

# Identification of Native FHB Resistance QTL in the SRW Wheat Cultivar Jamestown

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## ABSTRACT

*Fusarium* Head Blight (FHB) is a devastating fungal disease of wheat (*Triticum aestivum* L.) and results in significant economic losses due to reductions in grain yield and the accumulation of mycotoxins, such as deoxynivalenol (DON) and nivalenol (NIV). As a result, breeding programs have been working to identify resistance genes in wheat varieties known to be resistant to FHB. Some of the major quantitative trait loci (QTL) for FHB resistance identified to date have been from exotic sources such as ‘Sumai3’ and the Chinese landrace Wangshuibai, and native resistance has been identified in North American cultivars such as Ernie and Truman which are being used in breeding programs. This study was conducted to characterize and map QTL for resistance to FHB in the soft red winter wheat cultivar Jamestown and to identify tightly linked DNA markers associated with those QTL so that marker-assisted selection (MAS) can be used in pyramiding these and other known QTL into elite backgrounds. Types of resistance assessed in this study include: Type I (resistance to initial infection; incidence), Type II (resistance to spread in wheat spike; severity), and decreases in mycotoxin accumulation (DON) and percentage of *Fusarium* damaged kernels (FDK). A population composed of 186 F<sub>5:7</sub> recombinant-inbred lines (RILs) from the cross Pioneer Brand ‘25R47’ / Jamestown were used to evaluate these traits in six environments (MD, NC, and VA in 2011 and 2012). This study identified a QTL for resistance to DON accumulation and FHB severity on the wheat chromosome 1B. The QTL accounted for 12.7% to 13.3% of the phenotypic variation in DON accumulation and 26.1% of

the phenotypic variation in FHB severity. The most diagnostic marker for the QTL on chromosome 1B associated with resistance to FHB severity and DON accumulation is *Xwmc500.6* located 7.2 cM from the QTL peak and flanked by markers *Xwmc500.7* and *Xgwm273.2* (28.2 cM interval).

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# Chapter 1

## Literature Review

## INTRODUCTION

*Fusarium* head blight (FHB), commonly referred to as Scab, caused by the pathogen *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae*), is a devastating fungal disease of wheat (*Triticum aestivum* L.) and results in significant economic losses due to reductions in grain yield and quality. McCullen et al. (1997) estimated that monetary losses due to FHB between 1993 and 1997 were \$3 billion in the U.S., and Nganje et al. (2004) reported an estimated loss of \$2.7 billion from 1998 to 2000 in the Midwest. In the United States, FHB epidemics have increased in occurrence and severity during the past 20 years, particularly in the Midwest and Eastern states where environmental conditions are favorable for perpetuation of FHB (Stack, 1999). These epidemics have resulted in an increased focus on breeding for resistance to FHB, which has been emphasized greatly in the past two decades following a major epidemic in the U.S. in 1993 (McCullen et al., 1997).

### ***Fusarium* Head Blight Pathogens and Disease Cycle**

There are at least 17 different species of *Fusarium* that cause FHB in small grains, some of the more common ones include: *F. graminearum*, *F. culmorum*, *F. poae*, *F. avenaceum*, and *Microdochium nivale* (Osborne and Stein, 2007; Parry et al., 1995; Sutton, 1982). The most common species present world-wide are *F. graminearum* and *F. culmorum*, depending on the environmental conditions one may be more prevalent in a given region (Mesterházy, 2003). The most common species in recent FHB epidemics of North America is *F. graminearum* (Stack, 1999). Though *F. graminearum* and *F. culmorum* are the most common species, multiple studies support the idea that there are no race-specific genes and that a given resistance quantitative trait

loci (QTL) may be just as effective against one *Fusarium* spp. as another (Mesterházy, 1995, 2003, 2005).

Sources of inoculum include soil and plant residue from previous crops (Dill-Macky and Jones, 1999). The greater source of inoculum is in plant residue from previous crops, specifically in areas where maize (*Zea mays* L.) and other susceptible grasses are grown, which allow *Fusarium* spp. to over-winter as *Fusarium* is a saprophytic fungus (Atanasov, 1920; Burgess, 1981; Dill-Macky and Jones, 1999; Osborne and Stein, 2007; Shaner, 2003). With the increasing cultural practice of reduced tillage, crop residue is more common in recent years (Dill-Macky and Jones, 1999; Osborne and Stein, 2007; Mesterházy, 2003). Several fungal structures can infect wheat including: chlamydospores, macroconidia, ascospores and mycelium (Sutton, 1982; Osborne and Stein, 2007).

*Fusarium* is a necrotrophic fungus, which means once it infects its preferred host it results in the death of host cells for continued growth. This is different from other common fungal pathogens of wheat such as: powdery mildew (*Blumeria graminis*), leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis*), which are biotrophic fungi and require the host to remain alive for them to grow. The disease cycle of *Fusarium* is depicted in figure 1 (adapted from Sutton, 1982). *Fusarium* head blight (FHB) is a monocyclic disease, meaning infection only occurs once on the host crop in a season (Gilbert and Tekauz, 2000; Miedaner, 1997; Muthomi, et al., 2002). Due to the saprophytic nature of *Fusarium* spp., they have the ability to over-winter in crop debris, most often in the form of chlamydospores (thick-walled fungal structures) or macroconidia (asexual spores) (Dill-Macky and Jones, 1999; Osborne and Stein, 2007; Parry et al, 1995; Sutton, 1982). Optimal conditions including warm temperatures, from about 23°C to 28°C, and moisture are needed for perithecia (fruiting body) to develop and produce ascospores

(sexual spores) (Doohan et al., 2003; Shaner, 2003; Sutton, 1982). Ascospores are forcibly discharged from perithecia and can be dispersed by wind (Schmale and Bergstrom, 2003) and carried in a splash of water (Shaner, 2003; Sutton, 1982). According to Schmale et al. (2006) the majority of ascospore dispersal occurs during the night, from about 12:00 pm until 6:00 am. Ascospores and macroconidia (asexual spores) can serve to infect the wheat when the optimum temperatures are between 15°C to 30°C and under a relatively moist environment (Schmale and Bergstrom, 2003). Because wheat and/or barley infected by FHB is present about the time when corn is silking and tasseling, the FHB infected crop and or crop debris serves as a source of inoculum for corn which in turn serves as a source of inoculum for wheat (Schmale and Bergstrom, 2003; Shaner, 2003; Sutton, 1982).

*Fusarium* head blight symptoms are characterized by prematurely bleached spikes which are often accompanied with other visual symptoms such as black perithecial structures and may have pink sporodochia, a dense mass of hyphae (McMullen et al., 1997; Osborne and Stein, 2007; Parry et al. 1995). The fungus initially develops in the wheat spikelet when ascospores or macroconidia come into contact with the spikelet and germinate there. Infection is more likely if this occurs during anthesis, though, infection can occur any time between anthesis (flowering) and the dough stage, and can spread throughout the spike (Sutton, 1982; Osborne and Stein, 2007; Buerstmayr et al., 2009, 2012). Initially, the infection can appear as a brown spot on the glume or palea of the spikelet, but it can spread and result in a completely blighted spikelet (Chen, 2006). Spread of the fungus through the rachis can result in nutrients being unable to reach the upper portion of the spike results in premature senescence (Buerstmayr et al., 2012). Early maturation leads to shrunken and/or shriveled seed (tombstones) which results in low test weight and yield loss (Parry et al., 1995). Depending on the timing of initial infection in the

wheat head, seed embryos may be aborted resulting in yield loss (Buerstmayr et al., 2012). Once the seeds have become infected with *Fusarium*, over time the pathogen is believed to breakdown starch granules, storage proteins, and cell walls (Bechtel et al., 1985), this is one cause for tombstone seed and light-weight, chalky seed with pink coloring referred to as “scabby” seed (Parry et al., 1995). Scabby seed are the chief cause of decreased yield and test weight and are indicative of the presence of mycotoxins (Buerstmayr et al., 2009, 2012). It is important to note that seed that appear to be healthy may still contain mycotoxins.

## **Mycotoxins**

*Fusarium* spp. produce mycotoxins that accumulate in the seed including deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEN), as well as others. It has been hypothesized that mycotoxins produced by *Fusarium* spp., such as deoxynivalenol, may actually serve as a virulence factor (Bai and Shaner, 2004), allowing the fungus to spread throughout the spike after initial infection. Deoxynivalenol, a common mycotoxin of *Fusarium graminearum* encountered in the United States, is also referred to as vomitoxin due to its adverse effects in animals and humans. Pigs are particularly sensitive to deoxynivalenol, and depending on the level of DON in their diet, it can induce emesis (vomiting), feed refusal, and reduced weight gain (Miller and Trenholm, ed., 1994). In extreme cases of toxicoses some symptoms include reproductive problems and immune system suppression as well as others (<http://www.enviroligix.com/library/mycobook.pdf>). With the increasing regularity and severity of FHB epidemics in the United States, the Food & Drug Association (FDA) has set a limit of 1 ppm (mg/kg) concentration on the amount of DON allowed in finished wheat products for humans (FDA-Food, 2010). In addition, there is a 5 to 10 ppm advisory limit for animals (FDA-

Food, 2010). This regulation is important due to the fact that DON accumulates in humans and animals, which can lead to harmful side effects mentioned previously.

### **Economic Implications**

The FDA regulation of 1 ppm (mg/kg) concentration of DON in finished products for human consumption affects the milling and malting companies in that they have to carefully monitor the concentration of DON in the grain they use. Though milling companies are able to take grain that is contaminated with FHB, they have to balance it out with non-contaminated grain so as to remain below the FDA regulation (Sullins, 2011). Sullins (2011) of ADM Milling Company pointed out that if the majority of the wheat grown around a milling company is infected with FHB, then the milling company has to pay extra to have non-contaminated grain shipped to the mill which increases the cost of finished products (Cowger and Sutton, 2005; Sullins, 2011). Use of fungicides alone is not effective as losses due to FHB may only be reduced by 50% to 60% and, thus, are not particularly economical if DON levels are still unacceptable (Stack, 1999). In Virginia, the state wheat yield average in 1998 ( $3026.7 \text{ kg ha}^{-1}$ ) was  $1547.0 \text{ kg ha}^{-1}$  lower than that of 1997 which was a record yield year and  $908.0 \text{ kg ha}^{-1}$  less than the average yield of the previous six years. If wheat is expected to compete with corn then the magnitude of these losses must be minimized to an acceptable level. Resistance to FHB in wheat must be identified and markers found that are tightly linked to resistance QTL, so breeders can utilize them in marker-assisted selection. Several varieties released by Virginia Tech and documented to have resistance to FHB include Roane, Jamestown, and Tribute (Liu et al., 2012). Once a wheat line has been identified as having some level of resistance to FHB the next step is to characterize and map the resistance QTL.



## Management Practices for FHB

The use of fungicides is a common method of FHB control. Bravo<sup>®</sup> 500 (Chlorothalonil, Syngenta), Caramba<sup>®</sup> (Metaconazole, BASF), Folicur<sup>®</sup> 3.6 F (Tebuconazole, Bayer Crop Science), Proline<sup>®</sup> 480 SC (Prothioconazole, Bayer Crop Science), and Prosaro<sup>®</sup> 421 SC (Prothioconazole and Tebuconazole, Bayer Crop Science) are fungicides that are registered for FHB in wheat (Jones, 2000; Mesterházy, 2003c; Paul et al., 2008; Shaner and Buechley, 2003; Steffenson, 1998). While fungicide applications as a seed treatment or at anthesis can provide a level of protection, the cost and optimum time of application may make controlling FHB with fungicides difficult (Bai and Shaner, 2004; Mesterházy, 2003b). However, fungicide application coupled with use of cultivars with FHB resistance may result in a better form of control (Cromey et al., 2001). Another method for reduction in FHB epidemics would be tilling the fields to encourage decomposition of the previous crop debris and thereby minimize the amount of inoculum present in the environment.

Plants and pathogens are constantly evolving mechanisms to combat one another as described by Flor (1971) in a gene-for-gene system which is problematic when the crops are struggling to keep up. One of the advantages in breeding for resistance is that multiple resistance QTLs can be incorporated into a genome and allow the plant an advantage over the pathogen. This incorporation of multiple resistance QTLs into a wheat genome is highly effective for wheat, particularly for FHB because single genes conferring complete resistance to FHB have not been identified in wheat (Buerstmayr et al., 2009; Dodds and Rathjen, 2010). As evident with other diseases such as stem rust, it is ill advised to depend solely on one or even a few resistance genes. Durable resistance breeding requires “pyramiding” resistance QTL (Griffey et al., 2008). Resistance to FHB is not isolate-specific (Bai and Shaner, 2004) nor does it involve a gene for

gene interaction such is the case with other diseases like stem rust. However, some *Fusarium* isolates can result in more or less infection depending on the wheat genotype. There are multiple types of resistance to FHB and its mycotoxins which can be incorporated into a wheat genome and thereby minimize the impact of an epidemic.

### **Types of FHB Resistance**

There are multiple types of resistance to FHB which can be incorporated into a wheat genome, as defined by Mesterházy (1995): (1) Type I, resistance to initial infection (Schroeder and Christensen, 1963), (2) Type II, resistance to spread of infection in the spike (Schroeder and Christensen, 1963), (3) Type III, resistance to kernel infection (Mesterházy, 1995), (4) Type IV, tolerance to yield loss (Mesterházy, 1995), (5) Type V, resistance to mycotoxin accumulation (Miller et. al., 1985; Wang and Miller, 1988; Mesterházy, 1995). Type I and Type II resistance are always associated with FHB incidence and FHB severity, respectively. The remaining types of resistance are not always referred to in the same way, for example, Type III is listed as resistance to kernel infection by Mesterházy (1995) and yet in Miller et. al. (1985) it is referred to as resistance to mycotoxin accumulation. The terminology more commonly used for the remaining types of resistance refers simply to the trait being assessed, such as resistance to *Fusarium* damaged kernels (FDK) or DON accumulation.

### **Sources of Plant Resistance to FHB**

Because resistance to *Fusarium* head blight has been shown to be inherited quantitatively, it is important to identify resistance sources and map the resistance QTL in order to incorporate them into breeding lines (Buerstmayr et al., 2009; Dodds and Rathjen, 2010). Major sources of resistance to FHB have been found in Asian landraces and cultivars, spring wheat lines such as

Sumai 3, Ning7840, W14, and Wangshuibai. However, introgression of this resistance into wheat varieties grown in other diverse environments is difficult as unfavorable agronomic traits (low yield and susceptibility to other diseases of wheat) tend to be associated with FHB resistance (Bai and Shaner, 2004). The best source of Type II resistance was identified in ‘Sumai 3’ on chromosome 3BS (Buerstmayr et al., 2009), now referred to as gene *Fhb1*. Wangshuibai is an example of a Chinese landrace that is a good source of resistance to FHB incidence on chromosome 5A and to FHB severity on 7A, 3BS and 5B (Jia et al., 2005; Lin et al. 2004; Lin et al., 2006; Mardi et al., 2005; Yu et al., 2008; Zhou et al., 2004; Bai and Shaner, 2004). Since the 1980s, the cultivars Alsen and Glenn in addition to a few breeding lines ND2710, INW0412, and VA01W-476 have been developed using the Asian resistant lines and are being utilized in breeding programs (Wilcoxson, 1992; Chen et al., 2006). During the past two decades extensive efforts have been made by wheat breeders to utilize the QTLs found in Asian sources in addition to finding “native” North American sources of resistance (Griffey et al., 2008). More moderate resistance has been identified and confirmed in the Brazilian cultivar Frontana on chromosome 3A for Type II resistance (Mardi et al., 2006; Steiner et al., 2004). Swiss cultivar Arina has confirmed Type II resistance on chromosome 6B (Draeger et al., 2007; Semagn et al., 2007). There are several North American cultivars having native resistance to FHB including: Ernie, Goldfield, Freedom, Truman, Bess, Roane, and Jamestown, among others which have moderate to high levels of resistance (Griffey et al., 2008). Of the North American native sources listed above, QTL have only been mapped in the cultivars Ernie, Goldfield, Freedom and Truman.

### **FHB QTL Identified**

According to a review by Buerstmayr et al. (2009) and a meta-analysis paper by Liu et al. (2009), FHB resistance QTL have been reported on all wheat chromosomes. Buerstmayr et al.

(2009) reviewed 52 different mapping studies which composed over 100 identified QTL on all chromosomes except for 7D; of the QTL listed, the most reproducible QTL were on chromosomes 3BS (*Fhb1*), 5AS (*Fhb5*, or *Qfhs.ifa-5A*) and 6BS (*Fhb2*). In the meta-analysis by Liu et al. (2009), forty-five mapping studies were compared, containing 249 QTL for FHB resistance, 209 of which were able to be mapped together through common markers. The review by Buerstmayr et al. (2009) and meta-analysis by Liu et al. (2009) included mostly similar studies, with 16 additional studies included in the meta-analysis adding 53 new QTL. A majority of the resistance QTL (130) identified have been associated with Type II resistance (Liu et al., 2009).

Resistance to FHB in wheat has been identified in: Australian line Gamenya; Canadian line AC Foremost; Chinese lines Huapei 3, Ning894037, ND2603, Wuhan 1, Sumai 3, and its derivatives W14, Ning7840, Ning8026, BW278, CJ9306, DH181, CM82036; Japanese lines Nyubai, Fukuhokomugi, and Tokai-66; Korean line Chokwang; Dutch line Romanus; French lines Apache, Renan, Rubens, Soissons; German lines Biscay, Cansas, Dream, G16-92, History, Hussar, Pelikan, Pirat, Remus, Ritmo; Norwegian line NK93604; Romanian lines Fondulae201R, Sincron; Swiss lines Arina, Forno; United Kingdom lines Lynx, Rialto, Riband, Spark; Brazilian line Frontana; CIMMYT lines Seri82, Alondra; United States lines Becker, Ernia, Freedom, Goldfield, IL-94-1653, Massey, Patterson, Patton, SD97060, Stoa, and Wheaton (Buerstmayr et al., 2009; Liu et al., 2009). Table 1 contains QTL that have been confirmed in multiple populations for an FHB resistance QTL.

Additional mapping studies have been conducted since 2009, including a study by Chu et al., (2011) which identified two FHB resistance QTL on 5A in wheat accession PI277012, most notably these QTL have been successfully transferred into durum wheat (*Triticum turgidum*

subsp. *durum*). Jayatilake et al. (2011) identified a novel QTL on chromosome 7A from a Chinese Spring–Sumai 3 chromosome 7A disomic substitution line (CS-Sumai 3-7ADSL). This 7A QTL may have originated from the Italian cultivar Funo (Jayatilake et al., 2011). In a Chinese wheat landrace, Haiyanzhong (HYZ), Li et al. (2011) detected five FHB resistance QTL. A major QTL was identified on chromosome 7DL of HYZ for Type II resistance, and the remaining QTL on chromosome 6BS (two QTL), 5AS and 1AS had minor effects (Li et al., 2011). Three QTL were identified in the hard wheat cultivar Heyne, on chromosomes 3AS, 4DL, and 4AL for FHB severity in greenhouse point inoculation tests (Zhang et al., 2012). Liu et al. (2012) reported FHB resistance QTLs in VA00W-38, which is a descendent of the cultivar Roane and known to have resistance to FHB. Several unique QTL were identified in VA00W-38 which included regions on chromosomes: 2A (2AS, 2ASc, and 2A), 5B and 6A (Liu et al., 2012). A study of two mapping populations (Becker/Massey and Ernie/MO94-317) resulted in the identification of 8 QTL and 5 QTL, respectively, on chromosomes 1AS, 1DS, 2DS, 2BL, 4BS, 4D, 5AL and 6AL (Liu et al., 2013). Identification of FHB resistance in the cultivar Truman is in progress, and it is suspected that the QTL reside on chromosomes, 1BSc, 2BL, 2DS and 3BSc, of which only 2D is speculated to be novel (McKendry, 2012).

Currently, the QTL on chromosome 1B (Table 2) have been identified in wheat genotypes: Cansas, CM82036, Romanus, History, Biscay, Pirat, Seri82, Arina, Fundulea201R, Rialto, Wangshuibai, Alondra, Remus, and Lynx. The cultivars Alondra, Cansas, Fundulea201R, Lynx, Rialto and Sincron are known to have the T1BL.1RS translocation, and therefore the source of the resistance is unclear. The Chinese lines, CM82036 and Wangshuibai, as well as Arina seem to have QTL from the same region of 1B apart from the translocation. The QTL from the German lines: Biscay, History, and Pirat are separate and unique from the other two groups. All

of the QTL identified on 1B are associated with Type 2 resistance, apart from the one identified in Kansas which is associated with Type I resistance, and they account for 3% to 19.6% of the variation.

### **Marker Assisted Selection**

Marker assisted selection (MAS) has the ability to improve the speed and efficacy at which cultivars can be developed with resistance to FHB. In order to apply MAS effectively, especially in breeding for enhanced FHB resistance, the associated QTL must be reliable and diagnostic. Confirmed QTL are more reliable than QTL which have only been identified in one population or in one environment. Guidelines for identification of reliable QTL which are diagnostic include: the need for chromosome saturation with polymorphic markers, markers that are tightly linked to the QTL, little to no segregation distortion and reliable phenotypic data (Babu, 2004). Markers should be validated in multiple populations to guarantee their use in MAS. If the markers and associated QTL are reliable, there are many advantages to using MAS including: a rapid output of genotypic information that does not rely wholly on phenotypic data and an increasingly inexpensive way to introgress and pyramid desirable traits into a population. The effectiveness of MAS should be confirmed in environments where phenotypic expression of the trait(s) of interest is readily feasible and it is possible to differentiate between phenotypes and genotypes. Marker-assisted selection for FHB resistance is more likely to be effective earlier in the breeding process with screening of parental lines and progeny in early generations for the presence of QTL inferring resistance. According to Collard and Mackill (2008), MAS is often easier than phenotypic selection because there is less expenditure of time, energy and resources. This would be particularly useful as FHB epidemics are variable over environments and result in inconsistent disease data due to differences in FHB severity of epidemics from year to year.

Early selection via MAS can be deployed in the seedling stage to identify lines with FHB resistance and thus enrich breeding populations. Such benefits of MAS can expedite the breeding process and accelerate the development of FHB resistant varieties (Dreher, et al., 2003, Ribaut and Hoisington, 1998).

Applications of MAS to plant breeding suggested by Collard and Mackill (2008) include: 1) marker-assisted evaluation of breeding material – MAS can assist in identifying lines that are true to type which is important in breeding programs. Identification of complementary parents and the level of genetic diversity present in the breeding program and or population. This would be useful in combining QTL for FHB resistance from Sumai 3 and native sources. 2) Marker-assisted backcrossing – MAS is an excellent way to employ markers in identification and successful introgression of FHB resistance traits of interest. 3) Marker-assisted pyramiding – MAS can assist in pyramiding genes, not only to check for presence of the genes in the parents but also to detect the presence of the genes after the cross has been made. In other words, the progeny can be screened and individuals that contain the combination of desired QTL can be advanced in the breeding population. 4) Combined MAS and phenotypic assessment – this refers to the use of MAS and phenotypic data in selection and validation of FHB resistance in breeding populations, lines, and varieties.

### **Proposed Research**

Data from breeding and FHB nursery trials conducted over many environments have provided evidence and validation of FHB resistance in the SRW wheat cultivar Roane and more recently in Roane's offspring, the SRW cultivar Jamestown (Griffey et al., 2001; Griffey et al., 2010; Murphy and Navarro, 2009). Jamestown (Roane / Pioneer Brand '2691') has resistance to

multiple diseases including: FHB, leaf rust (*Puccinia triticina*), stripe rust (*Puccinia striiformis*) and Hessian fly (*Mayetiola destructor*), and has been widely used as a parent in the wheat breeding programs of the Mid-Atlantic and Southern states (Griffey et al., 2010). Though Jamestown is commonly used as a parent, no studies have been conducted to date to identify and/or validate its resistance genes and/or QTL for the aforementioned diseases. The objectives of this study are to: 1) characterize and map quantitative trait loci for resistance to *Fusarium* Head Blight in Jamestown and; 2) identify tightly linked DNA markers associated with FHB resistance that can be used in marker assisted selection (MAS) and pyramiding of resistance genes.



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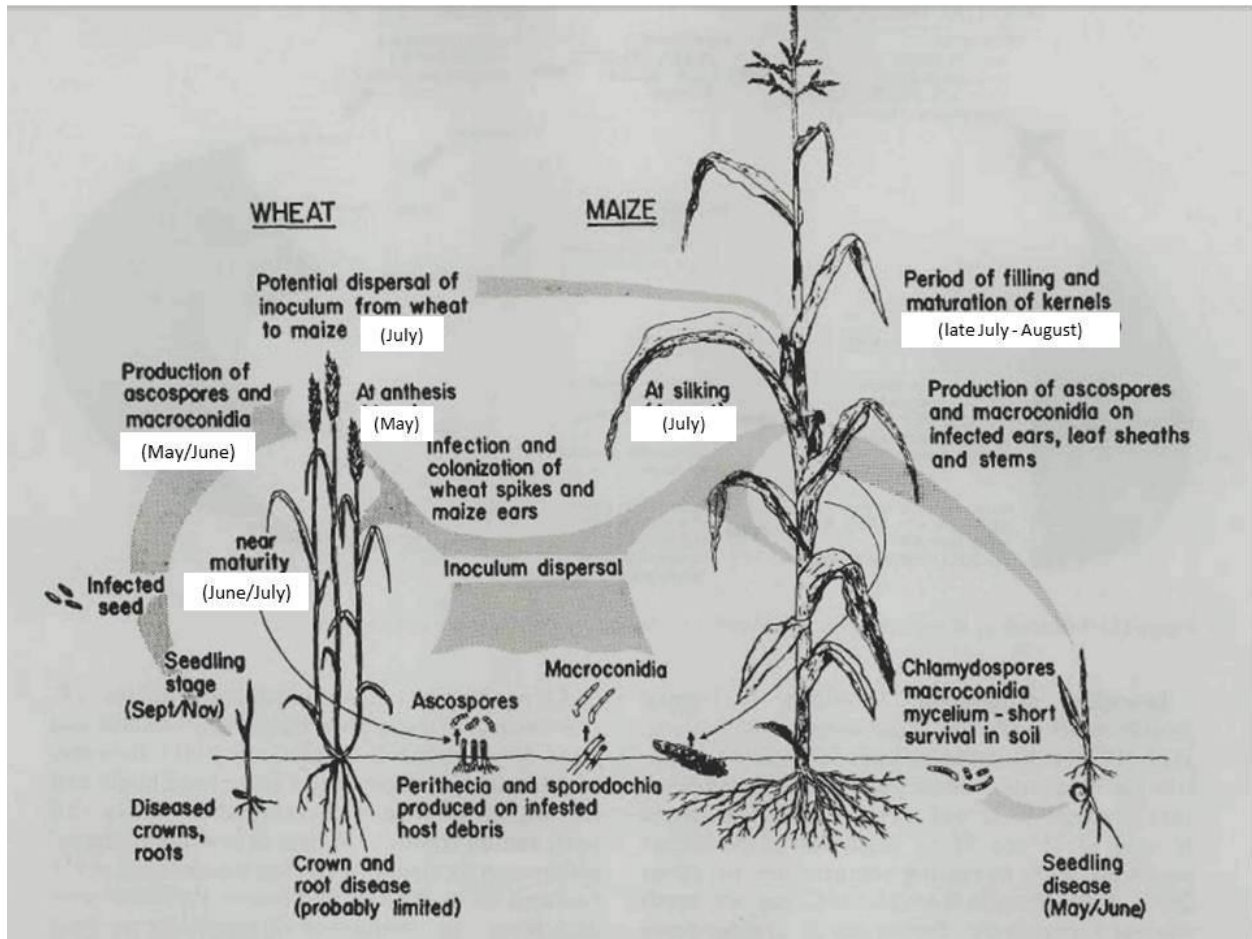


Figure 1: Disease cycle of *Fusarium* head blight. Adapted from “Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*” (Sutton, 1982), used with permission, 2014

Table 1.1: Confirmed QTL for Resistance to Fusarium Head Blight in Wheat

Resistance Trait	Chromosome	Original Source	Molecular Markers Associated with QTL	References
Severity <sup>†</sup>	3A	Frontana <sup>#</sup>	( <i>Xgwm720-Xgwm1121</i> ); ( <i>Xdupw227- Xgwm720</i> )	Mardi et al., 2006; Steiner et al., 2004
Incidence <sup>‡</sup>	5A	Sumai 3 <sup>††</sup>	( <i>Xgwm304- Xgwm156</i> ); ( <i>Xbarc117-barc186</i> ); ( <i>Xgwm293</i> )	Buerstmayr et al., 2003; Chen et al., 2006; Yang et al., 2005
Severity	5A	Sumai 3 <sup>††</sup>	( <i>Xgwm304- Xgwm156</i> ); ( <i>Xbarc117-barc186</i> )	Buerstmayr et al., 2003; Chen et al., 2006
DON <sup>§</sup>	5A	Sumai 3 <sup>††</sup>	( <i>Xbarc117- Xbarc186</i> ); ( <i>Xgwm425- Xbarc186</i> )	Chen et al., 2006; Jiang et al., 2007
Incidence	5A	Wangshuibai <sup>‡‡</sup>	( <i>Xgwm304- Xbarc56</i> ); ( <i>Xbarc180</i> )	Lin et al., 2006; Yu et al., 2008
Severity	7A	Wangshuibai <sup>‡‡</sup>	<i>Xwms1083</i>	Yu et al.,2008; Zhou et al., 2004
Severity	1B	Wangshuibai <sup>‡‡</sup>	( <i>Xgwm018- Xbarc181</i> ); ( <i>Xwms759</i> )	Lin et al., 2004; Zhou et al., 2004
Incidence	3BS	Sumai 3 <sup>††</sup>	( <i>Xgwm533- Xgwm493</i> ); ( <i>Xbarc133- Xgwm493</i> ); ( <i>Xgwm533</i> )	Buerstmayr et al., 2003; Chen et al., 2006; Yang et al., 2005
Severity	3BS	Sumai 3 <sup>††</sup>	( <i>Xgwm533- Xgwm493</i> ); ( <i>XSTS3B80- XSTS3B142</i> )	Anderson et al.,2001; Buerstmayr et al., 2003; Chen et al., 2006; Cuthbert et al., 2006; Jiang et al., 2007 Liu et al., 2006; Yang et al. 2005; Zhou et al. 2002
DON	3BS	Sumai 3 <sup>††</sup>	( <i>Xbarc133- Xgwm493</i> ); ( <i>Xgwm533b- Xgwm493</i> )	Chen et al., 2006; Jiang et al., 2007
FDK <sup>¶</sup>	3BS	Sumai 3 <sup>††</sup>	( <i>Xbarc133- Xgwm493</i> ); ( <i>Xgwm533b</i> )	Chen et al., 2006; Yang et al., 2005
Severity	3BS	Wangshuibai <sup>‡‡</sup>	<i>Xgwm533- Xgwm493</i>	Jia et al., 2005; Lin et al. 2004; Mardi et al., 2005; Yu et al., 2008; Zhou et al., 2004
Severity	5B	Wangshuibai <sup>‡‡</sup>	( <i>Xgwm335- Xgwm371</i> ); ( <i>Xwmc113- Xgwm544</i> )	Jia et al., 2005; Lin et al. 2004
Incidence	6BS	Sumai 3 <sup>††</sup>	( <i>Xgwm133- Xgwm644</i> ); ( <i>Xwmc397</i> )	Cuthbert et al., 2007; Yang et al., 2005
Severity	6BS	Sumai 3 <sup>††</sup>	( <i>Xbarc101/Xbcd1383</i> ); ( <i>Xgwm133- Xgwm644</i> ); ( <i>XP75M60.563 - Xgwm644</i> ); ( <i>Xwmc397</i> )	Anderson et al.,2001; Cuthbert et al., 2007; Haberer et al., 2009; Yang et al. 2005
FDK	6BS	Sumai 3 <sup>††</sup>	( <i>Xgwm133- Xgwm644</i> ); ( <i>Xwmc397</i> )	Cuthbert et al., 2007; Yang et al., 2005
Severity	6B	Arina <sup>§§</sup>	( <i>Xpap3131</i> );( <i>XP46/M62-107-XP45/M60-265</i> )	Draeger et al., 2007; Semagn et al., 2007
Severity	2DL	Sumai 3 <sup>††</sup>	( <i>Xgwm157- Xwmc243a</i> ); ( <i>Xwmc144- Xgwm539</i> )	Jiang et al., 2007; Yang et al., 2005

<sup>†</sup>Severity = resistance to spread of FHB in the wheat spike

<sup>‡</sup>Incidence = resistance to initial infection of FHB

<sup>§</sup>DON = resistance to deoxynivalenol accumulation in the seed

<sup>¶</sup>FDK = resistance to *Fusarium* damaged kernels

<sup>#</sup> South American - Brazil

<sup>††</sup> Asian - Sumai 3 or derivatives

<sup>‡‡</sup> Asian - Chinese landrace

<sup>§§</sup> European – Swiss

Table 1.2: QTL Identified on Wheat Chromosome 1B for Resistance to Fusarium Head Blight and Deoxynivalenol (DON)

Resistance Trait	Original Source	Molecular Markers Associated with QTL	References
Severity <sup>†</sup>	Alondra <sup>¶</sup>	<i>XeTCG-mAGC.7 - XeACCGmCT-C.7</i>	Zhang et al., 2004
Incidence <sup>‡</sup>	Cansas <sup>¶</sup>	<i>XE38M52.378 - Xgwm131</i>	Klahr et al., 2007
Severity	Fundulea201R <sup>¶</sup>	<i>Xbarc8</i>	Shen et al., 2003
Severity	Lynx <sup>¶</sup>	<i>Xp78m51.237- Xs26m23.356 (Xgwm18.iag95)</i>	Schmolke et al. 2005
Severity	Rialto <sup>¶</sup>	<i>XwPt-0705</i>	Srinivasachary et al., 2008
Severity	Seri82 <sup>¶</sup>	<i>Xe38m50_10</i>	Mardi et al., 2006
Severity	Sincron <sup>¶</sup>	<i>Gli-R1</i>	Ittu et al., 2000
Severity	Arina_1	<i>XS16M14.400</i>	Draeger et al., 2007
Severity	Romanus	<i>XP70M56.308</i>	Holzapfel et al., 2008
Severity	CM82036	<i>XgluB1</i>	Buerstmayr et al., 2002
Severity	Wangshuibai_1	<i>Xgwm018- Xbarc181</i>	Lin et al., 2004
Severity	Wangshuibai_2	<i>Xwms759</i>	Zhou et al., 2004
DON <sup>§</sup>	Wangshuibai_3	<i>Xwms759</i>	Yu et al., 2008
Severity	Arina_2	<i>XP43/M62-400 - XwPt-3475</i>	Semagn et al., 2007
Severity	History	<i>XP64M51.190</i>	Holzapfel et al., 2008
Severity	Biscay	<i>XP64M51.190</i>	Holzapfel et al., 2008
Severity	Pirat	<i>XP64M51.190</i>	Holzapfel et al., 2008
Incidence & Severity	Remus	<i>Xs12m25.14 - Xs24m17.2</i>	Steiner et al. 2004

<sup>†</sup>Severity = resistance to spread of FHB in the wheat spike

<sup>‡</sup>Incidence = resistance to initial infection of FHB

<sup>§</sup>DON = resistance to deoxynivalenol accumulation in the seed

<sup>¶</sup>T1BL.1RS translocation

## Chapter 2

### Identification of Native FHB Resistance QTL in the SRW Wheat Cultivar Jamestown

## ABSTRACT

*Fusarium* Head Blight (FHB) is a devastating fungal disease of wheat (*Triticum aestivum* L.) and results in significant economic losses due to reductions in grain yield and the accumulation of mycotoxins, such as deoxynivalenol (DON) and nivalenol (NIV). As a result, breeding programs have been working to identify resistance genes in wheat varieties known to be resistant to FHB. Some of the major quantitative trait loci (QTL) for FHB resistance identified to date have been from exotic sources such as ‘Sumai3’ and the Chinese landrace Wangshuibai, and native resistance has been identified in North American cultivars such as Ernie and Truman which are being used in breeding programs. This study was conducted to characterize and map QTL for resistance to FHB in the soft red winter wheat cultivar Jamestown and to identify tightly linked DNA markers associated with those QTL so that marker-assisted selection (MAS) can be used in pyramiding these and other known QTL into elite backgrounds. Types of resistance assessed in this study include: Type I (resistance to initial infection; incidence), Type II (resistance to spread in wheat spike; severity), decreases in mycotoxin accumulation (DON) and percentage of *Fusarium* damaged kernels (FDK). A population composed of 186 F<sub>5:7</sub> recombinant-inbred lines (RILs) from the cross Pioneer Brand ‘25R47’ / Jamestown were used to evaluate these traits in six environments (MD, NC, and VA in 2011 and 2012). This study identified a QTL for resistance to DON accumulation and FHB severity on the wheat chromosome 1B. The QTL accounted for 12.7% to 13.3% of the phenotypic variation in DON accumulation and 26.1% of the phenotypic variation in FHB severity. The most diagnostic marker for the QTL on chromosome 1B associated with resistance to FHB severity and DON accumulation was the simple sequence repeat (SSR) *Xwmc500.6* located 7.2 cM from the QTL peak and flanked by SSR markers *Xwmc500.7* and *Xgwm273.2* (28.2 cM interval).

## ACKNOWLEDGEMENTS

This study was a collaborative effort, the cooperators in this project include, J.P. Murphy and his team at North Carolina State University, Jose Costa and Aaron Cooper with their team at the University of Maryland. Aside from preparing the seed for planting and post-harvest analysis (DON and FDK), the cooperators did a majority of the work in relation to collecting the phenotypic data: field management, plot preparation and planting, inoculations, collecting FHB incidence and FHB severity data, harvesting and sending seed back to VA for further analysis. In addition, Humphrey Wanjugi, Michael Grosz, and their team at Monsanto Company did all the work performing the assay with the Illumina 9K SNP chip for all of the SNP data presented in this study. This study would not have been possible without the time and effort contributed by these cooperators.

## INTRODUCTION

*Fusarium* Head Blight, a major disease in wheat (*Triticum aestivum* L.) and commonly referred to as scab, caused by the pathogen *Fusarium graminearum* Schwabe, is most effectively controlled by deployment of cultivars having multiple pyramided resistance genes. In order to efficiently pyramid resistance genes, QTL must first be mapped and confirmed with tightly linked markers so that they might be utilized in marker-assisted selection (Collard and Mackill, 2008). Because resistance to FHB is quantitative and not isolate-specific, there are multiple forms of resistance that can be identified and incorporated into wheat lines in order to enhance their resistance (Bai and Shaner, 2004; Somers et al., 2003). Types of resistance, as defined by Mesterházy (1995), include: Type I, resistance to initial infection (as measured by FHB

incidence (INC)); Type II, resistance to spread of infection in the spike (as measured by FHB severity (SEV)); Type III, resistance to kernel infection (measured as % *Fusarium* damaged kernels); Type IV, tolerance to yield loss; and Type V, resistance to mycotoxin accumulation (e.g. deoxynivalenol concentration) (Miller et. al., 1985; Wang and Miller, 1988; Mesterházy, 1995; Schroeder and Christensen, 1963). In studies where both traits are assessed FHB severity and DON accumulation are generally positively correlated traits and, as one of the most devastating aspects of FHB is mycotoxin contamination of grain, resistance to these traits are most often desired in varieties (Chen et al., 2006a). Whereas at least 130 QTL associated with reduced FHB severity have been identified, only 25 QTL have been identified for reduction in DON accumulation (Liu et al., 2009). While many QTL associated with FHB have been identified in diverse wheat sources worldwide (Buerstmayr et al. 2009; Liu et al. 2009; Löffler et al. 2009), few of these QTL have been confirmed and/or fine mapped in subsequent studies. Most of the confirmed QTL are in Asian sources including Sumai 3 and Wangshuibai, with two QTL for resistance to DON accumulation confirmed in Sumai 3 (Buerstmayr et al., 2009; Liu et al., 2009). Though the Asian sources have the highest level of resistance currently identified e.g. gene *Fhb1*, the incorporation of such QTL into other breeding programs has often resulted in the simultaneous transfer of other undesirable traits such as low yield and decreased seed quality particularly in environments where Sumai 3 and other sources of resistance are not adapted. The objectives of the current study are to characterize and map quantitative trait loci (QTL) for resistance to FHB in the native soft red winter wheat cultivar Jamestown and to identify diagnostic markers to implement marker-assisted selection for incorporation and pyramiding these resistance genes into elite wheat backgrounds.

## MATERIALS AND METHODS

### Plant Material

The soft red winter (SRW) wheat cultivar Jamestown (PI 653731; Jtwn), which is moderately resistant to *Fusarium* head blight (FHB), was crossed with the moderately susceptible wheat line Pioneer Brand ‘25R47’ (PI 631473; P47) to develop a recombinant inbred line (RILs) mapping population (P47/Jtwn). A set of 186 RILs, derived from Pioneer Brand 25R47/Jamestown, were randomly selected from the F<sub>5</sub> breeding population developed at Virginia Tech. Jamestown was derived from the cross ‘Roane’ (PI 612958) / Pioneer Brand ‘2691’ (PI 590941) at Virginia Tech (Griffey et al., 2010). Roane (Griffey et al., 2001) is also moderately resistance to FHB and, therefore, it is likely that Jamestown inherited some or all of its resistance from Roane.

Seed of each of the 186 Pioneer Brand 25R47 / Jamestown RILs used in the 2010-11 experiments was derived from individually harvested F<sub>5:6</sub> head rows, and seed used in the 2011-12 experiments was derived from 20 representative heads selected and harvested from four F<sub>5:7</sub> head row plots grown at Warsaw, VA. The seed sample of each RIL used in these experiments were treated with Raxil-MD<sup>®</sup> (Tebuconazole, Metalaxyl and Imazalil, Bayer Crop Science) and Gaucho<sup>®</sup> XT insecticide (Imidacloprid, Bayer Crop Science) to limit infection of plants in the mapping populations with glume blotch (*Stagonospora nodorum* formerly *Septoria nodorum*) and Barley yellow dwarf (pathogen *Barley Yellow Dwarf Virus*). Subsamples of this seed were used to plant tests in VA and also sent to cooperators in NC and MD for their tests in 2010-11 and 2011-12 growing seasons.

### Inoculum



Inoculum of *Fusarium graminearum* used in these studies was comprised of a mixture of isolates from the state in which the respective tests were conducted. Isolates of *Fusarium* spp. used in VA field and greenhouse studies were obtained from Dr. David Schmale, department of Plant Pathology, Physiology, and Weed Science at Virginia Tech. The isolates were: GPS13#4, GPS8#12, GPS12#8, GPS11#9, KBTappN5, KBTappN10 and KBTappN27. *Fusarium graminearum* was cultured on full-strength potato dextrose agar (PDA) media (39 g potato dextrose agar in 1L water; Becton, Dickinson and Company, Sparks, MD 21152, USA). Corn (*Zea mays* L.) kernels were soaked overnight, placed in autoclave bags (Fisher Scientific, catalog no. 01-814-3) and sterilized by steaming for a 24-hour period in a steamer. Fungal mycelium were cut into 1 cm<sup>2</sup> pieces and put on the corn kernels in the autoclave bags, which were rotated and shaken frequently to disperse and mix the corn kernels and inoculum, and incubated at room temperature (21°C) for 21 days. Then corn spawn was sun dried in the greenhouse for 5 days. Macroconidia used to produce inoculum solutions that were used in greenhouse and field tests were grown on media composed of a one-quarter strength PDA (6 g potato dextrose broth and 15 g granulated agar in 1L water; Becton, Dickinson and Company, Sparks, MD 21152, USA). Macroconidia were harvested and inoculum solutions, adjusted to a concentration of 50,000 spores mL<sup>-1</sup> using distilled water, were prepared each day of inoculation for field and greenhouse tests.

### **Field Tests**

In 2010-11 and 2011-12 growing seasons, 186 RILs (F<sub>5:7</sub> and F<sub>5:8</sub>, respectively), parental lines and susceptible checks were evaluated for FHB assessment traits at three locations; Salisbury, MD (latitude 38° 21' 38" N and longitude 75° 35' 59" W; 1 replication, 1 head row/entry), Kinston, NC (latitude 35° 15' 45" N and longitude 77° 34' 54" W; 1 replication, 1

head row/entry), and Blacksburg, VA (latitude 37° 13' 1" N and longitude 80° 35' 52" W; 2 replications, 2 head rows/entry). The SRW wheat cultivars Pioneer Brand 26R46 (PI 612154) and Coker 9835 (PI 548846) are very susceptible to FHB and were included as early and late heading checks, respectively.

### **Field tests at Blacksburg, VA**

In 2010-11 and 2011-12 scab nursery tests at Blacksburg, VA the field plots were comprised of two replications arranged in a randomized complete block design and included the 186 Pioneer Brand 25R47/Jamestown RILs and the parental lines and susceptible checks present four times throughout the field for a total of 202 entries. Each experimental unit was comprised of a two-row plot, 1.2 m in length with 0.3 m space between the rows. In 2010-11, the plots were planted October 1, 2010. In 2011-12, the plots were planted September 30, 2011. Field management practices for 2010-11 included: fall pre-plant fertilizer 30-50-60 (N-P-K) and 3.923 t ha<sup>-1</sup> Lime; Harmony Extra SG<sup>®</sup> (Thifensulfuron methyl) herbicide was applied at 0.04 L ha<sup>-1</sup> on November 11, 2010; at GS 25 (Zadoks et al., 1974) a top dress application of 56.0 kg ha<sup>-1</sup> of nitrogen was applied as UAN 32-0-0 (32% Urea ammonium nitrate solution) in conjunction with an application of Harmony Extra SG<sup>®</sup> herbicide at 0.06 L ha<sup>-1</sup> on March 22, 2011; the fungicide Tilt<sup>®</sup> (Propiconazole) was applied at 0.3 L ha<sup>-1</sup> on April 21, 2011 to control glume blotch. Field management practices for 2011-12 included: fall pre-plant fertilizer 30-46-60 (N-P-K) and 2.24 t ha<sup>-1</sup> Lime; Harmony Extra SG<sup>®</sup> herbicide was applied at 0.05 L ha<sup>-1</sup> on December 12, 2011; at GS 30 a top dress application of 67.3 kg ha<sup>-1</sup> of nitrogen was applied as UAN 32-0-0 in conjunction with an application of Harmony Extra SG<sup>®</sup> herbicide at 0.04 L ha<sup>-1</sup> on March 8, 2011.

Corn spawn (25 g per 2-row head row plot) was applied at the boot stage during both years of the study. In 2011, each plot was spray-inoculated once at 50 percent anthesis using a CO<sub>2</sub>-pressurized sprayer (R and D Sprayers, Opelousas, LA 70570, USA) with an 80 mL conidial suspension (50,000 spores mL<sup>-1</sup>). For three weeks, overhead mist irrigation was applied daily at 1-hour intervals from 8:00 to 9:00 a.m. and from 4:00 to 5:00 p.m., except in the event of precipitation (Chen et al., 2006). In 2012, macroconidia spores were sprayed twice using a CO<sub>2</sub>-pressurized sprayer (R and D Sprayers, Opelousas, LA 70570, USA) with an 80 mL conidial suspension (50,000 spores mL<sup>-1</sup>), once at 50 percent anthesis and again one week after the initial inoculation. Mist-irrigation was applied at 15 minute intervals from 8:00 to 9:00 a.m. and from 6:00 to 7:00 p.m., except in the event of precipitation. In both test years, 20 spikes were evaluated 21 days post-inoculation (50 percent anthesis) in each of the parental, susceptible check and RIL plots. Each line was evaluated for phenotypic traits including 50% anthesis date, FHB incidence (INC), severity (SEV) and index (IND). FHB incidence (%) was calculated as (number of spikes infected/20) x 100%. FHB severity (%) was calculated as (number of infected spikelets/total number of spikelets) x 100% for each infected spike out of the 20 spikes evaluated per plot (Chen et al., 2006). The 2010-11 test was not harvested due to very low levels of FHB infection and severity. The 2011-12 test was harvested June 21-22 at maturity ( $\leq$ 16% moisture) using Wintersteiger plot combines (Wintersteiger, Salt Lake City, UT), with fans set on a low setting to minimize the loss of scabby kernels. Grain samples were cleaned using a screen cleaner without air and subsamples were used to assess FDK and DON toxin concentration.

### **Field tests at Salisbury, MD**

In 2010-11 and 2011-12 scab nursery tests at Salisbury, MD, comprised of one replication of 202 entries (parent lines, susceptible checks, and RILs) arranged in a randomized

design, was grown both years and planted in 1.2 m long 1-row plots with 0.3 m spacing between rows. In 2010-11, the plots were planted October 13, 2010. In 2011-12, the plots were planted October 8, 2011. Field management for 2010-11 included: fall pre-plant fertilizer 15-0-15 (N-P-K), S (5%), and B (0.2%) at 336.3 kg ha<sup>-1</sup> was applied on October 5, 2009 ; Harmony Extra XP<sup>®</sup> herbicide applied at 0.02 L ha<sup>-1</sup> with 820 Surf (Alcohol ethoxylate, Alkylphenol ethoxylate) at 1.2 L ha<sup>-1</sup> on November 9, 2009; a top dress application of 336.3 kg ha<sup>-1</sup> of 15-0-15 (N-P-K), S (5%), and B (0.2%) applied on March 2, 2011; a bulk Hi-Mg lime was applied at 2.24 t ha<sup>-1</sup> on March 9, 2010; the herbicide Harmony Extra XP<sup>®</sup> applied at 0.04 L ha<sup>-1</sup> in conjunction with another top dress application of 30% UAN at 174.9 L ha<sup>-1</sup> on March 25, 2010; the pesticide Warrior (Lambda-cyhalothrin) was applied at 0.27 L ha<sup>-1</sup> for cereal leaf beetles on May 7<sup>th</sup> and 10<sup>th</sup>, 2010; Goose Chase (Methyl Anthranilate) pesticide was applied at 1.2 L ha<sup>-1</sup>. Field management for 2011-12 included: fall pre-plant fertilizer 15-0-15 (N-P-K), S (5%), and B (0.2%) at 392.3 kg ha<sup>-1</sup> was applied on October 6, 2010; Harmony Extra XP<sup>®</sup> herbicide applied at 0.02 L ha<sup>-1</sup> with 820 Surf at 1.2 L ha<sup>-1</sup> on November 1, 2010; a top dress application of 448 kg ha<sup>-1</sup> of 15-0-15 (N-P-K), S (5%), and B (0.2%) applied on March 3, 2011; the herbicide Harmony Extra XP<sup>®</sup> applied at 0.04 L ha<sup>-1</sup> in conjunction with another top dress application of 30% UAN at 149.7 L ha<sup>-1</sup> on March 30, 2011. Colonized corn was applied at GS30. The inoculum was provided by Dr. A. Grybauskas, Department of Plant Science and Landscape Architecture, University of Maryland at College Park. Mist-irrigation was applied four times daily for one-hour intervals at 3 a.m., 6 a.m., 9 p.m., and 12 a.m. until maturity. FHB incidence was assessed as the percentage of the plot infected with FHB. Ten heads from each entry were used to calculate FHB severity. FHB incidence was calculated as the percentage of heads infected with FHB, and FHB severity was calculated as the average percentage of infected

spikelets. In 2010-11 and 2011-12, ten heads were harvested on June 17, 2011 and June 28, 2011, respectively, and sent to Virginia Tech for DON testing and FDK assessment.

### **Field tests at Kinston, NC**

In Kinston, NC a single replication of the 202 entries (parental lines, susceptible checks and RILs) were grown both years in one row field plots that were 1.2 m in length with 0.15 m spacing between the rows. Field plots were planted October 22, 2011. Field management for 2010-11 and 2011-12 scab nurseries included: pre-plant 33.6 kg ha<sup>-1</sup> of nitrogen was applied with an appropriate amount of phosphorus and potassium, according to soil test recommendations; top dress N at 112.1 kg ha<sup>-1</sup> was applied at GS 30.

Corn spawn was spread at a rate of 330 g per 80 head row plots (4g per head row) in the test at three different time points: three weeks prior to heading, two weeks before heading, and at heading (2012: March 16, 22, and 30, 2012). *Fusarium* cultures were developed using samples collected from *Fusarium*-infected wheat fields throughout the state of North Carolina (Murphy, personal communication). A backpack sprayer was used to spray macroconidia (50,000 spores mL<sup>-1</sup>) at anthesis. Overhead mist irrigation was run from 8:00 a.m. to 8 p.m. for 3 minutes at 20 minute intervals. Twenty-one days after the final inoculation, 50 to 100 heads per plot were evaluated for FHB incidence (number of spikes infected / number of spikes evaluated for that plot) x 100% and FHB severity was calculated as the percent of each head infected in accordance with the Stack and McMullen (2010) visual scale method. At maturity, a handful of arbitrarily selected heads was harvested (May 23, 2012) and sent to Virginia Tech for FDK assessment and DON testing.

### **FDK and Mycotoxin Evaluation**

Wheat heads harvested from tests in MD and NC were sent to Virginia Tech for FDK and DON analysis. The samples from MD were comprised of 15 to 20 heads per plot and were threshed using a small Vogel head thresher (Almaco, Nevada, IA 50201, USA). Larger samples containing more heads were sent from the tests in NC and these were threshed in a Wintersteiger plot combine (Wintersteiger, Salt Lake City, UT) using a low fan setting to limit loss of lighter weight and *Fusarium* damaged kernels. Once the samples had been threshed and hand cleaned using screens, 100 seeds were set aside for assessing the number of FDK. *Fusarium* damaged kernels were calculated as a percentage of scabby and/or tombstone seed out of a sample of 100 seeds. A subsample from the remaining seed (100 g from NC and VA locations and about 10 grams from MD) was sent to Dr. Schmale's lab at Virginia Tech for DON analysis using a Shimadzu QP2010 GC/MS (gas chromatography/mass spectrometry; Shimadzu Corporation, Kyoto, Japan; Tacke and Casper, 1996).

### **Greenhouse – FHB Type II resistance screening**

Parents and the 186 RILs of the Pioneer Brand 25R47/Jamestown population were evaluated for FHB type II resistance (resistance to spread in the spike) in the greenhouse (GH). The experiment was conducted in a greenhouse (temperature at 25°C ±3 and supplemental light for 12 h (Phillips metal halide fixtures, 400 W, high pressure bulbs) and repeated three times during spring 2011 (F<sub>5:7</sub>), fall 2011(F<sub>5:7</sub>), and fall 2012 (F<sub>5:8</sub>). One, three, and five plants were planted for FHB type II resistance evaluations during spring 2011 (GHS11), fall 2011 (GHF11), and fall 2012 (GHF12), respectively. A point inoculation method was used as described by Dill-Macky (2003); wherein, 10 µL of a conidial suspension (about 10,000 spores mL<sup>-1</sup>) of *Fusarium graminearum* was injected with a pipette between the lemma and palea of the 6<sup>th</sup> or 11<sup>th</sup> spikelet depending on the total number of spikelets of the individual head. The inoculated spikes were

then covered with plastic Ziploc® brand Snack bags (S. C. Johnson & Son, Inc., Racine, WI) to create a warm and moist environment for disease development. An average of five heads per line was inoculated with macroconidia spores and covered with a bag for 72 hours to provide high humidity for infection. In spring 2011, twenty-eight days after inoculation, each head was evaluated for FHB severity on the basis of percentage of infected spikelets [(total number of spikelets infected / total number of spikelets) x 100%]. The mean FHB severity for each line was calculated as the average of the five infected heads. In fall 2011, twenty-one days after inoculation, bleached and symptomatic spikelets were counted and FHB severity was calculated as in spring 2011. In fall 2012, bleached and symptomatic spikelets were counted after 7, 14, and 21 days after inoculation (dai). The FHB severity was calculated as in spring 2011. Area under disease progress curve (AUDPC) was calculated based on the following formula:

$$AUDPC = \sum_{i=1}^{N-1} N - 1 \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

where,  $y_i$  = disease severity at the  $i$ th observation

$t_i$  = time at the  $i$ th observation

$N$  = total number of observations

### **DNA Collection**

The F<sub>5:8</sub> and F<sub>5:9</sub> RILs were grown in the greenhouse and leaf tissue samples were collected at the two leaf stage. The tissue samples were placed in 2 mL test tubes containing a steel bead for grinding. A second steel bead was added after the tissue was in the tube to aid in the grinding step. The tissue samples were then placed in a -80 °C freezer overnight until frozen. The samples were placed in liquid nitrogen to ensure that the samples were completely frozen before being placed in a Spex CertiPrep 2000 Geno-Grinder (Metuchen, NJ) operated at

350 rpms for 30 seconds or until finely ground. DNA isolation was conducted using the CTAB extraction method as described by Saghai-Marroof et al. (1984, 1994).

## **Genotyping**

Two types of markers were used in this study; simple sequence repeats (SSRs) and single-nucleotide polymorphisms (SNPs). Selective genotyping with SNPs markers was done on the parents and a subset (42 RILs) of the 186 RILs. These forty-two RILs, selected based on their FHB severity ratings for the three 2010-11 test locations (MD, NC, and VA), included 21 susceptible lines and 21 resistant lines in order to preform BSA on this population. The 42 RILs and parental tissue were sent to Monsanto Company for SNP analysis on a 9K SNP chip using a combination of public SNP markers and proprietary Monsanto Company SNP markers. The 9K SNP assays or markers refer to 9K INFINIUM CHIP. The Infinium SNP genotyping is as detailed by ILLUMINA (This information is available on their website, <http://www.illumina.com/>).

Bulk segregant analysis (BSA, Michelmore et al., 1991) was conducted on this population using 170 SSR markers that were polymorphic between the parental lines, and 35 SSR markers were identified as significant between the bulks. These markers were used to identify putative QTL and elucidate the novelty of QTL identified in Jamestown, compared with previously reported QTL for FHB and/or DON. Similar to BSA described by Trick et al. (2012), DNA from three of the most resistant and three of the most susceptible RILs, selected on the basis of the first year data, were pooled in equal amounts to form the resistant and susceptible bulks, respectively. As all markers identified with BSA were previously run on the population, an additional 40 markers, which were identified as polymorphic between the parents, were run on the population in order to create a reliable linkage map. A combination of M13 and direct-



labeled SSRs were used in screening the population. The SSR markers consisted of GWM (Röder, et al., 1998), BARC (Song, et al., 2005), WMC (Gupta, et al., 2002, Somers, et al., 2004), CFA (Sourdille, 2001), CFD (Guyomarc'h, et al., 2002), GDM (Pestsova, et al., 2000), and NAU/STSBCD135-1 and NAU/STSBCD135-2 (Jianhui, et al., 2007). Finally, the markers that were identified as being significantly associated with FHB severity via BSA were used to analyze all of the 186 RILs in the population.

The M13-primer polymerase Chain Reaction (PCR) (Schuelke, 2000) was conducted in a volume of 10  $\mu$ L containing: 40 ng template (dried DNA); 1.0  $\mu$ M forward primer; 10.0  $\mu$ M reverse primer; 10.0  $\mu$ M M13 dye labeled primer; 1X standard reaction buffer, 1.5 mM MgCl<sub>2</sub>; and 5.0 U of *Taq* polymerase (Apex, Genesee Scientific, San Diego, CA). The florescent dyes: FAM (blue), VIC/HEX (green), NED (yellow), and PET (red) were added to the reaction with the indirect M13 tail (5' -CACGACGTTGTAAAACGAC - 3'). The reaction was processed sequentially: 1) 95°C for 5 min, 1 cycle; 2) 95°C for 45sec, 60°C for 45sec, 72°C for 1.5 min, 5 cycles; 3) 95°C for 45sec, 58°C for 45sec, 72°C for 1.5 min, 5 cycles; 4) 95°C for 45sec, 56°C for 45sec, 72°C for 1.5 min, 4 cycles; 5) 95°C for 45sec, 50°C for 45sec, 72°C for 1.5 min, 24 cycles; and 6) final extension at 72°C for 5 min, 1 cycle. The annealing temperatures varied and were optimized for specific primer pairs.

Direct-label PCR reactions were conducted, used a modified version of Röder et al., (1998), at 10  $\mu$ L volume containing: 40 ng template (dried DNA); 1 x standard reaction buffer, 1.5 mM MgCl<sub>2</sub>, 0.20 mM dNTP, 0.20  $\mu$ M forward primer, 0.20  $\mu$ M reverse primer, 0.5 U *Taq* polymerase (Apex, Genesee Scientific, San Diego, CA). The florescent dyes attached to the direct-labeled primers included: FAM, VIC/HEX, NED, and PET (Applied Biosystems, Foster City, CA). The reaction was processed sequentially: 1) 95°C for 5 min, 1 cycle; 2) 95°C for

45sec, 60°C for 1min, 72°C for 1.5 min, 34 cycles; and 3) a final extension at 72°C for 5 min, 1 cycle. The annealing temperatures varied and were optimized for specific primer pairs.

A 14 µL solution consisting of 9.85 µL of 95% formamide (Applied Biosystems, Foster City, CA), 0.15 µL of Gene Scan™ – 500 LIZ ® size standard (Applied Biosystems, Foster City, CA) and 1 µL each of FAM, VIC, NED, and PET PCR product was analyzed using a Applied Biosystems 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). GeneMarker V1.6 (SoftGenetics, State College, PA, USA) was used to call alleles for each marker, based on allele fragment size.

## **Statistical analysis**

### **Phenotypic data**

Phenotypic data was analyzed using SAS (version 9.3; SAS Institute, Cary, NC). Normality was tested using PROC UNIVARIATE. Means and standard deviation of means were calculated by PROC MEANS. Analysis of variance (ANOVA) was calculated for all data using PROC GLM. Pairwise correlations between FHB INC, SEV, DON, and FDK were estimated using PROC CORR. In addition, pairwise correlations between the three greenhouse experiments was conducted, for SEV, and were estimated using PROC CORR.

### **Linkage map: QTL intervals and LOD graphs**

MapDisto software (Lorieux, 2012) was used with Kosambi mapping function (Kosambi, 1944) and an LOD score 4.0. Marker order was confirmed using consensus maps by Somers et al. (2004), Marone et al. (2013), and Sourdille et al. (2004). QTL analysis was performed by QTL Cartographer (WinQTLCart2.5) and ICIMapping (Li et al., 2007; Wang, 2009). Composite interval mapping was performed with forward and backward stepwise regression in QTL

Cartographer. A thousand permutations were done to estimate the threshold LOD for QTL significance in both QTL Cartographer and ICIMapping. MapChart 2.2 (Voorrips, 2002) was used for the construction of the linkage maps. Single ANOVA was used to identify markers associated with FHB incidence, FHB severity, FDK and DON, for the selective genotyping.

## RESULTS

### **Phenotypic analysis of Pioneer 25R47/Jamestown RIL population**

Phenotypic assessments of FHB INC, FHB SEV, DON and FDK displayed differences between the parents, Pioneer 25R47 (P47) and Jamestown (Jtwn), in response to *F. graminearum* in Salisbury, MD (Figure 2.1). In Kinston, NC the parents responded differently to *F. graminearum* for all FHB phenotypic assessments except for FDK (Figure 2.2). In Blacksburg, VA the parents displayed differences in the 2012 experiment for DON and FDK (Figure 2.3). The 2011 experiment at Blacksburg was not harvested due to a low level of FHB infection. The parents differed significantly for FHB severity, assessed using a point inoculation method, in all three greenhouse experiments and for AUDPC calculated from the 2012 fall greenhouse (GHF12) severity data (Figure 2.4).

The combined analysis of variance for INC and SEV for Maryland, North Carolina, and Virginia for 2011 and 2012 are presented in Table 2.1. Location was used as the replication as North Carolina and Maryland had one replication each year of the experiment. Each location, line (RIL), and year were significantly different ( $P < 0.001$ ) for FHB INC and FHB SEV; whereas line by year was only significantly different ( $P < 0.001$ ) for FHB INC. The combined

analysis of variance for DON and FDK consisted of 2011 and 2012 data from Maryland and North Carolina, and only 2012 data for Virginia (Table 2.2). Replication, line, year, and line by year were all significantly different ( $P < 0.001$ ). The ANOVA tables for the greenhouse Type II FHB severity experiments are as follows: spring 2011 (Table 2.3), in which replication and line were highly significant ( $P < 0.001$ ), fall 2011 (Table 2.4), in which lines differed significantly ( $P < 0.05$ ) for two of the three severity measurements, fall 2012 (Tables 2.5 – 2.7), in which lines differed significantly ( $0.05 \leq P \leq 0.001$ ) in FHB severity 7, 14, and 21 days after inoculation, and AUDPC from fall 2012 data (Table 2.8), in which differences among lines were highly significant ( $P < 0.001$ ).

FHB incidence and severity means for MD in 2012 were significantly correlated ( $P > 0.05$ ) to incidence and severity, respectively, in NC and VA in at least one year of this experiment (Table 2.9). The 2011 Maryland data for INC, SEV, and FDK generally were not significantly correlated with FHB trait data from either NC or VA, which may be due to the lack of significant variation among lines for FHB in the 2011 MD test. FHB incidence in the 2011 NC test was nearly 100% for every line in the population, and was not significantly correlated with incidence data from any other environment, which likely was due to the lack of variation among lines for incidence (Tables 2.9 and 2.10). Therefore, it is unlikely that QTL will be identified for FHB INC in 2011 NC. Correlations between the 2011 and 2012 VA data for FHB INC and SEV were not significant, and likely was the result of low FHB levels at Blacksburg, VA in 2011 (Table 2.11). The irrigation in VA 2011 scab nursery was set-up later in the season than it should've been in order to receive a better infection that year. In general, correlations were stronger between severity assessments of Type II resistance within than between the three independent greenhouse experiments, which is expected due to inevitable variation in

greenhouse environments during and between years in addition to differing isolates and sample in these studies (Tables 2.12 and 2.13).

### **QTL Analysis**

Among 420 SSR primer pairs screened, 170 were polymorphic between the parents, Pioneer Brand 25R47 and Jamestown, but only 35 of those were polymorphic between the resistant and susceptible bulks. Initially, 35 SSR markers identified with BSA were run on the population. An additional 40 SSR markers were added according to the regions identified to be significant based on single ANOVA. Eighteen of the 75 polymorphic markers were removed from linkage analysis because of segregation distortion ( $\chi^2 \geq 15\%$ ), and the remaining 57 markers were used to construct the final genetic maps. Eight linkage groups (1B, 2A(3), 3BS, 5A, 5DL, and 6D) were constructed from the genetic maps, and the identity of the chromosomes were confirmed using the consensus maps by Somers et al. (2004), Marone et al. (2013), and Sourdille et al. (2004). The 1B linkage map (Figure 2.5) contains an FHB resistance QTL identified in cultivar Jamestown for FHB severity and DON accumulation. Two QTL in Jamestown, associated with increased FHB susceptibility, were identified on chromosomes 3BS and 5A (Table 2.14). Fragment sizes of diagnostic markers for identified QTL are presented in Table 2.15.

A combination of the 75 SSRs and 477 single nucleotide polymorphisms (SNPs) markers were run on the parental lines and a subsample (42 out of 186 RILs) of this population. The 9K SNP assay performed on the 42 lines and parents resulted in 3,118 SNPs called, of these only 532 SNPs were polymorphic between the parents. SNP markers with chi-square value of 12 or higher were removed resulting in a total of 477 SNP data points used in the analysis. Single

ANOVA of the SSRs and SNPs was used to identify possible regions associated with FHB (Table 2.16). Results from the single ANOVA supported the regions identified in the SSR map of Pioneer Brand 25R47/Jamestown population and identified significant SNP markers located on chromosomes 1B, 2A, 2D, 3A, 3B, 4A, 4B, 5A, 6B, and 7B (Table 2.16). In this study, 40% of the SSR markers were polymorphic between the parents whereas only 5.9% of the SNP markers were polymorphic.

### **QTL Mapping**

All of the QTL identified were associated with Jamestown (Table 2.14). On chromosome 1B the marker region, *Xwmc500.6-Xgwm273.2*, was significantly (LOD 4.4 - 7.9) associated with lower FHB severity and DON accumulation in the 2011 NC trial as well as with the two year (2011 and 2012) mean data for these traits in NC. The flanking markers for DON\_NC11 and DON\_NC11\_12 were *Xwmc500.7-Xgwm273.2*. Though the QTL region spans the interval from *Xwmc500.7* to *Xgwm273.2* (28.2 cM apart), the most diagnostic marker, *Xwmc500.6*, resides between these flanking markers and is 9.8 cM from *Xwmc500.7* and 18.4 cM from *Xgwm273.2*. Significance of this QTL in 2011 and over year's analyses of the NC data indicates that the QTL on 1B has a consistent effect under certain environmental conditions. The QTL explained between 13.9% and 26.1% of the variation in FHB severity and between 12.7% and 13.3% of the variation in DON accumulation.

Two other QTL associated with increased susceptibility to FHB were identified in Jamestown (Figure 2.6 and Table 2.14). In the 2012 MD test, a QTL on chromosome 3BS was associated with increased FDK in RILs carrying alleles from Jamestown, and accounted for 9% of the phenotypic variation. The flanking markers were *Xgwm376-Xgwm285* with the highest

QTL peak at 1 cM and the closest marker *Xgwm285* at 1.5 cM. The QTL on chromosome 5A was associated with increased FHB severity in the 2012 NC test in RILs carrying Jamestown alleles, and explained 11% of the phenotypic variation. The flanking markers for this region include *Xbarc180-Xgwm304.1* with the closest marker *Xgwm304.1* at 30 cM and the highest peak of the QTL at 31 cM.

## DISCUSSION

In previous studies, QTL associated with FHB resistance have been identified on all of the wheat chromosomes (Liu et al., 2009; Buerstmayr et al., 2009). Fourteen mapping studies have identified QTL for FHB resistance on chromosome 1B in hexaploid wheat (Buerstmayr et al., 2002; Draeger et al., 2007; Holzapfel et al., 2008; Ittu et al., 2000; Klahr et al., 2007; Lin et al., 2004; Mardi et al., 2006; Schmolke et al. 2005; Semagn et al., 2007; Shen et al., 2003; Srinivasachary et al., 2008; Steiner et al. 2004; Yu et al., 2008; Zhang et al., 2004; Zhou et al., 2004). However, QTL on 1B associated with FHB resistance previously have not been identified in North American wheat cultivars. Cultivars in which QTL have been identified on 1B include eleven cultivars from Europe (Arina, Biscay, Cansas, Fundulea 201R, History, Lynx, Remus, Rialto, Romanus, Pirat, and Sincron), two lines from Asia (CM82036 and Wangshuibai) and one cultivar (Seri82) from CIMMYT (Table 2.17). Of the seven cultivars having a QTL for FHB resistance on chromosome 1B, six are known to have the T1BL.1RS translocation (Alondra, Cansas, Fundulea 201R, Lynx, Rialto and Sincron) and, therefore, the source of FHB resistance is unclear (Buerstmayr et al., 2009). Jamestown does not have the T1BL.1RS translocation.

Linkage maps for the QTL on 1B from the previous studies were usually created and compared to either the consensus map by Röder, et al. (1998) or Somers et al. (2004). Linkage maps reported in mapping studies including the cultivars Fundulea 201R, Arina, Rialto, Romanus, History, Biscay, and Pirat had at least one marker in common with the 1B linkage map in the Pioneer Brand 25R47/Jamestown population. Comparisons were made between the 1B linkage map in the Pioneer Brand 25R47/Jamestown population with those from previous studies, and to Somers et al. (2004) consensus map to determine whether the QTL regions were similar. When compared to the consensus map, QTL found in Rialto, Romanus, and Arina were close to the 1B QTL region identified in Jamestown. The linkage map for Cansas did not have any markers in common with the Pioneer Brand 25R47/Jamestown linkage map, but when compared to the Somers et al. (2004) consensus map it is possible that the QTL identified in Cansas is in a similar region as the Jamestown QTL. However, further studies would be needed to determine whether the 1B QTL identified in Jamestown is the same as those identified in previous studies. Cultivars Rialto, Romanus, and Arina have a QTL on 1B that is associated with lower FHB severity, and these accounted for 13.4%, 3% and 10.1% of the phenotypic variation, respectively (Liu et al.,2009). The Jamestown QTL on 1B associated with FHB severity accounted for 26.1% of the phenotypic variation. Cansas is the only cultivar reported to have a QTL on 1B that likely resides in a region close to the QTL identified in Jamestown. However the QTL in Cansas was associated with a reduction in FHB incidence rather than severity. Jamestown is the only cultivar in which a QTL associated with lower DON accumulation has been identified in this region (*Xwmc500.7-Xgwm273.2*) of chromosome 1B. The Chinese landrace, Wangshuibai, was reported to have a QTL on 1B for DON accumulation, when



compared to Somers consensus map (2004) and the map in the review by Buerstmayr (2009), this region is similar to the QTL identified on Jamestown.

The QTL on 3BSc identified in Jamestown was associated with increased FDK and explained 9.1% of the variation. The QTL identified in Jamestown on 3B is not associated with *Fhb1*. When comparing the Pioneer Brand 25R47/Jamestown mapping population linkage map to those published in other papers, the QTL identified on 3B is associated with the same marker (*Xgwm285*) as that found in: the Japanese landrace Nyubai (Somers et al., 2003), the SRW cultivar Ernie (Liu et al., 2007; Abate et al., 2008), and the Chinese landrace Wangshuibai (Yu et al., 2008). The QTL in Nyubai was associated with resistance to FHB severity, explaining 4% of the phenotypic variation. In Ernie, all of the QTL on 3B were identified in greenhouse studies. The 3BSc QTL in Ernie accounted for 12.9%, 14% and 18% of the phenotypic variation associated with reduction of FHB severity, DON accumulation, and FDK, respectively. The QTL identified in Wangshuibai was associated with decreased FHB severity and accounted for 8.1% of the phenotypic variation. It appears that Jamestown is carrying the susceptible allele(s) in this QTL region; however, more markers should be run on the Pioneer Brand 25R47/Jamestown population to confirm that these are indeed the same QTL regions.

On chromosome 5A, a QTL was identified in Jamestown that was associated with increased FHB severity in NC 2012 and accounted for 11% of the variation. In other studies, QTL have been identified in a similar region, and most often associated with either *Xgwm304* and or *Xgwm415* which are the most closely linked markers to the QTL identified in Jamestown. A QTL identified in the following wheat genotypes were associated with at least one of these markers: CM-82036 (Buerstmayr et al., 2002; 2003), Wangshuibai (Lin et al., 2006), Fundulea201 (Shen et al., 2003), and Ernie (Liu et al., 2007; Abate et al., 2008). Almost all of

these lines or cultivars have more than one trait associated with these QTL markers. CM-82036, a descendant of Sumai3, has two QTL in the region associated with *Xgwm304* and lower FHB incidence and severity resistance (23.2% and 10.9% variation explained, respectively). In Wangshuibai, a QTL associated with lower FHB incidence, FHB severity, DON accumulation and FDK, is located in a similar region as the QTL identified in Jamestown. These QTL accounted for 27%, 3.5%, 5.1% and 16.4% of the phenotypic variation, respectively. In Fundulea201, a QTL associated with lower FHB severity, account for 2.1% of the variation. The QTL identified in Ernie was associated with lower FHB severity, DON accumulation and FDK (17.4%, 10%, and 18% variation accounted for, respectively). As noted for the QTL on 3B, it appears that Jamestown has alleles conferring FHB susceptibility at the 5A QTL region; however, additional markers need to be evaluated to confirm whether these two QTL regions are the same.

A comparison of mean QTL (1B, 3BS, and 5A) effects for groups of RILs having different combinations of parental alleles for diagnostic markers for each QTL are presented in Table 2.18. Significance of QTL effects was determined using a T-test for LSD. There was no significant difference between the FHB disease assessment traits for RILs having Jamestown alleles at 1B QTL (resistant) and 3BS QTL (susceptible) versus the Jamestown allele at 1B and Pioneer 25R47 allele at 3BS. This suggests that the 3BS QTL in Jamestown has a minor effect on susceptibility. In contrast, RILs have Jamestown marker alleles for all three QTL were more susceptible to FHB than those having Jamestown marker alleles at 1B and Pioneer Brand 25R47 marker alleles at 5A. Not surprisingly, lack of the Jamestown marker allele at 1B and the presence of the Jamestown marker alleles at 3BS and 5A QTL results in a higher disease.

## CONCLUSION

This study characterized FHB resistance in the soft red winter wheat cultivar Jamestown. A common QTL on chromosome 1B was associated with reduced FHB severity and DON accumulation in NC in 2011 and with mean data for both traits over years (2011-2012). In this study, the QTL on chromosomes 3BS and 5A identified in Jamestown were associated with FHB susceptibility. While presence of Jamestown alleles at the 3BS marker locus had a small or even a negligible effect on FHB susceptibility, presence of Jamestown alleles at the 5A marker locus significantly increased FHB susceptibility. The QTL for DON and FHB severity on chromosome 1B may be unique, but further studies in different genetic backgrounds are needed to verify these QTL. Markers that are common with those in other studies identifying a QTL on 1B should be tested for polymorphism in the Pioneer 25R47 / Jamestown population, and screened to determine whether these regions are similar. Once validated, the most diagnostic markers identified in this study can be used in MAS to incorporate and pyramid these and other QTL and resistance genes.

## FUTURE RESEARCH

Further research should include genotyping of all RILs in the Pioneer Brand 25R47/ Jamestown population using polymorphic SNP markers, identified among the 42 (out of 186) RILs, that are significantly ( $P < 0.001$  and  $P < 0.0001$ ) associated with FHB (Table 2.16). Currently, a 90K SNP assay is being run on 142 of these RILs, which should allow for more markers to be added to existing linkage maps and to potentially identify additional QTL on other linkage groups that are associated with FHB resistance. Once SNP data is available,

polymorphic markers will be used initially for BSA and then SNP markers associated with FHB resistance will be used in QTL analysis of the entire population to map QTL for FHB resistance.

Once QTL for FHB resistance are identified in the Pioneer Brand 25R47/ Jamestown population, the next step is to validate the QTL in other populations. There are two other Jamestown populations, FG95195 x Jamestown (170 RILs) and Jamestown x LA97UC113-124 (77 RILs) for which two years (2011 and 2012) of phenotypic data has already been collected in four locations (Arkansas, Louisiana, Georgia, and Virginia scab nurseries). In addition, FHB data has been collected over multiple years and locations for three Roane populations (Roane is a parent of Jamestown): Roane x Allegiance (33 RILs), Roane x KY93C-1238-17-1 (18 RILs), and Roane x KY94C-0094-11-2 (23 RILs). Markers linked to QTL associated with FHB resistance in the Pioneer Brand 25R47/Jamestown population will be genotyped in these populations (if polymorphic) to validate the 1B QTL and any additional QTL identified using the 90K SNP analysis data in the Pioneer Brand 25R47/Jamestown population. Once the QTL in Jamestown have been identified and validated, fine mapping of these regions may be needed to identify more tightly linked markers to make MAS for these QTL more reliable and successful.

In addition, to two years of phenotypic data, there is genotypic data for the most resistant and susceptible lines for the remaining Jamestown populations and the three Roane populations. Selective genotyping will be performed on these populations as well, and markers linked to the QTL from these populations will also be validated on the rest of the Jamestown and Roane populations. Additional markers linked to previously published QTL will be run on these populations to confirm the novelty of the QTL.

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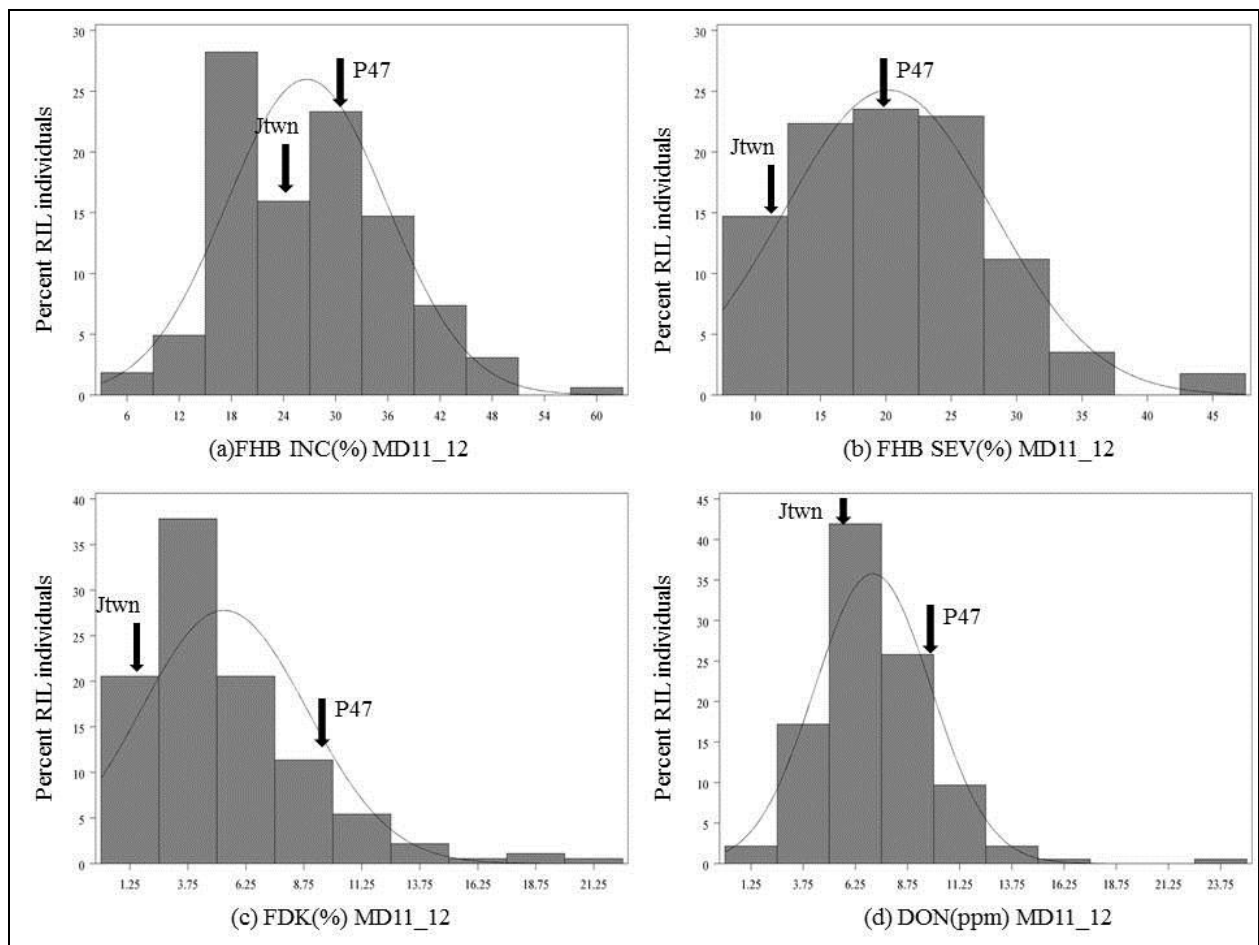
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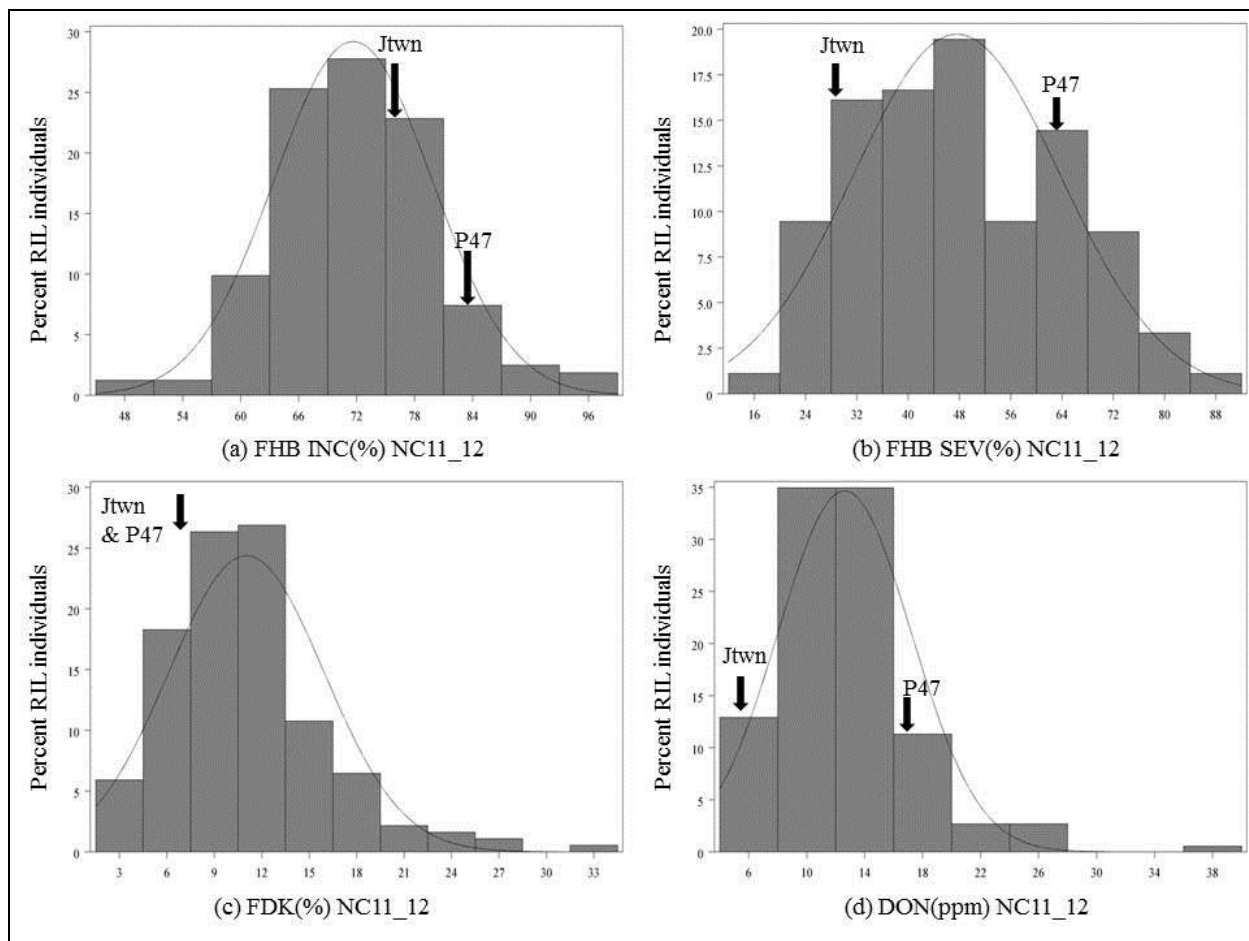
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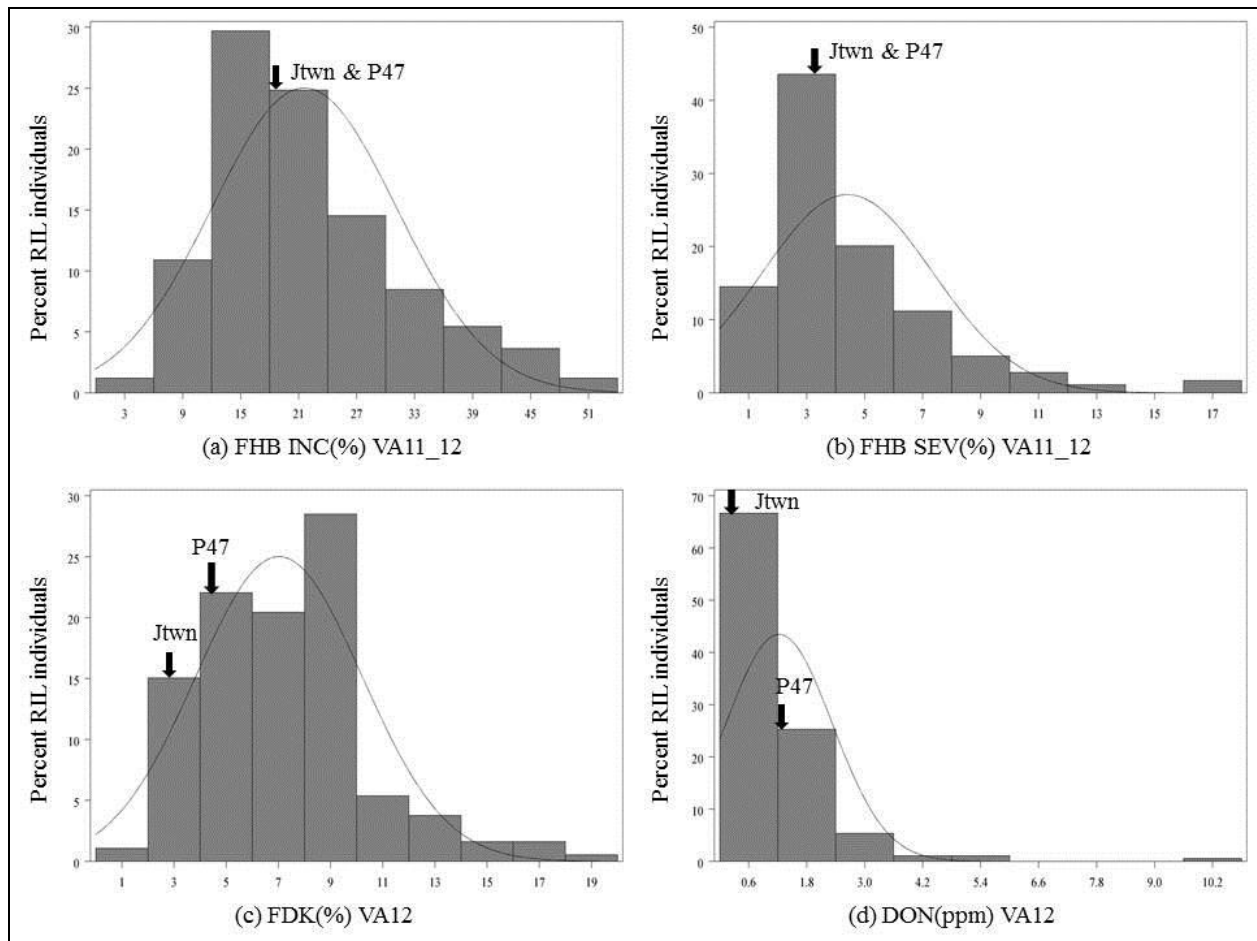
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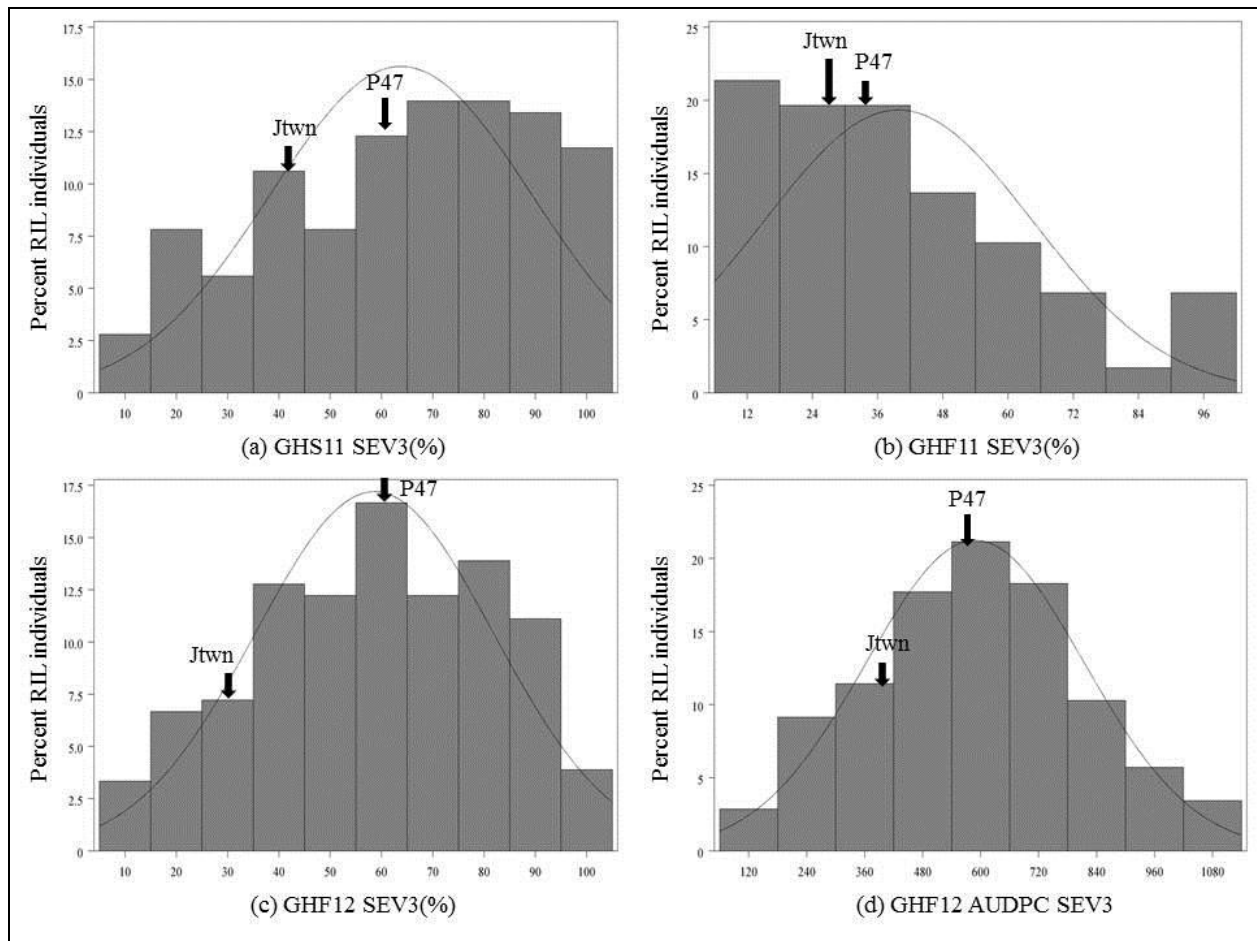
**Figure 2.1. Frequency distributions of parents and 186 RILs derived from the cross Pioneer 25R47 x Jamestown for *Fusarium* head blight (FHB) assessment parameters at Salisbury, Maryland (MD) over 2011 and 2012.** All data displayed is from the mean of 2011 and 2012 data for the given measurement. (a) Mean FHB incidence (INC; % infected spikes per row) in MD. (b) Mean FHB severity (SEV; % infected spikelets) evaluated in the scab nursery in MD. (c) Percentage of *Fusarium* damaged kernels (FDK) evaluated in the population samples from MD. (d) Deoxynivalenol (DON) content in ppm from harvested seed samples from MD. Arrows indicate the average rating for the resistant parent Jamestown (Jtwn) and the susceptible parent Pioneer Brand 25R47 (P47) for a given trait.



**Figure 2.2. Frequency distributions of parents and 186 RILs derived from the cross Pioneer 25R47 x Jamestown for *Fusarium* head blight (FHB) assessment parameters at Kinston, NC (NC) over 2011 and 2012.** All data displayed is from the mean of 2011 and 2012 data for the given measurement. (a) Mean FHB incidence (INC; % infected spikes per row) in NC. (b) Mean FHB severity (SEV; % infected spikelets) evaluated in the scab nursery in NC. (c) Percentage of *Fusarium* damaged kernels (FDK) evaluated in the population samples from NC. (d) Deoxynivalenol (DON) content in ppm from harvested seed samples from NC. Arrows indicate the average rating for the resistant parent Jamestown (Jtwn) and the susceptible parent Pioneer Brand 25R47 (P47) for a given trait.



**Figure 2.3. Frequency distributions of parents and 186 RILs derived from the cross Pioneer 25R47 x Jamestown for *Fusarium* head blight (FHB) assessment parameters at Blacksburg, VA (VA) over 2011 and 2012.** Data for incidence (INC) and severity (SEV) displayed is from the mean of 2011 and 2012 data for the given measurement. Data for deoxynivalenol (DON) and *Fusarium* damaged kernels (FDK) is based on 2012 data. (a) Mean FHB incidence (% infected spikes per row) in VA. (b) Mean FHB severity (% infected spikelets) evaluated in the scab nursery in VA. (c) Percentage of FDK evaluated in the population samples from VA 2012. (d) DON content in ppm from harvested seed samples from VA 2012. Arrows indicate the average rating for the resistant parent Jamestown (Jtwn) and the susceptible parent Pioneer Brand 25R47 (P47) for a given trait.



**Figure 2.4. Frequency distributions of parents and 186 RILs derived from the cross Pioneer 25R47 x Jamestown for *Fusarium* head blight (FHB) Type II greenhouse (GH) experiments, point-inoculations with *Fusarium graminearum*.** (a) GHS11 SEV3 = Mean FHB severity (SEV3; % total infected spikelets) in the spring 2011 greenhouse experiment. (b) GHF11 SEV3 = Mean FHB severity (SEV3; % total infected spikelets) in the fall 2011 greenhouse experiment. (c) GHF12 SEV3 = Mean FHB severity (SEV3; % total infected spikelets) in the fall 2012 greenhouse experiment. (d) GHF12 AUDPC SEV3 = FHB severity (SEV3; % total infected spikelets) measured by area under the disease progress curve (AUDPC) in the fall 2012 greenhouse experiment. Arrows indicate the average rating for the resistant parent Jamestown (Jtwn) and the susceptible parent Pioneer Brand 25R47 (P47) for a given trait.

**Table 2.1.** Over years (2011 and 2012) and locations (Maryland, North Carolina, and Virginia) analysis of variance for *Fusarium* head blight traits (FHB) incidence (INC) and severity (SEV).

Source	df	Mean Squares	
		FHB_INC	FHB_SEV
Replication	6	141952.4***	78770.5***
Lines	185	361.4***	196.2***
Year	1	87383.9***	31828.1***
Lines x Year	185	332.6***	115.6 <sup>NS</sup>
Error		170.0	112.1

\* Significant at 0.05 probability level

\*\* Significant at 0.01 probability level

\*\*\* Significant at 0.001 probability level

<sup>NS</sup> Not significant at 0.05 probability level

**Table 2.2.** Over years and locations (2011 and 2012 Maryland and North Carolina, 2012 Virginia) analysis of variance for deoxynivalenol accumulation (DON) and *Fusarium* damaged kernels (FDK).

Source	df	Mean Squares	
		DON	FDK
Replication	4	7281.1 <sup>***</sup>	1463.8 <sup>***</sup>
Lines	185	33.2 <sup>***</sup>	55.4 <sup>***</sup>
Year	1	3970.2 <sup>***</sup>	1029.9 <sup>***</sup>
Lines x Year	185	20.6 <sup>***</sup>	37.7 <sup>***</sup>
Error		14.0	25.6

\* Significant at 0.05 probability level

\*\* Significant at 0.01 probability level

\*\*\* Significant at 0.001 probability level

<sup>NS</sup> Not significant at 0.05 probability level

**Table 2.3.** Analysis of variance of Pioneer 25R47 / Jamestown population for Greenhouse Type II reaction to Fusarium head blight, Spring 2011 experiment.

Source	df	Mean Squares		
		Severity_1 <sup>†</sup>	Severity_2 <sup>‡</sup>	Severity_3 <sup>§</sup>
Replication	9	21844.7 <sup>***</sup>	40316.4 <sup>***</sup>	22200.6 <sup>***</sup>
Lines	178	3178.8 <sup>***</sup>	3023.5 <sup>***</sup>	2407.3 <sup>***</sup>
Error		819.5	721.8	507.6

<sup>†</sup>Severity\_1 = severity at or below 9th spikelet 21 days after inoculation (dai)

<sup>‡</sup>Severity\_2 = severity at or above 11th spikelet 21 dai

<sup>§</sup>Severity\_3 = severity of total spike 21 dai

\* Significant at 0.05 probability level

\*\* Significant at 0.01 probability level

\*\*\* Significant at 0.001 probability level

<sup>NS</sup> Not significant at 0.05 probability level



**Table 2.4.** Analysis of variance of Pioneer 25R47 / Jamestown population for Greenhouse Type II reaction to Fusarium head blight, Fall 2011 experiment.

Source	df	Mean Squares		
		Severity_1 <sup>†</sup>	Severity_2 <sup>‡</sup>	Severity_3 <sup>§</sup>
Replication	6	1449.8 <sup>NS</sup>	2149.9 <sup>NS</sup>	1134.5 <sup>NS</sup>
Lines	116	1402.5 <sup>*</sup>	2297.0 <sup>NS</sup>	914.9 <sup>*</sup>
Error		949.2	2011.3	627.6

<sup>†</sup> Severity\_1 = severity at or below 10th spikelet 21 days after inoculation (dai)

<sup>‡</sup> Severity\_2 = severity at or above 12th spikelet 21 dai

<sup>§</sup> Severity\_3 = severity of total spike 21 dai

<sup>\*</sup> Significant at 0.05 probability level

<sup>\*\*</sup> Significant at 0.01 probability level

<sup>\*\*\*</sup> Significant at 0.001 probability level

<sup>NS</sup> Not significant at 0.05 probability level

**Table 2.5.** Analysis of variance of Pioneer 25R47 / Jamestown population for Greenhouse Type II reaction to Fusarium head blight, Fall 2012 experiment, 7 days after inoculation.

Source	df	Mean Squares		
		SeverityD1_1 <sup>†</sup>	SeverityD1_2 <sup>‡</sup>	SeverityD1_3 <sup>§</sup>
Replication	8	88.6 <sup>NS</sup>	859.0 <sup>NS</sup>	150.6 <sup>**</sup>
Lines	179	120.0 <sup>***</sup>	637.3 <sup>**</sup>	109.1 <sup>***</sup>
Error		67.6	482.4	59.3

<sup>†</sup> SeverityD1\_1 = severity at or below 10th spikelet 7 days after inoculation (dai)

<sup>‡</sup> SeverityD1\_2 = severity at or above 12th spikelet 7 dai

<sup>§</sup> SeverityD1\_3 = severity of total spike 7 dai

\* Significant at 0.05 probability level

\*\* Significant at 0.01 probability level

\*\*\* Significant at 0.001 probability level

<sup>NS</sup> Not significant at 0.05 probability level

**Table 2.6.** Analysis of variance of Pioneer 25R47 / Jamestown population for Greenhouse Type II reaction to Fusarium head blight, Fall 2012 experiment, 14 days after inoculation.

Source	df	Mean Squares		
		SeverityD2_1 <sup>†</sup>	SeverityD2_2 <sup>‡</sup>	SeverityD2_3 <sup>§</sup>
Replication	8	1614.7 <sup>NS</sup>	1215.7 <sup>NS</sup>	452.4 <sup>NS</sup>
Lines	176	1670.0 <sup>***</sup>	1561.1 <sup>*</sup>	834.5 <sup>***</sup>
Error		1116.5	1244.4	481.4

<sup>†</sup> SeverityD2\_1= severity at or below 10th spikelet 14 days after inoculation (dai)

<sup>‡</sup> SeverityD2\_2= severity at or above 12th spikelet 14 dai

<sup>§</sup> SeverityD2\_3= severity of total spike 14 dai

<sup>\*</sup> Significant at 0.05 probability level

<sup>\*\*</sup> Significant at 0.01 probability level

<sup>\*\*\*</sup> Significant at 0.001 probability level

<sup>NS</sup> Not significant at 0.05 probability level

**Table 2.7.** Analysis of variance of Pioneer 25R47 / Jamestown population for Greenhouse Type II reaction to Fusarium head blight, Fall 2012 experiment, 21 days after inoculation.

Source	df	Mean Squares		
		SeverityD3_1 <sup>†</sup>	SeverityD3_2 <sup>‡</sup>	SeverityD3_3 <sup>§</sup>
Replication	8	1644.6 <sup>NS</sup>	1880.0 <sup>NS</sup>	675.6 <sup>NS</sup>
Lines	176	1931.3 <sup>***</sup>	2270.1 <sup>**</sup>	1233.8 <sup>***</sup>
Error		1159.3	1574.1	618.6

<sup>†</sup> SeverityD3\_1= severity at or below 10th spikelet 21 days after inoculation (dai)

<sup>‡</sup> SeverityD3\_2= severity at or above 12th spikelet 21 dai

<sup>§</sup> SeverityD3\_3= severity of total spike 21 dai

\* Significant at 0.05 probability level

\*\* Significant at 0.01 probability level

\*\*\* Significant at 0.001 probability level

<sup>NS</sup> Not significant at 0.05 probability level

**Table 2.8.** Analysis of variance of Pioneer 25R47 / Jamestown population for Greenhouse Type II reaction to Fusarium head blight, Fall 2012 experiment, Area Under Disease Progress Curve (ADUPC).

Source	df	Mean Squares		
		AUDPC_SEV1 <sup>†</sup>	AUDPC_SEV2 <sup>‡</sup>	AUDPC_SEV3 <sup>§</sup>
Replication	8	192207.8 <sup>NS</sup>	203424.7 <sup>NS</sup>	73535.4 <sup>NS</sup>
Lines	176	216295.3 <sup>***</sup>	297290.6 <sup>***</sup>	128849.3 <sup>***</sup>
Error		125091.5	195725.6	60905.2

<sup>†</sup> AUDPC\_SEV1 = area under the disease progress curve for severity at or below 10th spikelet from 7 to 21 days after inoculation (dai)

<sup>‡</sup> AUDPC\_SEV2 = area under the disease progress curve for severity at or above 12th spikelet from 7 to 21 dai

<sup>§</sup> AUDPC\_SEV3 = area under the disease progress curve for severity of total spike from 7 to 21 dai

\* Significant at 0.05 probability level

\*\* Significant at 0.01 probability level

\*\*\* Significant at 0.001 probability level

<sup>NS</sup> Not significant at 0.05 probability level

**Table 2.9.** Correlation coefficients from pairwise means comparisons among Maryland (MD) *Fusarium* head blight (FHB) traits and with FHB trait data from North Carolina (NC) and Virginia (VA) for 2011 and 2012.

	INC_MD11 <sup>†</sup>	INC_MD12	INC_MD11_12	SEV_MD11	SEV_MD12	SEV_MD11_12	DON_MD11	DON_MD12	DON_MD11_12	FDK_MD11	FDK_MD12	FDK_MD11_12
INC_MD12	0.05 <sup>NS</sup>											
INC_MD11_12	0.81 <sup>***</sup>	0.62 <sup>***</sup>										
SEV_MD11	0.40 <sup>***</sup>	0.07 <sup>NS</sup>	0.37 <sup>***</sup>									
SEV_MD12	0.14 <sup>NS</sup>	0.57 <sup>***</sup>	0.45 <sup>***</sup>	0.18 <sup>*</sup>								
SEV_MD11_12	0.37 <sup>***</sup>	0.36 <sup>***</sup>	0.52 <sup>***</sup>	0.84 <sup>***</sup>	0.69 <sup>***</sup>							
DON_MD11	0.08 <sup>NS</sup>	0.13 <sup>NS</sup>	0.13 <sup>NS</sup>	-0.07 <sup>NS</sup>	0.19 <sup>**</sup>	0.06 <sup>NS</sup>						
DON_MD12	-0.17 <sup>*</sup>	0.07 <sup>NS</sup>	-0.07 <sup>NS</sup>	-0.02 <sup>NS</sup>	0.03 <sup>NS</sup>	-0.01 <sup>NS</sup>	-0.07 <sup>NS</sup>					
DON_MD11_12	-0.13 <sup>NS</sup>	0.12 <sup>NS</sup>	-0.01 <sup>NS</sup>	-0.05 <sup>NS</sup>	0.11 <sup>NS</sup>	0.01 <sup>NS</sup>	0.33 <sup>***</sup>	0.92 <sup>***</sup>				
FDK_MD11	-0.11 <sup>NS</sup>	0.37 <sup>*</sup>	0.13 <sup>NS</sup>	-0.14 <sup>NS</sup>	0.07 <sup>NS</sup>	-0.08 <sup>NS</sup>	-0.20 <sup>NS</sup>	-0.29 <sup>NS</sup>	-0.34 <sup>*</sup>			
FDK_MD12	0.10 <sup>NS</sup>	0.41 <sup>***</sup>	0.31 <sup>***</sup>	0.09 <sup>NS</sup>	0.34 <sup>***</sup>	0.25 <sup>**</sup>	0.24 <sup>***</sup>	-0.06 <sup>NS</sup>	0.04 <sup>NS</sup>	0.27 <sup>NS</sup>		
FDK_MD11_12	0.09 <sup>NS</sup>	0.42 <sup>***</sup>	0.31 <sup>***</sup>	0.08 <sup>NS</sup>	0.35 <sup>***</sup>	0.25 <sup>**</sup>	0.23 <sup>**</sup>	-0.06 <sup>NS</sup>	0.04 <sup>NS</sup>	0.37 <sup>*</sup>	1.00 <sup>***</sup>	
INC_NC11	-0.14 <sup>NS</sup>	0.08 <sup>NS</sup>	-0.07 <sup>NS</sup>	-0.13 <sup>NS</sup>	0.02 <sup>NS</sup>	-0.09 <sup>NS</sup>	0.06 <sup>NS</sup>	0.01 <sup>NS</sup>	0.03 <sup>NS</sup>	-0.13 <sup>NS</sup>	0.01 <sup>NS</sup>	0.01 <sup>NS</sup>
INC_NC12	-0.09 <sup>NS</sup>	0.10 <sup>NS</sup>	0.01 <sup>NS</sup>	0.11 <sup>NS</sup>	0.17 <sup>*</sup>	0.18 <sup>*</sup>	0.00 <sup>NS</sup>	0.05 <sup>NS</sup>	0.05 <sup>NS</sup>	0.31 <sup>NS</sup>	0.13 <sup>NS</sup>	0.14 <sup>NS</sup>
INC_NC11_12	-0.14 <sup>NS</sup>	0.13 <sup>NS</sup>	-0.01 <sup>NS</sup>	0.06 <sup>NS</sup>	0.18 <sup>*</sup>	0.15 <sup>NS</sup>	0.02 <sup>NS</sup>	0.05 <sup>NS</sup>	0.06 <sup>NS</sup>	0.28 <sup>NS</sup>	0.13 <sup>NS</sup>	0.14 <sup>NS</sup>
SEV_NC11	-0.17 <sup>*</sup>	0.26 <sup>***</sup>	0.04 <sup>NS</sup>	-0.13 <sup>NS</sup>	0.21 <sup>**</sup>	0.03 <sup>NS</sup>	0.20 <sup>**</sup>	0.03 <sup>NS</sup>	0.11 <sup>NS</sup>	-0.08 <sup>NS</sup>	0.16 <sup>*</sup>	0.17 <sup>*</sup>
SEV_NC12	-0.01 <sup>NS</sup>	0.28 <sup>***</sup>	0.17 <sup>*</sup>	0.09 <sup>NS</sup>	0.34 <sup>***</sup>	0.25 <sup>**</sup>	0.22 <sup>**</sup>	-0.01 <sup>NS</sup>	0.08 <sup>NS</sup>	0.21 <sup>NS</sup>	0.28 <sup>***</sup>	0.29 <sup>***</sup>
SEV_NC11_12	-0.13 <sup>NS</sup>	0.26 <sup>***</sup>	0.07 <sup>NS</sup>	-0.10 <sup>NS</sup>	0.26 <sup>***</sup>	0.07 <sup>NS</sup>	0.24 <sup>***</sup>	0.03 <sup>NS</sup>	0.12 <sup>NS</sup>	-0.01 <sup>NS</sup>	0.19 <sup>*</sup>	0.20 <sup>**</sup>
DON_NC11	0.02 <sup>NS</sup>	0.32 <sup>***</sup>	0.21 <sup>**</sup>	0.14 <sup>NS</sup>	0.36 <sup>***</sup>	0.29 <sup>***</sup>	0.26 <sup>***</sup>	0.06 <sup>NS</sup>	0.16 <sup>*</sup>	-0.08 <sup>NS</sup>	0.21 <sup>**</sup>	0.22 <sup>**</sup>
DON_NC12	0.02 <sup>NS</sup>	0.31 <sup>***</sup>	0.22 <sup>**</sup>	0.13 <sup>NS</sup>	0.32 <sup>***</sup>	0.28 <sup>***</sup>	0.24 <sup>***</sup>	0.11 <sup>NS</sup>	0.20 <sup>**</sup>	0.05 <sup>NS</sup>	0.35 <sup>***</sup>	0.35 <sup>***</sup>
DON_NC11_12	0.02 <sup>NS</sup>	0.39 <sup>***</sup>	0.26 <sup>***</sup>	0.16 <sup>*</sup>	0.42 <sup>***</sup>	0.35 <sup>***</sup>	0.31 <sup>***</sup>	0.09 <sup>NS</sup>	0.21 <sup>**</sup>	-0.03 <sup>NS</sup>	0.32 <sup>***</sup>	0.32 <sup>***</sup>
FDK_NC11	0.03 <sup>NS</sup>	0.23 <sup>**</sup>	0.15 <sup>NS</sup>	0.04 <sup>NS</sup>	0.28 <sup>***</sup>	0.17 <sup>*</sup>	0.19 <sup>*</sup>	-0.1 <sup>NS</sup>	-0.02 <sup>NS</sup>	0.30 <sup>NS</sup>	0.15 <sup>***</sup>	0.17 <sup>*</sup>
FDK_NC12	0.10 <sup>NS</sup>	0.16 <sup>*</sup>	0.14 <sup>NS</sup>	0.06 <sup>NS</sup>	0.16 <sup>*</sup>	0.12 <sup>NS</sup>	0.07 <sup>NS</sup>	0.00 <sup>NS</sup>	0.03 <sup>NS</sup>	0.25 <sup>NS</sup>	0.27 <sup>***</sup>	0.28 <sup>***</sup>
FDK_NC11_12	0.08 <sup>NS</sup>	0.26 <sup>***</sup>	0.19 <sup>*</sup>	0.06 <sup>NS</sup>	0.29 <sup>***</sup>	0.19 <sup>*</sup>	0.18 <sup>*</sup>	-0.07 <sup>NS</sup>	0.00 <sup>NS</sup>	0.33 <sup>NS</sup>	0.27 <sup>***</sup>	0.28 <sup>***</sup>
INC_VA11	0.03 <sup>NS</sup>	0.05 <sup>NS</sup>	0.07 <sup>NS</sup>	0.05 <sup>NS</sup>	-0.05 <sup>NS</sup>	0.03 <sup>NS</sup>	-0.07 <sup>NS</sup>	-0.12 <sup>NS</sup>	-0.15 <sup>NS</sup>	0.22 <sup>NS</sup>	-0.12 <sup>NS</sup>	-0.12 <sup>NS</sup>
INC_VA12	0.15 <sup>NS</sup>	0.41 <sup>***</sup>	0.37 <sup>***</sup>	0.08 <sup>NS</sup>	0.31 <sup>***</sup>	0.24 <sup>**</sup>	0.25 <sup>***</sup>	-0.02 <sup>NS</sup>	0.08 <sup>NS</sup>	0.31 <sup>NS</sup>	0.25 <sup>***</sup>	0.25 <sup>***</sup>
INC_VA11_12	0.09 <sup>NS</sup>	0.40 <sup>***</sup>	0.34 <sup>***</sup>	0.12 <sup>NS</sup>	0.24 <sup>**</sup>	0.25 <sup>**</sup>	0.17 <sup>*</sup>	-0.07 <sup>NS</sup>	0.00 <sup>NS</sup>	0.35 <sup>*</sup>	0.16 <sup>*</sup>	0.17 <sup>*</sup>
SEV_VA11	-0.04 <sup>NS</sup>	0.08 <sup>NS</sup>	0.03 <sup>NS</sup>	-0.02 <sup>NS</sup>	-0.02 <sup>NS</sup>	0.01 <sup>NS</sup>	-0.01 <sup>NS</sup>	-0.08 <sup>NS</sup>	-0.08 <sup>NS</sup>	0.14 <sup>NS</sup>	-0.08 <sup>NS</sup>	-0.08 <sup>NS</sup>
SEV_VA12	0.09 <sup>NS</sup>	0.41 <sup>***</sup>	0.32 <sup>***</sup>	0.10 <sup>NS</sup>	0.29 <sup>***</sup>	0.25 <sup>**</sup>	0.22 <sup>**</sup>	-0.01 <sup>NS</sup>	0.08 <sup>NS</sup>	0.31 <sup>NS</sup>	0.31 <sup>***</sup>	0.31 <sup>***</sup>
SEV_VA11_12	0.07 <sup>NS</sup>	0.34 <sup>***</sup>	0.28 <sup>***</sup>	0.12 <sup>NS</sup>	0.22 <sup>**</sup>	0.25 <sup>**</sup>	0.16 <sup>*</sup>	-0.07 <sup>NS</sup>	0.00 <sup>NS</sup>	0.30 <sup>NS</sup>	0.15 <sup>*</sup>	0.16 <sup>*</sup>
DON_VA12	0.12 <sup>NS</sup>	0.40 <sup>***</sup>	0.34 <sup>***</sup>	0.15 <sup>*</sup>	0.37 <sup>***</sup>	0.33 <sup>***</sup>	0.23 <sup>**</sup>	0.00 <sup>NS</sup>	0.1 <sup>NS</sup>	0.35 <sup>*</sup>	0.37 <sup>***</sup>	0.37 <sup>***</sup>
FDK_VA12	0.12 <sup>NS</sup>	0.21 <sup>**</sup>	0.22 <sup>**</sup>	0.20 <sup>**</sup>	0.26 <sup>***</sup>	0.29 <sup>***</sup>	0.18 <sup>*</sup>	0.05 <sup>NS</sup>	0.12 <sup>NS</sup>	0.15 <sup>NS</sup>	0.16 <sup>*</sup>	0.16 <sup>*</sup>

<sup>†</sup> Trait is formatted as Trait\_LocationYear where: INC = incidence, SEV = severity, DON = deoxynivalenol, FDK = *Fusarium* damaged kernels; years are 11=2011, 12=2012 and 11\_12= the average of 2011 & 2012.

\* Significant at 0.05 probability level

\*\* Significant at 0.01 probability level

\*\*\* Significant at 0.001 probability level

<sup>NS</sup> Not significant at 0.05 probability level

**Table 2.10.** Correlation coefficients from pairwise means comparisons among North Carolina (NC) Fusarium head blight (FHB) traits and with FHB trait data from Virginia (VA) for 2011 and 2012.

	INC_NC11 <sup>†</sup>	INC_NC12	INC_NC11_12	SEV_NC11	SEV_NC12	SEV_NC11_12	DON_NC11	DON_NC12	DON_NC11_12	FDK_NC11	FDK_NC12	FDK_NC11_12
INC_NC12	-0.02 <sup>NS</sup>											
INC_NC11_12	0.32 <sup>***</sup>	0.90 <sup>***</sup>										
SEV_NC11	0.37 <sup>***</sup>	0.11 <sup>NS</sup>	0.24 <sup>**</sup>									
SEV_NC12	0.04 <sup>NS</sup>	0.18 <sup>*</sup>	0.19 <sup>*</sup>	0.20 <sup>**</sup>								
SEV_NC11_12	0.30 <sup>***</sup>	0.19 <sup>*</sup>	0.28 <sup>***</sup>	0.80 <sup>***</sup>	0.70 <sup>***</sup>							
DON_NC11	0.09 <sup>NS</sup>	0.17 <sup>*</sup>	0.19 <sup>*</sup>	0.44 <sup>***</sup>	0.34 <sup>***</sup>	0.49 <sup>***</sup>						
DON_NC12	0.02 <sup>NS</sup>	0.27 <sup>***</sup>	0.27 <sup>***</sup>	0.13 <sup>NS</sup>	0.50 <sup>***</sup>	0.26 <sup>***</sup>	0.29 <sup>***</sup>					
DON_NC11_12	0.08 <sup>NS</sup>	0.25 <sup>**</sup>	0.26 <sup>***</sup>	0.40 <sup>***</sup>	0.48 <sup>***</sup>	0.49 <sup>***</sup>	0.91 <sup>***</sup>	0.66 <sup>***</sup>				
FDK_NC11	0.12 <sup>NS</sup>	0.09 <sup>NS</sup>	0.13 <sup>NS</sup>	0.39 <sup>***</sup>	0.18 <sup>*</sup>	0.35 <sup>***</sup>	0.38 <sup>***</sup>	0.15 <sup>*</sup>	0.36 <sup>***</sup>			
FDK_NC12	-0.02 <sup>NS</sup>	0.21 <sup>**</sup>	0.20 <sup>*</sup>	-0.01 <sup>NS</sup>	0.25 <sup>**</sup>	0.07 <sup>NS</sup>	0.21 <sup>**</sup>	0.42 <sup>***</sup>	0.35 <sup>***</sup>	0.15 <sup>*</sup>		
FDK_NC11_12	0.08 <sup>NS</sup>	0.18 <sup>*</sup>	0.20 <sup>**</sup>	0.28 <sup>***</sup>	0.28 <sup>***</sup>	0.30 <sup>***</sup>	0.40 <sup>***</sup>	0.35 <sup>***</sup>	0.46 <sup>***</sup>	0.82 <sup>***</sup>	0.69 <sup>***</sup>	
INC_VA11	0.05 <sup>NS</sup>	0.16 <sup>NS</sup>	0.16 <sup>NS</sup>	-0.03 <sup>NS</sup>	0.02 <sup>NS</sup>	0.03 <sup>NS</sup>	0.01 <sup>NS</sup>	-0.05 <sup>NS</sup>	-0.01 <sup>NS</sup>	-0.01 <sup>NS</sup>	0.08 <sup>NS</sup>	0.04 <sup>NS</sup>
INC_VA12	-0.02 <sup>NS</sup>	0.33 <sup>***</sup>	0.31 <sup>***</sup>	0.17 <sup>*</sup>	0.40 <sup>***</sup>	0.29 <sup>***</sup>	0.29 <sup>***</sup>	0.32 <sup>***</sup>	0.37 <sup>***</sup>	0.16 <sup>*</sup>	0.16 <sup>*</sup>	0.21 <sup>**</sup>
INC_VA11_12	0.01 <sup>NS</sup>	0.41 <sup>***</sup>	0.39 <sup>***</sup>	0.13 <sup>NS</sup>	0.32 <sup>***</sup>	0.24 <sup>**</sup>	0.21 <sup>**</sup>	0.23 <sup>**</sup>	0.27 <sup>***</sup>	0.11 <sup>NS</sup>	0.14 <sup>NS</sup>	0.16 <sup>*</sup>
SEV_VA11	0.10 <sup>NS</sup>	0.10 <sup>NS</sup>	0.13 <sup>NS</sup>	0.17 <sup>*</sup>	0.01 <sup>NS</sup>	0.18 <sup>*</sup>	0.09 <sup>NS</sup>	-0.08 <sup>NS</sup>	0.04 <sup>NS</sup>	-0.01 <sup>NS</sup>	0.05 <sup>NS</sup>	0.02 <sup>NS</sup>
SEV_VA12	-0.01 <sup>NS</sup>	0.34 <sup>***</sup>	0.32 <sup>***</sup>	0.15 <sup>*</sup>	0.40 <sup>***</sup>	0.26 <sup>***</sup>	0.37 <sup>***</sup>	0.36 <sup>***</sup>	0.45 <sup>***</sup>	0.20 <sup>**</sup>	0.29 <sup>***</sup>	0.31 <sup>***</sup>
SEV_VA11_12	0.05 <sup>NS</sup>	0.37 <sup>***</sup>	0.36 <sup>***</sup>	0.23 <sup>**</sup>	0.31 <sup>***</sup>	0.32 <sup>***</sup>	0.37 <sup>***</sup>	0.23 <sup>**</sup>	0.39 <sup>***</sup>	0.17 <sup>*</sup>	0.24 <sup>***</sup>	0.26 <sup>***</sup>
DON_VA12	-0.01 <sup>NS</sup>	0.33 <sup>***</sup>	0.32 <sup>***</sup>	0.18 <sup>*</sup>	0.42 <sup>***</sup>	0.24 <sup>**</sup>	0.37 <sup>***</sup>	0.41 <sup>***</sup>	0.47 <sup>***</sup>	0.25 <sup>***</sup>	0.23 <sup>**</sup>	0.31 <sup>***</sup>
FDK_VA12	-0.03 <sup>NS</sup>	0.17 <sup>*</sup>	0.15 <sup>NS</sup>	0.17 <sup>*</sup>	0.22 <sup>**</sup>	0.16 <sup>*</sup>	0.25 <sup>***</sup>	0.19 <sup>**</sup>	0.28 <sup>***</sup>	0.20 <sup>**</sup>	0.10 <sup>NS</sup>	0.20 <sup>**</sup>

<sup>†</sup> Trait is formatted as Trait\_LocationYear where: INC = incidence, SEV = severity, DON = deoxynivalenol, FDK = *Fusarium* damaged kernels; years are 11=2011, 12=2012 and 11\_12= the average of 2011 & 2012.

\* Significant at 0.05 probability level

\*\* Significant at 0.01 probability level

\*\*\* Significant at 0.001 probability level

<sup>NS</sup> Not significant at 0.05 probability level

**Table 2.11.** Correlation coefficients from pairwise means comparisons among Virginia (VA) Fusarium head blight (FHB) traits for 2011 and 2012.

	INC_VA11 <sup>†</sup>	INC_VA12	INC_VA11_12	SEV_VA11	SEV_VA12	SEV_VA11_12	DON_VA12
INC_VA12	0.06 <sup>NS</sup>						
INC_VA11_12	0.56 <sup>***</sup>	0.86 <sup>***</sup>					
SEV_VA11	0.79 <sup>***</sup>	-0.04 <sup>NS</sup>	0.41 <sup>***</sup>				
SEV_VA12	0.07 <sup>NS</sup>	0.90 <sup>***</sup>	0.79 <sup>***</sup>	0.00 <sup>NS</sup>			
SEV_VA11_12	0.55 <sup>***</sup>	0.70 <sup>***</sup>	0.85 <sup>***</sup>	0.62 <sup>***</sup>	0.79 <sup>***</sup>		
DON_VA12	-0.05 <sup>NS</sup>	0.68 <sup>***</sup>	0.55 <sup>***</sup>	-0.13 <sup>NS</sup>	0.79 <sup>***</sup>	0.56 <sup>***</sup>	
FDK_VA12	-0.16 <sup>*</sup>	0.47 <sup>***</sup>	0.30 <sup>***</sup>	-0.13 <sup>NS</sup>	0.45 <sup>***</sup>	0.30 <sup>***</sup>	0.50 <sup>***</sup>

<sup>†</sup> Trait is formatted as Trait\_LocationYear where: INC = incidence, SEV = severity, DON = deoxynivalenol, FDK = *Fusarium* damaged kernels; years are 11=2011, 12=2012 and 11\_12= the average of 2011 & 2012.

\* Significant at 0.05 probability level

\*\* Significant at 0.01 probability level

\*\*\* Significant at 0.001 probability level

<sup>NS</sup> Not significant at 0.05 probability level



**Table 2.12.** Correlation coefficients from pairwise comparisons among 2011 greenhouse (GH) *Fusarium* head blight severity means and with those from 2012 greenhouse experiment.

	GHS11_Sev1 <sup>†</sup>	GHS11_Sev2	GHS11_Sev3	GHF11_Sev1	GHF11_Sev2	GHF11_Sev3
GHS11_Sev2	0.72 <sup>***</sup>					
GHS11_Sev3	0.98 <sup>***</sup>	0.82 <sup>***</sup>				
GHF11_Sev1	0.41 <sup>***</sup>	0.36 <sup>***</sup>	0.41 <sup>***</sup>			
GHF11_Sev2	0.21 <sup>*</sup>	0.14 <sup>NS</sup>	0.19 <sup>*</sup>	0.39 <sup>***</sup>		
GHF11_Sev3	0.40 <sup>***</sup>	0.36 <sup>***</sup>	0.40 <sup>***</sup>	0.95 <sup>***</sup>	0.61 <sup>***</sup>	
GHF12_Sev1_D1	0.15 <sup>*</sup>	0.10 <sup>NS</sup>	0.15 <sup>*</sup>	0.08 <sup>NS</sup>	0.23 <sup>*</sup>	0.11 <sup>NS</sup>
GHF12_Sev2_D1	0.29 <sup>***</sup>	0.16 <sup>*</sup>	0.27 <sup>***</sup>	0.04 <sup>NS</sup>	-0.04 <sup>NS</sup>	0.02 <sup>NS</sup>
GHF12_Sev3_D1	0.25 <sup>***</sup>	0.19 <sup>*</sup>	0.25 <sup>***</sup>	0.10 <sup>NS</sup>	0.15 <sup>NS</sup>	0.11 <sup>NS</sup>
GHF12_Sev1_D2	0.24 <sup>**</sup>	0.10 <sup>NS</sup>	0.22 <sup>**</sup>	0.15 <sup>NS</sup>	0.19 <sup>*</sup>	0.14 <sup>NS</sup>
GHF12_Sev2_D2	0.14 <sup>NS</sup>	0.08 <sup>NS</sup>	0.14 <sup>NS</sup>	-0.03 <sup>NS</sup>	-0.02 <sup>NS</sup>	-0.03 <sup>NS</sup>
GHF12_Sev3_D2	0.23 <sup>**</sup>	0.10 <sup>NS</sup>	0.22 <sup>**</sup>	0.15 <sup>NS</sup>	0.15 <sup>NS</sup>	0.13 <sup>NS</sup>
GHF12_Sev1_D3	0.19 <sup>*</sup>	0.10 <sup>NS</sup>	0.18 <sup>*</sup>	0.19 <sup>*</sup>	0.22 <sup>*</sup>	0.19 <sup>*</sup>
GHF12_Sev2_D3	0.24 <sup>**</sup>	0.16 <sup>*</sup>	0.24 <sup>**</sup>	0.18 <sup>NS</sup>	0.15 <sup>NS</sup>	0.20 <sup>*</sup>
GHF12_Sev3_D3	0.24 <sup>**</sup>	0.13 <sup>NS</sup>	0.23 <sup>**</sup>	0.22 <sup>*</sup>	0.23 <sup>*</sup>	0.22 <sup>*</sup>
GHF12_AUDPC_Sev1	0.24 <sup>**</sup>	0.12 <sup>NS</sup>	0.23 <sup>**</sup>	0.17 <sup>NS</sup>	0.24 <sup>*</sup>	0.17 <sup>NS</sup>
GHF12_AUDPC_Sev2	0.24 <sup>**</sup>	0.14 <sup>NS</sup>	0.23 <sup>**</sup>	0.04 <sup>NS</sup>	0.01 <sup>NS</sup>	0.03 <sup>NS</sup>
GHF12_AUDPC_Sev3	0.27 <sup>***</sup>	0.14 <sup>NS</sup>	0.26 <sup>***</sup>	0.18 <sup>NS</sup>	0.21 <sup>*</sup>	0.17 <sup>NS</sup>

<sup>†</sup> Trait is formatted as LocationYear\_Trait where: GHS11 = greenhouse spring 2011 and Sev1= severity at or below 9th spikelet, Sev2 = severity at or above 11th spikelet, Sev3 = severity total spikelet; for GHF11 = greenhouse fall 2011 and GHF12 = greenhouse fall 2012, Sev1 = severity at or below 10th spikelet, Sev2 = severity at or above 12th spikelet, Sev3 = severity total spikelet. D1= 7 days after inoculation (dai), D2=14 dai, D3= 21 dai. AUDPC = area under the disease progress curve.

- <sup>\*</sup> Significant at 0.05 probability level
- <sup>\*\*</sup> Significant at 0.01 probability level
- <sup>\*\*\*</sup> Significant at 0.001 probability level
- <sup>NS</sup> Not significant at 0.05 probability level

**Table 2.13.** Correlation coefficients from pairwise comparisons among 2012 greenhouse (GH) *Fusarium* head blight severity means and Area Under Disease Progress Curve (AUDPC) values.

	GHF12_Sev1 D1†	GHF12_Sev2 D1	GHF12_Sev3 D1	GHF12_Sev1 D2	GHF12_Sev2 D2	GHF12_Sev3 D2	GHF12_Sev1 D3	GHF12_Sev2 D3	GHF12_Sev3 D3	GHF12 AUDPC_Sev1	GHF12 AUDPC_Sev2
GHF12_Sev1 D1											
GHF12_Sev2 D1	0.38***										
GHF12_Sev3 D1	0.84***	0.66***									
GHF12_Sev1 D2	0.40***	0.10 <sup>NS</sup>	0.36***								
GHF12_Sev2 D2	0.31***	0.61***	0.42***	0.24**							
GHF12_Sev3 D2	0.44***	0.28***	0.47***	0.93***	0.52***						
GHF12_Sev1 D3	0.34***	0.10 <sup>NS</sup>	0.27***	0.88***	0.26***	0.84***					
GHF12_Sev2 D3	0.31***	0.49***	0.38***	0.34***	0.75***	0.54***	0.38***				
GHF12_Sev3 D3	0.39***	0.27***	0.37***	0.81***	0.47***	0.87***	0.92***	0.68***			
GHF12 AUDPC_Sev1	0.51***	0.15 <sup>NS</sup>	0.44***	0.98***	0.28***	0.92***	0.94***	0.40***	0.88***		
GHF12 AUDPC_Sev2	0.38***	0.78***	0.54***	0.26***	0.94***	0.52***	0.29***	0.85***	0.53***	0.31***	
GHF12 AUDPC_Sev3	0.55***	0.37***	0.59***	0.89***	0.54***	0.97***	0.86***	0.63***	0.93***	0.92***	0.59***

†Trait is formatted as LocationYear\_Trait where: GHF12 = greenhouse fall 2012, Sev1 = severity at or below 10th spikelet, Sev2 = severity at or above 12th spikelet, Sev3 = severity total spikelet. D1= 7 days after inoculation (dai), D2=14 dai, D3= 21 dai. AUDPC = area under the disease progress curve.

\* Significant at 0.05 probability level

\*\* Significant at 0.01 probability level

\*\*\* Significant at 0.001 probability level

<sup>NS</sup> Not significant at 0.05 probability level

**Table 2.14.** Quantitative trait loci (QTL) associated with *Fusarium* head blight (FHB) in the soft red winter wheat cultivar Jamestown.

Trait <sup>†</sup>	Chromosome <sup>‡</sup>	Marker Interval <sup>§</sup>	Position (cM) <sup>¶</sup>	LOD	Variation (%) <sup>††</sup>	Additive <sup>¶¶</sup>
SEV_NC11	1B	<i>Xwmc500.6-Xgwm273.2</i>	17	7.9	26.1	-10.3
SEV_NC11_12	1B	<i>Xwmc500.6-Xgwm273.2</i>	17	4.4	13.9	-6.0
DON_NC11	1B	<i>Xwmc500.7-Xgwm273.2</i>	15	4.4	13.3	-2.6
DON_NC11_12	1B	<i>Xwmc500.7-Xgwm273.2</i>	7	4.8	12.7	-1.6
FDK_MD12	3BS	<i>Xgwm376-Xgwm285</i>	1	3.7	9.1	2.2
SEV_NC12	5A	<i>Xbarc180-Xgwm304.1</i>	31	4.0	11.0	5.3

<sup>†</sup> Trait is formatted as Trait\_LocationYear where: INC = incidence, SEV = severity, DON = deoxynivalenol, FDK = *Fusarium* damaged kernels; NC= North Carolina, MD = Maryland; years are 11=2011, 12=2012 and 11\_12= the average of 2011 & 2012.

<sup>‡</sup> Chromosome location of QTL for FHB trait

<sup>§</sup> Flanking markers for the QTL

<sup>¶</sup> Position of the highest LOD score in terms of genetic distance from the first marker of the support interval

<sup>††</sup> Percent variation explained by the individual QTL

<sup>¶¶</sup> Additive effect (negative number = QTL infers resistance; positive number = QTL infers susceptibility)

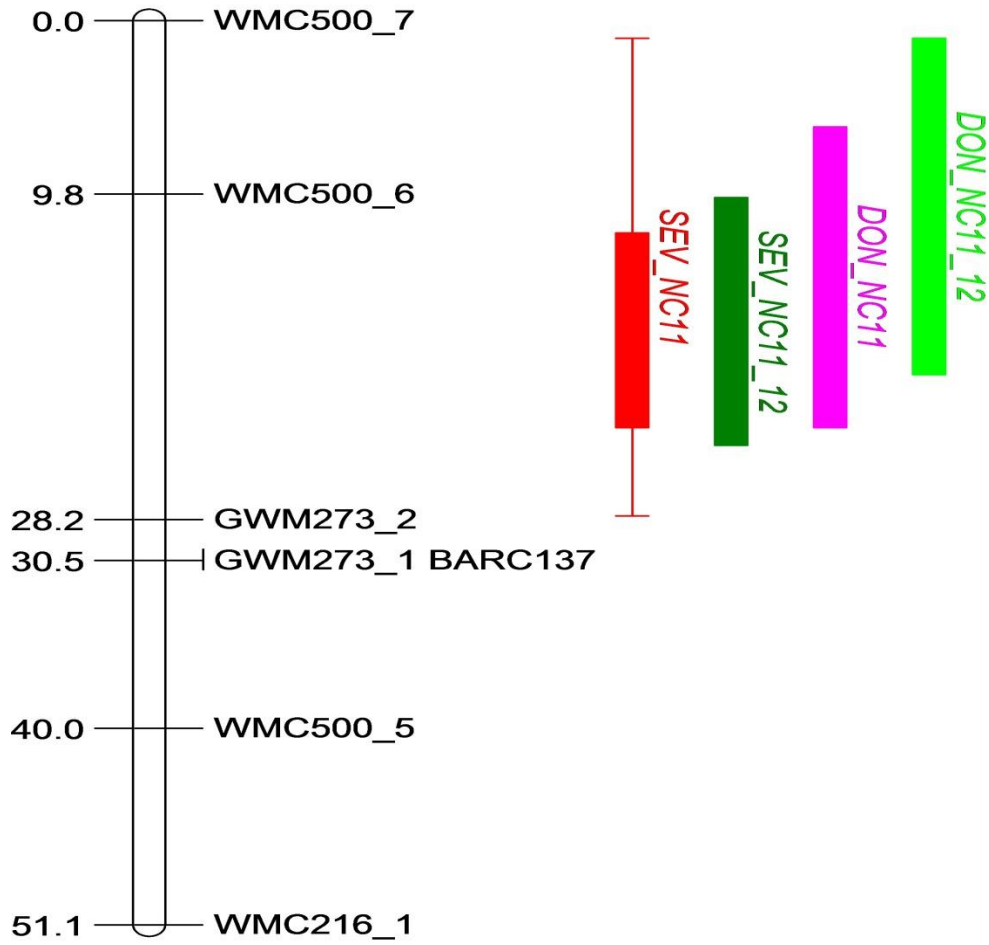
**Table 2.15.** Fragment sizes of Jamestown alleles for simple sequence (SSR) markers associated with quantitative trait loci (QTL) for resistance (1B) and susceptibility (3BS and 5A) to *Fusarium* head blight.

Chr <sup>†</sup>	Marker	Additive Effect	Fragment size <sup>‡</sup>
1B	<i>Xwmc500.6</i>	resistance	206
3BS	<i>Xgwm285</i>	susceptibility	246.6
5A	<i>Xgwm304.1</i>	susceptibility	200

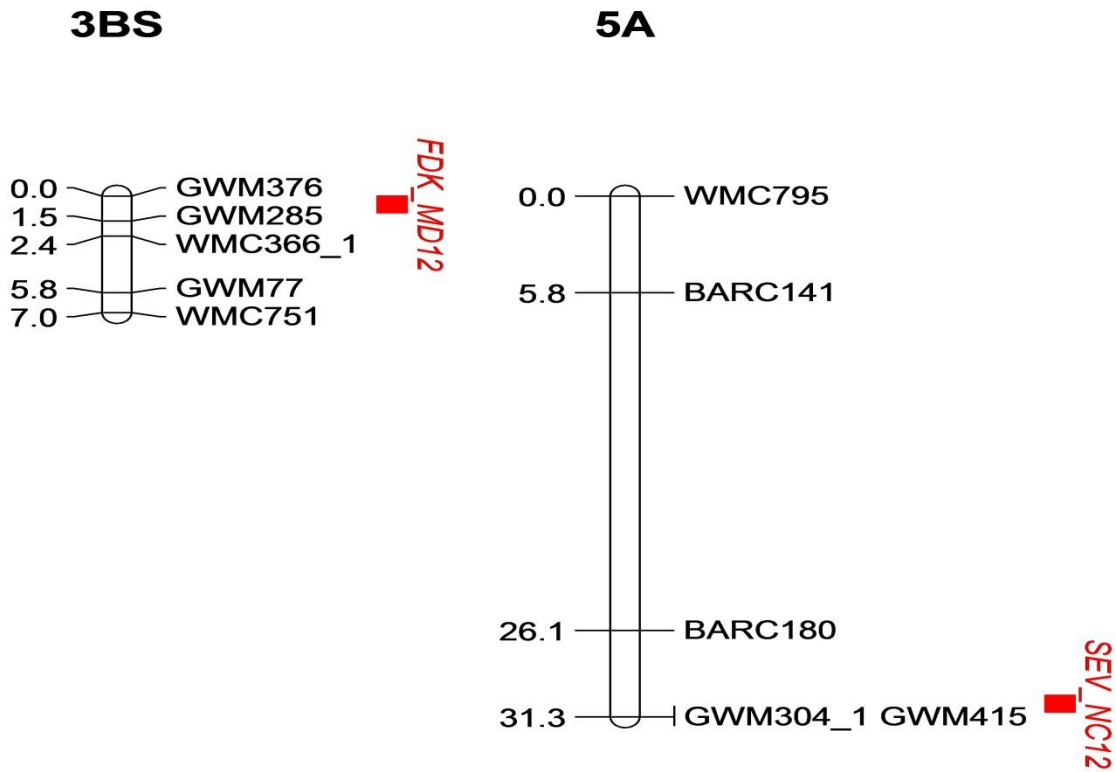
<sup>†</sup> Chromosome on which the simple sequence repeat (SSR) marker was mapped

<sup>‡</sup> The fragment size of the marker allele in Jamestown

# 1B



**Figure 2.5. Linkage map of chromosome 1B constructed from the Pioneer Brand 25R47/ Jamestown RIL population.** Putative quantitative trait loci (QTL) positions for FHB-associated traits are shown on the right of the linkage group. The QTL on 1B was identified to be associated with disease resistance with the Jamestown allele. The traits include: severity in North Carolina 2011 (SEV\_NC11), mean severity in North Carolina 2011 and 2012 (SEV\_NC11\_12), deoxynivalenol accumulation in North Carolina 2011 (DON\_NC11), and mean deoxynivalenol accumulation in North Carolina 2011 and 2012 (DON\_NC11\_12). The QTL peak is indicated by the thick bar and the confidence interval by the lines on either side of the bar. Genetic distances are shown in centimorgans to the left of the linkage group



**Figure 2.6. Linkage maps of chromosome 3B and 5A constructed from the Pioneer Brand 25R47/ Jamestown RIL population.** Putative quantitative trait loci (QTL) positions for FHB-associated traits are shown on the right of the linkage group. On chromosome 3B, the trait *Fusarium* damaged kernels (FDK) in Maryland 2012 (FDK\_MD12) was associated with disease susceptibility for the Jamestown allele. On chromosome 5A, the trait associated with increased disease is severity in North Carolina 2012 (SEV\_NC12) for the Jamestown allele. The QTL peak is indicated by the thick bar and the confidence interval by the lines on either side of the bar. Genetic distances are shown in centimorgans to the left of the linkage group.

**Table 2.16.** Single analysis of variance for SNP and SSRs identified as polymorphic for Pioneer 25R47 x Jamestown recombinant inbred lines for *Fusarium* head blight traits severity (SEV) and incidence (INC), as well as deoxynivalenol (DON) and *Fusarium* damaged kernels ( FDK).

Trait <sup>†</sup>	Chromosome <sup>‡</sup>	Marker Associated	Marker Type	Fvalue	Variation(%)
INC_VA11_12	1B	NTAES008348802 <sup>§</sup>	SNP	16.6***	29.3
INC_VA11_12	1B	NTAES008352626 <sup>§</sup>	SNP	19.5****	34.0
SEV_VA11_12				12.8***	25.0
DON_NC11	1B	wsnp_BE495786B-Ta_2_1-0_T_R_1891210776	SNP	12.8***	25.8
DON_NC11	1B	wsnp_BE495786B-Ta_2_2-0_B_F_1891198548	SNP	13.1***	24.6
SEV_NC11	1B	wsnp_Ex_c22364_31560642-0_T_R_1891204028	SNP	23.3****	36.8
SEV_NC11_12				16.0***	29.1
DON_NC11	1B	wsnp_Ex_c5679_9976893-0_B_R_1891204920	SNP	14.1***	25.8
SEV_NC11	1B	wsnp_Ex_c5947_10431109-0_B_F_1891202146	SNP	19.4****	34.8
SEV_NC11_12	1B	wsnp_Ex_c5947_10431109-0_B_F_1891202146	SNP	13.7***	28.0
DON_NC11	1B	wsnp_Ra_c19148_28288923-0_T_R_1891209275	SNP	13.9***	25.8
SEV_NC11	1B	wsnp_Ra_c21132_30487331-0_B_F_1891209292	SNP	21.3****	34.8
SEV_NC11_12				15.2***	28.0
DON_NC11	1B	wsnp_Ra_c29782_39115548-0_B_F_1891209348	SNP	13.9***	25.8
INC_VA11_12				18.5***	31.6
SEV_VA11	1B	Xwmc216.1	SSR	12.8***	23.5
SEV_VA11_12				14.7***	25.7
SEV_NC11	1B	Xwmc500.6	SSR	48.6****	54.9
SEV_NC11_12				35.5****	47.6
DON_NC11				15.3***	27.6
SEV_NC11	1B	Xwmc500.7	SSR	31.8****	44.3
SEV_NC11_12				24.6****	38.7
SEV_NC11	1Bc	Xbarc137	SSR	13.3***	27.9
SEV_NC11	1BL	Xgwm259	SSR	13.4***	21.7
SEV_NC11_12				14.9***	24.5
SEV_NC11	1BS, 6BL	Xgwm273.1	SSR	16.9***	29.7
SEV_NC11_12				12.8***	24.7
SEV_NC11	1BS, 6BL	Xgwm273.2	SSR	19.7****	33.0
SEV_NC11_12				16.7***	30.0
INC_VA11	2A	NTAES008349707 <sup>§</sup>	SNP	18.0***	34.6
INC_VA11	2A	NTAES008354979 <sup>§</sup>	SNP	18.0***	34.6
GHF11_Sev1	2A	wsnp_Ex_c19516_28482852-0_T_F_1891203907	SNP	14.0***	34.2
GHF11_Sev1	2A	wsnp_Ex_c19516_28483050-0_B_R_1891203910	SNP	14.0***	34.2
GHF11_Sev1	2A	wsnp_Ex_c19516_28483751-0_B_R_1891203912	SNP	14.0***	34.2

<sup>†</sup> Trait is formatted as Trait\_LocationYear where: INC = incidence, SEV = severity, DON = deoxynivalenol, FDK = *Fusarium* damaged kernels; MD = Maryland, NC= North Carolina, VA= Virginia, GH= Greenhouse; years are 11=2011, 12=2012 and 11\_12= the average of 2011 & 2012. Sev1= spread of infected spikelets below inoculated spikelet; Sev2= spread of infected spikelets above inoculated spikelet; Sev3= total number of infected spikelets on head. D3= rating taken at 21 dai. AUDPC= area under the disease progress curve for severity of total spike from 7 to 21 dai, point-inoculations preformed in the greenhouse.

<sup>‡</sup> Possible chromosome location

<sup>§</sup> Monsanto Proprietary SNP Marker

\*\*\* Significant at 0.001 probability level

\*\*\*\* Significant at 0.0001 probability level

**Table 2.16 (continued).** Single analysis of variance for SNP and SSRs identified as polymorphic for Pioneer 25R47 x Jamestown recombinant inbred lines for *Fusarium* head blight traits severity (SEV) and incidence (INC), as well as deoxynivalenol (DON) and *Fusarium* damaged kernels ( FDK).

Trait <sup>†</sup>	Chromosome <sup>‡</sup>	Marker Associated	Marker Type	Fvalue	Variation(%)
DON_NC11_12				17.7***	30.6
SEV_NC11_12	2A	wsnp_Ex_c2258_4232538-0_B_F_1891201022	SNP	27.8****	41.0
SEV_NC12				14.5***	26.6
INC_VA11	2A	wsnp_Ex_c3685_6723631-0_T_F_1891207864	SNP	16.5***	32.7
GHS11_Sev1	2AS, 2B, 2D, 5B	Xwmc149.2	SSR	13.8***	25.7
GHS11_Sev3				12.8***	24.3
INC_MD11	2B	wsnp_Ku_c16547_25454123-0_B_R_1891208826	SNP	13.3***	27.6
GHF12_AUDPC_Sev3				16.3***	31.2
GHF12_Sev2_D3	2B	wsnp_Ra_c407_862316-0_B_F_1891198138	SNP	14.5***	27.6
GHF12_Sev3_D3				17.4***	31.4
DON_MD12	3A	wsnp_Ex_c14400_22381382-0_T_F_1891200988	SNP	13.1***	24.7
DON_MD12	3A	wsnp_Ku_c8400_14280021-0_T_R_1891196297	SNP	13.1***	24.7
SEV_VA12	3AL, 3BS, 4BSc, 7ALc	Xwmc695	SSR	13.4***	25.9
SEV3_AUDPC_GHF12	3B	NTAES008352775 <sup>§</sup>	SNP	13.7***	25.5
SEV_NC12	3B	NTAES008350443 <sup>§</sup>	SNP	16.8***	30.7
DON_MD12	3B	NTAES008352775 <sup>§</sup>	SNP	13.2***	24.8
INC_VA11	3B	NTAES008353297 <sup>§</sup>	SNP	19.2****	36.1
INC_VA11	3B	wsnp_Ex_c6223_10857649-0_T_F_1891196173	SNP	18.6***	35.4
SEV_NC11_12	3B	wsnp_Ex_c8386_14127329	SNP	13.2***	24.9
INC_VA11	3B	wsnp_Ku_c26257_36216869-0_B_F_1891206418	SNP	19.2****	36.1
INC_VA11	3B	wsnp_Ra_c12935_20587578-0_T_F_1891201509	SNP	19.2****	36.1
FDK_MD11	3BS	Xgwm376	SSR	19.8****	79.9
FDK_NC11_12	3BS	Xwmc63	SSR	18.2***	27.7
INC_VA11				14.5***	29.9
INC_NC12	4A	NTAES008351895 <sup>§</sup>	SNP	16.6***	29.4
INC_VA11_12	5A	NTAES008350671 <sup>§</sup>	SNP	16.4***	29.1
INC_VA11_12	5A	NTAES008353227 <sup>§</sup>	SNP	18.4***	31.5
INC_VA11_12	5A	wsnp_Ex_c30969_39821293-0_B_R_1891204348	SNP	18.4***	31.5
FDK_VA12	5A	wsnp_Ex_c31154_39982416-0_B_F_1891199516	SNP	14.7***	26.9
DON_NC11_12				14.4***	26.4
DON_NC12				13.7***	25.5
SEV_NC11	5A	Xgwm304.1	SSR	14.4***	26.5
SEV_NC11_12				27.6****	41.5
SEV_NC12				17.0***	31.0

<sup>†</sup> Trait is formatted as Trait\_LocationYear where: INC = incidence, SEV = severity, DON = deoxynivalenol, FDK = *Fusarium* damaged kernels; MD = Maryland, NC= North Carolina, VA= Virginia, GH= Greenhouse; years are 11=2011, 12=2012 and 11\_12= the average of 2011 & 2012. Sev1= spread of infected spikelets below inoculated spikelet; Sev2= spread of infected spikelets above inoculated spikelet; Sev3= total number of infected spikelets on head. D3= rating taken at 21 dai. AUDPC= area under the disease progress curve for severity of total spike from 7 to 21 dai, point-inoculations performed in the greenhouse.

<sup>‡</sup> Possible chromosome location

<sup>§</sup> Monsanto Proprietary SNP Marker

\*\*\* Significant at 0.001 probability level

\*\*\*\* Significant at 0.0001 probability level



**Table 2.16 (continued).** Single analysis of variance for SNP and SSRs identified as polymorphic for Pioneer 25R47 x Jamestown recombinant inbred lines for *Fusarium* head blight traits severity (SEV) and incidence (INC), as well as deoxynivalenol (DON) and *Fusarium* damaged kernels ( FDK).

Trait <sup>†</sup>	Chromosome <sup>‡</sup>	Marker Associated	Marker Type	Fvalue	Variation (%)
DON_NC12				13.6***	25.4
SEV_NC11_12	5A	Xbarc180	SSR	29.2****	42.2
SEV_NC12				13.0***	24.6
DON_NC11_12				17.7***	30.7
DON_NC12	5A	Xgwm415	SSR	15.8***	28.3
SEV_NC11_12				26.0****	40.0
SEV_NC12				19.0****	33.3
GHS11_Sev1	5AL	Xbarc100.2	SSR	15.1***	27.5
GHS11_Sev3				14.1***	26.1
SEV_NC11_12	5B	NTAES00834635§	SNP	14.1***	26.6
DON_NC11	5B	wsnp_BE443187B_Ta_2_1-0_T_F_1891210647	SNP	13.3***	25.8
SEV_NC11	5B	wsnp_BE443187B_Ta_2_1-0_T_F_1891210647	SNP	14.0***	26.0
SEV_NC11_12	5B	wsnp_BE443187B_Ta_2_1-0_T_F_1891210647	SNP	13.0***	25.0
DON_NC11				13.1***	24.6
SEV_NC11	5B	wsnp_BE497820B_Ta_2_2-0_B_F_1891210805	SNP	14.0***	26.0
SEV_NC11_12				13.0***	25.0
INC_VA11_12	5B	wsnp_Ex_c3817_6937184-0_B_F_1891196606	SNP	13.7***	30.6
SEV_NC11_12	5B	wsnp_RFL_Contig4565_5399994-0_T_F_1891203236	SNP	14.1***	26.6
GHF11_Sev2	6B	wsnp_Ra_c7468_12851157-0_B_F_1891206964	SNP	16.6***	38.1
INC_VA11	7A	wsnp_CAP11_c1761_958064-0_T_R_1891203253	SNP	13.1***	27.8
INC_VA11	7A	wsnp_Ex_c47919_52987043-0_B_R_1891208029	SNP	13.3***	28.1
INC_VA11	7A	wsnp_Ku_c10202_16937059-0_B_F_1891208707	SNP	13.1***	27.8
INC_VA11_12	7B	NTAES00834481§	SNP	15.7***	33.6
GHF12_AUDPC_Sev1	7B	NTAES008348096§	SNP	12.8***	26.2
INC_VA11_12	7B	wsnp_JD_c4641_5774711-0_T_R_1891208626	SNP	21.5****	34.0
SEV_VA11_12	7B	wsnp_JD_c4641_5774711-0_T_R_1891208626	SNP	13.7***	25.0
GHF12_AUDPC_Sev3				16.3***	31.2
GHF12_Sev2_D3	unknown	NTAES00834484§	SNP	14.5***	27.6
GHF12_Sev3_D3				17.4***	31.4
INC_VA11	unknown	NTAES008346920§	SNP	19.5****	36.5
FDK_NC11_12	unknown	NTAES008347968§	SNP	15.3***	31.2
INC_VA11_12	unknown	NTAES00834843§	SNP	13.7***	30.6

<sup>†</sup> Trait is formatted as Trait\_LocationYear where: INC = incidence, SEV = severity, DON = deoxynivalenol, FDK = *Fusarium* damaged kernels; MD = Maryland, NC= North Carolina, VA= Virginia, GH= Greenhouse; years are 11=2011, 12=2012 and 11\_12= the average of 2011 & 2012. Sev1= spread of infected spikelets below inoculated spikelet; Sev2= spread of infected spikelets above inoculated spikelet; Sev3= total number of infected spikelets on head. D3= rating taken at 21 dai. AUDPC= area under the disease progress curve for severity of total spike from 7 to 21 dai, point-inoculations performed in the greenhouse.

<sup>‡</sup> Possible chromosome location

§ Monsanto Proprietary SNP Marker

\*\*\* Significant at 0.001 probability level

\*\*\*\* Significant at 0.0001 probability level

**Table 2.16 (continued).** Single analysis of variance for SNP and SSRs identified as polymorphic for Pioneer 25R47 x Jamestown recombinant inbred lines for *Fusarium* head blight traits severity (SEV) and incidence (INC), as well as deoxynivalenol (DON) and *Fusarium* damaged kernels (FDK).

Trait <sup>†</sup>	Chromosome <sup>‡</sup>	Marker Associated	Marker Type	Fvalue	Variation(%)
DON_NC11_12				17.7***	30.7
DON_NC12	unknown	NTAES008351197§	SNP	15.8***	28.3
SEV_NC11_12				26.0****	40.0
SEV_NC12				19.0****	33.3
FDK_MD11	unknown	NTAES008353073§	SNP	20.0****	83.8
INC_VA11	unknown	NTAES008355249§		24.8****	42.2
SEV_NC11			SNP	13.8***	25.6
FDK_MD11	unknown	wsnp_CAP11_rep_c4009_1892626-0_B_F_1891200960	SNP	20.0****	79.9
FDK_MD11	unknown	wsnp_Ex_c410_810806-0_B_R_1891207937	SNP	20.0****	79.9
DON_NC11				15.0***	27.3
SEV_NC11	unknown	wsnp_Ku_c30910_40695864-0_T_R_1891206446	SNP	26.5****	39.8
SEV_NC11_12				18.0***	31.6
INC_VA11_12	unknown	wsnp_Ku_c3826_7020960-0_T_R_1891206484	SNP	13.7***	30.6

<sup>†</sup> Trait is formatted as Trait\_LocationYear where: INC = incidence, SEV = severity, DON = deoxynivalenol, FDK = *Fusarium* damaged kernels; MD = Maryland, NC= North Carolina, VA= Virginia, GH= Greenhouse; years are 11=2011, 12=2012 and 11\_12= the average of 2011 & 2012. Sev1= spread of infected spikelets below inoculated spikelet; Sev2= spread of infected spikelets above inoculated spikelet; Sev3= total number of infected spikelets on head. D3= rating taken at 21 dai. AUDPC= area under the disease progress curve for severity of total spike from 7 to 21 dai, point-inoculations performed in the greenhouse.

<sup>‡</sup> Possible chromosome location

<sup>§</sup> Monsanto Proprietary SNP Marker

\*\*\* Significant at 0.001 probability level

\*\*\*\* Significant at 0.0001 probability level

**Table 2.17.** QTL Identified on Wheat Chromosome 1B for Resistance to Fusarium Head Blight and Deoxynivalenol (DON)

Resistance Trait	Source	Molecular Markers Associated with QTL	Variation(%)	References
Severity†	Alondra‡	<i>XeTCG-mAGC.7 - XeACCGmCT-C.7</i>	15.6	Zhang et al., 2004
Incidence‡	Cansas‡	<i>XE38M52.378 - Xgwm131</i>	16.5	Klahr et al., 2007
Severity	Fundulea201R‡	<i>Xbarc8</i>	18.7	Shen et al., 2003
Severity	Lynx‡	<i>Xp78m51.237- Xs26m23.356 (Xgwm18.iag95)</i>	12.0	Schmolke et al. 2005
Severity	Rialto‡	<i>XwPt-0705</i>	13.4	Srinivasachary et al., 2008
Severity	Seri82‡	<i>Xe38m50_10</i>	8.0	Mardi et al., 2006
Severity	Sincron‡	<i>Gli-R1</i>	-	Ittu et al., 2000
Severity	Arina_1	<i>XS16M14.400</i>	10.1	Draeger et al., 2007
Severity	Romanus	<i>XP70M56.308</i>	3.0	Holzapfel et al., 2008
Severity	CM82036	<i>XgluB1</i>	9.7	Buerstmayr et al., 2002
Severity	Wangshuibai_1	<i>Xgwm018- Xbarc181</i>	7.6	Lin et al., 2004
Severity	Wangshuibai_2	<i>Xwms759</i>	11.9	Zhou et al., 2004
DON§	Wangshuibai_3	<i>Xwms759</i>	7.8	Yu et al., 2008
Severity	Arina_2	<i>XP43/M62-400 - XwPt-3475</i>	19.6	Semagn et al., 2007
Severity	History	<i>XP64M51.190</i>	7.9	Holzapfel et al., 2008
Severity	Biscay	<i>XP64M51.190</i>	6.5	Holzapfel et al., 2008
Severity	Pirat	<i>XP64M51.190</i>	13.8	Holzapfel et al., 2008
Incidence & Severity	Remus	<i>Xs12m25.14 - Xs24m17.2</i>	5.5	Steiner et al. 2004

†Severity = resistance to spread of FHB in the wheat spike

‡Incidence = resistance to initial infection of FHB

§DON = resistance to deoxynivalenol accumulation in the seed

‡ T1BL.1RS translocation

**Table 2.18** Comparison of mean severity (SEV) in North Carolina (NC), mean deoxynivalenol (DON) accumulation in NC, and mean of *Fusarium* damaged kernels (FDK) in Maryland (MD) for Pioneer 25R47 x Jamestown recombinant inbred lines having different combinations of parental alleles for diagnostic markers associated with quantitative trait loci (QTL) located on chromosomes 1B, 3BS, and 5A.

QTL combination	Allele †	N§	SEV_NC11	N	SEV_NC12	N	SEV_NC11_12	N	DON_NC11	N	DON_NC11_12	N	FDK_MD12
1B+3BS+5A	AAB	8	43.1 <sup>F</sup>	8	21.1 <sup>D</sup>	8	32.1 <sup>E</sup>	8	9.6 <sup>F</sup>	8	8.7 <sup>F</sup>	7	9.4 <sup>C</sup>
1B+3BS+5A	ABB	38	46.8 <sup>FE</sup>	36	22.6 <sup>DC</sup>	37	35.2 <sup>ED</sup>	38	11.7 <sup>EF</sup>	38	9.7 <sup>FE</sup>	38	7.7 <sup>C</sup>
1B	A	84	53.0 <sup>FDE</sup>	72	23.7 <sup>DC</sup>	82	41.2 <sup>DC</sup>	84	13.5 <sup>ED</sup>	84	11.0 <sup>FDE</sup>	83	9.6 <sup>BC</sup>
5A	B	78	55.4 <sup>DEC</sup>	70	23.3 <sup>DC</sup>	75	41.0 <sup>DC</sup>	79	14.0 <sup>EDC</sup>	79	11.0 <sup>FDE</sup>	78	9.1 <sup>C</sup>
3BS	B	110	58.6 <sup>BDC</sup>	96	27.7 <sup>BDC</sup>	107	45.5 <sup>BC</sup>	111	15.2 <sup>EBDAC</sup>	111	12.1 <sup>BDEC</sup>	111	8.7 <sup>C</sup>
1B+3BS+5A	ABA	21	59.5 <sup>BDC</sup>	14	23.7 <sup>DC</sup>	20	48.9 <sup>BAC</sup>	21	14.4 <sup>EBDC</sup>	21	11.7 <sup>DEC</sup>	21	9.9 <sup>BC</sup>
1B+3BS+5A	AAA	12	62.1 <sup>BDAC</sup>	10	27.0 <sup>BDC</sup>	12	48.1 <sup>BAC</sup>	12	18.7 <sup>A</sup>	12	14.3 <sup>BA</sup>	12	14.6 <sup>A</sup>
3BS	A	68	64.8 <sup>BAC</sup>	60	31.6 <sup>BAC</sup>	66	50.0 <sup>BA</sup>	68	16.9 <sup>BDAC</sup>	68	13.5 <sup>BAC</sup>	67	13.4 <sup>BA</sup>
1B+3BS+5A	BBA	31	65.3 <sup>BAC</sup>	30	37.2 <sup>A</sup>	31	52.0 <sup>BA</sup>	31	17.4 <sup>BAC</sup>	31	14.4 <sup>BA</sup>	31	8.8 <sup>C</sup>
5A	A	104	65.5 <sup>BAC</sup>	90	33.8 <sup>BA</sup>	102	51.9 <sup>BA</sup>	104	17.1 <sup>BDAC</sup>	104	13.7 <sup>BAC</sup>	104	11.2 <sup>BAC</sup>
1B+3BS+5A	BAB	13	67.4 <sup>BA</sup>	9	24.7 <sup>BDC</sup>	12	52.8 <sup>BA</sup>	13	16.3 <sup>BDAC</sup>	13	12.7 <sup>BDAC</sup>	13	13.9 <sup>A</sup>
1B+3BS+5A	BBB	17	68.5 <sup>BA</sup>	15	24.9 <sup>BDC</sup>	16	48.7 <sup>BAC</sup>	18	18.9 <sup>A</sup>	18	13.4 <sup>BAC</sup>	18	8.4 <sup>C</sup>
1B	B	99	68.8 <sup>BA</sup>	89	33.5 <sup>BA</sup>	96	52.8 <sup>BA</sup>	100	17.9 <sup>BA</sup>	100	14.0 <sup>BAC</sup>	100	10.9 <sup>BAC</sup>
1B+3BS+5A	BAA	34	70.3 <sup>A</sup>	32	32.3 <sup>A</sup>	33	54.1 <sup>A</sup>	34	18.4 <sup>A</sup>	34	14.6 <sup>A</sup>	34	13.4 <sup>BA</sup>

† A allele = Jamestown ; B allele = Pioneer Brand 25R47.

‡ Mean phenotype of different quantitative trait loci (QTL) combinations sharing the same letter are not significantly different ( $P < 0.05$ ).

§ - The number of recombinant inbred lines having a given QTL combination based on parental marker alleles.