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A Genome Wide Association Study for Fusarium Head Blight Resistance in Southern Soft Red Winter Wheat

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A Genome Wide Association Study for Fusarium Head Blight Resistance in Southern Soft Red Winter Wheat

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ABSTRACT

Fusarium head blight (FHB) is a disease of small grains caused by the fungal pathogen *Fusarium graminearum*. FHB poses potential economic losses and health risks due to the accumulation of the mycotoxin deoxynivalenol (DON) on infected seed heads. The objectives of this study are: 1) evaluate soft red winter wheat (SRWW) lines for resistance to FHB in terms of resistance to initial inoculum (incidence); resistance to spread within the head (severity); resistance to DON accumulation; and resistance to Fusarium damaged kernels (FDK), 2) determine the frequency and effect of known FHB resistance genes and quantitative trait loci (QTL), and 3) identify novel resistance loci using a genome wide association (GWA) approach. From 2014-2017, 360 SRWW breeding lines were evaluated in inoculated misted FHB nurseries in Fayetteville and Newport, AR and Winnsboro, LA (2017 only) in a randomized complete block design. At all locations, lines were sown in two row plots, inoculated with *F. graminearum* infected corn (*Zea mays L.*) and overhead misted throughout the months of April and May to provide optimal conditions for FHB infection. In addition to visual ratings and DON analysis, lines were screened with KASP® markers linked to known FHB resistance genes, including *Fhb1*. The known resistance QTL, *Qfhb.nc-2B.1* (*Bess*), on chromosome 3B was significantly associated with a reduction in incidence, severity, and DON accumulation. Genome wide SNP markers generated through genotype by sequencing (GBS) were used to perform GWA in order to identify marker-trait associations for FHB resistance. The GWA analysis identified 58 highly significant SNPs associated with the four disease traits. The most highly significant SNP was found on chromosome 4A and the minor allele was found to significantly reduce incidence by 1.17%. Results from this study will facilitate the development of SRWW cultivars with improved resistance to FHB.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

FHB	*Fusarium* head blight
GWAS	Genome-wide association study
SRWW	Soft red winter wheat
FDK	*Fusarium* damaged kernels
DON	Deoxynivalenol
QTL	Quantitative trait loci
GBS	Genotype-by-sequencing
SNP	Single nucleotide polymorphism
HRW	Hard red winter (wheat)
HRS	Hard red spring (wheat)
SRW	Soft red winter (wheat)
SWW	Soft white winter (wheat)
HWW	Hard white winter (wheat)
NIV	Nivalenol
FDA	Food and Drug Administration
USWBSI	United States Wheat and Barley Scab Initiative
VDHR	Variety Development and Host Resistance
MGMT	*Fusarium* head blight Management
FST	Food Safety and Toxicology
GDER	Gene Discovery and Engineering Resistance
GC	*Galla chinesis*

TA	Tannic acid
K	Kinship
PC	Principle components
PCA	Principle components analysis
K-PC	Kinship and principle components
cMLM	Compressed mixed linear model
MAS	Marker assisted selection
SSR	Simple sequence repeat
STR	Short tandem repeat
MTA	Marker-trait association
AMP	Association mapping panel
FAY	Fayetteville
NPT	Newport
HD	Heading date
HT	Mature plant height
BLUPs	Best linear unbiased predictions
H^2	Broad sense heritability
DNA	Deoxyribonucleic acid
KASP	Kompetitive allele specific PCR
PCR	Polymerase chain reaction
UFHBN	United *Fusarium* head blight Nursery
GAPIT	Genomic Association and Prediction Integrated Tool
SMA	Single marker analysis

Inc	Incidence
Sev	Severity
Chr	Chromosome
Maf	Minor allele frequency

OBJECTIVES OF THIS PROJECT

The overall goal of this research is to produce marketable wheat cultivars that show resistance to FHB and are well suited for growth in Arkansas and to do this using both traditional and molecular breeding methods, including marker assisted selection and genomic selection. The data generated on these lines will be used in future studies as a training panel for genomic selection practices at the University of Arkansas. The major objective of this proposal was to evaluate the frequency and genetic effect of known FHB resistance genes within the Arkansas breeding material and to identify new sources of resistance alleles through genome wide association analysis (GWAS). The specific objectives are as follows:

Objective 1: Evaluate soft red winter wheat (SRWW) lines for resistance to FHB in terms of resistance to initial inoculum (incidence); resistance to spread within the head (severity); resistance to Fusarium damaged kernels (FDK); and resistance to accumulation of DON. This objective was accomplished through multi-year field evaluation of a panel of 360 genotypes for FHB resistance in misted and inoculated screening nurseries at two locations in Arkansas. Ratings included incidence, severity, FDK, and DON.

Objective 2: Determine the frequency and effect of known FHB resistance genes and native quantitative trait loci (QTL). This objective was accomplished through screening lines with molecular markers for known resistance genes and comparing the genetic analysis to phenotypic data for each line. Lines were screened for native resistance genes, including those from ‘Bess’, ‘Jamestown’, and ‘Neuse’ as well as the exotic resistance gene *Fhb1*.

Objective 3: Identify novel resistance loci using a genome wide association (GWAS) approach. To identify resistance genes unique to this population, we genotyped each line using a genotype by sequencing (GBS) approach and input both genotypic data as well as phenotypic data for each line into a GWAS analysis program in R software. Using a GWAS approach allowed us to discover both unique, single nucleotide polymorphisms (SNPs) as well as pleiotropic alleles which may be affecting more than one trait. For example, height dwarfing genes in wheat may lead to lower Type 1 resistance (Yan 2011). A genome wide study insures that we are able to evaluate the effect of all genes on resistance to FHB.

CHAPTER 1: LITERATURE REVIEW

Wheat Production

Wheat was first domesticated and cultivated in southwest Asia between the Tigris and Euphrates rivers in what is now present day Turkey, Syria, and Iraq. Today, wheat (*Triticum aestivum*) is produced on more hectares worldwide than any other cereal crop and is consistently the third highest yielding cereal crop behind corn and rice (FAO 2014). According to figures provided by the USDA, in the 2015-2016 season, wheat was grown on more than 220 million hectares worldwide and produced over 730 million metric tons of grain. In the United States, wheat is produced on over 19 million hectares and averages almost three metric tons per hectare (USDA 2016).

Wheat plays an important role in human food and animal feed. Wheat contains more protein per 100 grams than any other cereal (Gobbetti 2013). Two important types of protein in wheat, gliadins and glutenin, combine to form the complex gluten protein. The presence of gluten in the endosperm of wheat kernels allows wheat flour to retain its elasticity when combined with water to produce leavened bread. According to the National Association of Wheat Growers, wheat is responsible for approximately 20% of calories consumed by humans each day around the world (FAO 2014).

In addition to being recognized as the third most important crop worldwide behind corn and rice, wheat is also the third most important crop in the United States in terms of production following corn and soybean (USDA 2016). In the 2015-2016 growing season, the U.S. produced over 54 million metric tons of wheat (USDA 2016). The total production is sub-classified into six types of wheat based on kernel color, hardness, and growing season. The six types of wheat

include hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), soft white winter (SWW), hard white winter (HWW), and durum wheat (Associates 2013).

Classifications of Wheat

Each classification of wheat is grown in different regions in the United States and processed to produce a unique product. Hard red winter wheat is the most commonly grown classification in the U.S. and is primarily produced in the Great Plains of Kansas, Nebraska, Oklahoma, and Texas. Hard red winter wheat is milled to make flour for bread, tortillas, and cereal (Committee 2012). The second most commonly grown classification in the U.S. is hard red spring wheat, grown primarily in the Dakotas and Minnesota. Hard red spring flour is used in producing products such as croissants, bagels, and pizza crust (Associates 2013). Soft red winter wheat is the third most popular classification of wheat in the U.S. and is grown in the eastern regions of the country. Soft red winter wheat is used to make flat breads, cookies, and other pastries (Associates 2013). Soft white winter wheat is grown primarily in the northwestern states of Washington and Oregon and is used to produce finer, whiter flour for cakes, pastries, and Asian style noodles (Commission 2014). Hard white winter wheat is the newest classification of wheat and production of this classification is scattered throughout the United States and used primarily for Asian style noodles and whole wheat white flour. The final class of wheat is durum wheat. Durum wheat is the hardest of all classes and is grown mainly in North Dakota and southern California. Durum flour has a high gluten content and is used for pasta products (Commission 2014). Of these six classifications, HRW, HRS, and SRW made up about 85% of total U.S. wheat production in the 2015 harvest season (USDA 2016).

The primary classification of wheat produced in Arkansas is SRW wheat. In Arkansas, SRW wheat is planted in the fall and harvested in June the following year (Kelley 2016). It is

often found in rotation with summer annual crops such as corn, soybean, or rice and may also be used in animal production settings as a fall and winter forage or cover crop. In 2015-2016, SRW wheat was planted on over 188,000 hectares in Arkansas which is considerably lower than the 243,000-hectare average over the past few years provided by the Arkansas Extension Service (Kelley 2016). On average SRW wheat produces 58 bushesl/acre in Arkansas. Soft Red Winter wheat is produced in three main regions in Arkansas, the Mississippi River Delta, the Arkansas River Valley, and the Red River Valley in the eastern, western, and southern regions of the state, respectively (Kelley 2016).

Common Wheat Diseases

Of the many problems that Arkansas wheat producers face, one of the most prevalent is disease susceptibility. The most common diseases of wheat in Arkansas are stripe rust (*Puccinia striiformis*), leaf rust (*Puccinia triticina*), Fusarium head blight or head scab (*Fusarium graminearum*), *Septoria* leaf blotch (*Septoria tritici*), and bacterial streak (*Xanthomonas translucens*) (Spurlock 2015). Pathogens causing disease in wheat may attack the roots, stems, leaves, or seed head of the plant with some diseases, such as stripe rust, spreading from one part of the plant to another. Of all the Arkansas wheat diseases, stripe rust causes the most damage from year to year. However, given the right circumstances, Fusarium head blight (FHB) poses as much or more of a problem than stripe rust (Rudd 2001).

Fusarium Head Blight

Fusarium head blight, also known as head scab, is mainly caused by the fungal pathogen *Fusarium graminearum*. Other members of the *Fusarium* species may contribute to this disease or be more prevalent in other wheat growing regions, however, *F. graminearum* is the main causal agent in the Midwest and southern United States (Wegulo S. N. 2008). *F. graminearum*

belongs to the division of fungi known as Ascomycota. Ascomycetes are characterized by their the ascus which is a sack within the fruiting body containing eight sexually produced ascospores (Schmale III 2003). The sexual stage, or teleomorph, of *F. graminearum* is *Gibberella zeae*.

The environment plays a large role in the infection of small grains by *Fusarium* species. Chlamydospores, perithecia, or mycelia from this fungus must overwinter in the soil or on infected crop residue before maturing the next spring and releasing spores (Wegulo S. N. 2008). Sexually produced ascospores are formed in an ascus within a fruiting body known as a perithecium (Wegulo S. N. 2008). Ascospores make up the majority of primary inoculum each spring; however, fungal hyphae or asexually produced macroconidia may also contribute to primary inoculum. Macroconidia are produced in a sporodochium before being dispersed by to seed heads (Schmale III 2003). The majority of spores are distributed to seed heads by the wind, however, splash dispersal by water also plays a part in spore dispersal.

For infection to occur, conditions must be favorable for the pathogen. *F. graminearum* spores require warm temperatures (23-26ºC) and adequate moisture to infect the seed heads of small grains during flowering (Schmale III 2003). Under ideal conditions, spores begin to germinate 6-12 hours after initial infection. Infections occur mainly during flowering as the pollen and extruded anthers of the wheat flower serve as food for the germinating ascospores (Wegulo S. N. 2008). After germination, spores produce a germ tube that gives rise to hyphae that grows through the protective walls of the lemma, glume, and palea. The movement of the fungus from infected tissue to uninfected tissue occurs through either passive or direct penetration. An example of passive infection occurs when hyphae grow into the floret through natural openings such as stomata (Walter 2010). Active penetration is characterized by the use of hydrolyzing enzymes to degrade the host cuticle to allow hyphae to enter the floret. Fungal

hyphae spread from the initial site of infection to neighboring florets in one of two ways: through vascular tissue or stomata. Spread of hyphae and microconidia through vascular tissues not only helps quickly disperse the fungus, but also reduces flow of water and nutrients to maturing seeds interrupting normal seed set (Cai 2012).

Once infected, the seed head turns white in what is recognized as the characteristic "bleached" head associated with FHB (Wegulo S. N. 2008). Other recognizable traits of this disease include orange or pink spores, black perithecia, and shrunken, wrinkled kernels (Schmale III 2003). After infecting a seed head, this fungus begins to produce the mycotoxins deoxynivalenol (DON) and nivalenol (NIV). DON serves as a virulence factor for this fungus and causes host cell death and release of nutrients to the fungus (Walter 2010). Fusarium head blight poses a serious threat to producers' profit as well as consumer health because of the damage to the kernel and the production of mycotoxins (Pirgozliev 2003). Damaged and infected kernels may result in a drastically lower test weight thus decreasing yield and ultimately producer profits. In addition to a decreased yield, scabby seed may pose serious health risks to humans and animals alike because of the production of DON (FDA 2010).

Effects of Deoxynivalenol

If ingested by humans, DON may cause temporary nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever (Sobrova, Adam et al. 2010). If consumed in high levels by pregnant individuals, DON may lead to abortion of offspring. For this reason, the U.S. Food and Drug Administration (FDA) has restricted DON contamination to 1 ppm in products for human consumption such as flour, bran, and germ (FDA 2010). In addition to wheat products, animal products may also be a source of DON in the human diet. A study conducted in 2008 found animal products such as muscle and kidneys may contain low levels of DON if the animal had

been fed a contaminated diet (Sobrova, Adam et al. 2010). However, because of low levels of residual DON, it does not pose a significant risk to human health. Animals and humans are affected by DON in the same way, and to address this, the FDA also set acceptable limits to DON in animal feed. Since swine are the most susceptible to DON contamination, the acceptable limit set by the FDA for swine feed is 5 ppm with the recommendation that infected feed not exceed 20% of their diet. For contaminated feed destined for other animals, such as cattle and chickens, the allowable limit is 10 ppm with the recommendation that DON infected grain not surpass 50% of the animal's diet (FDA 2010).

Fusarium head blight poses a potential problem to producers around the world each year. Between 1990 and 2003, producers lost an estimated 3 billion dollars to epidemics of Fusarium head blight (Cowger 2005). This disease has been known to cause yield losses of up to 50% in severe cases (Rudd 2001). In the past twenty-five years, there have been several outbreaks of FHB in the United States. The most decimating of these outbreaks occurred during the 1993 growing season. An epidemic of FHB reduced yield by 40% and by 50% in North Dakota and northwest Minnesota, respectively, compared to 1992 (McMullen 1997). Since the outbreaks in the 1990s, some regions of the country have continued to experience epidemics in small grain production.

Data compiled by McMullen et al. in 2012 summarized the recent FHB epidemics of the United States. The northern states of North Dakota, South Dakota, and Montana recorded chronic losses of Hard Spring Wheat crops due to FHB from 1998-2010. The eastern states of Maryland, Virginia, and North Carolina experienced losses of SRW wheat to epidemics in 1998 and 2003 (McMullen 2012). Epidemics drastically reduced production of SRW wheat in Kentucky and Illinois in 2004 as well as in 2009 when this region, along with Arkansas, Indiana,

Tennessee, and Virginia experienced a second outbreak. Perhaps the most severe losses due to FHB occurred in the great plains states of Nebraska, Kansas, and Oklahoma in 2007, 2008, and 2009 (McMullen 2012). The most recent outbreaks of FHB in the southeast region of the country occurred in the 2014-2015 season. In addition to reducing yield, wheat harvested with extreme disease severity is further docked at grain mills resulting in an even lower profit for producers.

As a result of the early devastating epidemics, the United States Wheat and Barley Scab Initiative (USWBSI) was formed in 1997 (Anderson 2010). The USWBSI is made up of a collection of producers, researchers, and representatives of the food processing industry (millers, brewers, bakers, etc.) (Anderson 2010). The goal of the U.S. Wheat and Barley Scab Initiative is to develop as quickly as possible effective control measures that minimize the threat of Fusarium head blight (scab) to the producers, processors, and consumers of wheat and barley (Anderson 2010). The USWBSI provides research funding to 81 scientists in 24 different states. In addition, 129 state university research projects are made possible through the Initiative (Mason 2015). The primary categories of research through the USWBSI include Variety Development and Host Resistance (VDHR), FHB Management (MGMT), Food Safety and Toxicology (FST), and Gene Discovery and Engineering Resistance (GDER), as well as others.

Management of *Fusarium* Head Blight

Management of FHB can be broken down into two categories; prevention and control, with different types of management (biological, chemical, cultural, or genetic) falling into those two management categories (Pirgozliev 2003). Management practices such as planting genetically resistant seed, pre-planting seed treatments, adjusting crop rotation to prevent successive planting of FHB susceptible crops, and reducing the amount of infected residue left on the surface of the soil all fall into the prevention category. The control category is primarily

comprised of fungicide applications and biological control methods. Since resistance to FHB is a quantitative resistance, relying heavily upon the combined effects of several small-effect QTL, there are no completely resistant wheat varieties currently available (Pirgozliev 2003). Because of the lack of completely resistant varieties, other methods of FHB control must be used in conjunction with genetic resistance.

The most common complement to genetic resistance is a combination of cultural and chemical prevention. Cultural prevention practices such as crop rotation with non-host crops, such as soybean, and removal of infected crop debris through conventional tillage or burning are often used in conjunction with fungicide seed treatments and genetic resistance to reduce the potential of an FHB outbreak (Pirgozliev 2003). Cultural methods of control are effective only when the initial source of inoculum is from infected crop debris. The saprophytic capabilities of *F. graminearum* are dependent on the weather and a reduction occurs in inoculum available if it is not able to find another susceptible host within the next growing season (Wise 2015). However, if the initial inoculum is from infected neighboring fields or an infected seed source, cultural control alone will not prevent an outbreak of FHB.

Chemical methods of FHB management may fall into either management category. Pre-planting fungicide seed treatments are an example of preventative chemical control while application of fungicides at or after heading represent a form of chemical management that falls into the control category. The effectiveness of fungicides depends on three factors: application timing, spray coverage, and disease pressure (Wise 2015). Several fungicides are labeled for control of FHB; however, the most effective class of fungicides are the triazoles, while the least effective are fungicides containing a strobilurin (Wise 2015).

While the use of biologic control methods is not as popular as genetic, chemical, or cultural methods, in recent years more effort has been made to identify biologic agents to combat FHB. In research performed at Duke University, applications of solutions with 1% of Chinese galls (*Galla chinensis*, GC) or 1% of tannic acid (TA) inhibited germination of conidia or mycelium growth of *F. graminearum* by 98%–100% or by 75%–80%, respectively (Forrer 2014).

Known Resistance Genes and QTL

Resistance to FHB can be classified as either morphological (passive resistance) or physiological (active resistance) (Gilsinger 2005). Some examples of morphological resistance traits include plant height, heading date, presence of awns, and openness of flowers during anthesis. In contrast, physiological resistance involves a resistance pathway that inhibits infection and pathogen spread in some way (Gilsinger 2005). Resistance genes fall into this second category. There are four types of genetic resistance to Fusarium head blight, including: resistance to initial infection (Type 1); resistance to spread of disease within the seed head (Type II); resistance to Fusarium damaged kernels (Type III); and resistance to accumulation of mycotoxins (DON) (Type IV) (Rudd 2001). The most common form of resistance among resistant cultivars is Type II resistance or resistance to spread of disease within the seed head. Each of these types of resistance is the result of combined efforts of several minor genes or QTL. Resistance to FHB is a quantitative resistance meaning that it is a product of several resistance genes contributing to the overall resistance level of the plant. Known resistance genes for FHB fall into one of two categories: native or exotic.

The most common example of exotic resistance comes from the *Fhb1* gene first discovered on the short arm of chromosome 3B in the Chinese wheat cultivar Sumai 3. Exotic

resistance confers a high level of resistance in wheat to Fusarium head blight. *Fhb1* provides the plant with Type II resistance or resistance to spread within the seed head after initial infection. In some studies, *Fhb1* has been shown to reduce the impact of FHB and FHB symptoms by 20-25% overall (Anderson 2007). In comparison to exotic resistance, native resistance delivers a lower level of resistance. Some native sources of FHB resistance include those derived from 'Bess', 'Truman', 'Ernie', 'Jamestown', and 'Neuse' (Griffey 2008).

Genome-Wide Association Studies

Genome-wide association studies (GWAS) have historically been used for human genetics research, where segregating populations cannot be created. More recently, they have become popular for identifying molecular markers for utilization in plant breeding and as a step in genomic selection, which aims to predict performance of crosses (Hamblin 2011). GWAS have been used extensively in wheat and other small grains for quantitative traits such as yield, disease resistance, milling quality, and plant structure (Brachi 2011). In comparison to quantitative trait loci (QTL) mapping, which is performed on bi-parental mapping populations, a GWAS approach is capable of handling large panels of unrelated individuals (Brachi 2011). The use of unrelated lines results in increased variation among individuals due to past recombination events. As a result, higher mapping resolution is expected with GWAS compared to biparental QTL mapping (Myles 2009).

Several research projects have already made use of GWAS for studying disease resistance in wheat. One example screened 273 SRW wheat lines from the University of Illinois wheat breeding program and found ten significant SNPs associated with resistance to FHB (Arruda 2016). A similar study consisting of 455 European winter wheat lines was conducted by

Miedaner et al. and 9 significant SNPs were found to be related to FHB resistance (Miedaner 2011).

CHAPTER 2: GENOME-WIDE ASSOCIATION ANALYSIS OF *FUSARIUM* HEAD BLIGHT RESISTANCE IN SOFT RED WINTER WHEAT USING A GENOTYPE-BY-SEQUENCING APPROACH

Abstract

Fusarium head blight (FHB) is a disease of wheat caused by the fungal pathogen *F. graminearum.* FHB poses potential economic losses and health risks due to the accumulation of the mycotoxin deoxynivalenol (DON) on infected seed heads. Genetic resistance to FHB is quantitative and relies on several small-effect QTL to provide partial resistance. A genome-wide association study (GWAS) was conducted on 360 soft red winter wheat (SRWW) inbred lines adapted to the southern US to identify novel QTL for FHB resistance. From 2013-2017, the association mapping panel (AMP) was evaluated for incidence, severity, *Fusarium* damaged kernels, and DON accumulation in inoculated misted FHB nurseries in a randomized complete block design. Genotype-by-sequencing (GBS) identified 71,428 high quality single nucleotide polymorphisms (SNP) markers across all twenty-one wheat chromosomes. To determine significant marker-trait associations, a K-PC model accounting for the kinship (K) matrix and the first three principle components (PC) was included in a compressed mixed linear model (cMLM) using the GAPIT function in R software. Fifty-eight highly significant ($p < 0.0001$) SNPs were associated with one of four disease-related phenotypic traits. Highly significant SNPs were identified in the 1A, 2D, 3B, 4A, 4B, 7A, and 7D chromosomes. The minor allele for the most significant SNP associated with incidence was responsible for a reduction in incidence of 12.2% and an additive effect was observed when in combination with the favorable alleles from the second and third most significant SNPs associated with incidence. Overall, our results

demonstrate the potential of these SNPs for marker assisted selection for increased resistance to FHB in the University of Arkansas wheat breeding and genetics program.

Introduction

Fusarium head blight is a disease of small grains caused by fungal pathogens of the *Fusarium* species. *Fusarium graminearum* is the main causal organism in the United States, however, *F. culmorum* and *F. avenaceum* though less common, may also contribute to disease outbreaks. In the 2015-2016 growing season, economic losses incurred by U.S. wheat producers due to *Fusarium* head blight were estimated at $4.2 billion dollars (Nganje 2017). Economic losses may be due to yield decrease, mycotoxin accumulation, loss of grain quality, or failed preventative efforts such as late fungicide application.

Accumulation of mycotoxins such as deoxynivalenol (DON) may not only increase financial loss for producers, but is also a potential health risk to consumers. Consuming high levels of DON infected grain has several adverse effects on health of both humans and animals including diarrhea, nausea, vomiting, and weight loss in animals (Sobrova, Adam et al. 2010). Several studies have found that mycotoxins are not decomposed in current production methods used in the food processing industry (Hazel 2004). The U.S. Food and Drug Administration limits the amount of DON in finished human food to less than one part per million and in animal feed to less than ten parts per million with the added recommendation that contaminated grains not exceed more than 50% of the animal's diet (FDA 2010).

Due to the substantial economic losses associated with this disease, breeders are actively looking to incorporate sources of genetic resistance into their breeding programs. There are four types of resistance to FHB including I) resistance to initial infection (incidence), II) resistance to spread within the spike (severity), III) resistance to *Fusarium* damaged kernels (FDK), and IV) resistance to the accumulation of mycotoxins (DON). Resistance may come in the form of native or exotic resistance. The most commonly recognized exotic resistance gene is *Fhb1*. *Fhb1* was

first discovered in the Chinese cultivar Sumai3 and confers a type II resistance. Several native resistance quantitative trait loci (QTL) have been identified in the soft red winter wheat (SRWW) gene pool. Native resistance QTL which are currently in use in southern SRWW breeding programs include: 'Bess', 'Ernie', 'Jamestown', 'Neuse', and 'Truman' among others (McKendry 1995, Murphy 2004, McKendry 2005, McKendry 2007, Wright 2014) (Table 1). Once resistance is established in a breeding program, breeders may choose to take advantage of marker-assisted selection (MAS) in order to improve breeding efficiency. MAS is an effective tool to speed up classical breeding by indirectly selecting for desired traits by selecting for molecular markers associated with those traits (He 2014).

Marker assisted selection is a valuable tool for modern plant breeders as it saves time, money, and resources when compared to conventional breeding (Collard 2008). Markers are generated through linkage mapping studies. Several bi-parental linkage mapping studies have identified at least one QTL for FHB resistance on every wheat chromosome. An article published by Liu et al. in 2009 reviewed 249 resistance QTL to identify stable FHB resistance (Liu 2009). In comparison to bi-parental linkage mapping studies where all individuals are related, genome-wide association studies (GWAS) capitalize on the genetic variation due to historical recombination events in a group of unrelated individuals (Lin, et al. 2016). The use of unrelated individuals with random recombination events results in a higher mapping resolution. An important consideration in GWAS is that there is enough marker coverage on the genome so that desired alleles will be in linkage disequilibrium with at least one molecular marker (Lin, et al. 2016).

Several GWAS have been performed in wheat to identify resistance to FHB. In 2011, Miedaner et al. evaluated 455 European soft red winter wheat lines for FHB resistance using 115

simple sequence repeat (SSR) markers and found nine significant marker-trait associations (MTA) with two unique genomic regions on chromosomes 1D and 3A (Miedaner 2011). In comparison to the SSR marker technique, Kollers et al. used 732 short tandem repeats (STR) markers to identify MTA for FHB resistance in the form of incidence and severity (Kollers 2013). This study detected significant associations on all chromosomes except 6B, several of which coincide with regions evaluated by Buerstmayr et al. in 2009. Arruda et al. (2016) used a panel of 273 soft red winter wheat lines and 19,992 SNPs to identify marker-trait associations on every wheat chromosome including significant associations on chromosomes 1D, 3B, 4A, 4D, 6A, 7A, and 7D. Several of the SNPs found on chromosome 3B were associated with *Fhb1*.

This study accomplishes three objectives: 1) evaluate soft red winter wheat (SRWW) lines for resistance to FHB in terms of resistance to initial inoculum (incidence); resistance to spread within the head (severity); resistance to DON accumulation; and resistance to *Fusarium* damaged kernels (FDK), 2) determine the frequency and effect of known FHB resistance genes and quantitative trait loci (QTL), and 3) identify novel resistance loci using a genome wide association (GWA) approach.

MATERIALS AND METHODS

Plant Materials

An association mapping panel (AMP) of 360 SRWW lines was used in this study which consisted of 240 lines developed by the University of Arkansas Wheat Breeding Program, and 40 lines each from the University of Georgia, Louisiana State University, North Carolina State University (Appendix A). These public programs represent the majority of source germplasm used in the University of Arkansas wheat breeding program. In addition to the AMP, two checks were included: an FHB resistant check, ‘Bess’, and an FHB susceptible check, ‘Coker 9835’.

‘Bess’ shows native resistance to FHB in the form of type II resistance or resistance to spread within the head (severity). ‘Coker 9835’ shows susceptibility in terms of incidence and severity but to a lesser extent for accumulation of DON.

Field Screening for *Fusarium* Head Blight Resistance

The AMP was grown in inoculated and misted FHB disease nurseries in Fayetteville (FAY) and Newport (NPT) in Arkansas over four growing seasons from 2013-2017. In 2013-2014 and 2014-2015 only a partial set of the AMP (120 different Arkansas lines each year) were evaluated. In both 2015-2016 and 2016-2017, all 360 lines were grown. For field experiments, lines were drill seeded at a rate of six grams per square meter plot for a seeding rate of ~65kg/ha in two-row plots in a randomized complete block design with two replications. Plots were managed according to the recommendations for wheat in Arkansas (Kelley, 2012). The plots in Fayetteville, AR received 100 kg/ha of urea while plots in Newport, AR received 77 kg/ha of urea and were also supplemented with 24 kg/ha of ammonium sulfate. A combination of herbicides including Axial XL (Syngenta), Harmony Extra (DuPont), and Osprey (Bayer) was used each year to control weeds.

The disease nurseries were inoculated with *F. graminearum* infected corn (*Zea mays* L.). The inoculant consisted of seven different *F. graminearum* isolates collected at various research stations in Arkansas and grown each year on fermenting corn kernels. The isolates are stored from year to year at 4ºC and then grown at room temperature in petri dishes on potato dextrose agar to prepare the initial inoculum. To prepare the corn inoculum, kernels were first soaked in water and then autoclaved twice, 24 hours apart, to remove all other fungi and bacteria cells. After the corn was sterilized, it was divided among 14 metal trays and 1 cm^2 pieces of active *F. graminearum* isolates were scattered evenly throughout the tray. The trays were covered and the

fungus permitted to grow for three weeks with weekly stirring of the corn to spread fungus as evenly as possible throughout the tray. When all corn kernels were covered with the fungus, the kernels were spread out on drying racks and allowed to dry for three days before being returned to their trays to await dispersal. Initially, the isolates were grown in separate trays; later, all isolate infected corn was combined before spreading the infected corn in the field. Inoculum was spread by hand in the field at a rate of ~65 kernels m^{-2} when wheat reached a growth stage between 6 and 8 on the Feekes scale, allowing for colonization of corn kernels in the field and production of black perithecia before the wheat began to head (Feekes 10.1) (Larson, 2015).

Following the spread of inoculum, mist irrigation was set up every sixth row throughout the disease nursery to provide complete coverage. Mist irrigation commenced at the time perithecia were observed on the corn inoculum to provide optimal conditions for FHB infection and spread throughout the months of April and May. Duration of misting was adjusted for each location based on the available precipitation and dew point. In order for the fungus to spread it was important for the young seed heads to remain moist. Timing of misting as well as duration varied between locations and years. During a particularly dry spring, plots may be misted for longer or more often in comparison to a wet spring. In general, the Fayetteville location required less total misting time than the Newport location. In the 2014-2015 season, Fayetteville received a total of 720 minutes of misting while Newport received 784 minutes. Fayetteville received 480 minutes for both the 2014-2015 and 2015-2016 seasons. In those same seasons, Newport received 720 and 520 minutes, respectively. In the 2016-2017 season, Fayetteville required 544 minutes of misting while Newport totaled 704 minutes of misting.

Phenotypic Measurements

Data were collected for four FHB resistance traits: incidence, severity, *Fusarium* damaged kernels (FDK), and deoxynivalenol accumulation (DON). In addition, heading date (HD) and plant height (HT) were also recorded for each line in this panel. Heading date was recorded in Julian days (days since January 1) when 50% of the seed heads have extended 50% of the way out of the flag leaf. As there was variation in heading date between lines in the AMP, heading notes were recorded every other day from the onset of heading and continuing until all plots in the nursery were headed. At maturity, plant height was recorded in inches from the surface of the soil to the tip of the awn.

Ratings for FHB resistance were recorded beginning at one week after the average heading date (Feekes 10.5) to allow time for disease infection and spread. At each location, incidence and severity ratings were collected on the same day first for incidence and then severity. The first set of field ratings were generally collected beginning in mid-May at the FAY location with the second set of ratings collected about ten days later than the first. In comparison, field ratings in NPT typically began in late May. Incidence was recorded as a percentage of the total number of heads in a plot that showed any sign of infection regardless of how severe or contained the infection was. Severity was estimated as a percentage of total infected spikelets within each head within the plot. Both incidence and severity were recorded on a scale of 0-9% with 1% increments in plots with infection occurrence less than 10% and on a scale of 10-100% with increments of 5% for plots with initial infection levels higher than 10%. Two sets of severity and incidence ratings were recorded each year for the FAY plots and in NPT one set of incidence and severity ratings were recorded for each plot with a second, incomplete, rating recorded ten days to two weeks later. In general the second set of ratings were used for further

analysis excluding cases where the first produced a higher disease rating. Severity ratings were collected immediately after incidence ratings had been recorded at each location.

At maturity lines were hand harvested and threshed using a Vogel thresher. To retain as many damaged and shrunken kernels as possible, the thresher was set to a very low speed as seeds were collected. After the threshing of each plot, excessive chaff was removed by hand before threshing of the next plot began. Seeds were stored in labeled envelopes at room temperature before being evaluated for DON accumulation and *Fusarium* damaged kernels.

The total percentage of *Fusarium* damaged kernels (FDK) was evaluated after harvest. Samples from each line were compared to a set of standards to determine what percentage of kernels showed damage due to FHB. The standards ranged in percentage of FDK from 0-75% in increments of 10%. Standards were created by counting and combining damaged kernels with healthy kernels in a sample of 300 kernels for each increment of FDK.

The analysis of deoxynivalenol (DON) accumulation was conducted by the Plant Pathology Department at the University of Minnesota. Post-harvest, 50 g samples of grain from each of the 360 lines and both checks were sent to the University of Minnesota where DON accumulation was conducted by gas chromatography.

Phenotypic Data Analysis

For each of the four FHB-associated phenotypic traits, best linear unbiased predictions (BLUPs) were calculated using a mixed model approach following the model:

$Y_{ijk} = \mu + environment_i + rep\ (environment) + line_k + heading_{ijk} + (environment\ x\ line) + \varepsilon_{ijk}$

Where Y_{ijk} is the observed phenotype, μ is the overall mean, $environment_i$ is the random effect of the ith environment, $rep(environment)_{ij}$ is the random effect of jth rep within the ith environment, $line_k$ is the random effect of the kth line, $heading_{ijk}$ is a quantitative covariate

trait treated as fixed, consisting of the Julian day for the kth line in the jth rep within the ith environment, $environment\ x\ line_{ik}$ is the random effect of the interaction between the ith environment and the kth line, and ε_{ijk} is the random error term. Correlations were determined between all four disease-related traits and two phenological traits, heading date and mature plant height. A strong correlation between heading date and all four disease traits was observed. To account for this correlation, heading date was included as a covariate when calculating BLUPs. The plot mean-based broad-sense heritability (H^2) was calculated for each trait across long using the variance components estimated from the equation below.

Heritability values (H^2) were calculated using the following formula:

$$H^2Entry = \frac{\sigma^2 Genotype}{\sigma^2 Genotype + \frac{\sigma^2 Genotype\ x\ Location}{location} + \frac{\sigma^2 Error}{location\ x\ reps}}$$

The variances were estimated by analysis of variance (ANOVA) using PROC MIXED function in SAS v 9.4 (SAS Institute Inc. Cary. NC).

DNA Extraction

For DNA extraction, the 360 AMP lines were germinated and allowed to grow for two weeks before tissue was harvested. Two 100-mg tissue samples were harvested from each of the 360 lines in the AMP as well as from 2-3 positive controls for each of the five resistance QTL. Samples were immediately stored in Eppendorf tubes and placed in ice while sampling was taking place before being stored in -80ºC until extraction.

Frozen tissue samples were ground using titanium beads in the Qaigen TissueLyser before DNA extraction was performed using a modified version of the cetyl trimethylammonium bromide (CTAB) protocol (Pallotta 2003). DNA concentration was determined using the

NanoDrop 2000 (Thermo Fisher Scienctific) spectrophotometer, diluted to 20 ng/µL and stored at 4ºC.

KASP Genotyping

The AMP lines were screened using KASP assays for five known FHB resistance QTL using SNP markers designed by and currently being used to screen the Uniform FHB Nursery (UFHBN) at North Carolina State University (Murphy, personal communication). The five known FHB QTL included *Fhb1* and four native resistance QTL (*Qfhb.nc-2B.1* (*Bess*), *Qfhb.nc-3B.1* (*Bess*), *QTL_1B* (*Jamestown*), and *QTL_1A* (*Neuse*) (Table 2). The KASP protocol follows that developed by the AgriGenomics Lab at Texas A&M University. KASP reactions were performed in a total volume of 5µL, following the manufacturer's instructions with some modifications. Before addition of the KASP mix (master mix + primer mix), 5µL of sample DNA were plated and dried for one hour. After one hour, 5µL of the KASP mix were added to each well. The conditions for thermal cycling using the Bio-Rad CFX96™ Real-Time system (Bio-Rad, CA, USA) were as follows: 94°C for 15 min (hot-start activation); 94°C for 20 sec, and 65-58°C (decrement of 0.8°C per cycle) for 9 cycles; 94°C 20 sec and 57°C for one minute for 25 cycles; 35°C for 3 min and a plate read step. Additional thermal cycling was used as needed to improve accuracy and precision of clustering. The profile for the cycling step is: 94°C for 20 seconds followed by 57.0°C for one minute for 2 cycles; and 35°C for one minute plus a plate read step. A single marker analysis (SMA) was run in SAS v. 9.4 to determine the effects of each known resistance QTL.

Genotype-by-Sequencing

All accessions in the AMP were genotyped by using genotyping by sequencing (GBS) in collaboration with the USDA Eastern Regional Small Grains Genotyping Lab in Raleigh, NC.

DNA was extracted using Mag-Bind® Plant DNA Plus kit from Omega Bio-tek (Norcross, GA, USA), following the manufacturer's instructions. Genomic DNA was quantified using Quant-iT™ PicoGreen® dsDNA Assay Kit and normalized to 20 ng µl-1. GBS libraries were created using *Pst1-Msp1* and/or *the Pst1-Mse1* restriction enzyme combinations (Poland, 2012). The samples were pooled together at 192-plex to create libraries and each library was sequenced on a single lane of Illumina Hi-Seq 2500.

SNP calling was performed using the TASSEL 5 GBSv2 pipeline using 64 base kmer length and minimum kmer count of 5 (Bitbucket, 2017). Reads were aligned to wheat reference "IWGSC_WGAv0.4" using the alignment method of Burrows-Wheeler aligner (BWA) version 0.7.10 (Li, et al., 2009). Raw SNP data generated from the TASSEL pipeline were filtered to remove taxa with more than 90% missing data. Genotypic data were then filtered to select for biallelic SNPs with maf ≥ 5%, missing data ≤ 50% and heterozygosity ≤ 10% (Li et al. 2009). Fast and accurate short read alignment was performed with Burrows-Wheeler transformation. Remaining missing data were imputed using the LD-kNNi function in TASSEL.

Genome-Wide Association Analysis

The genome-wide association analysis was performed using the Genome Association and Prediction Integrated Tool (GAPIT) function in R software (Lipka 2012). Three types of GWAS models were used: 1) a naïve model, which used no principle components or kinship structure, 2) a kinship model using only the kinship structure, and 3) a K-PC model that took advantage of the kinship structure and the principle component analysis. In the K-PC model, which is the only model using the principle component analysis, the PCA was set equal to 3. The best fit model for this study was chosen by comparing the distribution of observed *p*-values to the cumulative *p*-value (Bordes 2013). A diagonal line formed by the comparison of the negative $\log_{10}$ of the

observed *p*-values and the cumulative *p*-values indicates the reliability of the model. Any variation from this diagonal line indicates that the model includes false associations. The significance of marker-trait associations was based on a *p*-value of <0.0001. A total of ten datasets were used for association mapping. In addition to a combined dataset where BLUPs were calculated across all environments, BLUPs were also calculated for the individual site years: FAY14, NPT14, FAY15, NPT15, FAY16, NPT16, FAY17, NPT17, and LSU17. SMA run in SAS v. 9.4 were used to determine the allelic and additive effects of highly significant SNPs identified by GWAS for each trait.

RESULTS

Phenotypic Data

Significant genotype (line) variation was observed for all four FHB traits, with a range of 0-100% observed for incidence in FAY16 and NPT17, severity in FAY16 and NPT17, and FDK in FAY16, NPT16, and LSU17. For DON, the largest single site-year range occurred in FAY16 from 0.385 to 26.9 ppm. The highest mean value for DON was found in the FAY15 site-year with a value of 29.5 ppm, nearly 30 times the FDA limit restriction of 1 ppm for direct human consumption (FDA 2010).

An analysis of variance showed a significant ($p<0.0001$) effect for genotype, environment, and genotype x environment for each of the four FHB traits (Table 3). A significant correlation ($p< 0.0001$) was observed between heading and incidence, severity, and FDK. DON was also correlated with heading at a $p<0.001$ significance level. Plant height was found to be significantly ($p<0.01$) correlated only with FDK (Table 4).

The highest heritability value across all nine-site years was observed for FDK ($H^2 = 0.82$) and the lowest for severity ($H^2 =0.38$) (Table 3). In general, moderately high heritability values

were determined with significant environment x line variation minimized by evaluating the population across nine site-years.

Native Resistance QTL

The frequency of known resistance QTL within the AMP was low, ranging from 0% for *Qfhb.nc-2B.1* (*Bess*) and *Fhb1* to 11% for *QTL_1B* (*Jamestown*) (Table 2). There were a total of 66 lines in our AMP homozygous for the favorable allele at one or more native resistance QTL. Single marker analysis showed the favorable allele of *Qfhb.nc-3B.1* (*Bess*) to significantly reduce incidence, severity, and DON accumulation (Table 2). *Qfhb.nc-3B.1* (*Bess*) was present in the AMP at a frequency of 6.9%. A significant effect was not observed for the additional known resistance QTL.

Population Structure

Genotype-by-sequence (GBS) identified 71,428 SNPs after the filtering criteria were applied. SNPs were distributed unevenly across the A, B, and D genomes with the highest number being from the B (33,530) followed by A (26,823) and the D (9,402) genomes with 1,673 SNPs left unassigned to a chromosome.

Principle Component Analysis (PCA) showed two main clusters, with lines from each breeding program present in both (Figure 1). While some sub-clustering based on the program origin of the lines was observed within the main clusters, it is hypothesized that the main effect was due to the presence or absence of the stem rust resistance gene *Sr36*, which is the result of a translocation from *Triticum timopheevii* (Nyquist 1962). Overall, the population structure in this mapping panel was low with the first three PCs accounting for only 6.0, 5.0, and 3.8% of the total genetic variation, respectively (Figure 1).

Marker-Trait Associations

When BLUPs calculated across all site-years were used for GWAS, the K model detected the greatest number of significant SNPs ($p<0.0001$) at ninety-one marker-trait associations (MTA). The naïve model followed the K model with eighty-two significant SNPs and the K-PC model detected the fewest number with fifty-eight significant SNPs spread across the four traits (Table 5). The K-PC model was chosen as the best fit model for this study after examining QQ-plots and further performing a comparison between the negative $\log_{10}$ of observed *p* values and cumulative *p* values.

Single Site-year Associations

In comparison to the dataset wherein BLUPs were calculated across all nine site-years, BLUPs calculated individually for each site-year gave a total of 289 significant MTA at a threshold of $p<0.0001$ spread out across eight of the nine site-years. Only FAY14 was found to have no significant marker-trait associations at a significance threshold of $p<0.0001$. Sixty-five of the 289 MTAs were highly significant for more than one site-year trait with forty-eight significant for two site-year traits, thirteen significant for three site-year traits, two significant for four site-year traits, and two significant for five site-year traits. SNPs significantly associated with two or more site-year traits were found in four site-years including: FAY16, FAY17, NPT16, and NPT17 (Table 6). Of the duplicated SNPs, FDK had the highest number of MTAs with 36 unique SNPs associated with this trait. In comparison to FDK, DON was found to have the fewest number of duplicated SNPs with only two SNPs found in association with 2 or more site-year traits. Both DON SNPs were significantly associated with DON in FAY17 and NPT17. There were two SNPs associated with five site-year traits located on chromosome 4B. The two

SNPs are in linkage and found to be highly significant for incidence (FAY16), severity (FAY16), and FDK (FAY16, NPT16, and NPT17) (Table 6).

FHB-Related Traits

Incidence

GWAS used to evaluate data across all nine site-years in a K-PC model identified 19 highly significant SNPs ($p<0.00001$) associated with incidence (Figure 2.A.). The most highly significant SNP, S4A_574518163 was on chromosome 4A and accounted for 14.7% of the variance for incidence within the population. The minor allele for S4A_574518163 was found at a frequency of 9.7% and is estimated to reduce incidence by 12.2% when compared to lines containing the unfavorable major allele (Table 7). The second and third most highly significant SNPs associated with incidence, S7A_11152072 and S2D_526929200, accounted for 13.4% and 13.3% of the variance in incidence. At both loci, the minor allele was found to be favorable and reduced incidence by 10.7% (S7A_11152072) and 13.3% (S2D_526929200) (Table 7). The minor allele was present a frequency of 11.8% for S7A_11152072 and 7.7% for S2D_526929200. An additive effect was observed when combining the favorable alleles (+) for each of the three most highly significant SNPs. A haplotype of '+ + +' resulted in a mean incidence rating of only 3.9% compared to the unfavorable haplotype (- - -) which resulted in a mean rating of 30.1% (Figure 3.A). Two other combinations of alleles, '+ + -' and '- + -', resulted in mean ratings of 21.5% and 16.5%, respectively, which were not significantly different from the mean rating of our favorable allele combination.

Severity

Eight significant SNPs were identified for severity using the K-PC model and BLUPs calculated across all nine site-years (Figure 2.B). All eight of the SNPs were located on chromosome 3B, with S3B_784540562, S3B_795306092, and S3B_784573154 being the most highly significant and accounting for 12.1- 12.3% of the variance in severity within the AMP. The minor allele frequency for S3B_784540562, S3B_795306092, and S3B_784573154 were 41.5%, 6.0%, and 41.6%, respectively (Table 7). In each case, the minor allele was responsible for higher severity and resistance was associated with the major allele. The major allele of the most significant SNP, S3B_784540562, reduced FHB severity by 4.6%. The second and third most significant SNPs, S3B_795306092 and S3B_784573154, were responsible for reductions of 10.8% and 4.2%, respectively. An additive effect was not observed when comparing the favorable haplotype (+ + +) with the unfavorable haplotype (- - -) (Figure 3.B).

Fusarium damaged kernels

GWAS utilizing the K-PC model identified one highly significant MTA for FDK (Figure 2.C.). The significant SNP, S3B_526480094, found on chromosome 3B and explained 10.8% of the variance for FDK within the panel. The minor allele in this case was responsible for a reduction in FDK of 7.7% and was present at a frequency of 5.7%.

Deoxynivalenol accumulation

A GWAS for DON was run with a limited set of environments (FAY15, NPT15, NPT16, and LSU17) due to low accumulation of DON in the other environments. Three SNPs identified on chromosomes 1A, 4B, and 7B were significantly associated with DON accumulation (Figure 2.D.). The SNPs, S1A_282055814, S4B_21625964, and 7B_595827248 explained an estimated

variance within the panel of 7.3 to 7.6%. The major allele of the most highly significant SNP, S1A_282055814, was found at a frequency of 5.5% and was responsible for an estimated reduction in DON accumulation of 1.83 ppm though this reduction was not statistically significant based on a standard t-test (Table 7). The minor allele of the second most significant SNP, S4B_21625964, was found at a frequency of 30.0% in the AMP and was responsible for an estimated reduction in DON of 0.83 ppm. This was the only significant contribution to DON accumulation as the major allele of the third SNP reduced DON by 1.63 ppm and did not qualify as a statistically significant reduction. An additive effect was not observed when comparing any combination of favorable and unfavorable alleles (Figure 3.C).

Discussion

FHB is an important disease in wheat as it causes yield losses due to shrunken, damaged kernels as well as economic losses due to the accumulation of DON. Breeding to develop resistant wheat varieties could help minimize the effects of this disease. Resistance to FHB is complex and relies on the combined effects of several resistance QTL. In this study it was hypothesized that there was untapped native resistance to FHB present in the mapping panel and that the resistance was controlled by SNPs. In order to test this hypothesis data were collected on four phenotypic disease traits and a genome-wide association analysis was used to associate lower levels of disease traits to the genome-wide SNP data which were generated through genotype-by-sequencing (GBS). A total of 71,428 SNPs were identified by GBS and used to determine MTAs. GWAS was performed on a mapping panel consisting of 360 soft red winter wheat lines from four breeding programs in the southeastern region of the United States.

Population structure can lead to false positives and accounting for this in GWAS is necessary (Rosenberg 2010). Population structure analyses based on principal components

revealed two major groups which did not cluster based on origin. A GWAS study relies on the historic recombination of semi-unrelated individuals, and neglecting to correct for relatedness or population structure present may lead to the detection of false positive marker-trait associations. The population structure was determined using a principle component analysis (PCA) where all SNPs were used. The PCA results showed the panel clustering into two main groups with the first three principle components contributing a total 14.8% to total genetic variance. A high level of relatedness was expected as the lines originated from four breeding programs that share germplasm. In addition, two thirds of the lines in the AMP were advanced breeding lines from the University of Arkansas breeding program. Lines do not seem to cluster based solely on origin, and instead seem to cluster based on the presence or absence of *Sr36*, a stem rust resistance gene translocated from the grass species *Triticum timopheevii*.

The best fit model was the K-PC which accounted for both kinship as well as principle components. Models lacking a correction for principle components and relatedness identified more MTAs than those that did not and thus I hypothesize that the additional MTAs identified by these models are most likely false positives. The four breeding programs contributing lines to this study did so as a part of a U.S. Wheat and Barley Scab Initiative (USWBSI) project and have been known to exchange germplasm in the past. There is an underlying relatedness factor in this study though it did not significantly impact our PCA. The inclusion of the kinship matrix in our GWA analysis was a vital component for controlling the underlying relatedness.

Correlations were calculated between two phenological traits, heading date and mature plant height, and four disease-related phenotypic traits. While significant correlations were observed between heading date and each of the four disease related traits, height was not significantly correlated with any of the disease traits in the 2015-2016 growing season and with

only FDK at the Winnsboro, LA location in 2016-2017. The lack of correlation between height and disease traits was unexpected as studies have shown a relationship between plant height and FHB infection where shorter plants are more likely to become infected due to their closer proximity to splash-dispersed spores (Jenkinson 1994).

Although researchers have described several QTL for FHB resistance, few of them are stable across environments and germplasms. Perhaps the most stable resistance QTL is *Fhb1* located on the short arm of chromosome 3B. KASP assays determined that there were no lines within the AMP that were homozygous for the favorable *Fhb1* allele; however, three very highly significant SNPs, S3B_784540562, S3B_795306092, S3B_783061490, located on chromosome 3B were identified which were significantly associated with severity. While it is assumed that these SNPs are not linked to *Fhb1* due to the low frequency of *Fhb1* in the AMP, two native resistance QTL, *Qfhb.nc-3B.1* (*Bess*) and *QTL_3BL* (*Massey*), are also located on chromosome 3B. KASP markers were used to determine that *Qfhb.nc-3B.1* (*Bess*) is present in 6.9% of the AMP. *Qfhb.nc-3B.1* (*Bess*) was responsible for a reduction in all four disease-related traits with a significant reduction in incidence, severity, and DON. Resistance in the AMP seems to be mainly type I and type II resistance. GWAS identified the majority of significant SNPs to be associated with incidence in this study. In addition to the sheer volume of significant SNPs associated with incidence and severity, significant additive effects were also evaluated for both of these traits (Table 3).

Incidence

The most significant SNPs showing a reduction in incidence due to the minor allele were S4A_574518163, S7A_11152072, and S2D_526929200, located on chromosomes 4A, 7A, and 2D, respectively. Both Buerstmayr et al. (2009) and Liu et al. (2009) published reviews of all

reported FHB resistance QTL to date and each showed QTL found on chromosomes 4A and 2D were responsible for a reduction in incidence. The QTL reviewed on chromosome 4A had been previously described by Steed et al. in 2005 and was known to reduce incidence (Steed 2005). Arruda et al. (2016) reported two SNPs on chromosome 4A and 7A responsible for a reduction in incidence. These two SNPs may be related to our SNPs found on the corresponding chromosomes as the panels used in the study share some background. However, given the new marker technology that we are utilizing it is difficult to make a direct comparison to historical FHB mapping studies.

Severity

Chromosome 3B contained all eight highly significant MTAs for severity. A similar study by Arruda et al. (2016) also found four highly significant SNPs associated with severity to be found in chromosome 3B. The SNPs found by Arruda et al. (2016) were located between 6.86cM and 18.32cM. In addition to the study conducted by Arruda et al. (2016), several other studies have also discovered significant SNPs associated with severity. Most significantly, Buerstmayr et al. (2013) discovered a QTL on chromosome 3B responsible for a reduction in severity of 29.1%. While the SNPs found in this study may not be related to *Fhb1* which is found on the short arm of chromosome 3B, there are other known resistance QTL located on 3B including *QTL_3BL* (*Massey*), located on the long arm of 3B, and *Qfhb.nc-3B.1* (*Bess*) which was found at a frequency of 6.9% in the AMP.

***Fusarium* Damaged Kernels**

This study discovered one SNP significantly associated with FDK found on chromosome 3B. A QTL associated with FDK has been previously described on this chromosome by Yang et al. (2006). Yang et al. (2006) screened a population of seventy-nine recombinant inbred lines

developed by crossing a resistant parent (Chokwang) and a susceptible parent (Clark). Yang et al. (2006) discovered a QTL on chromosome 3B with stable levels of type III resistance.

Deoxynivalenol Accumulation

This study identified three highly significant SNPs associated with DON accumulation located on chromosomes 1A, 4B, and 7B. Several previous studies have described QTLs found on both the long arm and short arm in chromosome 1A that contribute to type IV resistance. Jiang et al. (2007) reported a QTL located on the short arm of chromosome 1A which explained 4-6% of the variation for DON within the population. Also in 2007, a stable QTL was reported on chromosome 4B by McCartney et al. (2007) in a backcrossing population segregating for resistance genes contributed by FHB resistant line Wuhan-1. The significant SNP found in this study on chromosome 7B may potentially be unique to the AMP.

Conclusion

The complexity of the wheat genome combined with the quantitative nature of FHB resistance leads to difficulty when breeding for resistance. This study identified several SNPs on chromosomes where previously reported resistance QTL were located. Identification of MTA on chromosome 3B resulting in a reduction in severity, FDK, and DON suggests that known QTL are contributing resistance in this panel and are a worthwhile target for MAS. The overall goal for this research is to one day use the SNPs identified by this study for marker assisted and genomic selection in the University of Arkansas Wheat Breeding Program.

TABLES AND FIGURES

Table 1. Previously reported resistance QTL associated with Fusarium head blight in wheat.

Source	Type	Chromosome	Type of Resistance	Reference
Sumai 3	Exotic	3B (*Fhb1*)	Severity	Waldron et al., 1999
Bess	Native	2B, 3B	Severity	McKendry et al., 2007
Ernie	Native	2B, 3B, 5A	Severity	Liu et al., 2007
Goldfield	Native	2B, 7B	Incidence	Gilsinger et al. 2005
Jamestown	Native	1B, 6A	Inc, Sev	Griffey et al., 2010
Neuse	Native	1A, 4A, 6A	Inc, Sev, FDK	Murphy et al., 2004
Truman	Native	2B, 3B	Inc, Sev, FDK, DON	McKendry et al., 1995
Wuhan	Exotic	2D	Severity	Jiang et al., 2007

Table 2. Frequency and effect of known resistance QTL within the association mapping panel (AMP).

			Allelic Effect†			
QTL	**Chromosome**	**Frequency**	**Incidence**	**Severity**	**FDK**	**DON**
Qfhb.nc-2B.1 (Bess)	2B	0.0%	-	-	-	-
Qfhb.nc-3B.1 (Bess)	3B	6.9%	-5.8*	-6.0*	-5.8	-2.3*
Sumai 3_*Fhb1*	3B	0.0%	-	-	-	-
QTL_1B (Jamestown)	1B	11.1%	1.3	1.2	0.6	0.1
QTL_1A (Neuse)	1A	0.56%	-0.2	-1.8	2.0	3.9

†Allelic effect reported in reference to the minor allele
*Significant based on an LSD set at α=0.05

Table 3. Descriptive statistics and analysis of variance 360 soft red winter wheat lines.

						Means Squared		
Trait	**Mean**	**Min**	**Max**	**SD**	**$H^{2\dagger}$**	**Genotype**	**Environment**	**GxE**
Inc	28.9	0.8	80.0	35.4	0.78	4.26**	106.21**	3.19**
Sev	24.8	0	70.0	24.8	0.38	8.38**	26.76**	2.90**
FDK	31.5	3.2	83.0	29.7	0.82	15.93**	11.37**	4.18**
DON‡	10.1	1.7	27.6	11.4	0.79	13.05**	8.81*	3.90**

** Significant at p<0.0001.

*Significant at p<0.001

†Broad sense heritability (H^2) values calculated on an entry-mean basis for each of four phenotypic traits.

† Inc, incidence; Sev, severity; FDK, Fusarium damaged kernels;

‡ DON, deoxynivalenol accumulation. DON was measured in ppm, and the other parameters were measured in percentage.

Table 4. Correlations between physiological traits, heading date and mature plant height, and disease-related traits: incidence, severity, Fusarium damaged kernels, and deoxynivalenol accumulation.

Trait	Heading	Height	Incidence	Severity	FDK	DON
Heading	-					
Height	0.01	-				
Incidence	-0.37***	0.02	-			
Severity	-0.30***	0.03	0.55***	-		
FDK	-0.21***	0.14*	0.30***	0.37***	-	
DON	0.18**	0.01	-0.02	0.05	0.13	-

***Indicates significance at p<0.0001
**Indicates significance at p<0.001
*Indicates significance at p<0.01

Table 5. Comparison of three statistical models for amount of quantitative trait loci (QTL) associated with each FHB related phenotypic trait with significance set at $p<0.0001$. Marker-trait associations were determined using a panel of 360 advanced breeding lines genotyped with 71,428 single nucleotide polymorphisms.

Trait	Naïve	K	K-PC (cMLM)	Chromosomes
Incidence	55	66	46	2B, 2D, 3B, 4A, 5B, 6B, 7A,7B
Severity	23	17	8	3B
FDK	1	4	1	3B
DON	3	4	3	1A, 4B, 7B
Total	82	91	58	

‡ "naïve", statistical model with no control for population structure and relatedness; K, relatedness controlled using a marker-based kinship (K) matrix, treated as random; cMLM, compressed mixed linear model.

Table 6. The mean and ranges for disease-related traits in nine site-years.

	Incidence			Severity			FDK†			DON‡		
Site-yr	Mean	Range	Geno†	Mean	Range	Geno†	Mean	Range	Geno†	Mean	Range	Geno†
FAY14	-	-	-	25.5	2.5-57.5	***	-	-	-	-	-	-
NPT14	-	-	-	46.0	15-75	***	-	-	-	-	-	-
FAY15	-	-	-	56.1	18.3-98.3	***	68.1	30-97.5	***	29.5	14.4-54.5	***
NPT15	-	-	-	50.1	10-95	***	53.4	10-85	***	25.1	7.9-58.6	***
FAY16	6.7	0-100	***	8.2	0-100	***	12.2	0-100	***	4.7	0.39-26.9	***
NPT16	19.3	0-95	ns	36.2	0-85	ns	12.2	0-100	***	15.6	2.2-43.0	***
FAY17	7.6	0-82.5	***	4.0	0-72.5	***	35.9	2-100	***	0.88	0.13-4.1	***
NPT17	53.5	0-100	***	35.6	0-100	***	28.9	2-100	***	5.9	0.7-22.3	***
LSU17	77.2	10-100	***	26.1	10-80	***	55.2	0-100	***	15.5	0.82-50.9	***

***Significant at $p<0.0001$

ns not significant

† FDK, Fusarium damaged kernels; Geno, genotypic variation

‡ DON, deoxynivalenol accumulation. DON was measured in ppm, and the other parameters were measured in percentage

Table 7. Single-nucleotide polymorphisms (SNPs) associated with Fusarium head blight resistance in a panel of 360 breeding lines, chromosomal position, p-values, frequency, and effects of favorable alleles. Unit of phenotypical traits are percent except DON (ppm).

Trait	SNP	Chr	Allele	p-value	Maf	Allelic Effect‡	R^2
Inc	S4A_574518163	4A	A/T	6.31E-08	0.097	-1.17	0.147
Inc	S7A_11152072	7A	C/T	5.89E-07	0.118	-0.98	0.134
Inc	S2D_526929200	2D	C/A	7.05E-07	0.077	-1.11	0.133
Sev	S3B_784540562	3B	A/G	4.00E-05	0.415	0.30	0.123
Sev	S3B_795306092	3B	T/A	5.54E-05	0.060	-0.61	0.121
Sev	S3B_783061490	3B	T/C	9.74E-05	0.423	0.29	0.118
FDK	S3B_526480094	3B	G/T	9.53E-05	0.057	-0.75	0.108
DON	S1A_282055814	1A	T/G	4.72E-05	0.055	1.83	0.076
DON	S4B_21625964	4B	G/A	6.31E-05	0.300	-0.83	0.074
DON	S7B_595827248	7B	T/C	7.59E-05	0.078	1.63	0.073

*Significant at $\alpha = 0.05$ level

‡Allelic effect reported as a BLUP in reference to the minor allele.

† Inc, incidence; Sev, severity; FDK, Fusarium damaged kernels; DON, deoxynivalenol accumulation

Table 8. All significant SNPs (p<0.0001) identified by GWAS calculated in individual site-years associated with two or more site-year traits.

Trait	Site-year	SNP	Chr	p-value	Maf	Allele	Allelic Effect	R^2
Inc	FAY16	S2D_427477848	2D	6.36E-06	0.054	A/G	-11.133	0.176
Inc	FAY16	S2D_427477849	2D	6.36E-06	0.054	G/A	-11.133	0.176
Inc	FAY16	S2D_427477862	2D	6.36E-06	0.054	A/G	-11.133	0.176
Inc	FAY16	S2D_427477868	2D	6.36E-06	0.054	C/T	-11.133	0.183
Inc	FAY16	S2D_632044079	2D	9.98E-05	0.103	G/C	-8.398	0.180
Inc	FAY16	S2D_632044087	2D	9.98E-05	0.103	A/G	-8.398	0.185
Inc	FAY16	S2D_632044091	2D	9.98E-05	0.103	G/A	-8.398	0.179
Inc	FAY16	S3A_686904207	3A	2.68E-05	0.074	A/T	-6.816	0.178
Inc	FAY16	S3D_191783666	3D	3.1E-07	0.082	G/A	-9.502	0.194
Inc	FAY16	S4B_575510195	4B	9.87E-06	0.404	T/A	-5.164	0.177
Inc	FAY16	S4B_577008759	4B	2.63E-05	0.415	C/T	-5.121	0.182
Inc	FAY16	S4B_579987295	4B	5.8E-06	0.408	A/G	-5.489	0.118
Inc	FAY16	S4B_580353549	4B	5.06E-05	0.365	C/G	-4.479	0.293
Inc	FAY16	S6D_278102968	6D	1.47E-05	0.075	G/A	-7.974	0.360
Inc	FAY16	S6D_278102986	6D	1.47E-05	0.075	G/A	-7.974	0.370
Inc	NPT16	S3B_578568689	3B	1.02E-07	0.091	T/G	-20.657	0.178
Inc	FAY17	S1B_631080970	1B	3.52E-06	0.167	G/A	-4.380	0.185
Inc	FAY17	S1B_631081026	1B	3.52E-06	0.167	A/T	-4.380	0.185
Inc	FAY17	S4A_647100324	4A	1.46E-05	0.089	G/C	-5.114	0.175
Inc	FAY17	S4A_647100355	4A	1.46E-05	0.089	C/T	-5.114	0.174
Inc	FAY17	S4A_654802393	4A	3.54E-05	0.047	A/T	-7.040	0.174
Inc	FAY17	S4B_637387933	4B	2.57E-06	0.237	T/C	-3.988	0.281
Inc	FAY17	S4B_637388270	4B	2.43E-06	0.234	C/T	-4.019	0.281
Inc	FAY17	S4B_637576146	4B	1.99E-06	0.237	C/T	-3.990	0.175
Inc	FAY17	S4B_637576156	4B	1.99E-06	0.237	T/C	-3.990	0.111
Inc	FAY17	S6B_442425028	6B	1.95E-05	0.047	C/T	-8.187	0.362

Table 8. Cont.

Trait	Site-year	SNP	Chr	p-value	Maf	Allele	Allelic Effect	R^2
Inc	FAY17	S6B_482116417	6B	1.75E-05	0.088	G/T	-6.738	0.362
Inc	FAY17	S6B_482116427	6B	1.75E-05	0.088	G/T	-6.738	0.351
Inc	FAY17	S6B_694253050	6B	2.89E-06	0.106	A/G	-5.340	0.138
Inc	FAY17	S7B_559002698	7B	1.05E-05	0.082	G/A	-6.510	0.359
Inc	FAY17	S7B_559002717	7B	1.05E-05	0.082	T/C	-6.510	0.353
Inc	FAY17	S7B_576532318	7B	1.41E-05	0.042	T/C	-10.529	0.353
Sev	FAY16	S2D_427477848	2D	6.29E-05	0.054	A/G	-10.671	0.176
Sev	FAY16	S2D_427477849	2D	6.29E-05	0.054	G/A	-10.671	0.176
Sev	FAY16	S2D_427477862	2D	6.29E-05	0.054	A/G	-10.671	0.176
Sev	FAY16	S2D_427477868	2D	6.29E-05	0.054	C/T	-10.671	0.182
Sev	FAY16	S3A_686904207	3A	6.56E-05	0.074	A/T	-7.034	0.179
Sev	FAY16	S3B_817385698	3B	2.4E-06	0.060	C/A	-10.068	0.174
Sev	FAY16	S3B_817395079	3B	5.09E-06	0.061	C/T	-9.492	0.179
Sev	FAY16	S3B_817476155	3B	6.52E-06	0.065	A/C	-9.309	0.200
Sev	FAY16	S3B_817476198	3B	5.64E-06	0.061	C/G	-9.600	0.199
Sev	FAY16	S3B_817476221	3B	5.64E-06	0.061	T/C	-9.600	0.195
Sev	FAY16	S3D_191783666	3D	9.38E-05	0.082	G/A	-7.800	0.193
Sev	FAY16	S4B_575510195	4B	7.81E-06	0.404	T/A	-9.099	0.175
Sev	FAY16	S4B_577008759	4B	4.64E-06	0.415	C/T	-5.726	0.184
Sev	FAY16	S4B_579987295	4B	6.8E-07	0.408	A/G	-6.137	0.118
Sev	FAY16	S4B_580353549	4B	5.2E-05	0.365	C/G	-6.605	0.293
Sev	FAY16	S4B_81499209	4B	8.61E-05	0.086	G/A	-4.890	0.287
Sev	FAY16	S5B_16289326	5B	7.21E-05	0.043	C/T	-12.224	0.106
Sev	FAY16	S5B_16336584	5B	8.11E-05	0.043	C/T	-12.648	0.119
Sev	FAY16	S6D_278102968	6D	1.13E-05	0.075	G/A	-8.762	0.362
Sev	FAY16	S6D_278102986	6D	1.13E-05	0.075	G/A	-8.762	0.353
Sev	FAY16	S7A_4797458	7A	5.12E-05	0.099	A/T	-8.935	0.351

Table 8. Cont.

Trait	Site-year	SNP	Chr	p-value	Maf	Allele	Allelic Effect	R^2
Sev	FAY16	S7A_4797460	7A	5.12E-05	0.099	G/A	-8.935	0.351
Sev	FAY16	S7A_4797474	7A	5.12E-05	0.099	C/T	-8.935	0.122
Sev	FAY16	S7A_4797487	7A	5.12E-05	0.099	C/G	-8.935	0.359
Sev	NPT16	S3B_578568689	3B	2.1E-06	0.091	T/G	-9.761	0.180
Sev	FAY17	S1B_631080970	1B	8.77E-05	0.167	G/A	-2.825	0.219
Sev	FAY17	S1B_631081026	1B	8.77E-05	0.167	A/T	-2.825	0.219
Sev	FAY17	S4A_647100324	4A	2.06E-06	0.089	G/C	-4.350	0.174
Sev	FAY17	S4A_647100355	4A	2.06E-06	0.089	C/T	-4.350	0.174
Sev	FAY17	S4A_654802393	4A	1.99E-06	0.047	A/T	-6.277	0.173
Sev	FAY17	S4B_637387933	4B	1.8E-06	0.237	T/C	-3.267	0.281
Sev	FAY17	S4B_637388270	4B	1.57E-06	0.234	C/T	-3.296	0.287
Sev	FAY17	S4B_637576146	4B	1.49E-06	0.237	C/T	-3.252	0.306
Sev	FAY17	S4B_637576156	4B	1.49E-06	0.237	T/C	-3.252	0.111
Sev	FAY17	S5B_16289326	5B	9.65E-05	0.043	C/T	-5.541	0.119
Sev	FAY17	S5B_16336584	5B	7.22E-05	0.043	C/T	-5.665	0.352
Sev	FAY17	S6B_442425028	6B	9.85E-06	0.047	C/T	-6.937	0.361
Sev	FAY17	S6B_482116417	6B	4.38E-05	0.088	G/T	-5.326	0.362
Sev	FAY17	S6B_482116427	6B	4.38E-05	0.088	G/T	-5.326	0.138
Sev	FAY17	S6B_694253050	6B	5.8E-07	0.106	A/G	-4.439	0.138
Sev	FAY17	S7B_559002698	7B	8E-07	0.082	G/A	-5.670	0.353
Sev	FAY17	S7B_559002717	7B	8E-07	0.082	T/C	-5.670	0.353
Sev	FAY17	S7B_576532318	7B	3.1E-06	0.042	T/C	-8.987	0.143
FDK	FAY16	S1B_77632553	1B	3.55E-05	0.089	C/T	-10.596	0.178
FDK	FAY16	S1B_77632584	1B	3.55E-05	0.089	G/A	-10.596	0.178
FDK	FAY16	S2A_460777961	2A	6.41E-06	0.089	C/A	-10.594	0.187
FDK	FAY16	S2D_632044079	2D	5.33E-05	0.103	G/C	-11.825	0.182
FDK	FAY16	S2D_632044087	2D	5.33E-05	0.103	A/G	-11.825	0.179

Table 8. Cont.

Trait	Site-year	SNP	Chr	p-value	Maf	Allele	Allelic Effect	R^2
FDK	FAY16	S2D_632044091	2D	5.33E-05	0.103	G/A	-11.825	0.186
FDK	FAY16	S3B_817385698	3B	1.4E-05	0.060	C/A	-11.661	0.178
FDK	FAY16	S3B_817395079	3B	1.58E-05	0.061	C/T	-11.322	0.182
FDK	FAY16	S3B_817476155	3B	2.63E-05	0.065	A/C	-10.866	0.178
FDK	FAY16	S3B_817476198	3B	6.82E-06	0.061	C/G	-11.934	0.202
FDK	FAY16	S3B_817476221	3B	6.82E-06	0.061	T/C	-11.934	0.203
FDK	FAY16	S4A_159918210	4A	3.39E-05	0.067	A/T	-10.499	0.179
FDK	FAY16	S4A_159918229	4A	3.39E-05	0.067	C/T	-10.499	0.176
FDK	FAY16	S4B_453250321	4B	2.78E-05	0.116	T/C	-11.941	0.174
FDK	FAY16	S4B_542683613	4B	7.43E-05	0.419	G/A	-11.237	0.177
FDK	FAY16	S4B_543903651	4B	1.64E-05	0.421	C/T	-10.777	0.178
FDK	FAY16	S4B_570472978	4B	3.45E-05	0.337	C/T	-10.834	0.179
FDK	FAY16	S4B_575510195	4B	3.8E-07	0.404	T/A	-10.663	0.178
FDK	FAY16	S4B_577008759	4B	3.3E-07	0.415	C/T	-10.663	0.184
FDK	FAY16	S4B_579987295	4B	2.7E-07	0.408	A/G	-11.339	0.182
FDK	FAY16	S4B_580353549	4B	1.39E-06	0.365	C/G	-11.526	0.293
FDK	FAY16	S4B_81499209	4B	2.84E-05	0.086	G/A	-9.346	0.106
FDK	FAY16	S4B_82018887	4B	5.97E-05	0.086	G/C	-6.660	0.293
FDK	FAY16	S4B_82773235	4B	7.08E-05	0.088	A/G	-7.051	0.120
FDK	FAY16	S4B_84231602	4B	8.01E-05	0.086	G/A	-6.771	0.121
FDK	FAY16	S4B_84535336	4B	8.17E-05	0.088	C/T	-8.048	0.109
FDK	FAY16	S4B_84535373	4B	8.17E-05	0.088	G/T	-8.376	0.110
FDK	FAY16	S4B_84943413	4B	4.83E-05	0.086	G/A	-8.428	0.289
FDK	FAY16	S4B_85983933	4B	3.92E-05	0.086	G/C	-7.333	0.112
FDK	FAY16	S5B_115862443	5B	2.85E-05	0.064	A/C	-12.215	0.111
FDK	FAY16	S6A_21523672	6A	3.54E-05	0.039	G/A	-17.558	0.352
FDK	FAY16	S6D_428745237	6D	5.03E-05	0.091	G/A	-8.540	0.139

Table 8. Cont.

Trait	Site-year	SNP	Chr	p-value	Maf	Allele	Allelic Effect	R^2
FDK	FAY16	S7A_4797458	7A	1.25E-05	0.099	A/T	-12.661	0.140
FDK	FAY16	S7A_4797460	7A	1.25E-05	0.099	G/A	-12.661	0.352
FDK	FAY16	S7A_4797474	7A	1.25E-05	0.099	C/T	-12.661	0.120
FDK	FAY16	S7A_4797487	7A	1.25E-05	0.099	C/G	-12.661	0.122
FDK	NPT16	S1B_77632553	1B	3.65E-05	0.089	C/T	1.010	0.177
FDK	NPT16	S1B_77632584	1B	3.65E-05	0.089	G/A	2.478	0.177
FDK	NPT16	S2A_460777961	2A	6.88E-06	0.089	C/A	2.478	0.185
FDK	NPT16	S2D_632044079	2D	5.15E-05	0.103	G/C	2.259	0.181
FDK	NPT16	S2D_632044087	2D	5.15E-05	0.103	A/G	-5.042	0.186
FDK	NPT16	S2D_632044091	2D	5.15E-05	0.103	G/A	3.186	0.185
FDK	NPT16	S3B_817385698	3B	1.44E-05	0.060	C/A	-0.570	0.175
FDK	NPT16	S3B_817395079	3B	1.61E-05	0.061	C/T	2.522	0.181
FDK	NPT16	S3B_817476155	3B	2.66E-05	0.065	A/C	2.522	0.201
FDK	NPT16	S3B_817476198	3B	6.95E-06	0.061	C/G	1.152	0.200
FDK	NPT16	S3B_817476221	3B	6.95E-06	0.061	T/C	2.616	0.201
FDK	NPT16	S4A_159918210	4A	3.44E-05	0.067	A/T	2.616	0.178
FDK	NPT16	S4A_159918229	4A	3.44E-05	0.067	C/T	-4.960	0.175
FDK	NPT16	S4B_453250321	4B	3.27E-05	0.116	T/C	-4.960	0.173
FDK	NPT16	S4B_542683613	4B	7.5E-05	0.419	G/A	0.197	0.176
FDK	NPT16	S4B_543903651	4B	1.7E-05	0.421	C/T	3.078	0.177
FDK	NPT16	S4B_570472978	4B	3.25E-05	0.337	C/T	0.928	0.180
FDK	NPT16	S4B_575510195	4B	3.9E-07	0.404	T/A	3.307	0.178
FDK	NPT16	S4B_577008759	4B	3.4E-07	0.415	C/T	3.138	0.182
FDK	NPT16	S4B_579987295	4B	2.9E-07	0.408	A/G	3.138	0.184
FDK	NPT16	S4B_580353549	4B	1.42E-06	0.365	C/G	3.138	0.293
FDK	NPT16	S4B_81499209	4B	2.81E-05	0.086	G/A	-0.788	0.291
FDK	NPT16	S4B_82018887	4B	5.87E-05	0.086	G/C	-0.897	0.284

Table 8. Cont.

Trait	Site-year	SNP	Chr	p-value	Maf	Allele	Allelic Effect	R^2
FDK	NPT16	S4B_82773235	4B	6.94E-05	0.088	A/G	0.061	0.120
FDK	NPT16	S4B_84231602	4B	7.86E-05	0.086	G/A	1.005	0.121
FDK	NPT16	S4B_84535336	4B	8.1E-05	0.088	C/T	1.005	0.110
FDK	NPT16	S4B_84535373	4B	8.1E-05	0.088	G/T	1.005	0.119
FDK	NPT16	S4B_84943413	4B	4.73E-05	0.086	G/A	-1.940	0.289
FDK	NPT16	S4B_85983933	4B	3.85E-05	0.086	G/C	1.005	0.112
FDK	NPT16	S5B_115862443	5B	2.13E-05	0.064	A/C	2.438	0.106
FDK	NPT16	S6A_21523672	6A	3.29E-05	0.039	G/A	2.558	0.352
FDK	NPT16	S6D_428745237	6D	5.94E-05	0.091	G/A	0.054	0.140
FDK	NPT16	S7A_4797458	7A	1.28E-05	0.099	A/T	-1.066	0.140
FDK	NPT16	S7A_4797460	7A	1.28E-05	0.099	G/A	2.680	0.118
FDK	NPT16	S7A_4797474	7A	1.28E-05	0.099	C/T	-0.517	0.130
FDK	NPT16	S7A_4797487	7A	1.28E-05	0.099	C/G	-1.286	0.145
FDK	NPT17	S4B_577008759	4B	3.15E-05	0.415	C/T	-7.310	0.184
FDK	NPT17	S4B_579987295	4B	8.12E-05	0.408	A/G	-6.931	0.182
DON	FAY17	S6B_346973693	6B	2.95E-05	0.077	G/A	-0.109	0.352
DON	FAY17	S6B_346973713	6B	2.95E-05	0.077	A/C	-0.109	0.094
DON	NPT17	S6B_346973693	6B	8.69E-05	0.077	G/A	-1.794	0.352
DON	NPT17	S6B_346973713	6B	8.69E-05	0.077	A/C	-1.794	0.365

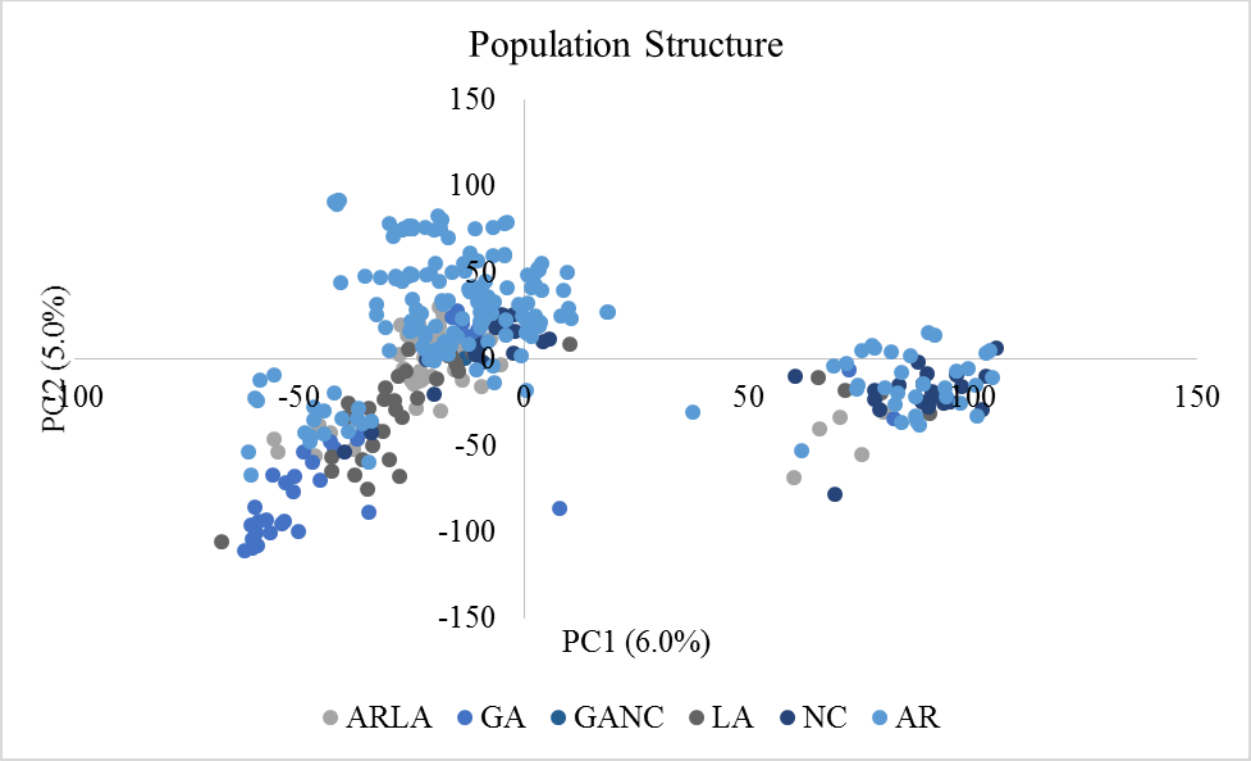

Figure 1. Population structure of 360 winter wheat breeding lines using 71,428 single-nucleotide polymorphisms. Colors represent the origin of the breeding lines. AR, University of Arkansas bred; ARLA, University of Arkansas and Louisiana State University backgrounds; GA, University of Georgia bred; GANC, University of Georgia and North Carolina State University backgrounds; LA, Louisiana State University bred; NC, North Carolina State University bred.

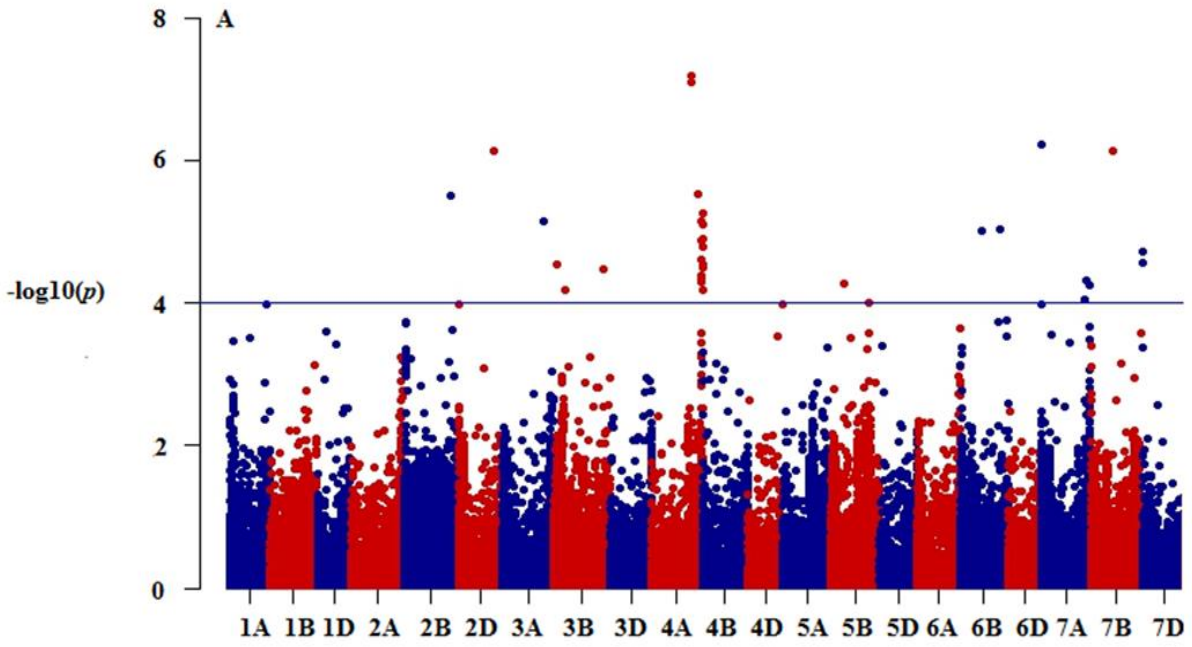

Figure 2. Manhattan plots for four phenotypic traits associated with *Fusarium* head blight resistance: (a) Incidence, (b) Severity, (c) *Fusarium* damaged kernels (FDK), and (d) Deoxynivalenol (DON) accumulation. The x-axis represents the 21 wheat chromosomes. The y-axis represents the *p*-value of the marker–trait association on a $-\log_{10}$ scale. The horizontal line represents the threshold for declaring a marker as significant (*p*-value < 0.0001).

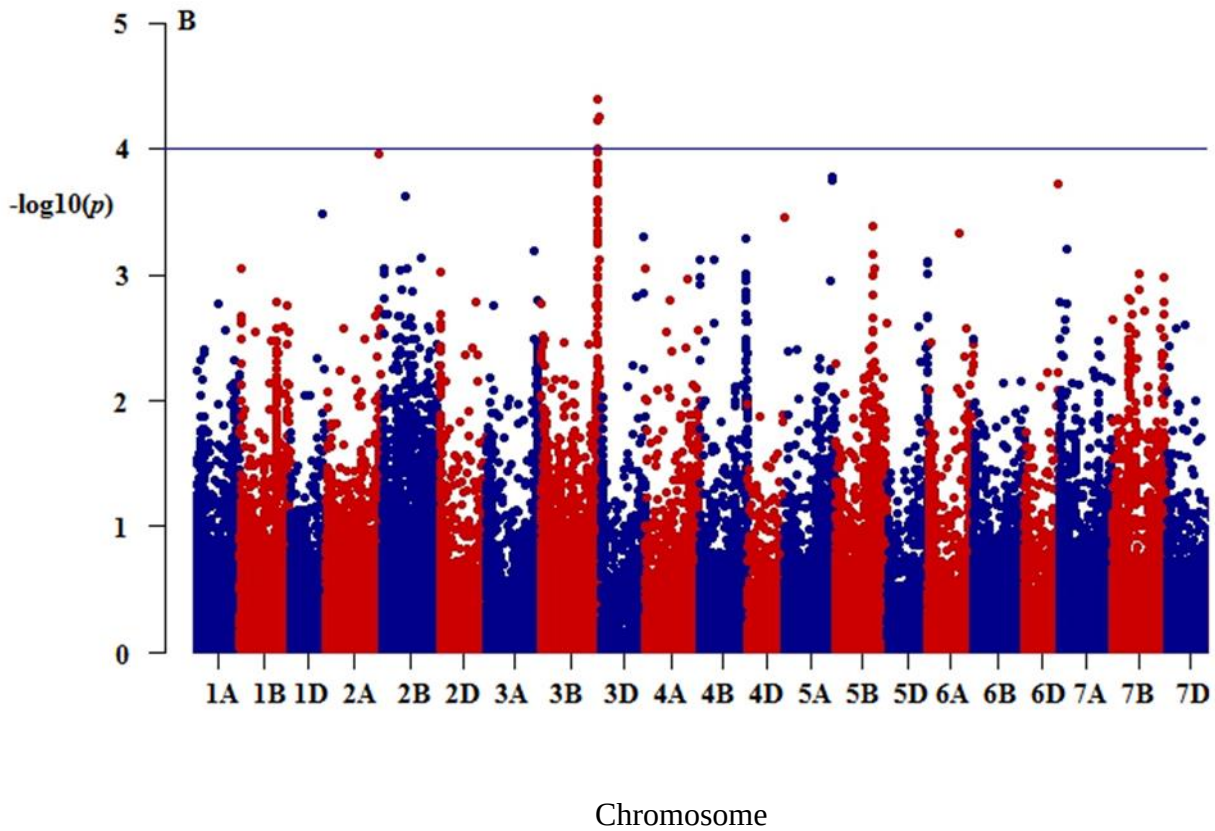

Figure 2. (Cont.) Manhattan plots for four phenotypic traits associated with *Fusarium* head blight resistance: (a) Incidence, (b) Severity, (c) *Fusarium* damaged kernels (FDK), and (d) Deoxynivalenol (DON) accumulation. The x-axis represents the 21 wheat chromosomes. The y-axis represents the *p*-value of the marker–trait association on a $-\log_{10}$ scale. The horizontal line represents the threshold for declaring a marker as significant (*p*-value < 0.0001).

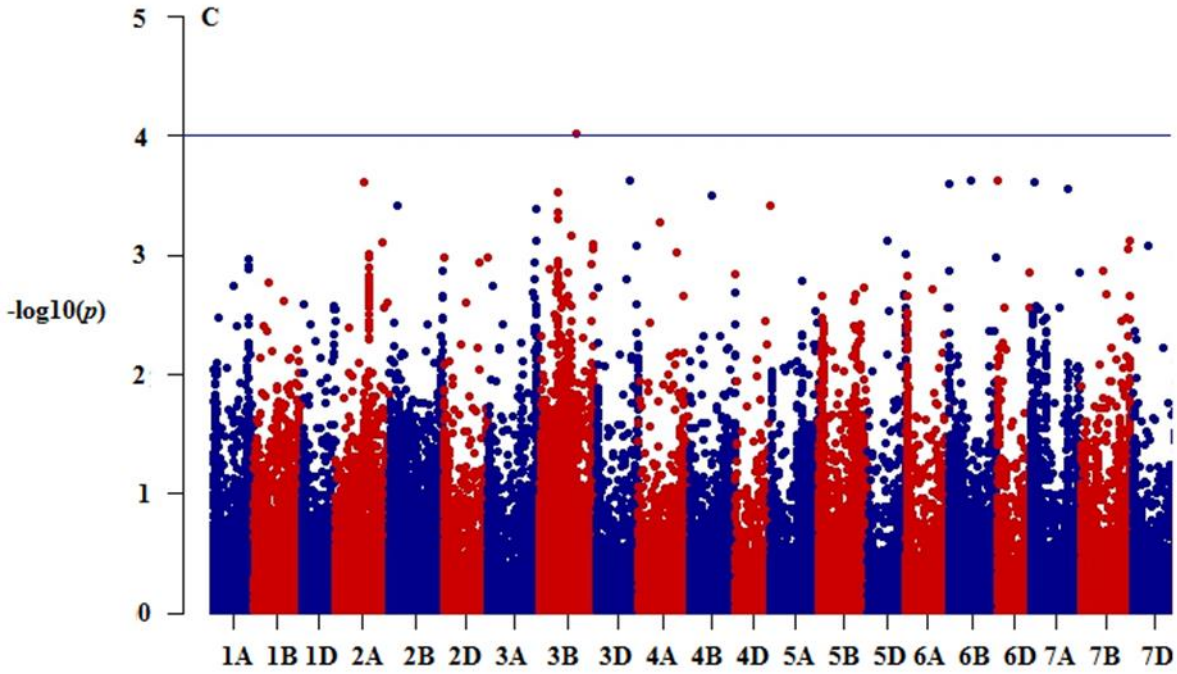

Figure 2. (Cont.) Manhattan plots for four phenotypic traits associated with *Fusarium* head blight resistance: (a) Incidence, (b) Severity, (c) *Fusarium* damaged kernels (FDK), and (d) Deoxynivalenol (DON) accumulation. The x-axis represents the 21 wheat chromosomes. The y-axis represents the *p*-value of the marker–trait association on a $-\log_{10}$ scale. The horizontal line represents the threshold for declaring a marker as significant (*p*-value < 0.0001).

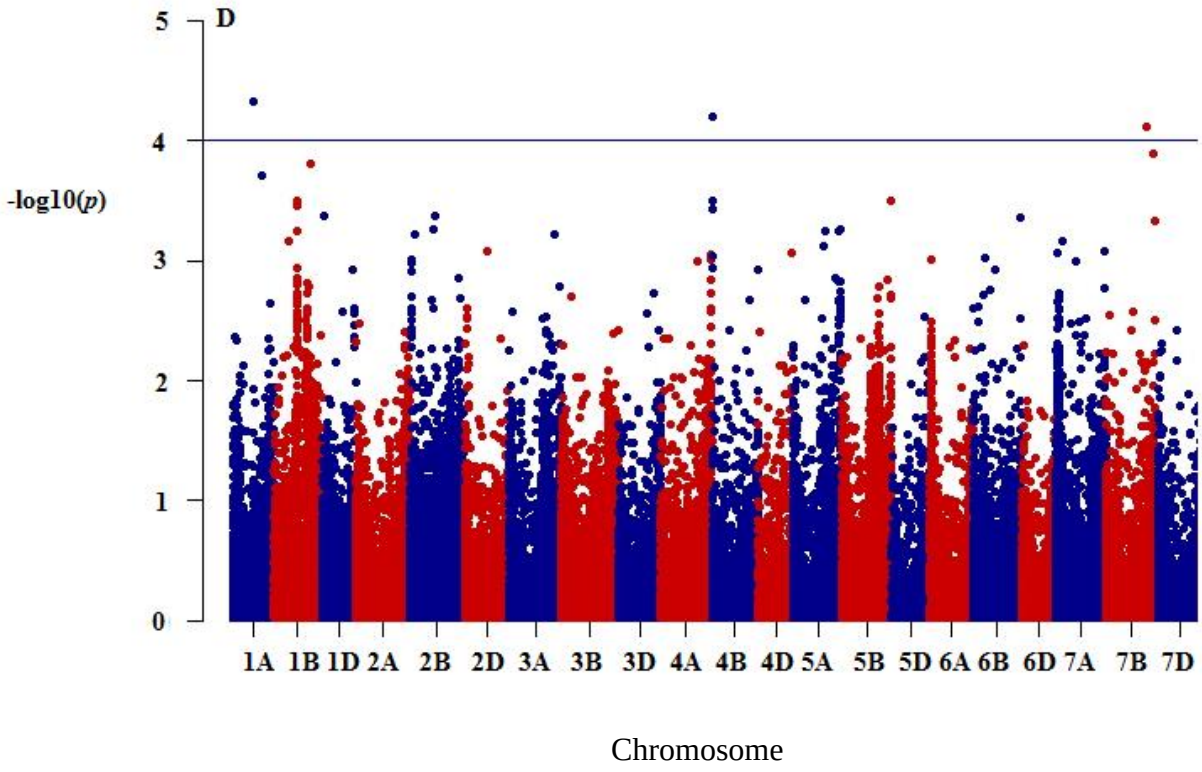

Figure 2. (Cont.) Manhattan plots for four phenotypic traits associated with *Fusarium* head blight resistance: (a) Incidence, (b) Severity, (c) *Fusarium* damaged kernels (FDK), and (d) Deoxynivalenol (DON) accumulation. The x-axis represents the 21 wheat chromosomes. The y-axis represents the *p*-value of the marker–trait association on a $-\log_{10}$ scale. The horizontal line represents the threshold for declaring a marker as significant (*p*-value < 0.0001).

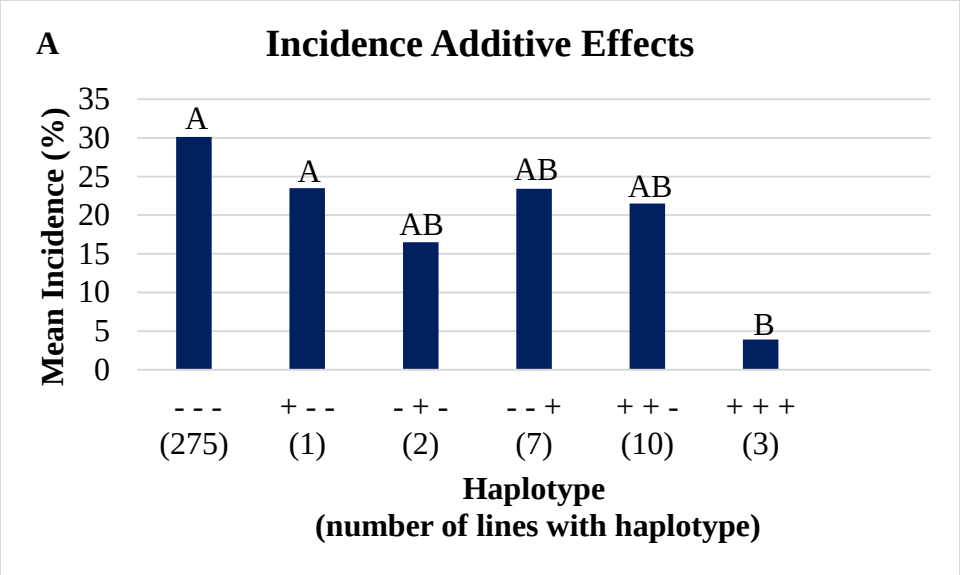

Figure 3. A simple T-test comparison of mean of (A) incidence, (B) severity, (C) and deoxynivalenol (DON) accumulation for different haplotypes within the association mapping panel (AMP). T-test does not account for relatedness or population structure within the panel. The haplotypes are present at different frequencies. The haplotype combinations represent the three most significant SNPs (beginning with the most significant SNP in order from left to right) associated with that trait. A plus (+) symbol represents the favorable allele while a minus (-) symbol represents the unfavorable allele for that SNP.

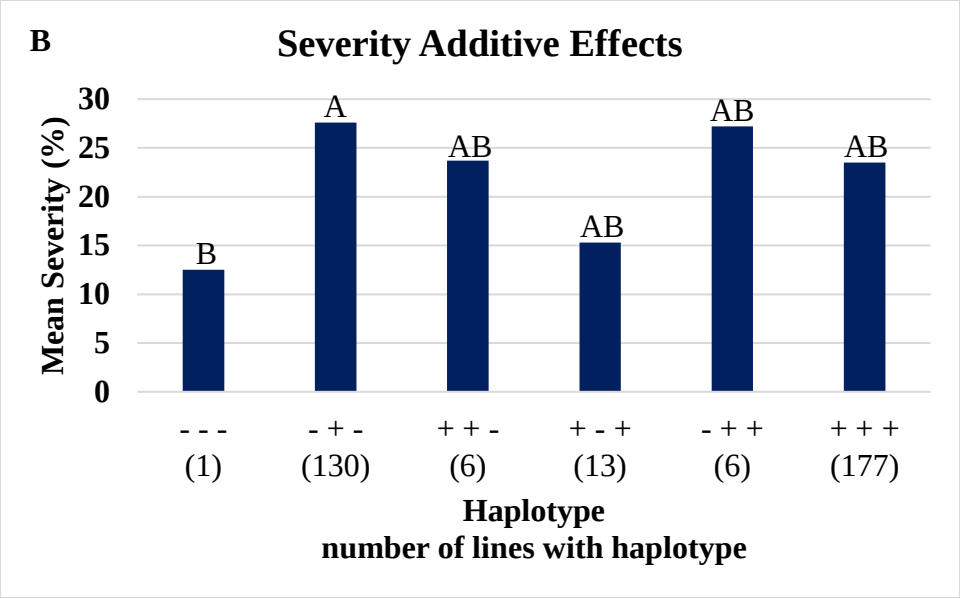

Figure 3. (Cont.) A simple T-test comparison of mean of (A) incidence, (B) severity, (C) and deoxynivalenol (DON) accumulation for different haplotypes within the association mapping panel (AMP). T-test does not account for relatedness or population structure within the panel. The haplotypes are present at different frequencies. The haplotype combinations represent the three most significant SNPs (beginning with the most significant SNP in order from left to right) associated with that trait. A plus (+) symbol represents the favorable allele while a minus (-) symbol represents the unfavorable allele for that SNP.

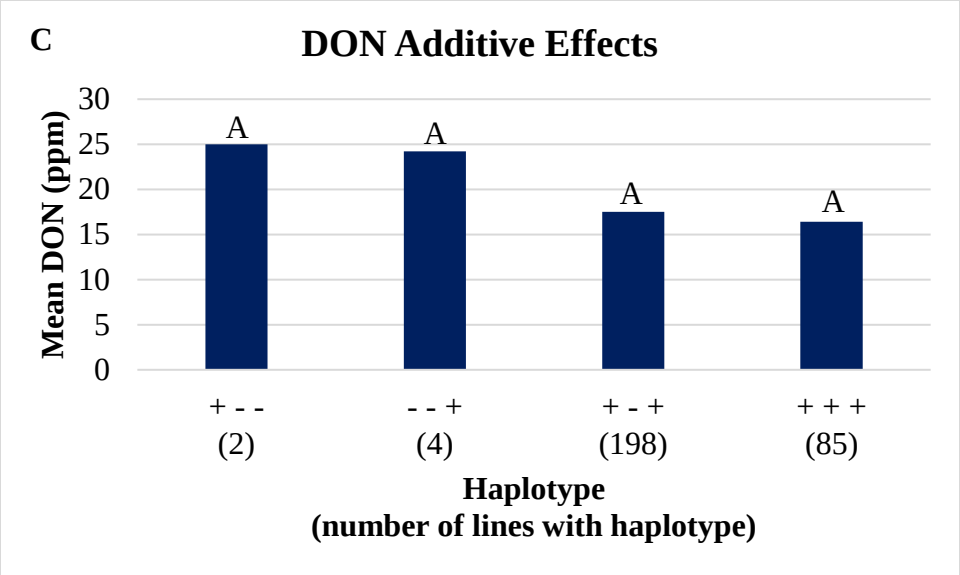

Figure 3. (Cont.) A simple T-test comparison of mean of (A) incidence, (B) severity, (C) and deoxynivalenol (DON) accumulation for different haplotypes within the association mapping panel (AMP). T-test does not account for relatedness or population structure within the panel. The haplotypes are present at different frequencies. The haplotype combinations represent the three most significant SNPs (beginning with the most significant SNP in order from left to right) associated with that trait. A plus (+) symbol represents the favorable allele while a minus (-) symbol represents the unfavorable allele for that SNP.

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Appendix 360 Soft red winter wheat lines included in the AMP.

Entry	Variety	Pedigree
ABB001	AR06004-5-1	AR01135/TERRAL TV8558
ABB002	AR06016-9-4	AR800-1-3-1/BESS
ABB003	AR06025-4-1	AR910-9-1/AR930035-4-1
ABB004	AR06025-8-2	AR910-9-1/AR930035-4-1
ABB005	AR06031-14-2	AR96077-7-2/AR01135
ABB006	AR06037-10-3	AR96077-7-2/VA00W526
ABB007	AR06048-16-2	CAYUGA/COKER 9553
ABB008	AR06049-10-4	COKER 9553/AR930035-4-1
ABB009	AR06050-12-1	COKER 9553/AR98084-4-1
ABB010	AR06075-13-1	UGA951216-2E26/AR02066
ABB011	AR06004-4-1	AR01135/TERRAL TV8558
ABB012	AR06004-13-4	AR01135/TERRAL TV8558
ABB013	AR06009-7-1	AR02136/AR930035-4-1
ABB014	AR06009-9-2	AR02136/AR930035-4-1
ABB015	AR06017-1-3	AR800-1-3-1/COKER 9663
ABB016	AR06021-8-4	AR800-1-3-1/VA00W-526
ABB017	AR06024-16-3	AR800-1-3-1/VA01W-476
ABB018	AR06029-4-1	AR96052-4-2/P98154A1-10-4-5-3
ABB019	AR06031-6-4	AR96077-7-2/AR01135
ABB020	AR06031-7-4	AR96077-7-2/AR01135
ABB021	AR06031-11-2	AR96077-7-2/AR01135
ABB022	AR06037-17-2	AR96077-7-2/VA00W526
ABB023	AR06040-3-4	AR97124-4-1/AR930035-4-1
ABB024	AR06042-4-4	AR97124-4-1/PAT
ABB025	AR06042-9-3	AR97124-4-1/PAT
ABB026	AR06045-3-1	BESS/AR97124-4-1
ABB027	AR06045-11-4	BESS/AR97124-4-1
ABB028	AR06046-10-3	BESS/PAT
ABB029	AR06050-5-1	COKER 9553/AR98084-4-1
ABB030	AR06050-6-2	COKER 9553/AR98084-4-1
ABB031	AR06050-7-2	COKER 9553/AR98084-4-1
ABB032	AR06061-8-1	P961341A3-1-2/VA01W-476
ABB033	AR06066-1-4	PAT/UGA971127-14-6-6
ABB034	AR06066-3-2	PAT/UGA971127-14-6-6
ABB035	AR06066-3-4	PAT/UGA971127-14-6-6
ABB036	AR06069-9-1	PIONEER 25W60/AR96077-7-2
ABB037	AR06072-11-1	TERRAL TV8558/VAN98W-342
ABB038	AR06080-3-4	UGA971127-14-6-6/BESS
ABB039	AR06081-11-1	VA00W-526/AR800-1-3-1
ABB040	AR06084-10-1	VA00W-526/PIONEER 25W33
ABB041	AR06004-4-2	AR01135/TERRAL TV8558

Appendix Cont.

Entry	Variety	Pedigree
ABB042	AR06004-6-1	AR01135/TERRAL TV8558
ABB043	AR06004-11-1	AR01135/TERRAL TV8558
ABB044	AR06004-12-1	AR01135/TERRAL TV8558
ABB045	AR06009-3-4	AR02136/AR930035-4-1
ABB046	AR06009-6-1	AR02136/AR930035-4-1
ABB047	AR06009-7-4	AR02136/AR930035-4-1
ABB048	AR06009-8-2	AR02136/AR930035-4-1
ABB049	AR06009-10-2	AR02136/AR930035-4-1
ABB050	AR06015-1-2	AR800-1-3-1/AR02066
ABB051	AR06015-3-4	AR800-1-3-1/AR02066
ABB052	AR06017-6-2	AR800-1-3-1/COKER 9663
ABB053	AR06025-4-4	AR910-9-1/AR930035-4-1
ABB054	AR06025-5-4	AR910-9-1/AR930035-4-1
ABB055	AR06028-11-3	AR96052-4-2/P961341A3-1-2
ABB056	AR06029-9-2	AR96052-4-2/P98154A1-10-4-5-3
ABB057	AR06031-8-2	AR96077-7-2/AR01135
ABB058	AR06031-8-3	AR96077-7-2/AR01135
ABB059	AR06031-17-2	AR96077-7-2/AR01135
ABB060	AR06045-10-4	BESS/AR97124-4-1
ABB061	AR06045-14-1	BESS/AR97124-4-1
ABB062	AR06049-1-3	COKER 9553/AR930035-4-1
ABB063	AR06049-2-2	COKER 9553/AR930035-4-1
ABB064	AR06049-7-1	COKER 9553/AR930035-4-1
ABB065	AR06049-7-2	COKER 9553/AR930035-4-1
ABB066	AR06050-1-2	COKER 9553/AR98084-4-1
ABB067	AR06050-12-4	COKER 9553/AR98084-4-1
ABB068	AR06061-11-1	P961341A3-1-2/VA01W-476
ABB069	AR06066-2-3	PAT/UGA971127-14-6-6
ABB070	AR06066-4-3	PAT/UGA971127-14-6-6
ABB071	AR06066-5-4	PAT/UGA971127-14-6-6
ABB072	AR06066-6-4	PAT/UGA971127-14-6-6
ABB073	AR06068-5-1	PIONEER 25W60/AR930035-4-1
ABB074	AR06068-5-2	PIONEER 25W60/AR930035-4-1
ABB075	AR06069-2-3	PIONEER 25W60/AR96077-7-2
ABB076	AR06069-4-2	PIONEER 25W60/AR96077-7-2
ABB077	AR06069-12-3	PIONEER 25W60/AR96077-7-2
ABB078	AR06075-12-1	UGA951216-2E26/AR02066
ABB079	AR06100-6-1	PIONEER26R61/AR96077-10-1
ABB080	AR06100-6-2	PIONEER26R61/AR96077-10-1
ABB081	AR06005-12-2	AR02066/AR839-10-1-1
ABB082	AR06005-14-1	AR02066/AR839-10-1-1

Appendix Cont.

Entry	Variety	Pedigree
ABB083	AR06006-3-3	AR02066/AR97002-2-1
ABB084	AR06006-8-2	AR02066/AR97002-2-1
ABB085	AR06012-6-3	AR02136/UGA951216-2E26
ABB086	AR06012-7-2	AR02136/UGA951216-2E26
ABB087	AR06013-4-2	AR02136/VA00W-526
ABB088	AR06022-5-1	AR800-1-3-1/VA00W-526
ABB089	AR06023-4-2	AR800-1-3-1/VA00W-526
ABB090	AR06023-9-2	AR800-1-3-1/VA00W-526
ABB091	AR06024-7-2	AR800-1-3-1/VA01W-476
ABB092	AR06025-3-1	AR910-9-1/AR930035-4-1
ABB093	AR06025-10-3	AR910-9-1/AR930035-4-1
ABB094	AR06026-4-3	AR910-9-1/BESS
ABB095	AR06027-6-4	AR96052-4-2/AR01135
ABB096	AR06033-4-1	AR96077-7-2/AR930035-4-1
ABB097	AR06035-2-4	AR96077-7-2/AR98084-4-1
ABB098	AR06040-8-1	AR97124-4-1/AR930035-4-1
ABB099	AR06042-6-4	AR97124-4-1/PAT
ABB100	AR06042-11-4	AR97124-4-1/PAT
ABB101	AR06045-2-4	BESS/AR97124-4-1
ABB102	AR06045-16-4	BESS/AR97124-4-1
ABB103	AR06046-5-2	BESS/PAT
ABB104	AR06048-7-1	CAYUGA/COKER 9553
ABB105	AR06049-6-3	COKER 9553/AR930035-4-1
ABB106	AR06059-13-2	P961341A3-1-2/AR96052-4-2
ABB107	AR06061-3-1	P961341A3-1-2/VA01W-476
ABB108	AR06061-5-1	P961341A3-1-2/VA01W-476
ABB109	AR06075-2-1	UGA951216-2E26/AR02066
ABB110	AR06075-3-1	UGA951216-2E26/AR02066
ABB111	AR06075-11-1	UGA951216-2E26/AR02066
ABB112	AR06075-11-3	UGA951216-2E26/AR02066
ABB113	AR06083-6-1	VA00W526/AR96077-7-2
ABB114	AR06084-12-4	VA00W-526/PIONEER 25W33
ABB115	AR06085-3-1	VAFE24-4-6/AR01135
ABB116	AR06085-5-2	VAFE24-4-6/AR01135
ABB117	AR06098-8-1	LA95181BUB40-1/VA02W-713
ABB118	AR06099-8-1	LA98113DWF/LA96140BUA70-2
ABB119	AR06099-10-2	LA98113DWF/LA96140BUA70-2
ABB120	AR06100-3-4	PIONEER26R61/AR96077-10-1
ABB121	GA07592-G1-3-2	051396 / 991371-6E11
ABB122	GA07192-1-16-4	D 00*6874-9 / SS 8641 // 991336-6E9
ABB123	GA06489-12-6-1	Panola / SS 8641

Appendix Cont.

Entry	Variety	Pedigree
ABB124	GA06489-12-6-2	Panola / SS 8641
ABB125	GA071703-15-5-4	991371 / 06069
ABB126	GA071260-24-5-3	991371 / 01170 // 991336
ABB127	GA101678-G1-G3-I1	AGS2000*3/ EL SYNTH #1-2// AGS 2020/3/ HAAS 797/ 3* AGS2020
ABB128	GA10041-G1-G1-G1-I2	AGS2020*2/021715//HAAS 797/2*AGS2020/3/AGS 2020
ABB129	GA061050-6-6-4	AGS 2031/Truman//SS8641
ABB130	GA07026-14LE4	AGS 2020*2/ USG 3555
ABB131	GA081628-14LE6	BALDWIN*4//051666/051717
ABB132	GA06489-14LE8	PANOLA / 96229-3A41//96229-3A41
ABB133	GA07506-14LE16	991371-14-4-8 / VA 02W433 // SS 8641
ABB134	GA07103-14LE17	SS 8641 / 98401-29-2-5 // Baldwin
ABB135	GA061530-14LE21	D 00*6874 / SS 8641
ABB136	GAJT 152-14LE37	JAMESTOWN/ AGS 2026
ABB137	061349-13E4	SS8641*2/981622-1-4-4
ABB138	071614-12-2-2-3	991371-6E13 *2 / AGS 2031 (Sr36)
ABB139	071621-1-7-1-3	991371-6E11 / 051740-G1-G1-G4
ABB140	071621-1-7-7-5	991371-6E11 / 051740-G1-G1-G4
ABB141	LA06020E-P16	DK(Westbred W1-0814 = TV8558)/PIO26R61
ABB142	LA05145D-16	JAMESTOWN/LA97113UC-124
ABB143	LA05038F-P1	SS8641/P26R61
ABB144	LA08005C-33	AGS2026/LA841
ABB145	LA08009C-40	AGS2026/VA02W-500
ABB146	LA08041C-P2	AR98003-7-1/LA821
ABB147	LA08173C-6	(LA99005UC-31-3/VA05W-500)LA07133,F1/LA07039,F1(AGS2026/LA99005UC-31-3)
ABB148	LA08184C-14	(NC03-5921/SS8641)LA07142,F1/LA841
ABB149	LA08221C-37	LA841/LA07045,F1(GA9811622-1-40-1/APCB02-8486)
ABB150	LA08254C-9	LA99005UC-31-3/LA07124,F1(LA98094BUB-58-5/VA03W-211)
ABB151	LA09062UB-48	LA01029D-139-3-C / AGS2026
ABB152	LA09080C-P5	LA01139D-56-1 / GA 041619-1-1-2-7-2-2-11
ABB153	LA09081C-P8	LA01139D-56-1 / GA001170-7E26
ABB154	LA09130UB-18	LA95135 / GA 051754-G1-1-8-1-
ABB155	LA08062C-19	GA00138-31-1-3/LA821
ABB156	LA08080C-40	GA951298-6E44/LA07177,F1(JAMESTOWN/LA841)
ABB157	LA08200C-6	(JAMESTOWN/LA95135)LA07178,F1/GA9811622-1-40-1

Appendix Cont.

Entry	Variety	Pedigree
ABB158	LA08201C-54	(JAMESTOWN/LA95135)LA07178,F1/GA991227-6A33
ABB159	LA05057D-115	(LA04026)95283CA78-1-2/GA941208E35//LA841
ABB160	LA08015C-1	AGS2060/LA07004,F1(AGS2060/LA98094BUB-58-5)
ABB161	NC12-20850	GA951395-3E27 / NC01-27750 // USG 3555
ABB162	NC12-20859	GA951395-3E27 / NC01-27750 // USG 3555
ABB163	NC12-21568	IL96-6472/VA04W-561 // SS8641
ABB164	NC12-22686	McCORMICK / SS 8641 // C 9553
ABB165	NC12-21940	NC04-15460 / VA04W-259
ABB166	NC12-22768	NC99BGTAG11G / C 9511 // Jamestown (Pm37)
ABB167	NC12-22844	NC-Neuse / C 9511 // NC02-11158
ABB168	NC12-22846	NC-Neuse / C 9511 // NC02-11158
ABB169	NC12-21164	NC-Neuse / C 9511 // NC02-11158
ABB170	NC10014-1	NC06-19896 / NC08-140 (Bdv2)
ABB171	NC10034-11	NC-Yadkin / Shirley
ABB172	NC10080-10	GA05052-G1-21 / Jamestown
ABB173	NC10157-2	GA051714-G112 / NC-Yadkin
ABB174	NB26	NC-Neuse / Bess
ABB175	NC10-24889	C9184 / NC00-14622 // C9553
ABB176	NC11-22233	C9511 / / NC96BGTD3 / NC-NEUSE
ABB177	NC11-20725	NC99BGTAG11G / AGS 2010 / / NC01-27764
ABB178	NC11-21960	NC-NEUSE / AGS 2010 / / C9553
ABB179	NC9485-14	VA05-510 / Bess
ABB180	NC8401-5	NC03-11465 / VA04W-264
ABB181	AR07016-2-1	AR97044-10-1/Magnolia
ABB182	AR07020-14-3	AR97044-10-2/AR99097W-11-2
ABB183	AR07022-10-1	AR97044-10-2/GA96693-4E16
ABB184	AR07023-1-1	AR97044-10-2/GA96693-4E-16
ABB185	AR07027-1-1	AR97124-4-2/AR96052-4-3
ABB186	AR07031-7-4	AR97124-4-2/AR99110W-12-1
ABB187	AR07034-5-4	AR97124-4-3/TERRAL LA482
ABB188	AR07035-17-4	AR97139-11-2/Magnolia
ABB189	AR07037-11-1	AR97139-11-2/TERRAL LA482
ABB190	AR07039-3-1	AR98001-5-1/AR96077-7-2
ABB191	AR07047-13-2	98023-5-1/98068-4-1
ABB192	AR07066-8-4	99110W-12-1/AGS 2000 2x
ABB193	AR07068-10-4	99110W-12-1/DK 9577
ABB194	AR07076-20-1	99122W-5-1/LA978UC-36-1-1-B
ABB195	AR07078-10-4	AGS2000/DK9577 2x
ABB196	AR07090-1-4	DK9577/97044-10-1
ABB197	AR07114-3-4	GA96693-4E16/LA978UC-36-1-1-B 2x

Appendix Cont.

Entry	Variety	Pedigree
ABB198	AR07116-20-1	GA96693-4E-16/DK9577
ABB199	AR07117-12-1	GA96693-4E16/PAT
ABB200	AR07119-1-4	LA978UC-36-1-1-B/98001-5-1 2x
ABB201	AR07121-16-4	LA978UC-36-1-1-B/99012W-1-3
ABB202	AR07122-1-4	LA978UC-36-1-1-B/DK9577
ABB203	AR07122-16-1	LA978UC-36-1-1-B/DK9577
ABB204	AR07124-1-4	Magnolia/97124-4-3 2x
ABB205	AR07154-16-3	TERRAL LA482/DK9577
ABB206	AR07155-16-2	TERRAL LA482/MAGNOLIA
ABB207	AR07155-7-1	TERRAL LA482/MAGNOLIA
ABB208	ARLA05001F-16-1	COKER 9553/LA841
ABB209	ARLA05009F-1-4	DK 9577/LA841
ABB210	ARLA05104F-18-1	LA96408D-89-3-2-B/NC03-5921
ABB211	ARLA05111F-7-1	LA97113UC-124-3-B/SS8641
ABB212	ARLA06016E-3-2	DKGR9108/LA95135
ABB213	ARLA06027E-3-1	AGS2010/AGS2060
ABB214	ARLA06030E-19-4	AGS2010/LA95135
ABB215	ARLA06040E-13-4	GA951216-2E26/LA841
ABB216	ARLA07053C-14-4	GA98244-1-14-5-4/MAGNOLIA
ABB217	ARLA07058C-4-4	GA98401-44-1-6/PIO/26R61
ABB218	ARLA07072C-13-4	LA06018,F1/LA841
ABB219	ARLA07080C-3-4	LA06090,F1/AR98003-7-1
ABB220	ARLA07084C-10-1	LA06101,F1/LA99005UC-31-3-C
ABB221	AR07025-2-1	AR97044-10-2/LA978UC-36-1-1-B
ABB222	AR07035-7-1	AR97139-11-2/Magnolia
ABB223	AR07039-1-4	AR98001-5-1/AR96077-7-2
ABB224	AR07049-2-1	98023-5-1/DK 9577
ABB225	AR07067-1-4	UNKNOWN
ABB226	AR07078-7-4	AGS2000/DK9577 2x
ABB227	AR07079-1-4	UNKNOWN
ABB228	AR07114-20-3	GA96693-4E16/LA978UC-36-1-1-B 2x
ABB229	AR07115-13-4	UNKNOWN
ABB230	AR07116-11-4	GA96693-4E-16/DK9577
ABB231	AR07116-1-3	GA96693-4E-16/DK9577
ABB232	AR07116-8-4	GA96693-4E-16/DK9577
ABB233	AR07117-1-4	GA96693-4E16/PAT
ABB234	AR07117-2-1	GA96693-4E16/PAT
ABB235	AR07117-5-4	GA96693-4E16/PAT
ABB236	AR07119-9-1	LA978UC-36-1-1-B/98001-5-1 2x
ABB237	AR07122-14-4	LA978UC-36-1-1-B/DK9577
ABB238	AR07122-17-1	LA978UC-36-1-1-B/DK9577

Appendix Cont.

Entry	Variety	Pedigree
ABB239	AR07122-9-1	LA978UC-36-1-1-B/DK9577
ABB240	AR07130-16-1	UNKNOWN
ABB241	AR07139-11-1	PAT/LA978UC-36-1-1-B 2X
ABB242	AR07140-11-1	UNKNOWN
ABB243	AR07140-1-4	UNKNOWN
ABB244	AR07157-10-1	UNKNOWN
ABB245	AR07158-1-4	TERRAL TV8558/98001-5-1
ABB246	AR07158-19-1	TERRAL TV8558/98001-5-1
ABB247	ARLA05001F-17-1	COKER 9553/LA841
ABB248	ARLA05001F-2-1	COKER 9553/LA841
ABB249	ARLA05001F-7-1	COKER 9553/LA841
ABB250	ARLA05009F-19-1	DK 9577/LA841
ABB251	ARLA05009F-4-1	DK 9577/LA841
ABB252	ARLA05111F-11-4	LA97113UC-124-3-B/SS8641
ABB253	ARLA05111F-4-1	LA97113UC-124-3-B/SS8641
ABB254	ARLA06007E-12-1	APCK B02-8443/LA95135
ABB255	ARLA06030E-3-4	AGS2010/LA95135
ABB256	ARLA06040E-5-1	GA951216-2E26/LA841
ABB257	ARLA07040C-1-4	SS8641/APC B02-8486
ABB258	ARLA07053C-7-1	GA98244-1-14-5-4/MAGNOLIA
ABB259	ARLA07058C-17-4	GA98401-44-1-6/PIO/26R61
ABB260	ARLA07072C-6-1	LA06018,F1/LA841
ABB261	AR07006-5-4	AR96077-7-2/AR96052-4-3
ABB262	AR07011-11-1	AR96077-10-1/TX02D5406
ABB263	AR07037-15-4	AR97139-11-2/TERRAL LA482
ABB264	AR07041-19-4	AR98001-5-1/AR97124-4-2
ABB265	AR07051-14-3	98023-5-1/Magnolia 2X
ABB266	AR07053-13-1	98068-4-1/97124-4-2
ABB267	AR07061-4-1	99097W-11-2/ORHO10920
ABB268	AR07073-18-4	99122W-5-1/98001-5-1
ABB269	AR07080-7-1	AGS 2000/DK GR9108
ABB270	AR07091-15-4	DK9577/98068-4-1
ABB271	AR07092-20-1	DK9577/99122W-5-1
ABB272	AR07108-6-1	FFR8302/BESS
ABB273	AR07110-14-4	GA96693-4E16/96052-4-3
ABB274	AR07128-4-1	UNKNOWN
ABB275	AR07138-2-1	PAT/DK GR9108
ABB276	AR07152-1-1	TERRAL LA482/98023-5-1
ABB277	ARLA05027F-20-1	GA951395-3E27/PIO26R61
ABB278	ARLA05038F-2-1	SS8641/P26R61

Appendix Cont.

Entry	Variety	Pedigree
ABB279	ARLA05066F-4-3	(LA04059)LA98007BUB-37-4-C/AWD00*6832,F1//LA841
ABB280	ARLA05066F-7-1	(LA04059)LA98007BUB-37-4-C/AWD00*6832,F1//LA841
ABB281	ARLA05104F-14-3	LA96408D-89-3-2-B/NC03-5921
ABB282	ARLA06053E-20-1	SS8641/PIO26R61
ABB283	ARLA06064E-1-1	LA95135/AGS2060
ABB284	ARLA06096E-1-1	LA97113UC-124-3/GA951216-2E26
ABB285	ARLA06102E-13-4	LA98094BUB-58-5-B/LA99003UC-59-3
ABB286	ARLA06117E-5-1	LA99042E-62/LA841
ABB287	ARLA06128E-6-4	NC02-4518/AGS2060
ABB288	ARLA07019C-20-4	AR98003-7-1/AGS2060
ABB289	ARLA07023C-18-1	AR98003-7-1/MAGNOLIA
ABB290	ARLA07025C-3-1	ARGE97-1060-5-5/LA01096D-98(ND2928/LA841)
ABB291	ARLA07048C-11-1	GA9811622-1-40-1/LA98205D-17-2-4
ABB292	ARLA07056C-10-1	GA98401-44-1-6/AGS2060
ABB293	ARLA07095C-17-1	LA06112,F1/VA02W-713
ABB294	ARLA07097C-5-1	LA06113,F1/AR98003-7-1
ABB295	ARLA07102C-2-4	LA06146,F1/LA841
ABB296	ARLA07128C-1-3	LA98205D-17-2-4/LA01110D-88
ABB297	ARLA07128C-17-1	LA98205D-17-2-4/LA01110D-88
ABB298	ARLA07133C-19-4	LA99005UC-31-3/VA05W-500
ABB299	ARLA07133C-2-4	LA99005UC-31-3/VA05W-500
ABB300	ARLA07133C-3-4	LA99005UC-31-3/VA05W-500
ABB301	GA07323-19-4-4	991371 / AGS 2020
ABB302	GA081018-3-1	011177-9-4-5 / AGS 2035
ABB303	GA081104-24-1	991371-6E13 / 011177-9-4
ABB304	GA081625-4-6-8	061654-G7(BDV3) / 07393-G5-G3(IN97219)
ABB305	GA081176-24-6	Baldwin / 00219-7-4-8
ABB306	GA071468-1-3	Jamestown / 991371-6E12
ABB307	GA071092-B-5-3	USG 3120 *2 / Jamestown
ABB308	GA071171-6-3-2	Jamestown / 991371-6E12
ABB309	GA08028-21-5	02197-4-8 / AGS 2035
ABB310	GA081018-15-4	011177-9-4 / AGS 2035
ABB311	GA081305-22-12	02207-18-9 / 01129-1-3
ABB312	GA081627-5-1	991227-6A33 /4/ 00440 *2 // 03144(IN97219) / 97186 /3/ 2* 051396
ABB313	GA081067-24-5	02148-46-6 / 011177-9-4
ABB314	GA05450-28-8	96229-3E39 / C 9553
ABB315	GA061157-5-3	AGS 2020 *2 / 96229-3E39
ABB316	GA10365-G1-G1-G1-2	071728 /3/ 07498 // AGS 2000 *3 / EL SYN #1-2

Appendix Cont.

Entry	Variety	Pedigree
ABB317	GA08250-15-1	Jamestown / 991336-6E9
ABB318	GANC 10014-38	NC 06-19896 / NC 08-140
ABB319	GANC9337-25	Jamestown / SS 8641
ABB320	GA081248-1-2	031238-7E34 / AGS 2020
ABB321	LA05038F-P1	SS8641/P26R61
ABB322	LA05102C-8-8	.
ABB323	LA08090C-26-3	GA991336-6E9/AGS 2060
ABB324	LA08172D-2	(LA95135/GA011341-1LA07121)F1/LA07047,F1(GA9811622-1-40-1/LA95135)
ABB325	LA08249D-24	LA821-B/LA07174,F1(JAMESTOWN/AGS2026)
ABB326	LA08249D-29	LA821-B/LA07174,F1(JAMESTOWN/AGS2026)
ABB327	LA09021UB-6-3	CROPLAN 8302 / LA482
ABB328	LA09061UB-25-3	LA01*425 / LA95135
ABB329	LA09072UC-22	LA01034D-235-1-C /BALDWIN
ABB330	LA09082C-17	LA01139D-56-1 / GA081057,F1(LA821/MO050146)
ABB331	LA09135C-36	LA95135 / LA01172D-27-5-4
ABB332	LA09159C-7	NC05-21642 / LA01172D-27-5-4
ABB333	LA09163C-12	NC06-27 / LA01172D-27-5-4
ABB334	LA09164C-52	NC06-27 / LA08035,F1(AR98003-7-1/LA01164D-94-2)
ABB335	LA09164C-P4	NC06-27 / LA08035,F1(AR98003-7-1/LA01164D-94-2)
ABB336	LA09172C-10	P04287A1-10 / LA482
ABB337	LA09208C-5	ARS03-5358 / LA01029D-139-3
ABB338	LA09225C-33	LA01139D-56-1 / GA001492-7E9
ABB339	LA11154S-P1	LA04041D-10 / VA08W-176
ABB340	LANC9337-60	Jamestown / SS8641
ABB341	NC13-20227	NC06BGTAG12 / USG3209/ / Jamestown
ABB342	NC13-22726	BESS / SS 8641 / / NC-NEUSE
ABB343	NC13-22591	C 9553 / VA02W-513/ / AGS 2035
ABB344	NC13-20737	Jamestown / /NC-Neuse / NC03-11158
ABB345	NC13-22114	Jamestown / /USG 3555 / NC03-11158
ABB346	NC13-20698	Jamestown //NC03-11158 / SS 8641
ABB347	NC09-22206-3	NC00-16203 // P26R24 / NC96-13965
ABB348	NC13-22349	NC03-11457 / SS 8641/ / McCORMICK Fhb1 and 5A
ABB349	NC13-21682	NC04-15533 / VA05W-500 // VA05W-108
ABB350	NC10014-52	NC06-19896 / NC08-140 Bdv2
ABB351	NC13-21261	NC-NEUSE / Jamestown
ABB352	NC9356-23	NC-Yadkin / Oakes
ABB353	NC10034-13	NC-Yadkin / Shirley

Appendix Cont.

Entry	Variety	Pedigree
ABB354	NC10034-47	NC-Yadkin / Shirley
ABB355	NC13-21213	Oglethorpe / Jamestown
ABB356	NC13-21217	Oglethorpe / Jamestown
ABB357	NC11-5-16-21	USG3209 // GA081631-G2-G10 / NC06-27-11 /3/ USG3209 (Lr34, 46, Sr2)
ABB358	NC13-21445	VA04W-259 / Jamestown
ABB359	SS8641	Check
ABB360	Branson	Check