd/X117/3/VA54-429//92145	Purdue U.	2009	NUWWSN
P.0172A1-12-1	97395/981129	Purdue U.	2008	PNUWWSN
P.0175A1-37-4	981419/97397	Purdue U.	2008	PNUWWSN
P.0179A1-17	Fdm/Gfd//92829/Patton	Purdue U.	2008	NUWWSN
P.03112A1-7-3	97395//INW0315/99794	Purdue U.	2008	NUWWSN
P.03615A1-4-4	Ernie/INW0316//981358/97462	Purdue U.	2009	NUWWSN
P.03630A1-18	99751/INW0315//981358/97462	Purdue U.	2008	PNUWWSN
P.04281A1-4-5	INW0304/9811//92823/Ernie	Purdue U.	2008	PNUWWSN
P.04287A1-16	INW0316*2//INW0304//9346/CS5A	Purdue U.	2008	PNUWWSN
P.04704A1-2-1-1	INW0316*2//Ernie/9346	Purdue U.	2009	NUWWSN
P.0513A1-2-3	Truman/INW0731//Fdm/F201R	Purdue U.	2008	PNUWWSN
P.05218A1-6-31	INW0304/9346//97395/INW0411	Purdue U.	2009	PNUWWSN
P.0527A1-9-15	99751/2754//97462/INW0412	Purdue U.	2009	PNUWWSN
P.0537A1-7-12	INW0411/2754//INW0412/98134	Purdue U.	2009	NUWWSN
P.053A1-6-7	2754/INW0412/Truman/INW0303	Purdue U.	2009	NUWWSN
P.0570A1-7-6	9017/92823//F201R/04302	Purdue U.	2009	PNUWWSN
P.992192A1-5-4-5-81	92145//201R/Patton	Purdue U.	2008	PNUWWSN

Appendix A Cont.

Entry	Pedigree	Developing Program	Year Evaluated	Nursery Evaluated
AJAX	T63/PION2737W	Sunbeam Extract	2009	PNUWWSN
ARENA	NASW84-345/Coker9835//OH419/OH389	Sunbeam Extract	2009	NUWWSN
BDLS.HONEY-6	NASW84-345/Coker9835//OH419/OH389	Sunbeam Extract	2009	PNUWWSN
CANON	MV 17/RUBY	Sunbeam Extract	2009	NUWWSN
LINUS	5-TIEGANMAI/PION 25R26	Sunbeam Extract	2009	PNUWWSN
MOCHA	OH489/OH490	Sunbeam Extract	2009	NUWWSN
MONDO	ROANE/IL95-3245	Sunbeam Extract	2010	NUWWSN
OKIE	F285N3-111/65343(spelt)	Sunbeam Extract	2009	PNUWWSN
PENZO	5-TIEGANMAI/PION 25R26	Sunbeam Extract	2009	PNUWWSN
PROBE	MENDON/GR915	Sunbeam Extract	2010	NUWWSN
RUBIN	MO800071-56/PION2545//KY88C	Sunbeam Extract	2009	NUWWSN
RUMOR	HOPEWELL/HONEY	Sunbeam Extract	2010	NUWWSN
SHAVER	NASW84-345/Coker9835//OH419/OH389	Sunbeam Extract	2009	NUWWSN
SILAS	OH546/SE1694-12	Sunbeam Extract	2009	PNUWWSN
TABOO	L930605/ASHLAND	Sunbeam Extract	2010	NUWWSN
03M1539#019	GIBSON/92226E2-5-3	Syngenta	2010	NUWWSN
03M1539#031	GIBSON/92226E2-5-3	Syngenta	2010, 2009	NUWWSN NUWWSN, USFHB
03M1599#0007	M99*3038/Pioneer 25R49	Syngenta	2009	NUWWSN, USFHB
B0390207	BL931167/Pioneer 2643 CK68-19 // CK61-19*3 / IN4946A4-18-2-10-2 /4/ Bb /3/ CK65-20*5 / W17-TRANS // TIFT /5/ P 2550	Syngenta	2009	USFHB
COKER9835		Syngenta	2008, 2009	USFHB
M03-3616B	HOPEWELL/PATTON	Syngenta	2008	USFHB
M03-3616C	HOPEWELL/PATTON	Syngenta	2008	USFHB NUWWSN, USFHB
M04*5109	VA94-54-479/PIO2628	Syngenta	2008	USFHB PNUWWSN, USFHB
M04-4566	BRADLEY/ROANE	Syngenta	2008	USFHB PNUWWSN, USFHB
M04-4715	MASON/ERNIE	Syngenta	2008	USFHB NUWWSN, USFHB
M04-4802	FFR518/ELKHART/MV18	Syngenta	2008	USFHB PNUWWSN, USFHB
M05*1589	GA871339/PIO2540	Syngenta	2008	USFHB PNUWWSN, USFHB
M05-1172	M94-1048-1/IO2552	Syngenta	2008	USFHB PNUWWSN, USFHB
M05-1531	LA87167-D8-/P92118B4-2	Syngenta	2008, 2009	NUWWSN
MH06-2370	COOPER/SS550	Syngenta	2009	USFHB
MH06-2410	M98-1660//PATTON/Pioneer 2552	Syngenta	2009	PNUWWSN
ML06-2097	BENTON/M98-1569	Syngenta	2010	NUWWSN
ML07*7571	VA98W-586/HONEY	Syngenta	2009	USFHB
W1104	HOPEWELL/M94-1107	Syngenta	2010	NUWWSN
NE05459	IN92823A1-1-4-5/NE92458	U. of Nebraska	2009	NUWWSN
NE06469	Pedigree lost	U. of Nebraska	2010, 2009	NUWWSN

Appendix A Cont.

Entry	Pedigree	Developing Program	Year Evaluated	Nursery Evaluated
NI04420	NE96644(=ODESSKAYA P./CODY)//PAVON/*3SCOUT66/3/WAHOO SIB	U. of Nebraska	2009	NUWWSN
NI04427	KS98HW22//W95-615W/N94L189	U. of Nebraska	2009	NUWWSN
WESLEYFHB1	No pedigree	U. of Nebraska	2010	NUWWSN

Appendix B. Entry and dendogram cluster assignment of 251 lines from the Uniform Southern Fusarium Head Blight Nursery (USFHBN), Northern Uniform Winter Wheat Scab Nursery (NUWWSN), and Preliminary Northern Uniform Winter Wheat Scab Nursery (PNUWWSN) grown over the 2008, 2009, and 2010 seasons.

Entry	Cluster
AR97002-10-2	2
AR97002-2-1	2
AR97124-4-2	2
AR99028-1-1	8
AR99039-5-2	9
AR99054-4-1	8
AR99071-7-2	9
AR99254-7-1	2
AR99263-7-1	8
ARGE97-1048-6	5
GA031307-DH14	5
GA031354-DH30	8
GA031454-DH31	8
GA031454-DH38-7	8
GA031454-DH7	8
GA981621-5E34	5
GA991109-1-G1	8
GA991109-6E8	8
GA991209-6E33	5
GA991371-6E12	5
LA01141D-98-6-2	5
LA01162D-131-8	5
LA01162D-136-8	5
LA01164D-43-7-B	4
LA01164D-94-2	5
LA03187C-2	5
NC05-19896	10
NC05-20671	10
NC05-21090	10
NC05-21937	10

Appendix B Cont.

Entry	Cluster
NC05-25066	10
NC06-20288	10
NC05-23015	10
NC07-22927	10
NC07-23170	10
B030543	9
D05*6437	8
ARS03-3806	5
ARS03-4736	5
ARS03-5358	10
ARS04-1249	5
ARS05-0242	8
ARS05-0443	5
ARS05-1044	5
ARS05-1234	5
MD01W233-06-16	2
MD01W255-08-1	2
MD02W81-08-2	4
MD02W81-08-6	4
MD03W61-09-1	8
MD03W91-09-8	2
MD99W483-06-11	10
TRIBUTE	2
VA04W-360	10
VA04W-90	9
VA05W-425	9
VA05W-510	9
VA05W-534	8
VA05W-640	10
VA05W-641	10
VA05W-777	9
VA06W-553	9
VA06W-558	2
VA06W-561	8
VA06W-575	9
VA06W-578	9

Appendix B Cont.

Entry	Cluster
VA06W-587	9
VA06W-608	8
VA06W-612	10
VA06W-615	10
VA06W-622	2
VA07W-568	2
VA07W-580	2
VA07W-591	10
VA07W-594	10
VA07W-600	10
VA07W-601	10
VA07W-607	2
VA07W-643	2
VA07W-672	10
VA08W-734	2
NY03179FHB-10	7
NY03179FHB-12	7
NY03180FHB-10	7
NY88046-7088	7
NY93246SP-9070	7
NY94052-9340	7
NY99045-3110	7
NY99068-3251	7
NYCalresel-L	7
NYW103-102-9103	7
NYW103-21-9183	7
NYW103-70-9232	7
ACF12004	5
ACF126103	4
ACF213003B	4
DH22/24	8
DH22/8	5
DHF/SF,23	7
GS-0-EM0614	5
GS-0-EM0681	5
GS-1-EM0362	5

Appendix B Cont.

Entry	Cluster
MSULineE3024	7
MSULineE5011	7
MSULineE5011B	7
MSULineE5024	7
MSULineE6001	4
MSULineE6002	5
MSULineE6003	5
MSULineE6012	7
MSULineE6059	8
MSULineE7035R	5
MSULineE8052	8
IL01-34159	2
IL02-19463	2
IL04-10118	2
IL04-10721	2
IL04-10741	2
IL04-11003	2
IL04-17204	2
IL04-17762	2
IL04-24668	2
IL04-7874	2
IL04-7942	2
IL04-8445	2
IL05-15079	3
IL05-27333	2
IL05-27522	2
IL06-14262	2
IL06-7550	2
IL79-002T-B-B	2
KY00C-2059-16	8
KY00C-2059-19	8
KY00C-2143-08	10
KY00C-2515-02	10
KY00C-2567-01	8
KY00C-2755-03	8

Appendix B Cont.

Entry	Cluster
KY02C-3005-25	3
KY02C-3005-44	3
KY02C-3006-46	3
KY02C-3007-45	3
KY02C-3008-01	3
KY03C-1192-34	8
KY02C-3004-04	3
KY02C-3005-25	3
KY02C-3005-44	3
KY02C-3006-46	3
KY02C-3007-45	3
KY02C-3008-01	3
KY03C-1192-34	8
KY03C-2170-24	3
KY04C-2151	3
KY97C-0321-05-2	8
KY99C-1205-06-1	8
BESS	6
ERNIE	2
MO041020	6
MO041687	6
MO050101	6
MO050143	6
MO050144	8
MO050219	6
MO050261	8
MO050617	2
MO050699	6
MO050771	6
MO050917	6
MO050921	6
MO080104	9
MO080864	6
MO081652	9

Appendix B Cont.

Entry	Cluster
TRUMAN	6
FREEDOM	4
OH02-12678	4
OH02-12686	4
OH02-13567	2
OH02-7217	2
OH03-235-2	4
OH03-41-45	2
OH04-176-29	2
OH04-213-39	4
OH04-264-58	8
OH04-268-39	4
OH05-101-1	4
OH05-152-68	8
OH05-164-76	3
OH05-200-74	4
OH05-248-38	8
OH05-249-32	8
OH05-72-6	10
PIONEER2545	8
01946A1-16-48-5	1
059A1-2-4-3	1
99691A2-5-4-16-1	2
MO051150	6
MO071411	2
MO071722	6
P.011010A1-15	1
P.0128A1-22-22	2
P.0172A1-12-1	2
P.0175A1-37-4	2
P.0179A1-17	1
P.03112A1-7-3	1
P.03615A1-4-4	1
P.03630A1-18	1
P.04281A1-4-5	1
P.04287A1-16	1

Appendix B Cont.

Entry	Cluster
P.0513A1-2-3	3
P.05218A1-6-31	1
P.0527A1-9-15	4
P.0537A1-7-12	1
P.053A1-6-7	1
P.0570A1-7-6	1
P.0558A1-5-5	1
P.992192A1-5-4-5-81	7
P.99600A2-4-93	2
AJAX	10
ARENA	8
BDLS.HONEY-6	8
CANON	5
LINUS	8
MOCHA	4
MONDO	7
OKIE	3
PENZO	8
PROBE	10
RUMOR	4
SHAVER	8
SILAS	4
TABOO	3
03M1539#019	3
03M1539#031	8
03M1599#0007	8
B0390207	8
COKER9835	8
M03-3616B	4
M03-3616C	4
M04*5109	8
M04-4566	4
M04-4715	4
M04-4802	8
M05*1589	9
M05-1172	8

Appendix B Cont.

Entry	Cluster
MH06-2370	3
MH06-2410	3
ML06-2097	3
ML07*7571	3
ML07-7758	3
W1104	4

Appendix C. Presence or absence of *Sr36*, *Ppd-D1a*, *Rht-B1b*, *Rht-D1b*, 1RS•1BL, 1RS•1AL, *Fhb1*, *Qfhs.crc-2DL*, and *Qfhs.ici-5AL*.

Entry	Sr36	Ppd-D1a	Rht-B1b	Rht-D1b	Rht8	1RS•1AL	1RS•1BL	Fhb1	2DL Wuhan	5A Ning
AR97002-10-2	No	No	Yes	No	No	No	No	No	No	het
AR97002-2-1	Yes	No	Yes	No	No	No	No	No	No	.
AR97124-4-2	No	.	Yes	No	No	No	No	No	No	No
AR99028-1-1	No	No	Yes	No	No	No	No	No	No	No
AR99039-5-2	No	Yes	No	Yes	No	No	No	No	No	No
AR99054-4-1	No	No	No	No	No	No	No	No	No	No
AR99071-7-2	No	Yes	No	Yes	No	No	No	No	No	No
AR99254-7-1	Yes	No	No	No	No	No	No	No	No	No
AR99263-7-1	No	No	Yes	No	No	No	No	No	No	No
ARGE97-1048-6	No	No	No	Yes	No	No	Yes	No	No	No
GA031307-DH14	No	Yes	No	Yes	No	No	No	No	No	No
GA031354-DH30	No	.	No	Yes	.	No	No	No	No	No
GA031454-DH31	No	No	No	Yes	No	No	No	No	No	No
GA031454-DH38-7	No	Yes	No	Yes	.	No	No	No	No	No
GA031454-DH7	No	No	No	Yes	.	No	No	No	No	No
GA981621-5E34	No	No	No	Yes	No	No	Yes	No	No	No
GA991109-1-G1	No	No	Yes	No	No	No	No	No	No	No
GA991109-6E8	No	Yes	Yes	No	Yes	No	No	No	No	No
GA991209-6E33	No	No	No	Yes	Yes	No	Yes	No	No	No
GA991371-6E12	No	Yes	No	No	No	Yes	No	No	No	No
LA01141D-98-6-2	No	Yes	No	Yes	No	No	No	No	No	No
LA01162D-131-8	No	Yes	No	Yes	No	No	No	Yes	No	No
LA01162D-136-8	Yes	No	No	Yes	No	No	No	Yes	No	Yes
LA01164D-43-7-B	No	Yes	No	Yes	No	No	Yes	No	No	Yes
LA01164D-94-2	Yes	Yes	No	Yes	No	No	No	Yes	No	Yes
LA03187C-2	No	Yes	No	Yes	No	No	No	No	Yes	No
NC05-19896	Yes	Yes	No	Yes	No	No	No	No	No	No
NC05-20671	Yes	Yes	No	Yes	No	No	No	No	No	No
NC05-21090	Yes	Yes	No	Yes	No	No	No	No	No	No
NC05-21937	Yes	No	No	Yes	No	No	No	Yes	No	No
NC05-23015	Yes	Yes	No	Yes	.	No	No	No	No	No
NC05-24112	Yes	No	No	Yes	No	Yes	No	No	No	No
NC05-25066	Yes	No	No	Yes	No	No	No	Yes	No	No
NC06-20288	Yes	No	No	Yes	No	No	No	No	No	No

Appendix C Cont.

Entry	Sr36	Ppd-D1a	Rht-B1b	Rht-D1b	Rht8	IRS•1AL	IRS•1BL	Fhb1	2DL Wuhan	5A Ning
NC07-23170	Yes	No	No	Yes	No	No	No	No	No	No
B030543	Yes	Yes	No	Yes	No	No	No	No	No	No
D05*6437	No	No	No	Yes	No	No	No	No	No	No
ARS03-3806	No	No	Yes	No	No	Yes	No	No	No	No
ARS03-4736	No	.	Yes	No	No	Yes	No	No	No	No
ARS03-5358	Yes	No	No	No	No	No	No	No	No	No
ARS04-1249	No	.	Yes	No	No	Yes	No	No	No	No
ARS05-0242	Yes	.	No	Yes	No	No	No	No	No	No
ARS05-0443	No	No	Yes	No	.	No	Yes	No	No	No
ARS05-1044	No	.	Yes	No	No	Yes	No	No	No	No
ARS05-1234	No	No	Yes	No	No	No	No	No	No	No
MD01W233-06-16	No	No	No	Yes	No	Yes	No	No	No	No
MD01W255-08-1	No	No	No	Yes	No	Yes	No	No	No	No
MD02W81-08-2	Yes	No	Yes	No	No	No	Yes	Yes	No	Yes
MD02W81-08-6	Yes	No	Yes	No	No	No	Yes	No	No	No
MD03W61-09-1	No	Yes	No	Yes	No	No	Yes	Yes	No	No
MD03W91-09-8	No	Yes	No	Yes	No	No	No	No	No	No
MD99W483-06-11	Yes	Yes	No	Yes	No	No	No	No	No	No
TRIBUTE	No	Yes	No	Yes	No	No	No	No	No	No
VA04W-360	Yes	No	No	No	No	No	No	No	No	No
VA04W-90	No	Yes	No	Yes	No	No	No	No	No	No
VA05W-425	No	No	Yes	Yes	No	No	No	No	Yes	.
VA05W-510	Yes	Yes	No	Yes	No	No	No	No	No	No
VA05W-534	No	Yes	Yes	No	No	Yes	No	No	No	No
VA05W-640	het	Yes	No	Yes	No	No	No	No	No	No
VA05W-641	nd	Yes	Yes	No	No	No	No	No	.	.
VA05W-777	No	.	No	Yes	No	No	No	No	No	No
VA06W-553	No	.	Yes	Yes	No	No	No	No	Yes	het
VA06W-558	No	No	Yes	No	No	Yes	No	No	No	No
VA06W-561	No	.	No	Yes	Yes	No	No	No	No	No
VA06W-575	Yes	Yes	No	Yes	No	No	No	No	No	No
VA06W-578	Yes	Yes	No	Yes	.	No	No	No	No	No
VA06W-580	Yes	Yes	No	Yes	No	No	No	No	No	No
VA06W-587	No	Yes	No	Yes	No	No	No	No	No	No
VA06W-608	Yes	.	No	Yes	No	No	No	No	No	No
VA06W-612	Yes	No	No	Yes	No	No	No	No	No	No
VA06W-615	Yes	No	No	Yes	No	No	No	No	No	No
VA06W-622	Yes	.	Yes	No	No	No	No	No	No	No

Appendix C Cont.

Entry	Sr36	Ppd-D1a	Rht-B1b	Rht-D1b	Rht8	IRS•1AL	IRS•1BL	Fhb1	2DL Wuhan	5A Ning
VA07W-568	No	No	No	Yes	No	No	No	No	No	No
VA07W-591	Yes	No	No	Yes	No	No	Yes	No	No	No
VA07W-594	Yes	Yes	No	Yes	No	No	Yes	No	No	No
VA07W-600	Yes	No	No	Yes	No	No	No	No	No	No
VA07W-601	Yes	No	No	Yes	No	No	No	No	No	No
VA07W-607	No	No	No	Yes	No	No	No	No	No	No
VA07W-643	No	No	No	Yes	No	Yes	No	No	No	No
VA07W-672	het	Yes	No	Yes	No	No	No	No	No	No
VA08W-734	No	No	Yes	No	No	Yes	No	No	No	No
NW07505	No	No	Yes	No	.	No	No	No	No	No
NY03179FHB-10	No	No	No	Yes	No	No	No	Yes	No	No
NY03179FHB-12	No	No	No	Yes	No	No	No	Yes	No	No
NY03180FHB-10	No	No	No	Yes	No	No	No	Yes	No	No
NY88046-7088	No	No	No	Yes	No	No	No	No	No	No
NY93246SP-9070	No	Yes	No	Yes	No	No	No	No	No	No
NY94052-9340	No	No	No	Yes	No	No	No	No	No	No
NY99045-3110	No	No	No	Yes	No	No	No	No	No	No
NY99068-3251	No	No	No	Yes	No	No	No	Yes	No	No
NYCalresel-L	No	Yes	No	Yes	No	No	No	No	No	No
NYW103-102-9103	No	No	No	No	No	No	No	No	No	No
NYW103-21-9183	No	No	No	No	No	No	No	No	No	No
NYW103-70-9232	No	Yes	No	Yes	No	No	No	No	No	No
ACF12004	No	Yes	No	No	Yes	No	No	No	No	No
ACF126103	No	Yes	Yes	No	Yes	No	Yes	No	No	No
ACF213003B	No	No	Yes	No	Yes	No	Yes	No	No	No
DH22/24	No	.	Yes	No	No	No	No	Yes	No	No
DH22/8	No	.	No	No	No	No	No	Yes	No	No
DHF/SF,23	No	No	No	Yes	No	No	No	Yes	No	No
GS-0-EM0614	No	No	No	Yes	.	No	Yes	No	No	No
GS-0-EM0681	No	No	No	Yes	.	No	Yes	No	No	No
GS-1-EM0362	No	No	No	Yes	No	No	Yes	No	No	No
RCUOGtr35	No	No	No	No	No	Yes	No	No	No	No
MSULineE3024	No	No	No	Yes	No	No	No	No	No	No
MSULineE5011	No	No	No	Yes	No	No	No	No	No	No
MSULineE5011B	No	Yes	No	Yes	No	No	Yes	No	No	No
MSULineE5024	No	No	No	Yes	No	No	Yes	No	No	No
MSULineE6001	No	No	No	Yes	No	No	Yes	Yes	Yes	Yes
MSULineE6002	No	No	No	Yes	Yes	No	No	No	No	No

Appendix C Cont.

Entry	Sr36	Ppd-D1a	Rht-B1b	Rht-D1b	Rht8	1RS*1AL	1RS*1BL	Fhb1	2DL Wuhan	5A Ning
MSULineE6003	No	No	No	Yes	No	No	No	Yes	No	.
MSULineE6012	No	Yes	No	No	No	No	No	No	No	No
MSULineE6059	No	Yes	No	Yes	No	No	No	No	No	No
MSULineE7035R	No	Yes	No	Yes	No	No	Yes	Yes	No	No
MSULineE8052	No	Yes	No	Yes	No	No	No	No	No	No
IL01-34159	No	No	Yes	No	No	No	No	Yes	No	No
IL02-19463	No	No	Yes	No	No	No	No	No	No	No
IL04-10118	No	Yes	Yes	No	No	Yes	No	No	No	No
IL04-10721	No	No	Yes	No	No	No	No	No	No	No
IL04-10741	No	No	Yes	No	No	No	No	No	No	No
IL04-11003	No	No	Yes	No	No	No	No	No	No	No
IL04-17204	Yes	.	Yes	No	No	No	No	No	No	No
IL04-17762	No	No	Yes	No	No	No	No	No	No	No
IL04-24668	No	Yes	Yes	No	No	No	No	No	No	No
IL04-7874	No	No	Yes	No	No	No	No	No	No	No
IL04-7942	No	No	Yes	No	No	No	No	No	No	No
IL04-8445	No	No	Yes	No	No	No	No	No	No	No
IL05-15079	No	No	Yes	No	No	No	Yes	No	No	No
IL05-27333	No	No	Yes	No	No	No	No	No	No	No
IL05-27522	No	No	Yes	No	Yes	No	No	No	No	No
IL06-14262	No	No	Yes	No	No	No	No	No	No	No
IL06-7550	No	No	Yes	No	No	No	No	No	No	No
IL79-002T-B-B	No	No	Yes	No	No	No	No	No	No	No
KY00C-2059-16	No	No	No	Yes	No	No	No	No	No	No
KY00C-2059-19	No	Yes	No	Yes	No	No	No	No	No	No
KY00C-2143-08	Yes	Yes	Yes	No	.	No	Yes	No	No	No
KY00C-2515-02	No	No	No	Yes	No	No	Yes	No	No	No
KY00C-2567-01	No	Yes	No	Yes	No	No	No	No	No	No
KY00C-2755-03	No	Yes	No	Yes	No	No	No	No	No	No
KY02C-3004-04	No	.	No	Yes	No	No	Yes	Yes	No	No
KY02C-3005-25	No	No	Yes	No	No	Yes	No	Yes	.	No
KY02C-3005-44	No	No	No	No	No	No	Yes	Yes	No	No
KY02C-3006-46	No	Yes	Yes	No	No	No	Yes	Yes	No	No
KY02C-3007-45	No	Yes	Yes	No	No	No	Yes	Yes	.	No
KY02C-3008-01	No	Yes	Yes	Yes	No	No	Yes	No	No	No
KY03C-1192-34	No	Yes	No	No	No	No	No	No	No	No
KY03C-2170-24	het	Yes	Yes	No	Yes	No	Yes	No	het	No
KY04C-2151	No	Yes	No	No	No	No	Yes	Yes	het	No

Appendix C Cont.

Entry	Sr36	Ppd-D1a	Rht-B1b	Rht-D1b	Rht8	1RS•1AL	1RS•1BL	Fhb1	2DL Wuhan	5A Ning
KY97C-0321-05-2	No	Yes	No	Yes	No	No	No	No	No	No
KY99C-1205-06-1	Yes	No	No	Yes	No	No	No	No	No	No
BESS	No	.	Yes	No	No	No	No	No	No	No
MO041020	No	Yes	Yes	No	No	No	No	No	No	No
MO041687	No	No	Yes	No	No	No	No	No	No	No
MO050101	No	No	Yes	No	No	No	No	No	No	No
MO050143	No	No	Yes	No	No	No	No	No	No	No
MO050144	No	No	No	Yes	No	No	No	No	No	No
MO050219	No	No	No	Yes	No	No	No	No	No	No
MO050261	No	.	No	Yes	No	No	No	No	No	No
MO050617	No	No	Yes	No	No	No	No	No	No	No
MO050699	Yes	Yes	Yes	No	No	No	No	No	No	No
MO050771	No	No	Yes	No	No	No	No	No	No	No
MO050917	No	Yes	Yes	No	No	No	No	No	No	No
MO050921	No	No	Yes	No	No	No	No	No	No	No
MO051150	No	No	Yes	No	No	No	No	No	No	No
MO071411	No	Yes	Yes	No	No	No	No	No	No	No
MO071722	Yes	No	Yes	No	No	No	No	No	No	No
MO080104	No	Yes	No	No	No	No	No	No	No	No
MO080864	No	No	Yes	No	No	No	No	No	No	No
MO081652	No	Yes	No	No	No	No	No	No	No	No
TRUMAN	No	No	Yes	No	No	No	No	No	No	No
FREEDOM	Yes	No	Yes	No	No	No	Yes	No	No	No
OH02-12678	No	.	Yes	No	No	Yes	No	No	No	Yes
OH02-12686	No	No	Yes	No	No	Yes	No	No	No	No
OH02-13567	No	Yes	Yes	No	No	No	No	No	No	No
OH02-7217	No	.	Yes	No	No	No	No	No	No	No
OH03-235-2	No	Yes	Yes	No	No	No	No	No	No	No
OH03-41-45	No	No	Yes	No	No	Yes	No	No	No	No
OH04-176-29	No	No	Yes	No	No	No	No	No	No	No
OH04-213-39	No	No	Yes	No	No	No	No	No	No	No
OH04-264-58	No	Yes	No	Yes	No	No	No	No	No	No
OH04-268-39	No	No	Yes	No	No	No	No	No	No	No
OH05-101-1	No	Yes	Yes	No	No	No	No	No	No	No
OH05-152-68	No	No	Yes	No	No	No	No	No	No	No
OH05-164-76	No	Yes	Yes	No	No	No	Yes	Yes	No	No
OH05-200-74	No	No	Yes	No	No	Yes	No	No	No	No
OH05-248-38	No	Yes	Yes	No	No	No	No	No	No	No

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Entry	Sr36	Ppd-D1a	Rht-B1b	Rht-D1b	Rht8	IRS•1AL	IRS•1BL	Fhb1	2DL Wuhan	5A Ning
OH05-249-32	No	Yes	Yes	No	No	No	No	No	No	No
OH05-72-6	Yes	Yes	Yes	No	No	No	Yes	No	No	No
PIONEER2545	No	Yes	No	Yes	No	No	No	No	No	No
01946A1-16-48-5	Yes	Yes	Yes	No	No	No	Yes	No	No	No
99691A2-5-4-16-1	No	Yes	Yes	No	No	No	No	No	No	No
P.011010A1-15	het	.	Yes	No	No	No	No	No	No	No
P.0128A1-22-22	No	No	Yes	No	No	No	No	Yes	No	No
P.0172A1-12-1	No	Yes	Yes	No	No	No	No	Yes	No	No
P.0175A1-37-4	Yes	No	Yes	No	No	No	Yes	No	No	Yes
P.0179A1-17	Yes	No	Yes	No	No	No	Yes	No	No	No
P.03112A1-7-3	Yes	No	Yes	No	No	No	Yes	No	No	No
P.03615A1-4-4	Yes	No	Yes	No	No	No	Yes	Yes	No	No
P.03630A1-18	No	No	Yes	No	No	No	Yes	Yes	No	No
P.04281A1-4-5	Yes	No	Yes	No	No	No	No	Yes	No	No
P.04287A1-16	Yes	Yes	Yes	No	No	No	Yes	Yes	No	No
P.04704A1-2-1-1	Yes	No	Yes	No	No	No	Yes	No	No	No
P.0513A1-2-3	No	Yes	Yes	No	No	No	No	No		
P.05218A1-6-31	Yes	Yes	Yes	No	No	No	No	Yes	No	No
P.0527A1-9-15	No	Yes	Yes	No	No	No	Yes	No	No	No
P.0537A1-7-12	Yes	Yes	Yes	No	No	No	No	Yes	No	No
P.053A1-6-7	n	Yes	Yes	No	No	No	No	No	No	No
P.0558A1-5-5	Yes	Yes	Yes	No	No	No	No	No	No	No
P.0570A1-7-6	Yes	Yes	Yes	No	No	No	No	No	No	No
P.992192A1-5-4-5-81	No	No	Yes	No	No	No	Yes	No	No	No
P.99600A2-4-93	No	No	Yes	No	No	No	No	No	No	No
AJAX	het	Yes	No	Yes	No	No	No	No	No	No
ARENA	No	Yes	No	Yes	No	No	No	No	No	No
BDLS.HONEY-6	No	No	Yes	No	No	No	No	No		
CANON	No	Yes	No	No	No	No	No	No	No	No
LINUS	No	Yes	Yes	Yes	No	No	No	No	No	No
MOCHA	No	Yes	Yes	No	No	No	No	No	No	No
MONDO	No	No	No	No	No	No	No	No	No	No
OKIE	No	Yes	Yes	No	Yes	No	Yes	No	No	No
PENZO	No	Yes	No	Yes	No	No	No	No	No	No
PROBE	Yes	No	No	Yes	Yes	No	No	No	No	No
RUBIN	No	No	Yes	No	Yes	No	Yes	No	No	No
RUMOR	No	No	No	Yes	No	No	No	No	No	No
SHAVER	No	Yes	No	Yes	No	No	No	No	No	No

Appendix C Cont.

Entry	Sr36	Ppd-D1a	Rht-B1b	Rht-D1b	Rht8	1RS•1AL	1RS•1BL	Fhb1	2DL Wuhan	5A Ning
SILAS	No	No	No	Yes	No	No	No	No	No	No
TABOO	No	No	Yes	No	Yes	No	Yes	No	No	No
03M1539#019	No	Yes	Yes	No	No	No	Yes	No	No	No
03M1539#031	No	Yes	Yes	No	No	No	No	No	No	No
03M1599#0007	No	Yes	Yes	No	No	No	No	No	No	No
COKER9835	Yes	No	No	No	No	No	No	No	No	No
M03-3616B	No	Yes	Yes	No	No	No	No	No	No	No
M03-3616C	No	No	Yes	No	No	No	Yes	No	No	No
M04*5109	No	No	Yes	No	No	No	No	No	No	No
M04-4566	No	Yes	Yes	No	No	No	Yes	No	No	No
M04-4715	No	.	No	Yes	No	No	Yes	No	No	No
M04-4802	No	No	No	Yes	No	No	No	No	No	No
M05*1589	No	No	No	Yes	No	No	No	No	No	No
M05-1172	Yes	Yes	No	Yes	No	No	No	No	No	No
M05-1531	No	No	Yes	Yes	No	No	Yes	No	No	No
MH06-2370	No	Yes	Yes	No	No	No	Yes	No	No	No
MH06-2410	No	Yes	No	Yes	No	No	Yes	No	No	No
ML06-2097	No	Yes	Yes	No	Yes	No	Yes	No	No	No
ML07*7571	No	No	Yes	No	No	No	No	No	No	No
ML07-7758	No	No	Yes	No	No	No	No	No	No	No
W1104	No	Yes	Yes	No	No	No	Yes	No	No	No
NE05459	No	Yes	Yes	No	No	No	No	No	No	No
NE06469	No	Yes	Yes	No	No	No	No	No	No	No
NE06471	No	Yes	Yes	No	No	No	No	Yes	No	No
NI04420	No	Yes	Yes	No	.	No	Yes	No	No	No
NI04427	No	No	Yes	No	.	No	No	No	No	No
WESLEYFHB1	No	No	Yes	No	.	No	No	Yes	No	No

Appendix D. Location of microsatellite, KASPar, and sequence tagged site markers targeted to reported QTL or flanking regions from reported literature

Marker	Chromosome	Position	QTL or Gene	Reference
gwm357	1A	54	<i>QFhs.nau-1AS</i>	Jiang et al. 2007a, Semagn et al. 2007
wmc716	1A	91		Jiang et al. 2007a, Semagn et al. 2007
barc17	1A	114.8		Semagn et al. 2007
gwm259	1A	106.8		Jiang et al. 2007a, Semagn et al. 2007
scm09	1AL	-		Ittu et al 2000
barc137	1B	34.3		Semagn et al. 2007
gwm124	1B	64.1		Somers et al. 2004
barc80	1B	106.3		Loffler et al. 2009, Somers et al. 2004
cfa2219	1B	124.1		Loffler et al. 2009
scm09	1BL	-	<i>Qfhs.jic-1b</i>	ittu et al 2000
barc99	1D	50.5		Yang et al 2005, Somers et al. 2004
barc76	2A	131		Loffler et al. 2009
wmc177	2A	28.3		Somers et al. 2004
wmc770	2B	42.6	<i>Qfhs.umc-2B</i>	Schmolke et al. 2008
gwm319	2B	63.2	<i>Qfhs.umc-2B</i>	Liu et al. 2007
wmc477	2B	63.4	<i>Qfhs.umc-2B</i>	Loffler et al. 2009
gwm374	2BS	60.7	<i>Qfhs.umc-2B</i>	Gervais et al. 2003
Ppd-D1	2D	0		Somers et al. 2004
Gwm261	2D	23.2		Shen et al. 2003
gwm608	2D	87.7	<i>QFhs.nau-2DL, QFhs.crc-2D</i>	Somers et al. 2004
gwm349	2D	93.2	<i>QFhs.nau-2DL, QFhs.crc-2D</i>	Somers et al. 2004
gwm157	2DL	73.1	<i>QFhs.nau-2DL, QFhs.crc-2D</i>	Jiang et al. 2007a,b
cfd233	2DL	78.7	<i>QFhs.nau-2DL, QFhs.crc-2D</i>	Somers et al. 2004
wmc144	2DS	67.1	<i>QFhs.nau-2DL, QFhs.crc-2D</i>	Yang et al 2005

Appendix D Cont.

Marker	Chromosome	Position	QTL or Gene	Reference
gwm539	2DS	90.9	<i>QFhs.nau-2DL, QFhs.crc-2D</i>	Jiang 2007 et al. a,b
wmc11	3A	0	<i>Qfhs.nau-3A</i>	Somers et al. 2004
wmc559	3A	83.3	<i>Qfhs.nau-3A</i>	Somers et al. 2004
gwm533	3B	5.6	<i>Fhb1</i>	Zhou et al. 2002
barc147	3B	7.09	<i>Fhb1</i>	Zhou et al. 2002
UMN10	3B	9	<i>Fhb1</i>	Bernardo et al. 2011
Xsnp3BS-8	3B	9.1	<i>Fhb1</i>	Bernardo et al. 2011
GWM493	3B	11.3	<i>Fhb1</i>	Anderson et al. 2001
cf79	3B	18.3	<i>Fhb1</i>	Somers et al. 2004
gwm264	3B	44.8	<i>Qfhs.umc-3B</i>	Liu et al. 2007
wmc231	3B	53.9	<i>Qfhs.umc-3B</i>	Somers et al. 2004
gwm285	3B	59.6	<i>Qfhs.umc-3B</i>	Liu et al. 2007
wmc1Fd	3B	64.4	<i>Qfhs.umc-3B</i>	Somers et al. 2004
wmc307	3B	64.43	<i>Qfhs.umc-3B</i>	Somers et al. 2004
wmc418	3B	74.4	<i>Qfhs.umc-3B</i>	Somers et al. 2004
wmc471	3B	81	<i>Qfhs.umc-3B</i>	Somers et al. 2004
wmc787	3B	82.2	<i>Qfhs.umc-3B</i>	Somers et al. 2004
wmc612	3B	57.7	<i>Qfhs.umc-3B</i>	Somers et al. 2004
Barc71	3D	78.6		Yu et al. 2008
wmc707	4A	39.5	<i>QFhs.fal-4AL</i>	Somers et al. 2003
wmc219	4A	87.6	<i>QFhs.fal-4AL</i>	Paillard et al. 2004
barc184	4A	176		Somers et al. 2004
wmc710	4B	11.2		Somers et al. 2004
Rht-B1	4B	12		Srinivasachary et al. 2008
wmc48	4B	23.9	<i>Qfhs.umc-4BL</i>	Somers et al. 2004
wmc657	4B	26.7	<i>Qfhs.umc-4BL</i>	Somers et al. 2004

Appendix D Cont.

Marker	Chromosome	Position	QTL or Gene	Reference
gwm165	4B	27.6	<i>Qfhs.umc-4BL</i>	Somers et al. 2004
gwm149	4B	30.9	<i>Qfhs.umc-4BL</i>	Liu et al. 2007
gwm495	4B	31.4	<i>Qfhs.umc-4BL</i>	Liu et al. 2007
barc163	4B	38.6	<i>Qfhs.umc-4BL</i>	Liu et al. 2007
gwm47	4B			Somers et al. 2004
barc20	4B	21.6		Somers et al. 2004
Rht-D1	4D	5	<i>Qfhs.jic-4d</i>	Draeger et al. 2007
wmc285	4D	9.9		Somers et al. 2004
gwm133	4D	37.1		Loffler et al.2010
wmc705	5A	57.6	<i>Qfhs.ifa-5A, Qfhs.umc-5A</i>	Somers et al. 2004
barc117	5A	58	<i>Qfhs.ifa-5A, Qfhs.umc-5A</i>	Chen et al. 2006a
barc186	5A	59.1	<i>Qfhs.ifa-5A, Qfhs.umc-5A</i>	Chen et al. 2006a
barc56	5A	60.7	<i>Qfhs.ifa-5A, Qfhs.umc-5A</i>	Liu et al. 2007
gwm304	5A	63.5	<i>Qfhs.ifa-5A, Qfhs.umc-5A</i>	Buerstmayer et al. 2002, Liu et al. 2007
barc40	5A	69.9	<i>Qfhs.ifa-5A, Qfhs.umc-5A</i>	Liu et al. 2007
barc45	5A		<i>Qfhs.ifa-5A, Qfhs.umc-5A</i>	Somers et al. 2004
gwm156	5A		<i>Qfhs.ifa-5A, Qfhs.umc-5A</i>	Buerstmayer 2003
wmc238	5A	25		Somers et al. 2003
wmc75	5B	89		Bourduncle et al. Somers et al 2004
barc3	6A	66		Somers et al. 2004, Anderson et al. 2001
gwm518	6B	27.3		Semagn et al. 2007, Cuthbert et al. 2007
wmc152	6B	49.1		Cuthbert et al. 2007
gwm219	6B	59.3		Cuthbert et al. 2007
Barc96	6D	91.7		Somers et al. 2004
cf49	6D	0	<i>Qfhs.fal-6D</i>	Paillard et al. 2004

Appendix D Cont.

Marker	Chromosome	Position	QTL or Gene	Reference
barc23	6D	57.5		Somers et al. 2004
gwm282	7A	99.8	<i>Qfhs.jic.7a</i>	Jia et al. 2005
wmc790	7A	102	<i>Qfhs.jic.7a</i>	Somers et al. 2004
wmc809	7A	131.2	<i>Qfhs.jic.7a</i>	Somers et al. 2004
gwm400	7B	40	<i>QFhs.nau-7BS</i>	Draeger et al. 2007
wmc506	7D	21		Somers et al. 2004