

ABSTRACT

Title of Document: **EVALUATION OF SCAB RESISTANCE
QUANTITATIVE TRAIT LOCI (QTL)
EFFECTS ON WHEAT**

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Fusarium Head Blight (FHB) of wheat (*Triticum aestivum*), caused by *Fusarium graminearum*, is a disease that periodically strikes the mid-Atlantic region of the USA. Breeding for resistant wheat cultivar is an effective method of disease control. McCormick, a genotype adapted to the mid-Atlantic region, was used in a backcross program with the Chinese cultivar Ning7840. Eight Near-Isogenic Lines (NIL) were developed by marker-assisted backcrossing. Three FHB resistance QTL on chromosomes 3BS, 2DL, and 5A were introgressed from non-adapted Ning7840 into the elite soft red winter wheat McCormick. The objective of this study was to evaluate the effects of QTL singly and in combination on FHB resistance. The 3BS+2DL NIL showed higher resistance and lower deoxynivalenol content than other NIL in both field and greenhouse studies. This suggests that the 3BS+2DL NIL can be used in the mid-Atlantic region to breed for improved FHB resistance.

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EFFECTS ON WHEAT.

By

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Chapter 1: Literature Review

Fusarium Head Blight (FHB) or scab is a devastating disease of small-grain cereals including wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*). It was first described by W. G. Smith in England as a fungal disease in 1884 (Smith, 1884) and was already recognized as a major threat to wheat and barley production in the early years of the twentieth century (Dickson, 1929). Recently, FHB disease has become more prevalent in many areas worldwide (Parry et al., 1995).

FHB can cause large economic losses by reducing wheat grain yield and quality. Infected kernels, also called tombstones, are blighted and lighter than healthy grains. In the United States, outbreaks of FHB on wheat and barley have resulted in about \$2.5 billion of cumulative direct economic losses and \$7.7 billion of total losses between 1993 and 2001 (Nganje et al., 2004). FHB has also become a threat to wheat and barley production in many other countries around the world. In China, up to 7 million hectares of wheat have been damaged by FHB during severe epidemics, resulting in estimated wheat production losses of over a million ton (Bai and Shaner, 2004).

Additionally, FHB infected grains are generally contaminated with mycotoxins, including deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEA), moniliformin (MON), beauvericin (BEA), and enniatins (EN), which are produced by the fungus (Desjardins and Proctor, 2007; Foroud and Eudes, 2009; Snijders, 1990c; Xu and Berrie, 2005). These toxins are hazardous to humans and animals. DON is the most common toxin in infected kernels. Wheat grains highly contaminated with DON are not suitable for human food or livestock feed. Different countries have their own specific limits on DON levels for wheat marketing. In the United States, the recommended tolerance level

of DON content is set to 1 ppm for human food products but is higher for animal feed (Chu, 1997).

Epidemiology of FHB

FHB pathogens

Several *Fusarium* species are responsible for FHB disease, including *F. graminearum*, *F. culmorum*, *F. poae*, *F. avenaceum*, *F. sporotrichoides*, and *Microdochium nivale*. The distribution and prevalence of these fungi depends largely on air temperature and the presence of moisture during flowering. *F. graminearum* is the predominant FHB pathogen in North America, China and many other countries, while *F. culmorum* is reported as a major causal agent of FHB in cooler areas around the world, especially in northern Europe and Canada (Osborne and Stein, 2007). In Europe, other species, such as *F. poae*, *F. avenaceum*, *F. sporotrichoides*, and *M. nivale*, are also associated with FHB of wheat and barley (Bottalico and Perrone, 2002; Nicholson et al., 2003; Xu et al., 2005). All the causal pathogens of FHB except *M. nivale* are capable of producing mycotoxins (Edwards, 2004; Xu and Berrie, 2005).

Life cycle

Crop residues, such as corn (*Zea mays*) stalks, wheat straw, and stems or roots of other crops, are the survival and overwintering sites of FHB pathogens. The asexual spores of the fungus, macroconidia, can be produced on the infested residues and be generally dispersed to other host by rain-splash (Horberg, 2002; Paul et al., 2004). With warm and wet weather conditions, perithecia, the sexual fruiting body of the fungus, may develop on the plant residue. The sexual spores, ascospores, are forcibly discharged from

mature perithecia into the air and are thought to be dispersed by wind. Both macroconidia and ascospores are primary inoculum that can infect wheat under favorable weather conditions (Fernando et al., 1997; Trail et al., 2002). In addition, chlamydospores and hyphal fragments may serve as inoculum in the infection process (Bai and Shaner, 1994). In greenhouse and field experiments, macroconidia are the most commonly used inoculum for FHB evaluation in wheat (Bai and Shaner, 1994).

Air temperature and moisture are the two critical factors for the production of macroconidia and ascospores. The favorable conditions vary for different FHB pathogens. Macroconidial production of FHB causal pathogens, including *F. avenaceum*, *F. culmorum*, *F. graminearum*, and *M. nivale*, were investigated on PDA media under constant temperature ranges (Rossi et al., 2002). Their results showed that the optimum temperature of macroconidial production for *F. culmorum* and *F. graminearum* was 32°C, whereas the optimum temperatures for *F. avenaceum* and *M. nivale*, were 28°C and 26°C, respectively. For ascospore production, the optimum temperature is 15 to 20°C, and soil moisture is 70 to 80% (Xu and Berrie, 2005).

Symptoms and signs

FHB symptoms in wheat, characterized as partially to fully blighted heads and a discolored brown or purple peduncle, appear shortly after anthesis. Later in the season, pink-black colored spores and/or bluish-black perithecia may appear on the infected heads, especially on the rachis and glumes (Schmale III and Bergstrom, 2006). FHB infected grains are often shrunken, rough, and with a bleached or pink appearance (McMullen et al., 1997; Parry et al., 1995).

Management of FHB

Several measures are available for FHB management, including cultural practices, fungicide application, biological control, and breeding for resistance. Each method has both advantages and limits. Appropriate cultural practices, like crop rotation, residue removal, and land preparation can help reduce the primary inoculum by eliminating survival sites of the fungus. However, the current prevalence of low- and no-till practices by farmers might make these options less attractive.

Fungicide control

Fungicide can have an effect when applied to wheat at anthesis. Currently, five fungicides, including propiconazole (Tilt, Bumper, and Propimax), prothioconazole (Proline), tebuconazole (Folicur), metconazole (Caramba), and a premix of tebuconazole and prothioconazole (Prosaro), are US EPA approved and labeled for FHB (Brown-Rytlewski and Naglekirk, 2008; McMullen et al., 2008a). According to the results from the North Central Regional Committee (NCERA-184) of Small Grain Pathologists, Proline, Caramba, and Prosaro are the most effective products (McMullen et al., 2008a).

Fungicide effects on DON accumulation vary from one study to another. Some studies showed that fungicide treated plots had less DON content than the untreated plots (Boyacioglu et al., 1992; Haidukowski et al., 2005; Homdork et al., 2000; Menniti et al., 2003; Pirgozliev et al., 2002). In other studies, fungicide had no effect or even opposite effects on DON accumulations (Milus and Parsons, 1994; Simpson et al., 2001; Siranidou and Buchenauer, 2001). Additionally, wheat usually flowers in a ten-day period, thus, it is difficult to determine the exact application time (Yuen and Schoneweis, 2007).

Moreover, the high cost and non-uniform spray patterns limit its application in the field (McMullen et al., 1999).

Biological control

Recently, research has been conducted on developing biological control for FHB disease. Several approaches are available (Snijders, 2004; Yuen and Schoneweis, 2007): application of microorganisms to crop residues; spraying of biological agents at wheat heads before or during the flowering period; and treatment of infected seeds with antagonists. However, no biological agent for FHB has been registered to date. Some bacteria and yeast strains have been reported to be effective for FHB control, such as *Bacillus*, *Lysobacter*, and *Cryotricoccus* spp. (Jochum et al., 2006; Khan et al., 2001; Schisler et al., 2002). Two isolates of *Paenibacillus polymyxa* inhibited *F. graminearum* colonization of wheat heads by 50% and reduced DON accumulation by 80% in greenhouse experiments (He et al., 2009).

Like fungicides, procedures and application timing are critical for biological agents and field performance may vary in different locations and at different application times (Yuen and Schoneweis, 2007). When combined with fungicide and host resistance, biological control is more effective (Khan et al., 2001).

Host resistance

To date, breeding for resistant wheat genotypes is the most effective and widely used method of FHB management (Gervais et al., 2003). Researchers have identified wheat sources with high levels of FHB-resistance in China, Japan, Brazil, and other countries (Snijders, 1990b; Snijders, 1994). Many of these sources have been extensively used in

breeding programs as resistant parents worldwide, such as ‘Sumai 3’ and ‘Wangshuibai’ from China, ‘Shinchunaga’ and ‘Nobeoka Bouzu’ from Japan, and ‘Frontana’ from Brazil.

When the favorite conditions for FHB are present, a single strategy usually fails because of severe FHB epidemics. McMullen et al. (2008b) have reported that multiple strategies are more effective than single method for FHB management in their study. Additionally, each management strategy has its limitations. Therefore, it requires the integration of all available strategies to achieve the goal of reducing FHB infection and mycotoxin contamination in wheat production (McMullen et al., 2008b).

FHB resistance in wheat

Resistance components

Resistance to FHB can be characterized as two main types: resistance to initial infection (type I) and resistance to pathogen spread in the infected spike (type II) (Schroeder and Christensen, 1963). Type II resistance has been widely studied in wheat and found in a number of wheat genotypes because it is relatively easy to evaluate in the greenhouse by single-floret inoculation. It is assessed by injecting a spore suspension into a single floret of a spike and counting the infected spikelets after a period of time. Type I resistance is evaluated by spraying a spore suspension over spikes at flowering and counting the diseased florets several days later. These procedures are usually done in a greenhouse with controlled conditions during infection. In addition, Type II resistance has been shown to be more stable and to be less affected by nongenetic factors than type I resistance (Bai and Shaner, 1994). In the field, corn grains cultured by *Fusarium*, which

serve as inoculum, can be scattered over the soil surface at the booting stage. Alternatively, wheat heads can be sprayed with a spore suspension at anthesis. However, it might be difficult to distinguish type II from type I resistance under field conditions.

Additionally, three other types of resistance have been proposed: resistance to toxin accumulation (type III), resistance to kernel infection (type IV), and yield tolerance (type V) (Mesterhazy, 1995; Mesterhazy et al., 1999). There are three possible mechanisms for type III resistance: 1) low levels of mycotoxin produced; 2) the degradation of mycotoxin by plant enzymes; 3) or the failure of mycotoxin to move into kernels (Bai and Shaner, 2004). Because of a lack of accurate methods for inoculation and evaluation, the remaining two resistance types, resistance to kernel infection and tolerance, have not been well accepted (Bai and Shaner, 2004).

Inheritance and stability of resistance

Resistance to FHB in wheat is quantitatively inherited and is under the control of several Quantitative Trait Loci (QTL). Type II resistance is relatively stable and highly inherited. It is controlled by a few major genes accompanied by some minor genes (Bai and Shaner, 1994; Buerstmayr et al., 2003; Van Ginkel et al., 1996). Additive gene action is prevalent in resistance, whereas nonadditive effects, including dominance and epistatic effects, might also be available in some cases (Bai et al., 2000; Snijders, 1990a). The significance of additive effects suggests that it is possible to enhance FHB resistance in wheat by pyramiding different resistance genes (Bai et al., 2000; Shi et al., 2008).

The number and location of FHB resistance genes in wheat have been extensively studied (Buerstmayr et al., 1999a; Buerstmayr et al., 2002; Snijders, 1990a; Van Ginkel et al., 1996). However, the results vary with the resistant genotypes studied, evaluation

methods used, and experimental conditions. Even the same resistant genotypes may have diverse numbers of resistance genes in different studies. Kolb et al. (2001) described some possible reasons for the inconsistent results from different studies. The reasons include polygenic inheritance of FHB resistance in wheat, genetic backgrounds, source of resistant parent, types of resistance evaluated, inoculation techniques applied, and genotype \times environment interactions (Kolb et al., 2001).

FHB resistance is quite durable in wheat. Resistant wheat genotypes showed consistent resistance to most isolates of *F. graminearum* worldwide (Miedaner, 1997). Sumai 3, for example, was first released in the 1970s in China (Bai and Shaner, 2004) and has been widely used in breeding programs worldwide. Sumai 3 and its derivatives have been extensively tested in China, Japan, the United States, and many European countries with *F. graminearum* isolates collected worldwide (Bai and Shaner, 2004; Ban, 2001; Kolb et al., 2001). Sumai 3 is still the best in the world for type II resistance. According to the resistance test results of genotypes to different species of *Fusarium*, Mesterhazy (1981) concluded that resistance to *Fusarium* in wheat was not strain- or species-specific. Therefore, it is estimated that the resistance genes in Sumai 3 and other FHB resistance sources will not be overcome by new *Fusarium* strains in the near future (Bai and Shaner, 2004).

Mechanisms of resistance

Passive and active mechanisms of FHB resistance have been identified in wheat (Mesterhazy, 1995). Passive resistance mechanisms are morphological features or structural differences that can help protect wheat from FHB infection. Active resistance,

also known as physiological resistance, inhibits pathogen growth by the production of host chemical compounds after infection.

Passive mechanisms of FHB resistance

Passive resistance mechanisms include several morphological traits, such as the presence of awns, plant height, earliness of maturity, and spikelet density. Snijders (1990e) first reported that FHB resistance was linked to the presence of awns in winter wheat. Recently, the linkage between one resistance QTL and the gene *BI* for the presence of awns has been confirmed (Gervais et al., 2003). However, the linkage is shown to be easily broken and the opposite result has also been reported that genotypes with awns tended to be naturally more severely infected by FHB than awnless ones in wheat (Mesterhazy, 1995). Additionally, a negative correlation has been observed between plant height or flowering date and FHB severity (Gervais et al., 2003; Mesterhazy, 1995). Gilsinger et al. (2005) reported that narrow flower opening is associated with reduction of FHB infection in a RIL population derived from Goldfield (with narrow flower opening) and Patterson (with wide flower opening).

Molecular mechanisms of FHB resistance

Mechanisms of wheat resistance to FHB at the molecular and biochemical level are still unknown. Research has attempted to investigate the differences in expression of induced chemical compounds between resistant and susceptible wheat genotypes. Chen et al. (1999) first reported that constitutive expression of a pathogenesis-related (PR) protein, a rice thaumatin-like protein, had enhanced FHB resistance in transgenic wheat. It was shown later that the expression of pathogenesis-related (PR) proteins PR-1, PR-2 (β -1,3-glucanases), PR-3 (chitinase), PR-4, and PR-5 (thaumatin-like protein) was

induced after *F. graminearum* infection in both susceptible and resistant wheat genotypes (Pritsch et al., 2000; Pritsch et al., 2001). This suggests that general defense response genes may play a role in resistance against *F. graminearum* infection (Kong et al., 2005). Additionally, it was proposed that overexpression of defense response genes in transgenic wheat enhanced the FHB resistance in both greenhouse and field tests (Mackintosh et al., 2007). In a recent study, Golkari et al. (2009) applied cDNA microarrays to identify the differences of gene expression in Sumai 3 and two susceptible Near-Isogenic Lines (NIL). The results showed that 25 genes were differentially expressed and genes encoding PR-2, PR-4 and PR-5 were upregulated in the genotypes with the 3BS region derived from Sumai 3.

In addition to PR proteins, other enzymes, including superoxide dismutase, catalase, phenylalanine ammonia-lyase, peroxidase, ascorbic acid peroxidase, and ascorbic acid oxidase, have also been related to FHB resistance in wheat (Chen et al., 2000). In the microarray study mentioned above, phenylalanine ammonia-lyase was significantly upregulated only in Sumai 3, suggesting that this protein may have an effect on FHB resistance (Golkari et al., 2009). In addition, Steiner et al. (2009) found several genes were differentially expressed in CM82036 (resistant line), Remus (susceptible line) and two lines derived from the cross of CM82036 and Remus. These genes were homologous to the genes encoding UDP-glucosyltransferase, phenylalanine ammonia-lyase, and PR proteins.

During *Fusarium* infection, DON produced by the fungus, is considered to be a virulence factor. Trichodiene synthase, encoded by *TRI5*, catalyzes the first step of trichothecene biosynthesis (Hohn and Beremand, 1989). Desjardins et al. (1996) found

that trichothecene-nonproducing mutants of *G. zeae* with the deletion of *TRI5* gene reduced disease severity in comparison to trichothecene-producing strains (*TRI5+*). Two studies further confirmed the virulence of DON in FHB infection and indicated that DON has an effect on the spread of FHB in a spike, but is not necessary for the initiation of FHB infection (Bai et al., 2002; Jansen et al., 2005). In addition, several studies showed that transgenic wheat with *TRI101*, a gene that reduces toxicity of trichothecene, has reduced disease severity after *Fusarium* inoculation (Alexander, 2008; Okubara et al., 2002).

In summary, FHB resistance in wheat is a complex and quantitatively controlled trait. The signaling pathway is an intricate network involving the interactions between causal pathogens and wheat genotypes. Further studies are necessary to elucidate the resistance mechanisms of FHB.

Molecular mapping of FHB resistance

Molecular marker techniques

Molecular markers include two major types: protein markers, like isozymes, and DNA markers. DNA markers have numerous advantages over protein markers as they are detectable and stable in all tissues and not limited to coding regions. DNA markers are DNA fragments that allow the identification of different genotypes by detecting the differences in their DNA sequences. DNA marker techniques can be divided into two classes: hybridization based techniques and polymerase chain reaction (PCR) based techniques (Agarwal et al., 2008).

Restriction fragment length polymorphisms (RFLP) are based on the differential hybridization of a labeled DNA probe to a DNA fragment digested by a restriction enzyme. DNA sequence polymorphisms occur because of nucleotide substitutions, or DNA rearrangements including insertion or deletion. RFLP markers are relatively highly polymorphic, codominant, highly reproducible, and highly specific. However, this technique is time-consuming, requires a large amount of high quality DNA, and uses radioactive reagents. These disadvantages limited the wide application of RFLP markers and improved the development of PCR-based techniques.

After the discovery of PCR technology, numerous molecular markers based on PCR have been developed. PCR-based molecular markers are superior to hybridization-based techniques. They are quick and easy to use and only a small amount of DNA is required. Several molecular markers are widely used in crops, including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR) or microsatellites, sequence tagged sites (STS), and single nucleotide polymorphisms (SNP). Molecular markers can be applied in the following areas: QTL detection, marker-assisted selection (MAS), germplasm characterization, genome study, and genetic diagnostics (Gupta et al., 1999; Korzun, 2002). It is estimated that at least 36 traits have been elucidated and mapped by molecular markers in wheat (Gupta et al., 1999).

QTL Mapping in wheat

FHB resistant sources have been reported all over the world, including China, Japan, Brazil, and many other countries in Europe. They show variable resistance levels to FHB, controlled by one or two major QTL and a few minor genes in different studies.

However, a genotype with complete resistance to FHB has not been discovered yet. It has been reported that resistance to FHB can be transgressive into progenies when crossing resistant parents (Buerstmayr et al., 1999b; Snijders, 1990d). Thus, it is possible to accumulate resistance genes in genotypes by breeding. So far, many QTL and their tightly linked markers have been identified in resistant genotypes, which can facilitate the breeding process.

QTL in Chinese resistant genotypes

In Chinese resistant sources, Sumai 3 and its derivatives are the most widely used in breeding programs and their resistance QTL have been well identified by many studies. Waldron et al. (1999) detected five QTL using RFLP markers in a population of recombinant inbred lines (RIL) derived from Sumai 3 and Stoa. Three of them were derived from Sumai 3 and two from Stoa. Two QTL on chromosomes 3BS (from Sumai 3) and 2AL (from Stoa) have major effects on FHB resistance. The other three QTL, one on 4BL from Stoa and two on 6BS from Sumai 3, respectively, are minor genes. Anderson et al. (2001) further studied the RIL population from Sumai 3 and Stoa with SSR markers and reported that a major QTL on 3BS explained 41.6% of resistance.

Using RAPD markers, two QTL were reported in a RIL population from the cross of Ning7840 (derived from Sumai 3) and Clark (susceptible) (Bai et al., 1995). In another study of the same population, Bai et al. (1999) found 11 AFLP markers closely linked to a major QTL. Later, this QTL was mapped to chromosome 3BS (Zhou et al., 2002). To facilitate MAS, one AFLP marker on 3BS was converted to a STS marker, a more breeder-friendly marker (Guo et al., 2003). Additionally, Zhou et al. (2002) identified two minor QTL on chromosomes 2BL and 2AS, respectively, from the same population.

CM-82036, also derived from Sumai 3, was studied in a double haploid (DH) population derived from CM-82036 and Remus (Buerstmayr et al., 2002; Buerstmayr et al., 2003). SSR and AFLP markers were used to map QTL for combined type I and II FHB resistance. They confirmed the major effect of the 3BS QTL, which explained up to 60% of the phenotypic variation for type II resistance and 29% for type I resistance. Another major QTL on 5A was also reported, which explained 20% of type I resistance. Thus, they concluded that the 3BS QTL was mainly responsible for FHB resistance to fungal spread and the 5A QTL was mainly associated with the resistance to initial fungal infection. These two major QTL were further validated with transcript-derived fragments (TDFs) in two sister lines from the CM-82036 and Remus cross (Steiner et al., 2009).

In addition to resistance sources from Sumai 3 and its derivatives, several other Chinese resistant genotypes have been mapped for FHB resistance QTL. In a DH population from the cross of W14 (resistant) and Pioneer2684, Chen et al. (2006) confirmed two known major QTL on chromosomes 3BS and 5AS, respectively. The 3BS QTL appeared to have a larger effect on resistance in greenhouse tests, while 5AS QTL had a larger effect in the field. With SSR markers, Somers et al. (2003) identified five QTLs on chromosomes 2DL, 3BS (2 QTL), 4B, and 5AS, respectively, in a DH population derived from Wuhan-1 (resistant) and Maringa. Lines with resistance alleles on 2DL and 3BS reduced fungal spread by 32% after single-floret inoculation. QTL on 3BS and 4B reduced the disease by 27% in the field, and QTL on 3BS and 5A reduced DON accumulation by 17%.

Furthermore, two QTL on chromosomes 3BS and 2DL were detected in resistant wheat Wangshuibai with AFLP and SSR markers (Mardi et al., 2005). Using SSR

markers, Shen et al. (2003b) identified one QTL on 3BS with major effect on FHB resistance and two minor QTL on 2B and 6D in Ning894037, respectively. In Huapei-57-2, Bourdoncle and Ohm (2003) mapped one major QTL on 3BS and three minor QTL on 3BL, 3A and 5B with SSR markers.

QTL in other resistance sources

Using RAPDs markers, Ban (1997) identified two QTL from the Japanese resistant genotype Fukuhokomugi. In a RIL population derived from Chokwang (a Korean resistant genotype) and Clark, one major QTL on chromosome 5AL was detected by SSR and target-region-amplified polymorphism (TRAP) markers (Yang et al., 2005). Two minor QTL on 4BL and 3BS, respectively, were also reported. Frontana is a Brazilian FHB resistant wheat genotype. In a DH population derived from Frontana and Remus, Steiner et al. (2004) mapped a major QTL on 3A, which accounted for 16% of the phenotypic variation for disease severity. Another QTL on 5A explained 9% of the phenotypic variation. In addition, six QTL with smaller effects were located on 1B, 2A, 2B, 4B, 5A, and 6B, respectively. This study also showed that QTL from Frontana primarily contributed to type I resistance.

Fundulea 201R (F201R) is a European FHB wheat resistant genotype. Shen et al. (2003a) detected four QTL located on chromosomes 1B, 3A, 3D, and 5A, respectively, in a RIL population from F201R and Patterson with SSR markers. QTL on 1B and 3A, derived from F201R, had large effects in this study. Another European resistant wheat, Renan, was mapped with SSR, AFLP, and RFLP markers under field conditions (Gervais et al., 2003). Nine QTL were detected in a RIL population derived from Renan and Récital using SSR markers. Three QTL (one on 2B and two on 5A), were stable over

three years of testing. These QTL explained between 6.9% and 18.6% of the resistance. Other minor QTL were located on chromosomes 2A, 3A, 3B, 5D, and 6D.

WSY is a pyramided line that was derived from three resistant parents: Sumai 3, Wangshuibai, and Nobeoka Bouzu (Shi et al., 2008). Sumai 3 and Wangshuibai are FHB resistant genotypes from China, while Nobeoka Bouzu is from Japan. WSY contains FHB resistant QTL from all the three parental genotypes: QTL on 1BL, 2BL, 5AS, and 7AL from Sumai 3; QTL on 2AS, 2DS, 3AS, and 6BS from Wangshuibai; and QTL on 3BS from Nobeoka Bouzu. Shi et al. (2008) concluded that it is possible to accumulate different resistant genes from different resistant genotypes into one wheat line and WSY showed higher FHB resistance than its three parents.

In summary, the QTL on 3BS, with a major effect on FHB type II resistance has been detected in most Chinese resistant genotypes. One QTL on 5A, reported in resistant wheat genotypes worldwide, may have a major effect on FHB type I resistance. Other QTL with smaller effects on FHB resistance have also been reported.

Strategies to develop FHB resistant wheat

Phenotypic selection

Breeding for FHB resistance is aimed at developing superior wheat genotypes with resistance to diseases (FHB and other diseases) and desirable agronomic traits. In the USA, most resistant wheat genotypes used for breeding are from China, Europe or other countries. They usually contain undesirable agronomic traits that are not adapted to the local environments. Additionally, FHB resistance is controlled by several genes and is

highly affected by environmental conditions. Thus, conventional breeding is time-consuming and largely depends on the environment.

Marker-assisted selection (MAS)

Molecular markers used in breeding can greatly help the selection of FHB resistance genes and shorten the length of the breeding period. The principle behind MAS is that a FHB resistant gene can be detected as the DNA sequence differences of diverse wheat genotypes by molecular markers. Once molecular markers are linked to an effective QTL, the QTL can be transferred into other genetic backgrounds by MAS. In addition, MAS may enhance the selection of combinations of multiple genes in one population. However, this new technology is limited by high costs such as those needed for personnel training and for expensive equipment and reagents. Anderson (2007) proposed three criteria for the use of MAS: 1) efficiency/gain must be higher than phenotypic selection; 2) markers must be effective and unique in breeding population; and 3) the cost must be less than that of the conventional breeding.

Transgenic wheat

Although breeding programs have largely improved the performance of wheat resistance to FHB, the resistance level is only partial (Kolb et al., 2001) because only type I and type II resistances are evaluated in most studies. Host resistance to FHB can be enhanced by genetic engineering that can transfer novel genes into wheat. Chen et al. (1999) first reported a transgenic wheat genotype, carrying a rice thaumatin-like protein gene (TLP) that conferred improved FHB resistance in greenhouse tests. Overexpression of PR genes, such as PR-2, PR-3, and PR-5 (TLP), has enhanced FHB resistance in

transgenic wheat (Anand et al., 2003; Mackintosh et al., 2007). For example, a transgenic wheat genotype carrying the *Arabidopsis thaliana* defense response NPR1 gene (AtNPR1), showed a better resistance to FHB (Makandar et al., 2006).

Although a number of wheat genotypes that are highly resistant to FHB have been identified worldwide, most of them are not adapted to the environment in the US mid-Atlantic region. It is urgent to develop and release local resistant wheat genotypes that are highly resistant to FHB and have desirable agronomic traits. The ultimate goal of this research is to develop a wheat cultivar that is locally adapted to the mid-Atlantic region with desirable agronomic traits and enhanced resistance to FHB.

Chapter 2: Evaluation of Scab Resistance Quantitative Trait Loci (QTL) Effects on Wheat

Introduction

Breeding for Fusarium Head Blight (FHB) resistance is one of the most efficient approaches to reduce FHB damage in wheat. Resistance to FHB in wheat is a quantitative trait controlled by several major and minor Quantitative Trait loci (QTL) (Bai and Shaner, 1994; Buerstmayr et al., 2003; Van Ginkel et al., 1996). Most FHB resistant wheat cultivars are of exotic origin and are not adapted to the local environment. They may display undesirable agronomic traits, such as tall plant height, low grain yield, or susceptibility to other diseases including powdery mildew and rusts. Furthermore, the severity of FHB is highly affected by the environment, especially air temperature and humidity. Therefore, it is difficult and time-consuming to develop wheat genotypes that are both locally adapted and highly resistant to FHB solely by using traditional breeding procedures. The use of molecular markers can complement and facilitate wheat breeding programs. For example, several QTL alleles from exotic sources have been introgressed into elite wheat backgrounds using molecular marker assisted selection (MAS) for improved resistance to FHB (Miedaner et al., 2006; Shi et al., 2008).

Ning7840 is a Chinese spring wheat genotype, derived from Sumai 3, which is highly resistant to FHB. Two major QTL have been identified on chromosomes 3BS and 5A in Ning7840 (Gupta et al., 2000). Additionally, a QTL on chromosome 2DL with a minor effect on FHB, found in Wangshuibai and Wuhan-1 (Mardi et al., 2005; Somers et al., 2003), may also be present in Ning7840. McCormick is an elite soft red winter wheat

cultivar adapted to the US mid-Atlantic region with moderate native resistance to FHB (unrelated to that of Sumai 3), high test weight, and high grain yield.

The ultimate goal of this research is to develop a wheat cultivar that is locally adapted to the mid-Atlantic region with desirable agronomic traits and enhanced resistance to FHB. The objective of this project was to study the effect of QTL on a common soft red winter wheat background. Eight Near-Isogenic Lines (NIL) were developed by marker-assisted backcrossing, with three exotic FHB resistance QTL located on chromosomes 3BS, 5A, and 2DL from Ning7840 introgressed into the adapted soft red winter wheat genotype McCormick. The effects on FHB disease and DON accumulation of these three QTL, singly and in combinations, were investigated from different environments that included greenhouse and field evaluations.

Materials and methods

Plant materials

The wheat genotype ‘Ning7840’ (PI 531188), derived from the cross Aurora/An Hui 11// Sumai 3, is a well-known resistance source for scab disease and was used as the QTL donor source of FHB resistance. ‘McCormick’ (PI 632691) (Griffey et al., 2005), a soft red winter wheat, was used as a recurrent parent. A backcross scheme was applied using MAS (Figure 1). McCormick was crossed with Ning7840 in 2004 in the greenhouse at College Park, Maryland. The F1 was backcrossed using McCormick as the female parent in the spring of 2005. Five hundred and sixty-four backcross one (BC1)F1 plants were screened for molecular marker polymorphism and recurrent parent selection. The BC1F1s with the QTL markers from Ning7840 and with the highest similarity to the

recurrent parent were selected. In 2006, the selected BC1F1s were used in crosses with McCormick to derive the BC2F1 generation. The BC2F1s with the Ning7840 QTL markers and with the highest McCormick background were selected for BC2F2s screening. BC2F2s plants that were homozygous for FHB resistance QTL were used to derive NIL. Eight NIL with single and all combinations of three known QTL as well as the two parents were used for field and greenhouse studies in the BC2F3 and BC2F4 generations.

Molecular marker Analysis

To further confirm the presence of the three QTL, young leaves from eight NIL and two parents were collected and sent to Dr. Gina Brown Guedira, USDA-ARS Raleigh (NC) National Genotyping Center for marker tests. Six simple sequence repeats (SSR) markers were used for detecting the donor-QTL alleles 3BS, 5A, and 2DL derived from Ning7840. Two SSR markers were used for each QTL, Umn10 and Gwn533 for QTL on 3BS, Barc186 and Gwm304 for QTL on 5A, as well as Gwm539 and Gwm608 for QTL on 2DL (Liu et al., 2008; Roder et al., 1998; Song et al., 2005).

Disease evaluation

Greenhouse study

A study to evaluate type II resistance of the eight NIL was conducted in April, 2009 in the greenhouse at College Park. Type II resistance was estimated as the spread of the pathogen from the point of inoculation within the spike. An aggressive isolate of *Fusarium graminearum* was provided by Dr. David Van Sanford, Department of Plant and Soil Sciences, University of Kentucky. Macroconidia was produced in liquid

CarboxyMethyl-Cellulose medium (Tuite, 1969). Spore concentrations were calculated using a hemacytometer and adjusted to the desired concentration with sterilized water. The conidia concentration used for inoculation was 50,000 spores per milliliter. A completely randomized design with four replications was used. Four heads of each NIL or the parental genotype was considered a replication.

The single-floret inoculation method was used (Wang and Miller, 1988). One or two heads close to anthesis in each pot were inoculated with 10 μ L of the inoculum. The inoculum was carefully injected into the basal floret of a central spikelet using a pipette. The inoculated spikes were tightly covered by plastic bags for 3 days to maintain high moisture.

At 21 days post-inoculation, plants were evaluated for FHB. The total number of spikelets in the inoculated head and the number of spikelets with FHB symptoms were recorded. Percentage of scabby spikelets (PSS) was calculated as the percentage of diseased spikelets from the total spikelets of the inoculated head. At maturity, inoculated heads for each NIL were carefully collected and threshed by hand. Grains of each NIL were rated for several measurements. One thousand seed weight (1000W) was calculated. *Fusarium*-damaged kernels (FDK) were determined as the percentage of infected seeds in samples. The percentage of scabby seeds by weight (PSW) was calculated by weighing infected seeds from the total seeds in samples. DON content of seed samples for each NIL was evaluated by Dr. Yanhong Dong, Department of Plant Pathology, University of Minnesota.

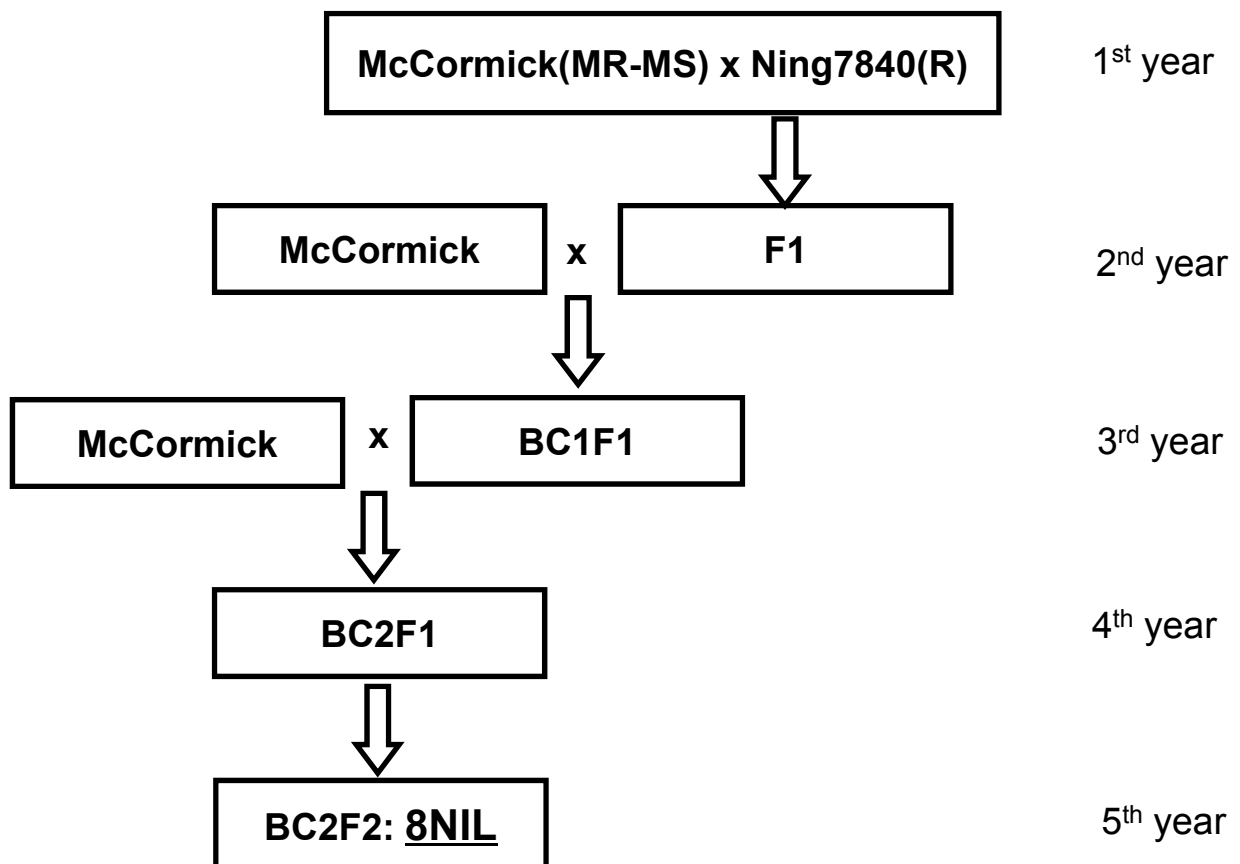


Figure 1. Breeding scheme to derive eight wheat near isogenic lines carrying different FHB QTL.

BC=backcross NIL=Near-isogenic lines

Field studies

In the 2007-2008 season, the BC2F3s were tested at Salisbury, Maryland. In the 2008-2009 season, the BC2F4s were tested at Salisbury, Maryland and Lexington, Kentucky. Thirty seeds of each NIL were sown in 1.2 m long 1-row plots. Planting dates were 4 December 2007 and 13 October 2008 in Maryland, and 21 October 2008 in Kentucky. The experimental design was a randomized complete block design with three blocks in the first year and four blocks in the second year.

Corn kernel inoculum was applied approximately 30 days before flowering in each year. The inoculum for Maryland was provided by Dr. Arvydas Grybauskas, Department of Plant Science and Landscape Architecture, University of Maryland at College Park. After inoculation, a misting system was applied daily to maintain high humidity for two hours (6 to 8 AM) in the morning and two hours in the evening (7 to 9 PM) until maturity.

For FHB evaluation, ten random heads in each row were visually rated for FHB incidence and severity. Incidence is reported as the percentage of heads showing symptoms. Severity is reported as the mean percentage of scabby spikelets of the infected heads in the ten heads. Incidence/severity/kernel damage index (ISK) was calculated by the formula: $0.3 \times \text{incidence} + 0.3 \times \text{severity} + 0.4 \times \text{FDK percentage}$. At maturity, ten heads per row were harvested and threshed manually. In the 2007/2008 Maryland study, all the harvested seeds were used for the measurements and analyses. Two hundred seeds were counted and weighed in 2008/2009. Grains of each NIL were rated for FDK, PSW, 1000W, and DON content.

Statistical analyses

Mean values of disease ratings in each experiment were used in statistical analysis. Least Significant Difference (LSD) values at 5% were used in the comparisons of the eight NIL. Simple correlation coefficients were calculated to estimate the relationships among measurements. Means of PSS and ISK followed a normal distribution and were directly used for correlation analysis. Means of DON contents in the greenhouse study had outliers that deviated from a normal distribution and were normalized by logarithmic transformation for correlation analysis. Statistical analyses were conducted on data using Proc GLM of the Statistical Analysis System version 9.1 (SAS Institute Inc., Cary, NC).

Results

Development of NIL with FHB QTL

Eight homozygous NIL derived from crossing Ning7840 and McCormick containing single and all combinations of three known QTL were detected and confirmed with SSR markers. Table 1 shows marker data of the parents and derived NIL. Additional screening with SSR markers (data not shown) indicated that the selected BC1F1 were 60% homozygous for McCormick background and the BC2F2, from which the NIL were derived, were 90% homozygous for McCormick background.

Greenhouse study

Eight NIL and two parental genotypes were evaluated for type II resistance to FHB in 2009. All the inoculated wheat lines showed FHB symptoms after single-point inoculation, indicating that the inoculation was successful. Significant variation among

the eight NIL was observed for all measurements including PSS, FDK, PSW, 1000W and DON content ($P < 0.0001$) (Table 2 and Table 3).

PSS is the percentage of scabby spikelets, which indicates the disease severity after inoculation PSS ranged from 10.6% to 89.6% for the eight NIL. NIL with at least one donor-QTL alleles showed significantly lower PSS than the NoQTL NIL. The 3BS+2DL NIL had the lowest value of PSS (10.6%), which represented a reduction of disease severity of 88% in comparison to the NoQTL NIL. Additionally, the 3BS and 3QTL NIL had similar PSS values as the 3BS+2DL NIL. Among the three NIL with a single QTL, the 3BS NIL had significantly lower PSS compared to the 2DL NIL. The 3BS NIL also showed lower PSS than the 3BS+5A, 5A+2DL, and 5A NIL, although this difference was not statistically significant.

The NoQTL NIL had significant higher DON content than any of the other lines. However, only the 3BS+2DL (0.4 ppm) and 3QTL (0.5 ppm) NIL had DON content lower than 1 ppm, which is the maximum allowed level set by FDA for human food products. In comparison to the NoQTL NIL, the 3BS+2DL NIL had a reduction of 99% in DON accumulation in the grains.

The results of PSW were similar to those for DON content. The NoQTL NIL had a significantly higher PSW compared to the other seven NIL. Additionally, the 3QTL and 3BS+2DL NIL had the lowest PSW (0.7%), which is only 1% of the value observed for the NoQTL NIL.

All the lines with donor-QTL allele from Ning7840 showed significant lower FDK values than the NoQTL NIL. The mean values of FDK ranged from 2.1% to 82.2%. The 3BS+2DL NIL had the lowest FDK value (2.1%), although it was not significantly

different to the 3BS and 3QTL NIL. The FDK value of the 3BS+2DL NIL represented a reduction of 97% compared to the NoQTL NIL.

The average values of 1000W ranged from 4.0g to 22.8g. Similar to the other measurements, all the seven NIL with at least one QTL resistant allele had significantly higher 1000W value than the NoQTL NIL. The three NIL, 3BS, 3BS+2DL, and 3QTL had the highest 1000W and no significant differences were observed among them.

To estimate the relationship between disease ratings and DON accumulation, simple linear correlations were conducted on PSS, FDK and DON content. Significant high positive correlations between disease ratings and DON accumulation were observed ($r = 0.82$, $P < 0.0001$ for PSS; and $r = 0.88$, $P < 0.0001$ for FDK) (Figure 2A and B). This suggests that higher DON content can be expected in the genotypes with higher PSS or FDK.

Field studies

Field studies were conducted for two years (2008 and 2009) in Maryland and for one year (2009) in Kentucky to evaluate the field resistance to FHB of eight wheat NIL. The two field studies in 2009 used the same rating system, which included disease incidence, disease severity, FDK, ISK, DON content (ppm), and 1000W (g). In the 2008 Maryland study, maturity masked the disease appearance because of late planting, thus FHB incidence and severity were not recorded.

Analysis of variance (ANOVA) was conducted for the three field studies including two years in Maryland and one year in Kentucky. Results of ANOVA for the three environments are presented in Table 4 and Table 5. Mean squares of the interaction

between NIL and the environments were significant for most measured FHB traits except for DON and 1000W.

Two-year study in Maryland: First year (2008)

Significant differences were observed among the eight NIL for PSW and DON content but not for FDK ($P = 0.0507$) and 1000W ($P = 0.1342$) (Table 6).

The mean values of PSW ranged from 4.0% to 13.6%. The 3BS+2DL NIL had the lowest PSW value (4.0%), which was significantly lower than the 2DL and NoQTL NIL. The 2DL NIL had the highest PSW value. It significantly differed from all the NIL with at least two QTL, but not from the lines with only one donor-QTL or no QTL.

The mean values of DON content varied from 1.4 to 6.8 ppm. The 3BS NIL had the highest DON content (6.8 ppm), which was similar to the 2DL NIL. In contrast, the 3BS+2DL NIL had the lowest DON content (1.4 ppm), but it differed only significantly from the NIL with 3BS, 2DL, or no QTL.

Second year (2009)

FHB incidence and severity were estimated visually in the field, while FDK, PSW, DON content, 1000W and ISK were calculated or analyzed in the lab. Eight NIL showed highly significant differences for all FHB-related traits (Table 7, Figure 3).

The mean values of FHB incidence ranged from 7.8% to 45.6%. The NoQTL NIL had the highest incidence (45.6%), although it was not significantly different from the 3BS and 3BS+5A NIL. All the other five NIL had significant lower incidence than the NoQTL line. Among them, the 3BS+2DL NIL had the lowest incidence (7.8%), which represented about 83% of disease reduction in comparison to the NoQTL NIL. The 3QTL NIL and the 2DL NIL were not significantly different from the 3BS+2DL NIL.

Table 1. Size (in base pairs) of DNA fragments derived from SSR markers of 8 NIL and 2 parents on three wheat chromosomes.

	3BS		5A		2DL	
	umn10	gwm533	barc186	gwm304	gwm539	gwm608
Ning 7840	239	145	213	217	126	152
McCormick	228	147	203	199	135	150
3BS	239	145	203	199	135	150
5A	228	147	213	217	135	150
2DL	228	147	203	199	126	152
3BS+5A	239	145	213	217	135	150
3BS+2DL	239	145	203	199	126	152
5A+2DL	228	147	213	217	126	152
3QTL	239	145	213	217	126	152
NoQTL	228	147	203	199	135	150

Table 2. Mean squares from the analysis of variance for FHB infection measured in the greenhouse at College Park (Maryland) in 2009.

Source of variation	df	PSS (%)	DON (ppm)	FDK (%)	PSW (%)	1000W (g)
NIL	7	2504***	2595***	2808***	1761***	140***
Error	24	174	267	58	118	16

***: Significant at $P < 0.001$

Table 3. Mean values of FHB traits for eight wheat NIL measured in the greenhouse at College Park (Maryland) in 2009.

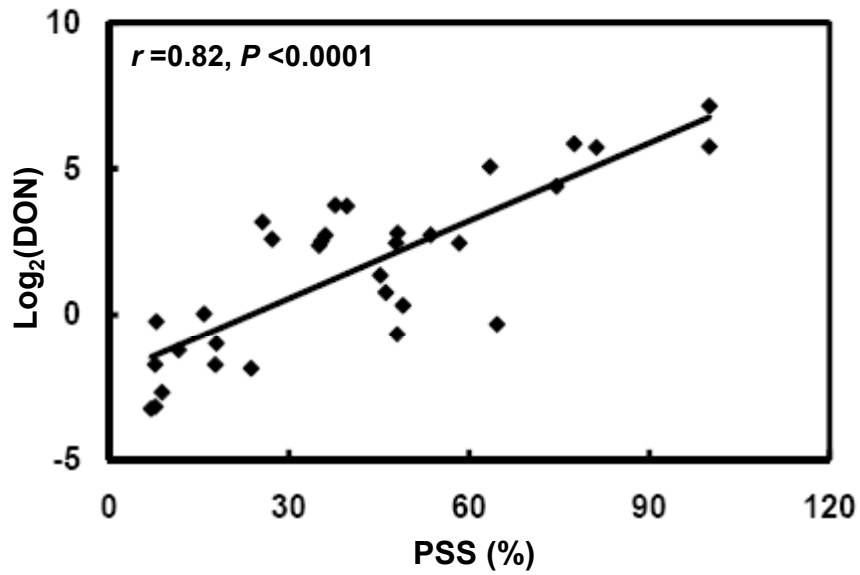
	PSS (%)[†]	DON (ppm)	FDK (%)	PSW (%)	1000W (g)
NIL:					
3BS	27.2	1.1	4.6	1.4	22.8
5A	45.9	9.8	34.5	10.4	12.2
2DL	58.3	6.0	13.3	7.6	13.7
3BS+5A	37.9	13.6	21.4	11.3	12.0
3BS+2DL	10.6	0.4	2.1	0.7	18.8
5A+2DL	43.2	6.0	23.9	7.1	13.2
3QTL	17.3	0.5	2.8	0.7	20.3
NoQTL	89.7	76.1	82.2	63.7	4.0
LSD[‡]	19.2	23.8	11.1	15.8	5.8
CV (%)[§]	31.9	115.1	33.0	84.5	27.2
Parents:					
Ning7840	5.8B	0.2B	1.8B	0.6B	37.4B
McCormick	97.8A	141.5A	88.1A	76.6A	5.7A

[†] : Means with the same letter are not significantly different at $P < 0.05$

[‡] : Least significant difference at $\alpha=0.05$

[§] : Coefficient of Variation

A



B

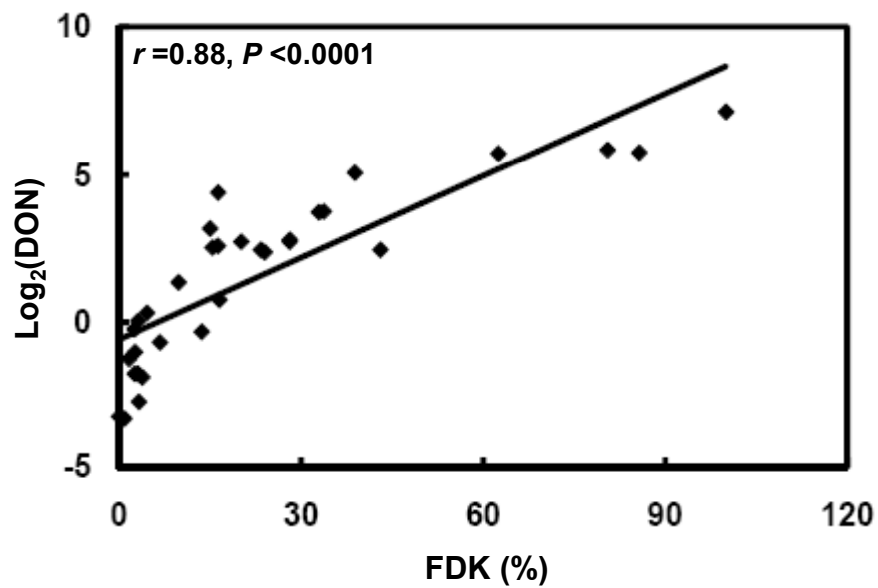


Figure 2. Association between FHB ratings A. PSS (%) and B. FDK (%) with DON content (ppm) in the greenhouse study (2009). The solid lines show the correlation.

Table 4. Mean squares from the analysis of variance for FHB infection measured in three field studies conducted in Maryland and Kentucky.

Source of variation	df	DON (ppm)	FDK (%)	PSW (%)	1000W (g)
NIL	7	37.9***	207***	122***	12.6
Environments (E)	2	656.6***	5486***	3783***	958.9***
NIL*E	14	9.3	114***	82***	9.6
Block(E)	8	30.9***	88***	39***	32.1***
Error	248	5.0	16	11	7.2

***: Significant at $P < 0.001$

Table 5. Mean squares from the analysis of variance for FHB infection measured in 2009 in Salisbury (Maryland) and Lexington (Kentucky).

Source of variation	df	Incidence (%)	Severity (%)	ISK
NIL	7	5528***	4109***	2447***
Location (L)	1	224096***	103598***	68522***
NIL*L	7	922***	1745***	226***
Block(L)	6	1888***	815***	453***
Error	234	237	187	60.5

***: Significant at $P < 0.001$

Table 6. Mean values of FHB traits for eight wheat NIL measured in the field in Maryland (2008).

QTL	DON (ppm)[†]	FDK (%)	PSW (%)	1000W (g)
NIL:				
3BS	6.8	13.6	9.0	15.8
5A	2.5	10.0	8.1	13.1
2DL	5.9	18.4	13.6	14.5
3BS+5A	2.9	6.4	4.0	13.6
3BS+2DL	1.4	6.1	4.0	15.0
5A+2DL	2.9	8.7	6.3	17.7
3QTL	2.3	7.5	4.9	17.3
NoQTL	4.5	14.1	11.3	17.0
LSD[‡]	2.0	NS [§]	5.9	NS
CV (%)[‡]	30.6	43.0	44.0	13.7
Parents:				
Ning7840	0.6B	5.8	3.7	14.7
McCormick	5.3A	12.2	8.7	15.8

[†] : Means with the same letter are not significantly different at $P < 0.05$

[‡] : LSD=Least significant difference at $\alpha=0.05$

[§] : NS=No significant differences were observed among NIL

[‡]: Coefficient of Variation

Table 7. Mean values of FHB traits for eight wheat NIL measured in the field in Maryland (2009).

QTL	Incidence (%)[†]	Severity (%)	DON (ppm)	FDK (%)	PSW (%)	1000W (g)	ISK
NIL:							
3BS	45.0	9.1	5.4	7.5	4.6	25.2	9.2
5A	31.3	6.9	4.4	7.1	5.0	22.6	14.3
2DL	17.8	4.0	4.5	7.3	4.9	23.2	9.5
3BS+5A	40.6	6.7	3.2	5.0	3.3	24.5	16.2
3BS+2DL	7.8	1.1	1.2	1.5	1.0	25.3	3.3
5A+2DL	25.0	6.8	4.4	8.0	5.4	25.3	12.7
3QTL	13.4	1.7	2.8	4.0	2.5	24.8	6.1
NoQTL	45.6	16.1	7.5	13.2	8.6	24.7	23.8
LSD[‡]	10.2	4.2	1.3	2.1	1.5	1.6	4.4
CV (%)[§]	51.2	91.4	45.7	44.3	49.1	9.2	47.3
Parents:							
Ning7840	2.5B	0.2B	0.4B	1.1B	0.6B	21.6	1.3B
McCormick	53.3A	17.0A	3.6A	7.8A	5.1A	23.8	24.2A

[†] : Means with the same letter are not significantly different at $P < 0.05$

[‡] : LSD=Least significant difference at $\alpha=0.05$

[§] : Coefficient of Variation

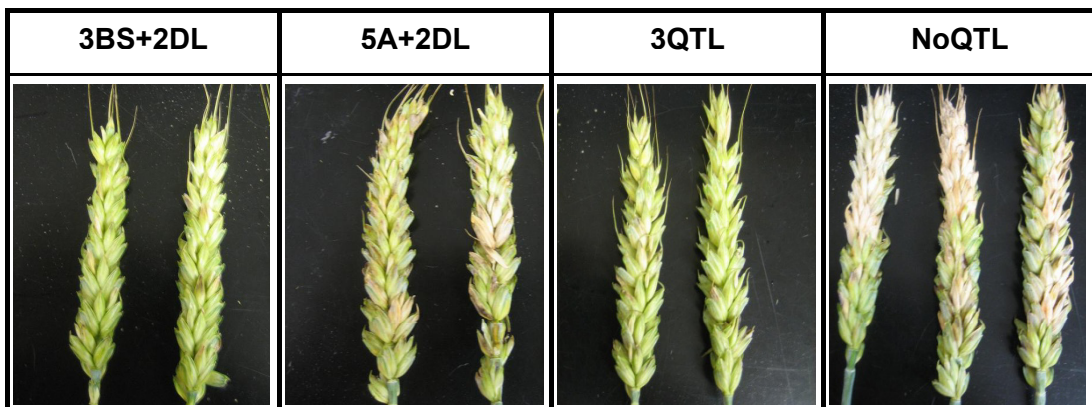
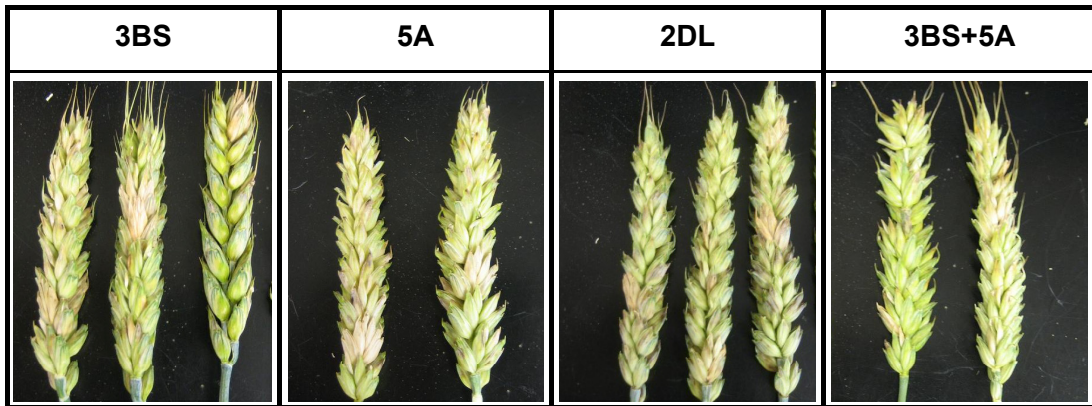


Figure 3. Infected heads collected from the field at Salisbury (Maryland) in June, 2009 for each wheat NIL.

The 3BS+2DL NIL had the lowest FHB severity (1.1%), reducing the disease by 93% in comparison to the NoQTL NIL. Among the eight NIL, all the lines with at least one QTL had significantly lower severity values than the NoQTL NIL. Two NIL, 3QTL and 2DL, showed similar resistance to the best line.

DON content ranged from 1.2 to 7.5 ppm. Similar to the results of FHB severity, NIL with at least one QTL were significantly different from the NoQTL NIL. Among them, the 3BS+2DL NIL had the lowest DON accumulation (1.2 ppm), reducing the DON content by 84% when compared to the NoQTL line. The second lowest DON content was observed in the 3QTL NIL (2.8 ppm).

One-thousand kernel weight ranged from 22.6 to 25.3 grams. A higher 1000W generally indicates that there are less blighted grains in a sample. The line with a single QTL on 5A had the lowest 1000W (22.6 g), which was similar to the 2DL NIL (23.2 g). All the other six NIL including the NoQTL one had higher 1000W than the 5A NIL.

The results of FDK and PSW were similar. The NoQTL NIL had the highest value for both FDK (13.2%) and PSW (8.6%). Additionally, all the lines with at least one QTL were significantly different to the NoQTL NIL for both FDK and PSW. The best line with the lowest FDK (1.5%) and PSW (1.0%) was the 3BS+2DL NIL, although it did not differ significantly from the 3QTL NIL in the case of PSW. The 3BS+2DL NIL had a disease reduction of 88% for both FDK and PSW compared to the NoQTL NIL.

ISK is an index that combines incidence, severity, and Fusarium damaged kernels. The mean ISK values ranged from 3.3 to 23.8. All the NIL with donor-QTL from the resistant parent showed reduced disease compared to the NoQTL NIL. The best line with

the lowest ISK value was the 3BS+2DL NIL (3.3). Additionally, the 3QTL NIL (6.1) had a similar effect on ISK to the 3BS+2DL NIL.

Significantly positive correlations were observed between DON content and disease ratings ($r=0.61$, $P < 0.0001$ for ISK; and $r=0.90$, $P < 0.0001$ for FDK) (Figure 4A and 5A). This result indicated that DON accumulation increased with more severe disease epidemics in the field.

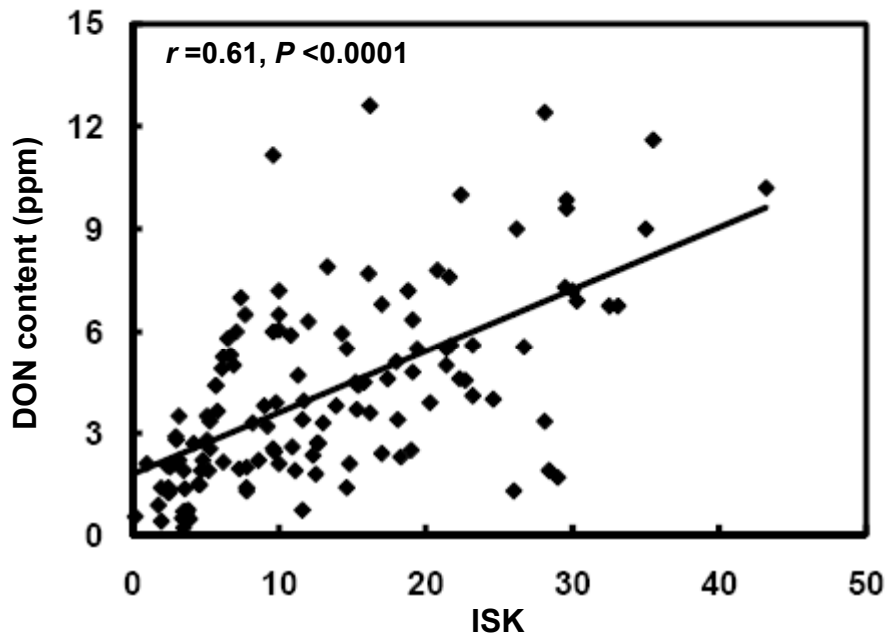
Field study in Kentucky (2009)

There were higher values for all FHB-related traits in Kentucky, but the trends were similar to those observed in Maryland (Table 8). Disease incidence ranged from 56.5% to 99.4%. The 3BS+2DL NIL had the lowest incidence (56.5%), which was significantly different from any of the other NIL. The NoQTL NIL showed the highest incidence (99.4%) in the field, which differed significantly from the 3QTL NIL, the 5A NIL, and the 3BS+2DL NIL. The most resistant line, 3BS+2DL, showed a reduction in disease incidence of 43% compared to the NoQTL NIL.

The means of FHB severity ranged from 13.9% to 69.3%. Among the eight NIL, the NoQTL and the 3BS NIL had significant higher disease severity than the other NIL. The best line was the 3BS+2DL (13.9%) that had an 80% reduction in severity compared with the NoQTL NIL. The 3QTL NIL was the second best line (27.3%), which showed better resistance than other lines except for the 3BS+2DL NIL.

The 3BS+2DL NIL (4.9 ppm) had the lowest DON content in its kernels, followed by the 3QTL NIL (6.4 ppm). Additionally, the 3BS+5A NIL (7.5 ppm) was not significantly different from the 3QTL NIL. Except for the 2DL NIL, all the other lines with at least one QTL had significantly lower DON accumulation than the NoQTL NIL (11.2 ppm).

A



B

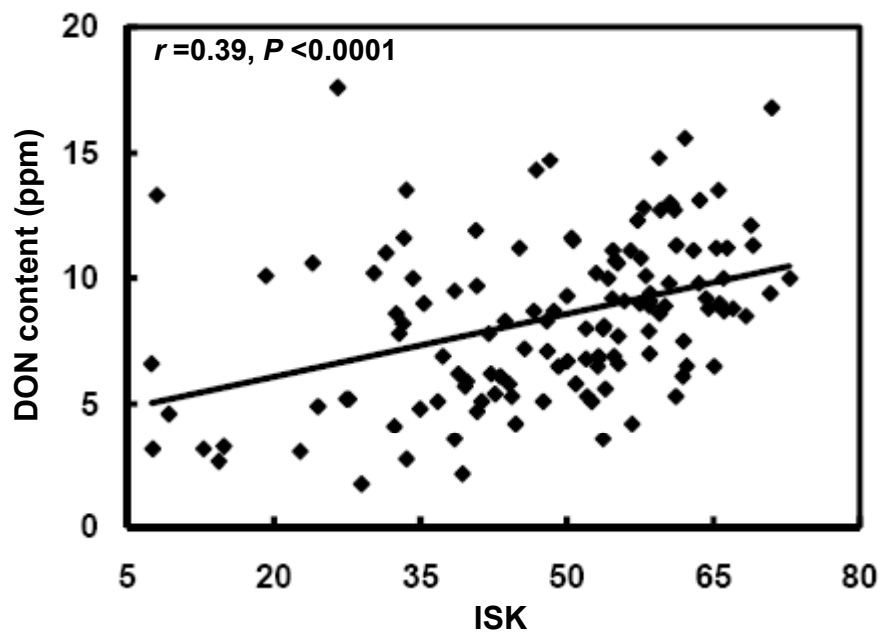
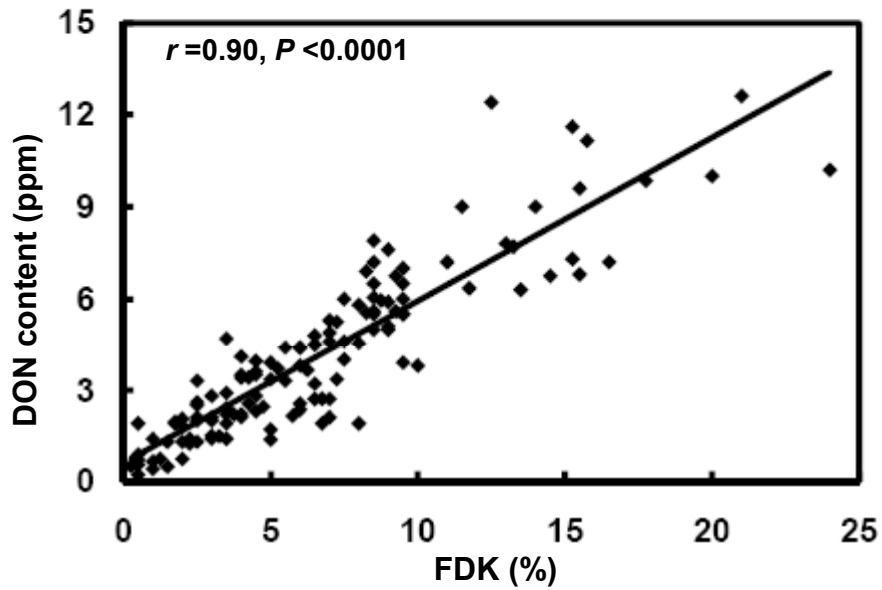


Figure 4. Association between ISK and DON content (ppm) for eight wheat NIL in the field study (2009) for A, Maryland and B, Kentucky. The solid lines show the correlation.

A



B

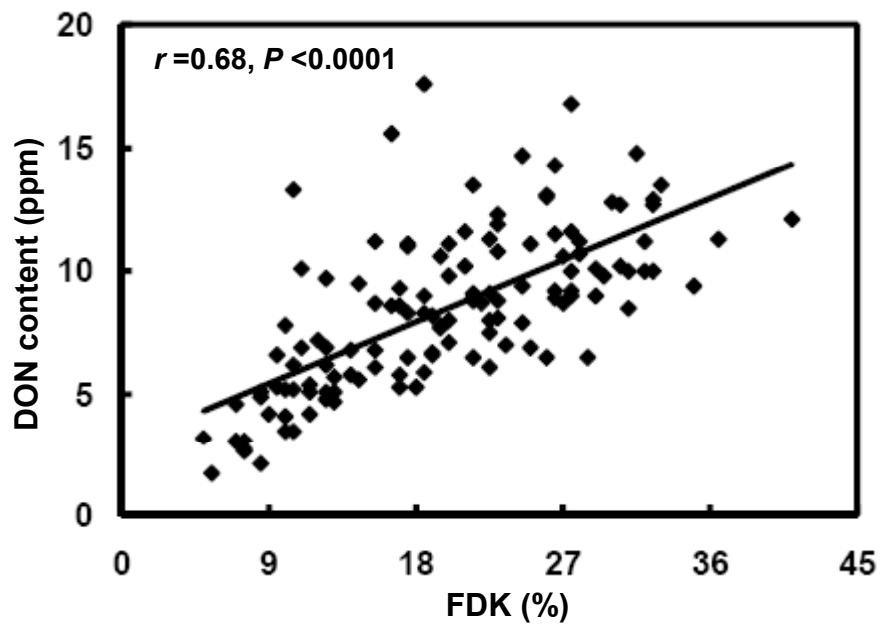


Figure 5. Association between FDK (%) and DON content (ppm) for eight wheat NIL in the field studies (2009) for A, Maryland and B, Kentucky. The solid lines show the correlation.

Table 8. Mean values of FHB traits for eight wheat NIL measured in the field in Kentucky (2009).

QTL	Incidence (%)[†]	Severity (%)	DON (ppm)	FDK (%)	PSW (%)	1000W (g)	ISK
NIL:							
3BS	98.8	64.8	8.4	19.7	15.5	23.0	56.9
5A	83.8	44.7	9.4	23.3	18.4	18.0	47.9
2DL	95.0	54.2	10.4	24.6	19.2	20.0	54.6
3BS+5A	91.8	53.9	7.5	15.9	12.3	20.0	50.1
3BS+2DL	56.5	13.9	4.9	9.2	6.6	22.7	24.8
5A+2DL	88.3	46.1	9.2	21.2	16.2	21.3	48.8
3QTL	86.3	27.3	6.4	13.1	9.7	22.4	39.3
NoQTL	99.4	69.3	11.2	30.5	23.8	20.8	62.8
LSD[‡]	11.6	12.8	1.7	3.1	2.8	1.9	6.7
CV (%)[§]	18.9	39.0	29.4	22.5	26.0	13.0	20.0
Parent[‡]:							
McCormick	81.5	40.3	8.7	20.8	15.2	20.4	44.8

[†] : Means with the same letter are not significantly different at $P < 0.05$

[‡] : LSD=Least significant difference at $\alpha=0.05$

[§] : Coefficient of Variation

[‡] : Ning7840 did not survive in the field

Similar to the results in the greenhouse and the Maryland field study, the best line with the lowest FDK was the 3BS+2DL NIL (9.2%) and the most susceptible was the NoQTL NIL (30.5%). Furthermore, both the best and worst lines showed significantly differences from the other NIL. Two NIL, 3QTL and 3BS+5A, had lower FDK values than other NIL except for the 3BS+2DL line.

PSW had similar trends as the those for FDK. The means of PSW ranged from 6.6% to 23.8%. The NoQTL NIL had the highest PSW value (23.8%). The 3BS+5A NIL and the 3QTL NIL had lower PSW than the other NIL except for the 3BS+2DL NIL. As observed before, the best genotype was the 3BS+2DL NIL (8.6%), which had significantly less PSW than all the other NIL.

The mean values of ISK ranged from 24.8 to 62.8. Except for the 3BS NIL, all the NIL with at least one QTL had significantly lower ISK values than the NoQTL NIL. The 3BS+2DL NIL had the lowest value of ISK (24.8) and was significantly different from all the other NIL. The 3QTL NIL (39.3) was the second best line.

Positive correlations that were similar to the 2009 field study in Maryland, were observed between DON content and disease ratings ($r=0.39$, $P<0.0001$ for ISK; and $r=0.68$, $P<0.0001$ for FDK) (Figure 4B and 5B).

Discussion

Eight newly-derived wheat NIL with different QTL combinations were evaluated for their resistance to FHB in four environments. Results were consistent across the one-year greenhouse study and two-year field studies at two locations. In the greenhouse study, the single-floret inoculation method was used. This method mainly evaluates type II resistance or resistance to pathogen spread (Wang and Miller, 1988). In the field studies,

Fusarium-infected corn kernels were used as the inoculum and a misting system was applied to favor the development of the disease. Field resistance comprises resistance to initial infection (type I resistance) and resistance to pathogen spread (type II resistance) (Miedaner et al., 2006).

Assessment of disease incidence and severity

In the greenhouse, three out of the four NIL with 3BS showed significant lower PSS than other NIL, indicating 3BS was the major QTL responsible for type II FHB resistance. Type II resistance is referred as prevention of pathogen spread in the infected spike (Schroeder and Christensen, 1963). Using SSR and AFLP markers, Zhou et al. (2004) mapped a major FHB resistance QTL on chromosome 3BS that is associated with type II FHB resistance in ‘Wangshuibai’ wheat. Similarly, the 3BS locus originated from the scab-resistant wheat ‘Sumai 3’ was characterized as the major type II FHB resistance QTL (Buerstmayr et al., 2002; Buerstmayr et al., 2003).

In contrast, when these NIL were evaluated in the field, 3BS exhibited only a relatively mild effect on FHB resistance. Although there was a small improvement in reduction of disease severity, no differences were observed in FHB incidence between the 3BS NIL and the NoQTL NIL in both field studies in 2009. In the wheat cultivar ‘W14’, 3BS only displayed a logarithm of odds or LOD score of ~2.85 in the field, which was significantly lower than the 5AL QTL of W14 (Chen et al., 2006).

Interestingly, the combination of two QTL, those on 3BS and 2DL, had the lowest FHB severity in both greenhouse and field studies, showing even less disease severity than the 3QTL NIL. To explain this result, there are several possible reasons: 1) 5A is not additive for FHB type II resistance; 2) there is an interaction between the 5A allele and

the other two QTL alleles; or 3) an epistatic effect is shown here. Similarly, Somers et al. (2003) also reported that lines carrying the 3BS+2DL QTL reduced disease spread by 32% compared to the mean of a segregating population after single-floret inoculation.

Effects of QTL on resistance to kernel infection

Fusarium-damaged kernels tend to be shriveled, small, light, and with white or pink coloration. FDK, which directly indicates the damage level of wheat kernels by FHB, is used to evaluate the resistance to kernel infection or type IV resistance. Lines carrying 3BS, 3BS+2DL, or all three QTL, showed similar results for both FDK and 1000W after single-floret inoculation. This indicates that the QTL on chromosome 3BS plays a major role in the resistance to kernel infection, whereas 2DL and 5A have minor effects to control kernel damage. Li et al. (2008) suggested that type I and type II resistance QTL may have effects on the resistance to kernel infection as well. This was also observed in this study. The result of FDK was consistent with that of the FHB severity measured by the PSS after single-floret inoculation.

In three field studies, the 3BS+2DL NIL had the lowest FDK value, which was even lower than the 3QTL NIL. This indicates that 5A had little effect or even a reverse effect on FDK. Additionally, the NIL with a single QTL either on 5A or on 2DL had the lowest values for 1000W, which were even lower than the NoQTL NIL. This indicates that 5A and 2DL may be associated with decreased 1000W. McCartney et al. (2007) also reported that the QTL on chromosome 5AS of Sumai 3 was related to 1000W reduction in their field studies.

Effects of QTL on resistance to DON accumulation

DON is a mycotoxin produced by *Fusarium* fungi in FHB infected kernels. The resistance to toxin (especially DON) accumulation is considered type III resistance (Mesterhazy, 1995). In this study, the effects of the three introduced QTL alleles on DON content were tested in both greenhouse and field studies. The DON contents of the NoQTL NIL were significantly higher than any of the other seven NIL in both greenhouse and the 2009 field studies in Maryland. These results indicated that any of the three QTL: 3BS, 2DL, or 5A, would increase the type III resistance in wheat.

A comprehensive study of the cultivars Wangshuibai and Wheaton identified 3BS as the major QTL associated with DON resistance (Yu et al., 2008). Also, a significant association was observed between DON resistance and 3BS in a field study (Lemmens et al., 2005). Similarly, my study showed that most NIL with 3BS had drastically reduced DON content in the infected kernels in comparison to the NIL with no QTL. One of the main toxic effects of DON in eukaryotic cells is the inhibition of protein synthesis (Rocha et al., 2005). It has been shown in *Arabidopsis thaliana* that the conjugation of DON with glucoside significantly reduced this toxic effect (Poppenberger et al., 2003). Therefore, these three FHB-resistance QTL may be involved in the conjugation and detoxification of DON. For example, 3BS has been proposed to encode a DON-glucosyltransferase based on the observation that this QTL was positively correlated with the DON-3-glucoside/DON ratio (Lemmens et al., 2005).

In this study, the other two QTL, 2DL and 5A, also contributed to DON resistance. In most cases, NIL containing either of these two QTL displayed significant reduction in DON content compared with the no-QTL genotype. These effects are consistent with the

results from other studies. For example, a QTL analysis of a recombinant inbred line population derived from the cross of Veery and CJ 9306, found that 2DL explained up to 20% of the phenotypic variation in DON content (Jiang et al., 2007). 5AS from Sumai 3 or Wuhan-1 was also identified as one of the major QTL associated with DON resistance (Miedaner et al., 2006; Somers et al., 2003).

Improved effects are usually observed in NIL containing two or more FHB-resistance QTL. Miedaner et al. (2006) showed that 3B+5A reduced DON accumulation by 78% in comparison to the susceptible line in the field. I observed the lowest DON contents in the 3BS+2DL NIL and 3QTL NIL across one greenhouse and three field tests. Furthermore, in my three field studies, the 3BS+5A NIL was similar to the 3QTL NIL.

Association between FHB ratings and DON accumulation

DON content was correlated with disease ratings in one greenhouse and two field studies. The results showed that both type II and field resistance were significantly correlated with DON accumulation. This result indicated that selection for type II or field resistance may simultaneously improve the resistance to DON accumulation. On the other hand, DON is proposed to be a virulence factor that can affect fungal spread in the spikes but is not required for disease initiation (Bai et al., 2002; Jansen et al., 2005). Additionally, Lemmens et al. (2005) reported that DON content was associated with type II resistance, but not with type I resistance. Because field resistance is the combination of both type I and type II resistance, it is unclear whether type I resistance is correlated with DON content. Therefore, NIL could be tested by spray inoculation in the greenhouse for type I resistance in the future to estimate the effects of different QTL on type I resistance.

Implications for breeding for FHB resistance

FHB resistance QTL alleles from exotic sources can be introgressed into common wheat backgrounds for improved resistance to FHB (Miedaner et al., 2006; Shi et al., 2008). However, the introgression of target QTL is often found to be associated with linkage drag from the donor (Jacobsen and Schouten, 2007). Therefore, wheat breeders search for the best allele combination that contains minimal exotic genetic background to develop new cultivars. In this study, the 3BS+2DL and 3QTL NIL had higher FHB resistance and lower DON content than the other NIL, but the 3QTL NIL did not show better FHB resistance than the 3BS+2DL NIL. Taken together, the results indicate that stacking 3BS+2DL would be beneficial for breeding wheat for FHB resistance without the need of having possible undesirable effects from linkage drag of 5A.

Conclusions

Eight NIL, with all the combinations of three resistance QTL alleles from Ning7840, were evaluated in a one-year greenhouse study, a two-year field study in Maryland, and a one-year field study in Kentucky. Taken together, the 3BS+2DL NIL showed higher FHB resistance and lower DON content in all studies. This suggests that the 3BS+2DL NIL can be used in the mid-Atlantic region to breed for improved FHB resistance. Moreover, positive correlations were observed between DON content and disease rating in both greenhouse and field studies. Therefore, the selection for type II or field resistance can improve the resistance to DON accumulation.

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