VALIDATION AND MARKER-ASSISTED SELECTION OF TWO MAJOR QUANTITATIVE TRAIT LOCI CONDITIONING FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

By

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Jianli Chen

ABSTRACT

Fusarium head blight (FHB) is one of the most destructive diseases of common wheat (*Triticum eastivum* L.) worldwide. Resistance to FHB is an ideal trait for which molecular marker assisted selection (MAS) would facilitate breeding and cultivar development efforts. Validation of quantitative trait loci (QTL) conferring FHB resistance is a prerequisite for MAS. This study was conducted to validate and evaluate the effect of two major QTL, previously reported on chromosomes 3BS and 5AS, on multiple FHB resistance components in two independent studies, one involving a mapping population derived from a cross between a known resistance source W14 and a susceptible soft red winter (SRW) wheat cultivar Pioneer2684, and the other involving seventy adapted SRW wheat lines. The first study confirmed that the 3BS and 5AS QTL were significantly associated with FHB resistance and further indicated that the 3BS QTL has a larger effect on three FHB resistance components (type II and III resistance and resistance to Fusarium Damaged kernels) evaluated in greenhouse experiments, while the 5AS QTL has a larger effect on type I resistance evaluated in a field experiment. Six simple sequence repeat (SSR) and two sequence targeted site (STS) markers associated with FHB resistance in the two QTL regions identified in the first experiment were then used to characterize FHB QTL marker haplotypes and their effect on FHB resistance in seventy wheat genotypes. Five main haplotype groups (1-5) were characterized among the elite lines on the basis of allelic differences of four

marker loci linked to the 3BS QTL and two marker loci linked to the 5AS QTL. Haplotype group 5 was comprised of marker allele combinations of both 3BS and 5AS QTL and elite lines with this haplotype have improved type I and type II resistance compared to the other haplotypes. This again validated the presence of QTL on chromosomes 3BS and 5AS, and illustrated the utility of SSR and STS markers in the two QTL regions in selection of FHB resistance in elite backgrounds. Four favorable marker alleles including two (Xbarc133 and XSTS3B142) on 3BS and two (Xbarc117 and Xbarc056) on 5AS are recommended for MAS of the two QTL for improved FHB resistance in wheat. Wheat lines having favorable marker alleles identified in the current study will provide breeding programs with a source of unique and adapted FHB resistant parents and some of the lines also may have potential for release as cultivars.

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CHAPTER I: LITERATURE REVIEW

Present and Future of Quantitative Trait Locus Analysis for Fusarium Head Blight Resistance in Wheat

ABSTRACT

Fusarium head blight (FHB) is one of the most destructive diseases of common wheat (*Triticum eastivum* L.) worldwide. Resistance to FHB is controlled by quantitative trait loci (QTL) that have been studied intensively during the past five years. Major progress made to date includes the identification and validation of three major QTL on chromosomes 3BS, 5AS, and 6BS in known resistance sources. The genetic map of 3BS QTL has been saturated with DNA markers and positional cloning has been initiated for this QTL. In addition to these QTL, an additional fifteen chromosome regions were postulated as conferring FHB resistance in very diverse backgrounds including known spring wheat resistance sources and adapted winter wheat sources (native sources) of different origin. The most significant applications of FHB QTL analysis seem to be marker assisted selection (MAS) in breeding and pre-breeding as well as QTL cloning. In order to successfully deploy MAS, it is critical to validate the known QTL in breeding populations and/or in near isogenic lines (NILs) of elite backgrounds. Such validation studies will be facilitated by combining QTL mapping with methods in functional genomics to develop markers from resistance genes themselves. This review will also discuss other areas envisioning where QTL analysis can contribute decisively in understanding and improving FHB resistance. These include new DNA markers, high density maps, and breeding strategies.

INTRODUCTION

Wheat is the most widely cultivated and important food crop in the world. It belongs to cereal grass *Gramineae* (*Poaceae*) family and the genus *Triticum.* Though grown under a wide range of climates and soils, wheat is best adapted to temperate regions with rainfall between 30 and 90 cm (12 and 36 inches). Winter and spring wheat are the two major types of the crop. Winter wheat is sown in the fall, while spring wheat is generally sown in the spring but also can be sown in the fall where winters are mild. More of the world's farmland is devoted to wheat than to any other food crop; in the late 20th century about 230,000,000 hectares (570,000,000 acres) were sown annually, with a total production of almost 600,000,000 metric tons. The world's largest wheat producer is China, with an estimated annual production of almost 114,400,066 metric tons. Other leading producers include India, the United States, France, Russia, Canada, Germany, Turkey, Kazakstan, Ukraine and Pakistan. The greatest portion of the wheat flour produced is used for making bread. Wheat grown in dry climates is generally hard wheat, having a protein content of 11-15% and strong gluten strength (elastic protein). Hard wheat flour is best suited for making bread. The wheat produced in humid areas is softer in grain texture with a protein content of about 8-10% and weaker gluten strength. Soft wheat flour is most suitable for cakes, crackers, cookies, and pastries. Durum wheat (*Triticum durum* L.) semolina is used for making pastas or alimentary pastes. While most wheat is grown for human consumption, about 10% is retained for providing seed, and for use by industries producing starch, paste, malt, dextrose, gluten, alcohol, and other products using small quantities of wheat. Inferior and surplus wheat and various milling by-products are used for livestock feeds. As a major source of energy in human diets, wheat grain varies in composition due to differences in climate and soil. On average, the wheat kernel contains 12% water, 70% carbohydrates, 12% protein, 2% fat, 1.8% minerals, and 2.2%

crude fibers. A pound (454 g) of wheat contains about 1,500 calories (100 grams contains about 330 calories). Thiamin, riboflavin, niacin, and small amounts of vitamin A are abundant in bran and germ which are usually removed during the milling process.

FUSARIUM HEAD BLIGHT OF WHEAT

Fusarium head blight (FHB) is one of the most destructive diseases of wheat (*Triticum aestivum* L. and *T. durum* L.) and barley (*Hordeum vulgare* L.) in warm and humid areas of the world (Schroeder and Christensen, 1963; Wang et al., 1982; Snijders 1990). It has been an increasingly serious problem in the north-central and eastern regions of the USA because of the emphasis on conservation tillage (Wilcoxson et al., 1988; Bai and Shaner, 1994), small grain rotations with maize (Windels and Kommedahl, 1984), and the lack of effective cultural and/or fungicide control (McMullen et al., 1997). FHB epidemics can cause significant yield losses, shriveled kernels, and deposition of vomitoxin (Deoxynivalenol (DON)) in the colonized seeds which renders the grain unsuitable for human consumption and animal feed (McMullen et al., 1997). Wheat and barley losses caused by FHB epidemics in the USA during the 1990s were estimated at close to $3 billion U.S. dollars (Windels, 2000).

Fusarium head blight (FHB) pathogens

Several species of the *Fusarium* genus are known to cause FHB (Wiese, 1987). In most areas of the world *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* Schw. (Petch)] is predominant, but *F. culmorum, F. avenaceum, F. sporotrichioides, F. poae and Microdochium nivale* also are important in specific areas (Mesterhazy et al., 2005). FHB severity of wheat genotypes to these different *Fusarium* species was reported to be very similar, indicating that

host resistance to *F. graminearum* is similar to that expressed to other *Fusarium* spp. (Mesterhazy et al., 2005). *F. graminearum* and *F. culmorum* are the primary *Fusarium* species causing DON contamination and reduced grain quality.

The fungus can survive as mycelium, ascospores, macroconidia and chlamydospores. Ascospores are the propagules of the sexual stage, and in soil the macroconidia or mycelium may be transformed into chlamydospores (Reis, 1990). *F. graminearum* survives between wheat crops in living or dead host tissues. Ascospores, macroconidia, chlamydospores and hyphal fragments all can serve as inoculum (Zhu and Fan, 1989). Ascospores and macroconidia are the principal source of inoculum because aerial dispersal is necessary for the fungus to reach the plant colonization site (Sutton, 1982). Fusarium colonized crop residues such as wheat, maize, soybean or rice on the soil surface are the most important sources of inoculum. In areas where wheat is planted after rice, rice stubble is a major source of inoculum for fusarium head blight (Zhu and Fan, 1989). Also, wheat planted after maize often has significantly more head blight than wheat planted after other crops. Therefore, reduced tillage in soil conservation systems increases the amount of inoculum present (Teich and Hamilton, 1985).

Ascospores produced on crop residue are the main source of inoculum for initial infection. Infection is initiated when airborne ascospores are deposited on wheat spikelets, and subsequently germinate and grow inside them. The fungus may also infect the glume, palea or rachila by direct penetration. Soon after infection dark brown spots appear on the colonized spikelets and later the entire spikelet becomes blighted (Bennett, 1931). If weather is favorable, aerial mycelium spread externally from one spikelet to another. If the fungus spreads internally,

brownish chlorotic symptoms extend up and down the entire spike and it dies. Visible pink mold appears on the spike when it is humid. Infected florets often fail to produce grain (Wiese, 1987). In nature, spike infection can occur any time after the beginning of flowering. Generally, small grains are most susceptible to infection at the flowering stage; however some cultivars are most susceptible at the milk or soft dough stages (Schroeder and Christensen, 1963). Anthesis is the predominant period for infection; therefore, the fungus is limited to one infection cycle per season (Strangge and Smith, 1987). The incubation period is as short as 2-3 days in the greenhouse or laboratory and 4-5 days in the field (Xiao et al., 1989). Primary infection may occur on several florets of a spike in the field, and the dark brown symptoms usually extend into the rachis. The pathogen mycelium invades parenchyma tissue as well as vascular tissue (Schroeder and Christensen, 1963). Clogging of vascular tissue in the rachis can cause the spike to senesce or die prematurely. If spikes are invaded extensively at an early stage, kernels may fail to develop entirely (Schroeder and Christensen, 1963). The optimum temperature for infection and development is 25°C, and little or no infection occurs at or below 15°C. Incidence increases as temperature increases from 20 to 30°C. The moist period required for infection ranges from 36 to 72 hours (Anderson, 1948).

Economic loss caused by FHB

FHB significantly reduces grain yield. Yield reduction results due to a shriveling of kernels, which may be light enough to be expelled from the combine with the chaff. In addition, the diseased grain that is not eliminated with the chaff has reduced volume weight because the kernels are light and shriveled. Also, FHB reduces seed germination and causes seedling blight and poor stands when colonized seed is sown (Bai and Shanner, 1994). FHB epidemics have

been reported worldwide. FHB epidemics have occurred in 26 U.S. states and five Canadian provinces, and contributed to yield losses exceeding 13.6 million tons during the 1990s (Rudd et al., 2001). Monetary losses due to FHB during the past decade have been valued at $3 billion (Windels, 2000; Van Sanford et al., 2001). Epidemics in China are most common and severe in Yangtze River Valley, and can affect more than seven million hectares of wheat. It is estimated in China that up to 2.5 million tons of grain may be lost to FHB in epidemic years (Wang, 1996). In Argentina, the worst outbreaks occurred in 1978, 1985 and 1993 where yield losses varied among regions but were estimated to average between 20-30% (Galich, 1996).

Toxins production by FHB pathogens

FHB causes additional economic loss as a result grain contaminated with potent mycotoxins produced by the fungus. The mycotoxins produced by *Fusarium graminearum* in diseased grain are detrimental to livestock and are a safety concern in human foods. The two most important mycotoxin produced by *Fusarium graminearum* are the estrogenic toxin zearalenone (ZEA) and the trichothecene deoxynivalenol (DON), a vomitoxin (Tuite et al., 1990). Grain with one or both toxins may be graded down or rejected entirely in commerce.

Control of FHB

There are various cultural control practices for reducing the prevalence and severity of FHB epidermics. Crop rotation coupled with plowing to bury infested crop residues and weed hosts can be effective. Also, use of appropriate methods for land preparation, good crop husbandry, timely harvest, proper grain storage and other practices can help reduce disease by reducing

primary inoculum. However, because of the ubiquitous nature and wide host range of *F. graminearum* adequate control by these methods may not be feasible (Reis, 1990).

Another way to control FHB disease is fungicide treatment. Seed-treatment fungicides reduce the spread of seed-borne inoculum and increase seedling vigor. Although, foliar application of fungicide at anthesis might provide some protection, there are many problems with such applications. Cost of treatment and difficulty of determining the optimum time of application also make this means of control less attractive to farmers. Highly effective fungicides are not available, and many fungicides cannot be legally applied after spike emergence. Even if a fungicide reduces direct yield loss, it may not reduce mycotoxin contamination to a tolerable level (Martin and Johnston, 1982).

FHB RESISTANCE TYPES

Resistance to FHB is complex, and has been delineated into five types: (1) type I, resistance to initial infection, (2) type II, resistance to disease colonization within a spike, (3) type III, decomposition or non-accumulation of mycotoxin, (4) type IV, resistance to kernel colonization, and (5) type V, tolerance to yield loss (Schroeder & Christensen, 1963; Wang & Miller, 1988; Mesterhazy, 1995). Three components of FHB resistance have generally been accepted and included type I, type II and type III (Somers et al., 2003). Type II resistance is the major type of resistance and has been studied most extensively. Type II resistance is usually assessed as FHB severity or Area Under the Disease Progress Curve (AUDPC) using single-floret inoculation methods conducted in greenhouse and/or in field experiments. Type I resistance is usually assessed as FHB incidence using spray-inoculation methods generally conducted in field

experiments and occasionally in greenhouse studies. Type III resistance is usually assessed as DON content of grains from naturally infected field trials or single-floret and spray-inoculated experiments conducted in both the greenhouse and field. Highly significant correlations were reported between FHB incidence, severity, and DON content using the same inoculation method and under same environment (Chen et al., 2000; Miedaner et al., 2004; Mesterhazy et al., 2005). Phenotypic selection on the basis of reduced FHB symptoms should result in a correlated selection response for low fungal biomass and low DON content in the grain (Miedaner et al., 2004). However, poor correlations often have been observed between FHB severity evaluated in greenhouse versus field experiments (Chen et al., 2005), and between FHB incidence, severity, and DON using different inoculation methods and/or experiments conducted in different years (Somers et al., 2003).

INHERITANCE OF FHB RESISTANCE

Many investigators considered FHB resistance to be quantitatively inherited and controlled by minor genes, whereas others provide evidence of oligogenic control. Chen (1989) proposed one dominant gene and some minor modifiers governing FHB resistance in Sumai 3, while Zhou et al. (1987) estimated two genes and Bai et al. (1989a) estimated three major resistance genes in Sumai 3. Bai (1995) confirmed the presence of three genes with major effects on FHB resistance in Sumai 3 and Ning7840 (a cultivar descended from Sumai 3). In the Brazilian cultivar Frontana, another important FHB resistance source, Singh et al. (1995) estimated the presence of at least three resistance genes, while Van Ginkel et al. (1996) reported two genes in Frontana and two genes in Ning 7840 with all four genes being different. During the same time, FHB resistance genes were assigned to several chromosomes using cytogenetic analysis (monosomic

analysis). Yu (1982) reported that Sumai 3 has FHB resistance genes on chromosomes 2A, 5A, 1B, 6D and 7D. Liao and Yu (1985) reported that the cultivar Wangshuibai has resistance genes on chromosomes 4A, 5A, 7A, and 4D. Yu (1990) reported that the genes for resistance to FHB in cultivar PHJZM are located on chromosomes 3B, 5B, 6B, 6D, and 7A,. Also, she indicated that the cultivar HHDTB has genes for resistance on chromosomes 1B, 4D, 5D, and 7B, and cultivar YGFZ has resistance genes located on chromosomes 3A and 4D. Using generation means analysis to study the resistance in crosses between resistant and susceptible cultivars several scientists have reported that resistance is controlled by additive genetic effects and that non-additive effects also may be significant (Chen, 1983; Bai et al., 1989b; Snijders, 1990; Bai et al., 1993). Within the non-additive components, dominance appeared to be most important (Bai et al., 1990; Snijders, 1990). Epistatic effects were significant in the study by Bai et al. (1993), while Zhuang and Li (1993) did not observed significant epistasis. The contradiction in these results could be due to the sources of resistance investigated, contribution of genes from susceptible parents, disease screening methods used, and/or the isolate of the pathogen used for evaluating phenotypes. All of these factors can influence the number of genes detected in inheritance studies (Kolb et al., 2001).

SOURCES OF FHB RESISTANCE IN WHEAT

Use of host-plant resistance is considered to be the most practical and effective means to control FHB (Schroeder and Christensen, 1963). Sources with complete resistance have not been found; however, sources with partial resistance have been identified in common wheat and can be classified into three groups. Group I consists of highly resistant sources, which mainly include spring wheat genotypes, such as Sumai3, Ning7840, Wangshuibai and W14 from China,

Nobeokabouzu-komugi and NyuBai from Japan. Sumai 3 and/or its derivatives are the most widely used FHB resistance source in the world. Sumai 3 has been used in Chinese breeding programs for at least 20 years. These sources have been characterized as having resistance to disease spread, called type II resistance. Genotypes in this group usually have 1-3 diseased florets per inoculated spike with little to no rachis colonization, and do not exhibit significant progression in colonization during disease development after inoculation (Chen et al., 2000). Since their introduction into the USA in late 1980s, these sources have been used extensively by both spring and winter wheat breeding programs (Wilcoxson, 1992) and have resulted in the development of a few cultivars such as Alsen and Glenn, and advanced lines, such as ND2710 (spring wheat), INW0412 (SRW wheat), VA01W-476 (SRW wheat). These lines are currently being used as parents in breeding programs. Problems associated with the use of Sumai 3 and its derivatives as parents include susceptibility to other prevalent diseases and low grain yield, therefore, few cultivars have been released with FHB resistance derived from Sumai 3 or its derivatives. However, improved germplasm derived from the Sumai 3, Ning 7840, W14 and other sources, currently being evaluated in wheat breeding programs, has greater potential for cultivar release (Griffey et al., 2004). Group II resistance sources include the Brazilian cultivar Frontana, which was postulated as having resistance to initial infection, called type I resistance, and DON resistance (Miller and Ewen, 1997).

Group III resistance sources include adapted winter wheat cultivars or lines of diverse origin that also are referred to as native resistance sources. Genotypes such as soft red winter (SRW) wheat cultivars Ernie (McKendry et al., 1995), Freedom (Gooding et al., 1997), and Roane (Griffey et al., 2001), have expressed variable ratings for FHB incidence, but generally have significantly low ratings in field experiments for FHB severity, yield and/or

test weight loss, fusarium damaged kernels (FDK), and DON content (Chen et al., 2000). Other winter wheat genotypes, such as the Swiss cultivar Arina (Paillard et al. 2004), the French cultivar Renan (Gervais et al. 2003), and the Romanian cultivar Fundulea 201R (Shen et al. 2003b), also were found to have FHB resistance. These lines have no ancestral relationship with lines in groups I and II on the basis of pedigree, and potentially offer complementary sources to groups I and II. Although FHB resistance of Group III sources is often only intermediate, these sources are very valuable and their use as adapted parents in crosses with non-adapted sources possibly will produce progeny that are more adapted and have better agronomic characteristics as well as enhanced FHB resistance. Group IV resistance sources include wild relatives of wheat, such as *T. tauschii* (*Coss*.) Schmal., *Roegneria kamoji* C. Koch, *Th. elongongatum* (Host), etc. (Oliver et al., 2005). These sources can play an important role in enriching the gene pool and providing novel and complementary sources of FHB resistance. Alien addition, substitution, and translocation lines are being developed by several programs (Cai et al., 2005; Oliver et al., 2005).

BREEDING FOR RESISTANCE TO FHB

Development and deployment of resistant cultivar is an environmentally safe and economically sound control measure, especially when considering the lack of available and economical chemical control at this time (Jones, 2000). Most breeders attempt to improve FHB resistance by crossing group I and/or group II sources with group III sources and simultaneously selecting for enhanced FHB resistance and desirable agronomic performance. The derived lines subsequently are crossed with other more adapted elite lines. This stepwise procedure has resulted in the development of a few adapted FHB resistant wheat lines in different breeding programs. In addition, significant progress has been obtained in deriving cultivars having moderate to high

levels of native FHB resistance, from traditional breeding populations, and include cultivars such as Roane, McCormick, Tribute, Neuse, Ernie, Truman, Goldfield, and Freedom. Top-crossing is the most common method used in breeding programs; however, a combination of top-crossing, backcrossing and doubled haploid methods has been applied in breeding programs to accelerate development of FHB resistant cultivars (Griffey et al., 2004).

TECHNIQUES FOR DISEASE DEVELOPMENT AND ASSESSMENT

Use of precise inoculation methods and reliable assessment parameters is fundamental for success in FHB research. Among a number of factors affecting disease development under field conditions, plant growth stage, inoculum source and application and availability of free-moisture are controllable factors, whereas temperature is non-controllable ones. The method used for disease establishment should correspond to specific research objectives and should reduce environmental and experimental variability to a minimum.

Scattering of Fusarium colonized grain as the primary source of inoculum is relatively easy, economical and not too laborious. Therefore, it has been used in many breeding programs to screen large numbers of pure lines and segregating populations. However, the incidence of disease resulting from this inoculation method can be affected greatly by plant height and growth stage (Chen et al., 2000) and, therefore, lacks precision in both uniformity and timing of infection. Spraying spikes with conidial suspensions according to optimal growth stage was found to be the most effective inoculation method under field conditions as it minimized variability due to differences in plant height as well as growth stage and provides for precise application of uniform inoculum. Because infection rate and uniformity can be more precisely

controlled and quantified, this method provides conditions for simultaneous assessment and selection of type I, type II and other types of resistance, which likely reflect overall field resistance (Chen et al., 2000). Single floret inoculation can be used effectively in both greenhouse and field evaluations to assess type II resistance. This inoculation method is not affected by plant height or growth stage and can be conducted under controlled environmental conditions, which are critical for conducting genetic and mapping studies. Because floret inoculation is laborious and time consuming, it is not practical for screening large numbers of pure lines or populations (Chen et al., 2000).

To evaluate field resistance, FHB incidence and severity are two commonly used measurements for type I and type II resistance. FHB index is widely used and is the product of incidence x severity x 100, which provides an estimate of overall resistance. To evaluate type II resistance, mediated by floret inoculation, several assessment methods have been used, such as infection type using a 1-5 incremental scale (Chen, 1989), percentage of infected spikelets (severity), AUDPC, and percentage of wilted spike (Buerstmayr et al., 2003). Severity is most widely used and accepted by researchers.

To evaluate DON resistance, early studies used a *F. graminearum* biomass assay and studied the mechanism of resistance in Frontana (Miller and Young, 1985; Miller and Arnison, 1986; Wang and Miller, 1987; Miller and Ewen, 1997). Current analysis is conducted via immunoassays (ELISA, Sinha and Savard, 1996) and gas chromatography/mass spectrometry (Mirocha et al., 1998) to obtain DON content of colonized grain. This analysis also is very costly and time consuming.

Percentage of fusarium damaged kernel (FDK) has been commonly used to evaluate kernel resistance. This is done either by actual counting or visually scoring of samples. This is time-consuming and is not a precise measurement in field experiment because not all of colonized seeds can be retained after combining, and the severely colonized spikes likely will not produce seeds at all.

FHB QTL MAPPING

QTL mapping of FHB resistance was initially conducted in 1999 when a major QTL on wheat chromosome 3BS was identified in a known resistance source Sumai 3 using a traditional RFLP marker system (Waldron et al., 1999). Subsequently, QTL mapping of other chromosome regions associated with FHB resistance was conducted using PCR-based marker systems, such as SSR and AFLP in recombinant inbred and doubled haploid mapping populations. To date, 18 wheat chromosomes regions have been reported to have a significant association with FHB resistance (Table 1). QTL on chromosomes 3BS, 5AS, and 6BS were reported in most mapping studies of Sumai 3 and its derivatives. QTL on chromosomes 2BS, 2D, 3BSc, and 3AS were reported in several studies of diverse sources. QTL in other chromosome regions were reported in one or a few studies in each case.

The QTL on chromosome 3BS (*Qfhs.ifa*-3B) in Sumai 3 was the first identified for FHB resistance (Waldron et al., 1999, Anderson et al., 2001). This QTL was subsequently identified and confirmed to be associated with type II resistance in most Sumai 3-derived spring type resistance sources including Ning7840 (Zhou et al., 2002), CM-82036 (Buerstmayr et al., 2002

& 2003), Ning894037 (Shen et al., 2003a), DH181 (Yang et al., 2005), W14 (Chen et al., 2005), Wangshuibai (Zhou et al., 2004; Zhang et al., 2004) and in the Japanese landrace NyuBai (miscorrectly identified as Maringa in Somers et al., 2003). The 3BS QTL also was reported as having association with type I and type III (DON) resistance in CM-82036, NyuBai, DH 181, and W14 (Buerstmayr et al., 2002 & 2003; Somers et al., 2003; Yang et al., 2005; Chen et al., 2005).

The QTL on chromosome 5AS (*Qfhs.ifa*-5A) was reported to be associated with type I, type II, and type III resistance in diverse sources including the Sumai 3 related sources CM-82036, DH181, and W14, Japanese landrace NyuBai, Brazilian cultivar Frontana, and SRW wheat cultivar Ernie (Buerstmayr et al., 2003, Yang et al., 2005; Chen et al., 2005; Somers et al., 2003; Steiner et al., 2004; McKendry et al., 2004).

The QTL on chromosome 6BS was reported to be associated with type I and/or type II resistance in Chinese sources and in Frontana (Waldron et al., 1999, Anderson et al., 2001; Shen et al., 2003a; Lin et al., 2004; Yang et al., 2005).

Each of the QTL on chromosomes 1B, 2A, 2BS, 2D, 3AS, 3BSc, 5BL and 7A were identified in one or more studies in spring wheat from China (Sumai3 and related sources), Japan (NyuBai), and Brazil (Frontana); winter wheat from France (Renan), Switzerland (Arina), and Romania (F201R); and/or in SRW wheat from USA (Ernie, Goldfield). Each of the QTL reported on five other chromosomes were identified in a single study in diverse sources from different countries.

MARKER ASSISTED SELECTION AND ITS APPLICATION FOR FHB RESISTANCE

Koebner and Summers (2003) summarized that MAS in wheat will be increasingly applied in four main areas in wheat breeding programs in the future: 1) for accelerated selection of a small number of traits that are difficult to phenotype, due to complex inheritance, low heritability, and confounding environmental effects; 2) for maintenance of recessive alleles in backcross breeding; 3) for pyramiding of disease-resistance genes; and 4) for selection of parents in crossing programs, to ensure minimal levels of duplication of alleles across sets of genes targeted for selection and to promote fixation. However, many markers identified in preliminary genetic mapping studies are not suitable for direct use in marker-assisted selection. High resolution mapping, validation of markers and possibly marker conversion are some steps required before use in MAS.

High resolution mapping utilizes a larger population size and a greater number of markers to identify tightly linked markers (at least <5 cM but ideally <1cM) that are ideal for MAS and also to discriminate between a single gene or several linked genes (Michelmore, 1995; Mohan et al., 1997). Validation of markers tests their linkage to and association with QTL and their effectiveness in postualtion and selection in the target phenotype in independent populations and different genetic backgrounds (Cakir et al., 2003; Collins et al., 2003; Jung et al., 1999; Langridge et al., 2001; Li et al., 2001; Sharp et al., 2001). Marker validation should be performed in diverse genetic backgrounds to ensure greater effectiveness in MAS (Sharp et al., 2001; Spielmeyer et al., 2003). Resistance to FHB is an ideal trait for marker-assisted selection as FHB is a spike disease, quantitatively inherited, and phenotypic evaluations require laborious and expensive screening systems, and are confounded by environmental factors. At present, 18

wheat chromosome regions have been reported to be associated with FHB resistance. However, few markers have actually been validated and implemented in wheat breeding programs for MAS. A primary reason for this lack of use is that many markers are not reliable in predicting the desired phenotype. In many cases, this can be attributed to low accuracy of QTL mapping studies in identifying and subsequently validating markers: MAS for 3BS QTL was evaluated in two DH populations (Yang et al., 2005), but only included two distal flanking markers Xgwm533 and Xgwm493 that are not tightly linked to the QTL. MAS for the 3BS QTL has only recently been applied in breeding populations and most of the remaining QTL have not been used in routine MAS programs. Lack of widespread adoption and routine application of MAS has been limited technically by a lack of both suitable markers (tightly linked markers) and high-throughput analytical platforms in most of breeding programs.

PERSPECTIVES FOR QTL ANALYSIS OF FHB RESISTANCE

High resolution mapping and marker validation

To date, several chromosome regions have been reported as having association with FHB resistance in two or more mapping studies. In order to successfully conduct MAS for these QTL, it is imperative to subsequently conduct high resolution mapping. High resolution mapping of FHB resistance QTL has been accelerated by comparative mapping of rice chromosomes. A fine molecular marker map of a major QTL on 3BS for FHB resistance has been developed (Liu and Anderson, 2003), and fine mapping of the 2DS FHB QTL, a QTL having negative effect on FHB resistance conferred in Sumai 3, was initiated using a comparative genomics approach in collaboration with three institutes in Japan (Handa et al., 2004) . It is expected that development of high resolution maps for these QTL will facilitate the isolation of actual genes (rather than

linked markers) via ‘map-based cloning’ (also ‘positional cloning’). The identification of genes controlling FHB resistance will enable us to predict gene function, isolate homologs and conduct transgenic experiments. High resolution mapping of the FHB QTL 5AS can be facilitated by incorporating new marker system, such as TRAP (target region amplification polymorphism, Hu and Vick, 2003), STS (sequence target site polymorphism), and SNP (single nucleotide polymorphism) as a synthetic map in rice is not well-developed.

Despite the large amount of QTL-mapping work reported on FHB resistance, the impact of QTL mapping on cultivar development has been minimal. Three primary factors contribute to limited deployment of markers in MAS. First, QTL discovery and cultivar development are currently separate processes, and often involve different types of parents and populations. This not only increases the time required for cultivar development, but also reduces the probability of successfully using QTL information to create a superior crop variety. Second, the 3BS QTL has been widely introduced into breeding programs but selection of this QTL to date has been based primarily on phenotypic selection. Finally, marker validation studies for other FHB QTL not only need to be conducted in independent mapping populations, but also need to be conducted using NIL and breeding populations of elite line in haplotyping studies (Liu and Anderson, 2003; Somers et al., 2003, Chen et al., 2004 a, b, Wilson et al., 2004).

New types of markers and high-throughput marker techniques should play an important role in high resolution mapping and marker validation studies. TRAPs may be a more efficient marker system than currently used AFLP (amplified fragment length polymorphisms, Vos et al., 1995) and SSR (simple sequence repeat, Beckman and Weber, 1992). Due to the abundance of single

nucleotide polymorphism (SNP) and development of sophisticated high-throughput SNP detection systems, it has recently been proposed that SNP markers will have a great impact on future mapping research studies and MAS (Rafalski, 2002; Koebner & Summers, 2003). Methods for detection and analysis of widely-used markers are becoming faster and more sophisticated, and many of these methods are automated (Ablett et al., 2003; Hori et al., 2003; Rampling et at, 2001; Warburton et al., 2002). One example of an improvement in the efficiency of marker analysis is multiplex PCR, which enables multiple marker loci to be tested simultaneously. Currently, the cost of utilizing markers is possibly the most important factor that limits the implementation of MAS. However, it is anticipated in the near future that novel applications and technology improvements will result in a reduction in the cost of deploying markers, which will subsequently lead to a greater adoption of routine MAS in plant breeding programs (Dreher et al., 2003). An example of such an improvement is use of multiplex PCR and high-throughput marker detection systems (Chao, personal communication).

To enhance the efficiency of MAS, knowledge of the DNA sequence of the desirable gene enables design of ‘perfect’ or ‘diagnostic’ markers, which are located within the actual gene sequence, thus eliminating the possibility of recombination between the markers and gene (Ellis et al., 2002; Ogbonnaya et al., 2001). However, DNA sequences for the majority of genes controlling agronomically important traits are unknown, and most probably, will remain unknown for sometime. In the meantime, plant scientists will continue to use QTL maps and molecular markers that tag genes of interest for many years to come for most selectable loci.

Combination of QTL mapping with functional genomics methods

Collard et al. (2005) indicated that the latest trend for QTL analysis is to combine QTL mapping with functional genomics. These methods include expressed sequence tag (EST) and microarray analysis, which can be utilized to develop markers from genes themselves (Gupta et al., 2001; Morgante and Salamini, 2003). The use of gene sequences derived from EST or gene analogs, described as the 'candidate gene approach' holds much promise in identifying the actual genes that control the desired traits (Cato et al., 2001; Pflieger et al., 2001; Yamamoto and Sasaki, 1997). These methods also can be utilized to identify SNP markers (Rafalski, 2002). EST-derived and SNP markers are usually integrated into existing maps that already have postulated the location of QTL (Hayashi et al., 2004; Ishimaru et al., 2001; Skiba et at, 2004; Wang et al.. 2001; Zhang et al., 2004). The number of EST and genomic sequences available in databases is growing rapidly (especially from genome sequencing projects e.g. rice), and the accumulation of these sequences will be extremely useful for the discovery of SNPs and data mining for new markers in the future (Gupta et al., 2001; Kantety et al., 2002). The development of more high-density (or saturated') maps that incorporate SNPs, EST-derived markers, and STSs will provide researchers with a greater arsenal of tools for QTL mapping and MAS.

Breeding strategies for FHB resistance

The QTL on chromosome 3BS has been validated, fine-mapped and incorporated into many adapted wheat backgrounds. However, previous and many current FHB resistant wheat lines were derived primarily by phenotypic selection. Until recently, MAS of the 3BS QTL was based on initially identified markers Xgwm389, Xgwm533, and Xgwm493 which are not tightly linked to 3BS QTL on the basis of a fine map recently developed by Liu and Anderson (2003). In

addition to 3BS, QTLs on chromosomes 3AS and 5AS likely confer FHB resistance in some of the Sumai 3 derived lines and in Frontana (Table 1). Markers (3AS-Xbarc067 and 5AS-Xbarc117) associated with these two QTL freqently have been found in adapted soft red winter wheat lines (McCartney et al., 2004; Wilson et al., 2004; Shen, personal communication) as Frontana and its relatives were often used as a source of leaf rust resistance in SRW wheat breeding programs (Griffey, personal communication). Therefore, an initial breeding goal is to incorporate the 3BS QTL from Sumai 3 derived lines into elite lines having 5AS and/or 3AS QTL using a combination of MAS and phenotypic selection. MAS for the 3BS QTL will be more effective via selection of the ideal haplotype than selection of single markers. The ideal haplotype of the 3BS QTL is comprised Sumai 3 alleles for markers Xbarc133 and XSTS142 or other STS markers tightly linked to these two markers. Selection based on markers Xgwm533 and Xgwm493 alone is not recommended as these distal flanking markers are not tightly linked to the 3BS QTL (Chen, unpublished data). The 5AS QTL region contains several markers that are co-segregating, such as Xbarc117, Xbarc56, Xgwm129, Xgwm293, and Xbarc186 (Buerstmayr et al., 2003; Chen et al., 2005). The effectiveness of these markers may vary among different genetic backgrounds. MAS for combining the 3BS QTL with either or both 3AS and 5AS QTL was more effective in enhancing FHB resistance than selection of only one QTL (Buerstmayr et al., 2004). Pyramiding of these three QTL with additional validated QTL likely will be the future goal of MAS for FHB resistance.

A second imperative goal is to characterize the effects of known QTL-marker alleles in frequently used wheat parents, released cultivars, and elite lines via NIL or haplotyping studies. Knowledge from such studies will facilitate effective pyramiding of different genes or QTL in

targeted breeding populations. Asins (2002) indicated that favorable alleles increasing trait value are present not only in the "good" parent but also in the "bad" parent. This applies to FHB resistance, where adapted wheat lines already possess some favorable QTL markers alleles, such as 5AS-Xbarc117 or Xgwm129, and 3AS-Xbarc67 (McCartney et al., 2004, Wilson et al., 2004). Known resistance sources also have some unfavorable alleles. Sumai 3 has alleles at 2D-Xgwm296 and 2DS-Xgwm261 that confer a negative effect on both type I and type II resistance (Handa et al., 2004).

The third goal is to integrate MAS with conventional breeding methods. Conventional breeding will continue to provide the foundation for development of FHB resistant cultivars and/or germplasm. However, MAS will greatly facilitate breeding efforts in selection of the best parents and progeny. Parents should be selected and crosses made on the basis of complementary traits and marker loci. The 3BS QTL should be included in at least one parent. Routine deployment of MAS in segregating populations will not be effective or feasible until tightly linked markers and high throughput equipment become available and accessible in most breeding programs.

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Table 1. Summary of QTL/markers significantly ($p \leq 0.05$) associated with type I, type II, and type III FHB resistance identified in known and native sources from 1999-2005.

QTL Location	Source	Flanking/closest markers	R^2 x100	Traits	Reference
3BS	Sumai 3	Xbcd907	15	II	Waldron et al., 1999
	Sumai 3	Xgwm533 Xgwm493	25-42	II	Anderson et al., 2001
	Sumai 3	XSTS3B-138 XSTS3B-142	51-55	II	Liu et al., 2003
3BS	CM-82036	Xgwm533 Xgwm493	57	II	Buerstmayr et al., 2002
	CM-82036	Xgwm533 Xgwm493	32	I & II*	Buerstmayr et al., 2003
3BS	Ning7840	Xbarc147 Xgwm493	18-48	II	Zhou et al., 2002
3BS	Ning894037	Xbarc133 Xgwm493	43	II	Shen et al., 2003a
3BS	NyuBai	Xgwm533 Xgwm493	13	II	Somers et al., 2003
	NyuBai	Xgwm533 Xgwm493	11	III	Somers et al., 2003
3BS	Wangshuibai	Xbarc147 Xgwm493	31-37	II	Zhou et al., 2004
	Wangshuibai	Xbarc147 Xgwm493	14-24	II	Zhang et al., 2004
3BS	DH181	Xgwm389 Xgwm533	7	I	Yang et al., 2005
	DH181	Xgwm389 Xgwm533	11	II	Yang et al., 2005
3BS	W14	Xbarc133 Xgwm493	10	I	Chen et al., 2005
	W14	Xgwm493 Xgwm533A	33	II	Chen et al., 2005
	W14	Xbarc133 Xgwm493	23	III	Chen et al., 2005

*Combination of FHB type I and II resistance was evaluated using spray-inoculation method.

Table 1, continue,

QTL Location	Source	Flanking/closest markers	R^2 x100	Traits	Reference
5AS	Frontana	Xgwm129 Xbarc197	9	II	Steiner et al., 2004
5AS	CM-82036	Xgwm293 Xgwm156	11	II	Buerstmayr et al., 2002
	CM-82036	Xgwm293 Xgwm156	23	I & II	Buerstmayr et al., 2003
5AS	NyuBai	Xgwm129 Xgwm96	6	III	Somers et al., 2003
5AS	Ernie	Xbarc56 Xbarc165	8-24	II	McKendry et al., 2004
5AS	W14	Xbarc117 Xbarc186	16-24	I	Chen et al., 2005
	W14	Xbarc117 Xbarc186	8	III	Chen et al., 2005
5AS	DH181	Xgwm293 Xgwm415	6	I	Yang et al., 2005
6BS	Sumai 3	Xbcd331	6	II	Waldron et al., 1999
	Sumai 3	Xbcd1383 Xbarc101	5-9	II	Anderson et al., 2001
6BS	Ning894037	Xgwm644	4	II	Shen et al., 2003a
6BS	Wangshuibai	Xgwmc539 Xbarc024	6-11	II	Lin et al., 2004
6BS	Frontana	Xs23m14_4	6-7	I & II	Steiner et al., 2004
6BS	DH181	Xumc397 Xgwm644	7	I	Yang et al., 2005
	DH181	Xumc397 Xgwm644	24	II	Yang et al., 2005

Table 1, continue,

QTL Location	Source	Flanking/closest markers	R^2 x100	Traits	Reference
2B	Ning7840	Xgwm120	4-6	II	Zhou et al., 2002
2B	W14	Xbarc18 Xgwm410	7	II	Chen et al., 2003
2B	Renan	Xgwm374	12	I & II	Gervais et al., 2003
2B	Frontana	Xs13m25_8 Xs24m15_6	6-9	I & II	Steiner et al., 2004
2B	Ernie	Xgwm271 Xgwm319	5-6	II	McKendry et al., 2004
2B	Goldfield	Xbarc200 Xgwm210	29	I	Gilsinger et al., 2005
2D	Sumai3	Xgwm539 Xwmc144	13	II	Xu et al., 2001
2D	Wuhan 1	Xgwm539 Xwmc144	13	II	Somers et al., 2003
2D	Ning894037	Xgwm296 Xgwm261	12	II	Shen et al., 2003a
2D	CASS94	Xgwm539 Xumc41	45-53	II	Lewis et al., 2004
2D	DH181	Xgwm539 Xwmc144	11	I	Yang et al., 2005
	DH181	Xwmc144	13	II	Yang et al., 2005
3BSc	NyuBai	Xgwm566 Xumc238	4	I & II	Somers et al., 2003
3BSc	DH181	Xumc517 Xwmc612	8	I	Yang et al., 2005
3BSc	Wangshuibai	Xbarc344	3-7	II	Zhou et al., 2004
	Wangshuibai	Xgwm285 XEtcg.Mctc-11	7-16	II	Zhang et al., 2004
3BSc	Ernie	Xgwm077 Xe8m1_1	5-11	II	McKendry et al., 2004
3AS	Frontana	Xgwm720 Xdupw227	12-13	I & II	Steiner et al., 2004
3AS	DH181	Xumc165	12	I	Yang et al., 2005
3AS	F201R	Xgwm674	13	II	Shen et al., 2003b

Table 1, continue,

QTL Location	Source	Flanking/closest markers	R^2 x100	Traits	Reference
1B	F201R	Xbarc8	19	II	Shen et al., 2003b
1B	Wangshuibai	Xwmc759	6-12	II	Zhou et al., 2004
2A	Freedom	NA	20	I & II	Gupta et al., 2001
2A	Ning 7840	Xgwm614	3-5	II	Zhou et al., 2002
2A	Renan	Xgwm311	6	I & II	Gervais et al., 2003
2A	Arina	Xgwm311	7	I & II	Paillard et al., 2004
3BL	Renan	Xgwm131b	4	I & II	Gervais et al., 2003
3BL	Arina	Xgwm131b	6	I & II	Paillard et al., 2004
4AL	Arina	Xgwm160	10	I & II	Paillard et al., 2004
4BS	Wuhan1	Xumc238 Xgwm192	12	I & II	Somers et al., 2003
4BL	Ernie	Xbarc495 Xgwm149	6-10	II	McKendry et al., 2004
4DL	DH181	Xwmc331 Xwmc473	13	I	Yang et al., 2005
5AL	Renan	Xgwm639b	19	I & II	Gervais et al., 2003
6DL	Arina	Xpsr915 Xcfd19a	22	I & II	Paillard et al., 2004
7A	Wangshuibai	Xgwm1083	3-7	II	Zhou et al., 2004
7BL	DH181	Xumc526 Xumc276	8	II	Yang et al., 2005

CHAPTER II

Validation of Two Major Quantitative Trait Loci for Fusarium Head Blight Resistance in Chinese Wheat Line W14

ABSTRACT

Fusarium head blight (FHB) is one of the most destructive wheat diseases worldwide. Resistance to *FHB* is an ideal trait for which molecular marker assisted selection (MAS) would facilitate breeding and cultivar development efforts. Validation of quantitative trait loci for FHB resistance is a prerequisite to MAS. This study was conducted to evaluate and validate the effect of two major QTL previously reported on chromosomes 3BS and 5AS in a doubled haploid population derived from a cross between a known Chinese resistance source W14 and a susceptible soft red winter (SRW) wheat cultivar Pioneer2684. Identity of QTL governing resistance to FHB initial infection (type I), disease spread (type II), deoxynivalenol (DON) accumulation (Type III), and fusarium damaged kernels (FDK), was characterized in ninety-six doubled haploid lines in two greenhouse and one field experiments. This study confirmed that the 3BS and 5AS QTL were significantly associated with FHB resistance and further documented that the 3BS QTL has a large effect on three components (type II, III and FDK) of FHB resistance evaluated in greenhouse test, while the 5AS QTL has a large effect on type I resistance evaluated in a field experiments. These two QTL together explained 37%, 33%, and 35% of the total phenotypic variation for type II, type III, and FDK resistance evaluated in the greenhouse experiments, respectively. In the field experiment, the two QTL explained 38% and 36% of the total phenotypic variation for type I and type II resistance, respectively. W14 has both QTL, which confer reduced initial infection, disease spread, kernel colonization, and DON accumulation. Therefore, marker-assisted selection (MAS) for both QTL should be implemented in incorporating W14 resistance into adapted wheat backgrounds. Flanking markers *Xbarc*133 and *XSTS3B-142* on 3BS and *Xbarc*117 and *Xbarc*56 on 5AS are recommended for MAS.

Keywords: *Triticum aestivum* - Fusarium head blight - Microsatellite - QTL mapping

INTRODUCTION

Fusarium head blight (FHB), commonly called scab, is one of the most destructive diseases of wheat (*Triticum aestivum* L.), and causes significant reductions in grain yield and quality. Mapping of QTL associated with FHB resistance and application of marker-assisted selection (MAS) can be used to accelerate the development of FHB resistant cultivars. Resistance to FHB is complex and comprised of three main components of resistance (Somers et al., 2003) including type I, resistance to initial infection, type II, resistance to spread of infection within a spike, and type III, decomposition or non-accumulation of mycotoxin, (Schroeder and Christensen, 1963; Wang and Miller, 1988). Extensive efforts have been made previously to map QTL for type II resistance and a major QTL (*Qfhs.ifa*-3B) on wheat chromosome 3BS has been identified and confirmed in a known Chinese source 'Sumai3' and related sources (Waldron et al., 1999; Anderson et al., 2001; Zhou et al., 2002; Buerstmayr et al., 2002; Shen et al., 2003; Somers et al., 2003; Zhou et al., 2004; Zhang et al., 2004; and Yang et al., 2005). In addition to 3BS, a QTL on chromosome 5AS (*Qfhs.ifa*-5A) has been identified for type I and type II resistance in two Sumai3-derived lines CM-82036 (Buerstmayr et al. 2002 & 03) and DH181 (Yang et al., 2005), one Japanese landrace NyuBai (Somers et al., 2003), one Brazilian cultivar Frontana (Steiner et al., 2004), and one soft red winter (SRW) wheat cultivar Ernie (McKendry et al., 2004). However, among all previously published papers on QTL mapping, none targeted all three components of FHB resistance in the same population, and very few papers explored type III resistance. Somers et al. (2003) elucidated two components of FHB resistance, type II and type III in a cross between one Sumai3 derivative (Wuhan 1) and one Japanese source

NyuBai, in which *Qfhs.ifa*-3B was significantly associated with type II and type III resistance, while the *Qfhs.ifa*-5A was only associated type III resistance (Somers et al., 2003). Yang et al. (2005) elucidated two components of FHB resistance, type I and type II in a cross between a Sumai 3 derivative (DH181) and one Canadian susceptible cultivar AC Foremost, in which *Qfhs.ifa*-3B was associated with type I and type II resistance, while the *Qfhs.ifa*-5A was only associated with type I resistance. Objectives of the current study were to characterize FHB resistance in Chinese wheat line W14 in comparison to QTL identified in previous studies and to determine the extent of allelic variation at the two known FHB QTL for three components of FHB resistance, type I, type II , and type III.

MATERIALS AND METHODS

Mapping population and FHB assessment

One double haploid (DH) population of wheat, comprised of 96 DH lines was developed using the wheat by maize hybridization method (Laurie and Bennett 1988) from a cross between W14 and Pioneer Brand 2684 (Pion2684). W14 was derived from a recurrent selection population comprised of 20 parents, including 15 FHB resistant cultivars, such as Sumai3, Ning7840, Zhen7495, Wangshuibai, Fanshanxiaomai, Shinchunaga, Frontana, Yangmai 4, etc. (Jiang, 1997, and personal communication). This line has improved FHB resistance compared to Sumai3 on the basis of lower observed disease spread, kernel colonization and deoxynivalenol (DON) production (Chen et al. 2000, Buerstmayr, personal communication). Pion2684 is a FHB susceptible SRW wheat cultivar.

Inoculum production

Isolation and purification of inoculum of *F. graminearum* was conducted on potato dextrose agar (PDA, Fisher, catalog no. 213400) medium two months before inoculation each year. Sporulation was induced on acidified PDA agar plates (pH = 4.5) and conidia were produced on half-strength PDA. A series of PDA plates were inoculated every other day with the same conidia stock solution in order to continuously produce uniform inoculum. The fresh conidia were harvested every other day and used for inoculation. Conidia concentration was determined with a hemacytometer (Fisher, catalog no. 1483) and adjusted to 5 x 10^{4} spores/ml with distilled water for inoculation. Tween 20 (Fisher, catalog no. 213400) was freshly added to the resulting spore suspensions at a rate of 1:100 ml suspension before inoculation. The same source of inoculum was applied in both greenhouse and field experiments.

Greenhouse experiments

The DH lines and parents were evaluated in two greenhouse (2001 and 2002) and one field (2004) experiments using single floret inoculation and spray-inoculation methods (Chen et al. 2000), respectively. In the greenhouse experiments, four inoculated plants per line received overhead mist-irrigation for 3 days at an interval of 45 seconds per half hour. FHB severity of one to three inoculated spikes per plant was evaluated on the 21st day after inoculation and was calculated using the formula: (number of infected spikelets/total number of spikelets) x 100. FHB severity for each line was based on a grand mean of all inoculated spikes. Percentage of fusarium damaged kernels (FDK) for each line was determined as the number of colonized kernels subdivided by the total number of kernels in the inoculated and hand-threshed spikes. These seeds were then assessed for DON concentration (ppm) using a Shimadzu QP2010

GC/MS system (Shimadzu Corporation, Kyoto, Japan, Mirocha et al., 1998). Severity data were collected from two independent experiments (2001 and 2002), whereas FDK and DON content were only evaluated in the 2001 experiment.

Field experiment

One field experiment was conducted during the 2003-2004 season in Montgomery County, VA. In the field experiment, the two parental and 96 doubled haploid lines were planted in single 1.5 m long rows with two replications arranged in a randomized complete block design. Each row was spray-inoculated twice, once at 100 percent spike emergence and again at 50 percent anthesis, with an 80 ml conidial suspension (50,000 spores/ml) using a CO_2-pressurized sprayer (R and D Sprayers, Opelousas, LA 70570, USA). Overhead mist-irrigation was applied at 1-hour intervals from 8:00 to 9:00 A.M. and again from 16:00 to 17:00 P.M. daily for three weeks when natural precipitation was absent. Ten spikes of each parental and DH line were evaluated on the 21st day after the second inoculation. FHB incidence for each line was calculated as (number of infected spikes/10) x 100. FHB severity for each infected spike was calculated as (number of colonized spikelets/total number of spikelets) x 100. FHB severity for each line was based on a grand mean of 10 colonized spikes per line.

Molecular marker analysis

Two bulked DNA samples were obtained by mixing an equal amount of DNA from 5 highly resistant and 5 highly susceptible DH lines, respectively. Resistant and susceptible lines were selected based on disease severity, FDK, and DON concentration. Four sets of SSR and one set of STS primers were used in the current study: 1) GWM primers were available from Röder et al.

(1998); 2) BARC primers were available from Shi et al. (2002) and Song et al. (2005); 3) UMC primers were obtained from published sequences (Gupta et al. 2002); 4) PSP primers (Bryan et al. 1997) were kindly provided by M. Gale, John Innes Centre, Norwich, UK; and 5) STS3B primers (Liu and Anderson, 2003) were kindly provided by Drs. Anderson and Liu, University of Minnesota, St. Paul, MN. The current validation study was based on linkage groups previously postulated as possessing QTL for FHB resistance on chromosomes 1B, 2B, 3B, 3A, 5A, 6B, and 7A. A total of 308 SSRs and 28 STSs on these chromosomes were used to survey DNA polymorphism among parents (W14 and Pion2684) and two extreme bulks using BSA analysis (Michelmore et al. 1991). Once putative association between a marker and FHB resistance was determined between two extreme bulks and/or between parents, the markers were genotyped for the whole population. DNA extraction, PCR amplification and SSR assays were conducted as previously described by Saghai Maroof et al. (1994) and Bryan et al. (1997).

Statistical Analysis

Phenotypic data

Analyses of variance of field and greenhouse data, and correlation and regression analysis were conducted using AGROBASE Version 1999 software (http://Agronomix.mb.ca).

Linkage analysis

Linkage maps were constructed using MAPMAKER 3.0b (Lander et al., 1987). A logarithm of odd (LOD) threshold of 3.0 was used for grouping. The most-likely marker orders were determined using the MAPMAKER 'ripple' command.

QTL mapping

QTL analysis was performed using yearly averages for FHB assessment data as well as a grand mean over years. FHB incidence (%), FHB severity (%), FDK, and DON concentration (ppm) were tested separately in the QTL analyses. Composite interval mapping method (CIM, Zeng, 1993 & 1994) was used via QTL Cartographer 2.0 software (http://statgen.ncsu.edu). The CIM analysis involves a single QTL model with multiple regression on selected marker loci outside the interval under consideration. A standard model including five SSR markers as cofactors in a forward regression method and a 10 cM window size (Basten et al. 2002) was applied to search for QTL. Significance threshold was determined by a permutation test with 1000 permutated samples (Doerge and Churchill 1996). A genome-wide significance level $P \leq 0.01$ for type I error with a logarithm of odd (LOD) threshold of 2.2 was used as a criterion to indicate putative QTL positions.

RESULTS

Phenotypic analysis of parental lines and their DH progeny

The parents, W14 and Pion2684, consistently displayed significant differences in response to *F. graminearum.* For all traits evaluated, except for FHB incidence, distribution of DH lines was skewed towards the resistant parent W14 (Fig. 1). High correlation coefficients were obtained for FHB severity between the two greenhouse experiments, and among FHB severity, FDK and DON in the 2001 greenhouse experiment ($r = 0.69 – 0.91$, $p \leq 0.0001$). A high coefficient of correlation also was observed between FHB incidence and FHB severity in the field experiment ($r = 0.78$, $p \leq 0.0001$); however, weaker correlations were obtained between disease data from

single floret inoculations (greenhouse) and spray inoculations (field) ($r = 0.27 – 0.37$, $p \leq 0.01$ to $p \leq 0.001$).

Bulked segregant analysis and genetic maps

Among 308 SSR and 28 STS3B primer pairs screened, 218 SSRs (71%) and 8 STS3B (29%) were polymorphic between parents W14 and Pioneer 2684, but only 18 SSRs and 3 STSs were polymorphic between two parents and two extreme bulks. When assessed in the entire population, sixteen of the 18 SSRs and 3 STSs retained significance at $p \leq 0.05$ on the basis of single marker analysis. These markers were located on five chromosomal regions 1B, 2B, 3B, 5A, and 7A. Additional markers flanking these SSR and STS markers were used to construct the genetic maps of these linkage groups. A total of 39 SSRs and 3 STSs were mapped in the DH population. Genetic maps of the 5 linkage groups were constructed, and chromosomal identities were determined via comparison with the SSR maps of wheat published by Röder et al. (1998) and Shi et al. (2002). Genetic maps of 3BS and 5AS, which contain the major FHB QTL, are presented in Fig. 2.

QTL Mapping

QTL in W14 for FHB resistance identified in greenhouse experiments

A total of four marker intervals in chromosomal 3BS region were significantly (LOD > 2.2) associated with FHB severity in at least one year (Table 1). One 3BS marker interval XSTS3B142-Xgwm493 was significant in both greenhouse experiments and explained the highest amount of phenotypic variation in FHB severity. The 3BS marker intervals Xgwm533A-Xbarc133 and Xbarc133-XSTS3B142 were associated with FHB severity in 2001 and the 2-year

mean; while the marker interval Xgwm493-Xcfd79B was only associated with FHB severity in 2002 (Table 1). The 3BS marker intervals Xbarc133-XSTS3B142 and XSTS3B142-Xgwm493 were significantly associated with type II, type III, and FDK resistance evaluated in the 2001 greenhouse experiment (Table 2). In addition to 3BS, another QTL on chromosome 5AS was significantly associated with type III and FDK resistance; however, this QTL explained much less of the phenotypic variation than the 3BS marker intervals Xbarc133-XSTS3B142 and XSTS3B142-Xgwm493 (Table 2).

QTL in W14 for FHB resistance identified in field experiment

The two 3BS marker intervals Xbarc133-XSTS3B142 and XSTS3B142-Xgwm493 and the 5AS marker interval Xbarc117-Xbarc186 identified in greenhouse experiments were also significantly associated with FHB incidence and severity in the field experiment (Table 3). On the basis of QTL estimates, the 5AS QTL had a much greater effect on overall field resistance than 3BS. In addition to 3BS and 5AS, another QTL on chromosome 7AL was associated with FHB severity. The resistance allele of 7AL was from Pioneer 2684.

DISCUSSION

Mapping efforts to date have reported nearly every wheat chromosomes as having association with FHB resistance. While the current study targeted five chromosome regions, only the presence of major FHB resistance QTL on chromosomes 3BS and 5AS were confirmed in W14. The two QTL together explained 38%, 37%, 33% and 35% of the total phenotypic variation for type I, type II, type III, and FDK resistance, respectively. QTL on chromosome 7AL was only associated with field severity and the resistant allele of this QTL was from Pioneer 2684. QTL

on chromosomes 1B and 2B indicated by single marker regression analysis were not significant on the basis of CIM method.

The two QTL in W14 were mapped to similar chromosomal positions on 3BS and 5AS in both greenhouse and field experiments. However, the 3BS QTL explained a greater proportion of the phenotypic variance for resistance to fungal spread in the greenhouse experiments than for FHB resistance in the spray inoculated field experiment, while the opposite was observed for the 5AS QTL in the greenhouse versus field experiments. This suggests that genetic control of type I resistance is likely different from that of type II resistance. It is also consistent with the moderate to low correlation values observed between FHB resistance data from greenhouse single floret-inoculation experiments and field spray-inoculation experiments (r = 0.27 – 0.39).

The 3BS QTL associated with type II resistance has been intensively studied and was confirmed to have a major effect on type II resistance in Sumai 3 (Waldron et al., 1999; Anderson et al. 2001), Ning7840 (Zhou et al. 2002), CM-82036(Buerstmayr et al. 2002 and 2003), Ning894037 (Shen et al., 2003), Wangshuibai (Zhou et al., 2004 and Zhang et al., 2004), and DH181 (Yang et al., 2005). These lines are all Sumai 3 derivatives. However, this QTL also was reported for type II and DON resistance in a Japanese landrace NyuBai (Somers et al., 2003). The current study is the first to demonstrate that the 3BS QTL flanked by markers Xbarc133 and XSTS3B142 in W14 is significantly associated with type I, type II, type III, and FDK resistance.

The 5AS QTL has been identified in diverse sources. This QTL was reported to have major effect on type I resistance in the Brazalian cultivar Frontana (Steiner et al. 2004), and in Sumai 3

related sources CM-82036 and DH181 (Buerstmayr et al. 2003; Yang et al., 2005). This QTL also was reported to have major effect on type II resistance in CM-82036 (Buerstmayr et al., 2002) and in the SRW wheat cultivar Ernie (McKendary et al., 2004), as well as association with DON resistance in NyuBai (Somers at al., 2003). The current study characterized the 5AS QTL, flanked by marker interval Xbarc117-Xbarc100, which was associated with type I, III, and FDK resistance in W14. An association of 5AS with type II resistance also was observed but not significant. Marker-assisted selection for the 3BS and 5AS QTL should be implemented to facilitate development of cultivars having overall effective FHB resistance via reduction of initial infection, FHB spread, kernel colonization, and DON production.

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Table 1. Putative QTL for type II FHB resistance (resistance to disease spread) detected using composite interval mapping method in a doubled haploid population from the cross W14 x Pioneer 2684 for greenhouse experiments in 2001, 2002, and the 2-year mean.

Chromosome	Map Interval	2-year mean		2001		2002	
		LOD	R^2	LOD	R^2	LOD	R^2
3BS	*Xgwm533A-Xbarc133*	8.18	0.33	7.14	0.28		
3BS	*Xbarc133-XSTS3B142*	9.18	0.33	7.31	0.26		
3BS	*XSTS3B142-Xgwm493*	10.27	0.37	7.78	0.28	6.72	0.27
3BS	*Xgwm493-Xcfd79B*					2.31	0.08

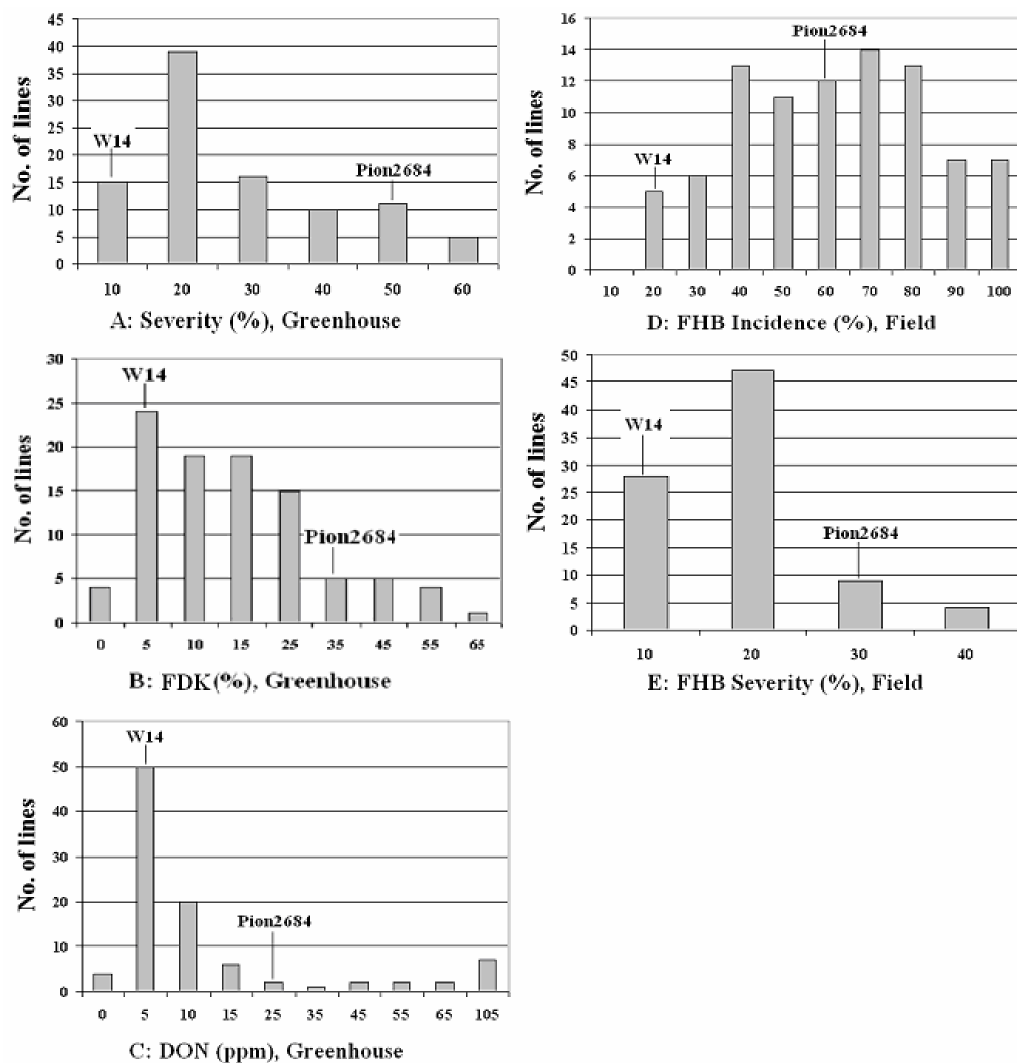
Table 2. Putative QTL for FHB resistance to type II (disease spread), type III (DON accumulation), and FDK detected using composite interval mapping method in a doubled haploid population from the cross W14 x Pioneer2684 evaluated in a greenhouse experiment in 2001.

Chromosome	Map Interval	Type II		Type III		FDK	
		LOD	R^2	LOD	R^2	LOD	R^2
3BS	*Xgwm533A-Xbarc133*	7.14	0.28				
3BS	*Xbarc133-XSTS3B142*	7.31	0.26	6.31	0.24	6.75	0.26
3BS	*XSTS3B142-Xgwm493*	7.78	0.28	6.42	0.24	7.29	0.28
5AS	*Xbarc117-Xbarc100*			2.58	0.09	2.24	0.07

Table 3. Putative QTL for FHB resistance to initial infection (type I) and disease spread (combination of type I and type II) detected using composite interval mapping method in a doubled haploid population from the cross W14 x PioneerBrand 2684 evaluated in a field experiment in 2004.

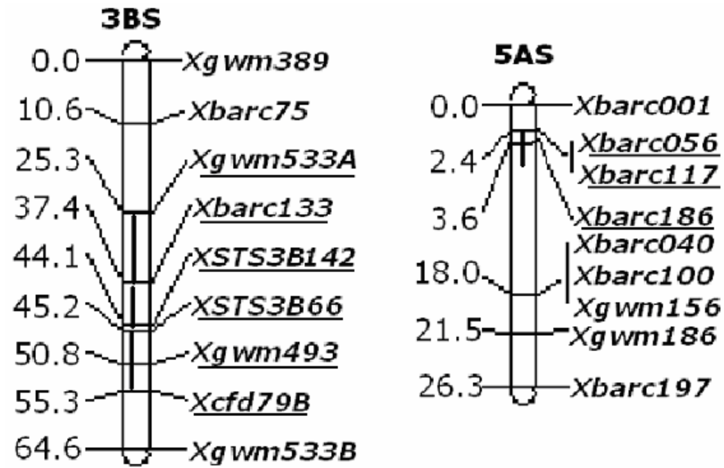
Chromosome	Map Interval	Type I		Type I & II	
		LOD	R^2	LOD	R^2
3BS	*Xbarc133-XSTS3B142*	2.53	0.09		
3BS	*XSTS3B142-Xgwm493*	1.73	0.06	2.67	0.09
5AS	*Xbarc117-Xbarc100*	7.67	0.29	5.13	0.18
7AL	*Xbarc121-Xbarc108*			2.84	0.09

Fig. 1. Frequency histograms of 96 DH lines derived from the cross of W14 x Pion2684.



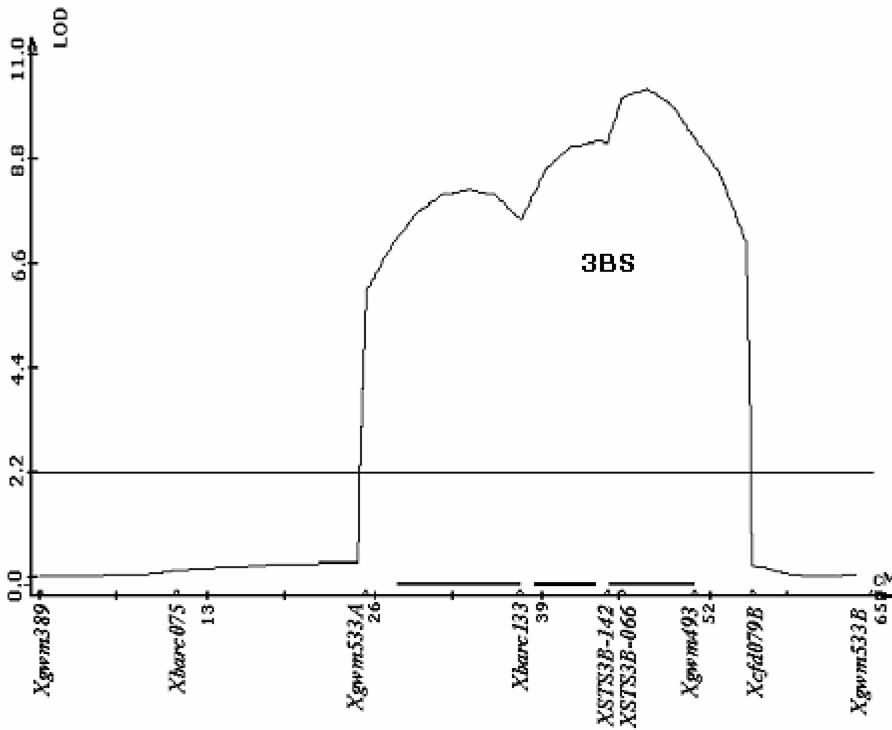
A: mean FHB severity (% infected spikelets) evaluated after single floret inoculation in two greenhouse experiments (2001, 2002). B: Percentage of fusarium damaged kernels (FDK) evaluated in a single floret inoculated greenhouse experiment in 2001. C: DON content in ppm of harvested seed samples from a single floret inoculated greenhouse experiment in 2001. D: FHB incidence (% infected spikes per row) evaluated after spray inoculation in a field experiment in 2004. E: FHB severity (% infected spikelets) evaluated after spray inoculation in a field experiment in 2004.

Fig. 2. Linkage maps of FHB QTL on chromosomes 3BS and 5AS generated using a DH wheat population of W14 x Pion2684.



Numbers at each marker represent the distance in cM. Dark area indicates the QTL positions based on the CIM method.

Fig. 3. Likelihood map showing multiple QTL in the 3BS QTL region identified by CIM and associated with FHB severity (2-year mean) evaluated in a DH population of W14 x Pion2684 in greenhouse experiments.



Markers and genetic distance (cM) of 3BS are presented on X-axis corresponding to Fig. 2. LOD scores are presented on Y-axis. The bold lines on X-axis represent the QTL position. LOD threshold 2.2 was determined by permutation test and is shown in the graph.

CHAPTER III

Haplotype Selection of Two Major Quantitative Trait Loci for Improved Fusarium Head Blight Resistance in Elite Wheat Backgrounds

ABSTRACT

This study was conducted to elucidate the potential use of marker assisted selection (MAS) for two major FHB resistance QTL previously reported on chromosomes 3BS and 5AS in seventy adapted soft red winter (SRW) wheat lines. Five haplotype groups (1-5) were classified among these lines on the basis of allelic differences of four marker loci linked to the 3BS QTL (*Xgwm533, Xbarc133, XSTS3B142, and Xgwm493)* and three marker loci (*Xbarc117, Xbarc056*, and *Xbarc186*) linked to the 5AS QTL. Genetic effects of the loci and haplotype groups for FHB resistance were analyzed on the basis of disease data collected in both greenhouse (type II resistance) and field experiments (type I and II resistance or field resistance). Haplotype group 5 was the best haplotype comprised of marker allele combinations of both 3BS and 5AS QTL and wheat lines included in this haplotype group have improved type I and type II resistance compared to the lines in the other haplotype groups. The favorable marker loci for selecting haplotype 5 include two markers (Xbarc133 and XSTS3B142) on 3BS and two markers (Xbarc117 and Xbarc056) on 5AS. This study supports results of the previous mapping study (Chapter II) in that the 3BS chromosome region may be comprised of multiple QTL governing FHB resistance. Three haplotype groups (2 to 4) were characterized based on the combinations of four marker alleles in the 3BS QTL region. Haplotype 4 comprised of two to four favorable marker alleles in the 3BS QTL region had better FHB resistance than haplotypes 3 and 2 comprised of Xgwm493 and Xgwm533 target marker alleles, respectively. This study also presents and discusses possible strategies for combining FHB resistance with high yield potential through MAS. Elite lines having desirable haplotypes identified in the current study will provide breeding programs with a source of unique and adapted FHB resistant parents and some of the lines also may have potential for release as cultivars.

INTRODUCTION

Fusarium head blight (FHB), commonly known as scab, is one of the most devastating diseases of wheat worldwide (Schroeder and Christensen, 1963; Snijders, 1990). Until recently, development of FHB resistant cultivars has been hindered and restricted by primary reliance on phenotypic selection (PS) in most of breeding programs. Three main factors inhibit phenotypic selection of FHB resistance from being highly effective and cost efficient. The complex inheritance of FHB resistance is a primary obstacle. Resistance to FHB has been delineated into five components (1) type I, resistance to initial infection, (2) type II, resistance to spread of infection within a spike, (3) type III, decomposition or non-accumulation of mycotoxin, (4) type IV, resistance to kernel colonization, and (5) type V, tolerance to yield loss (Schroeder & Christensen, 1963; Wang & Miller, 1988; Mesterhazy, 1995). Three components of FHB resistance have generally been accepted and included type I, type II and type III (Somers et al., 2003). Current knowledge indicates that resistance to FHB is controlled by multiple genes and is very quantitative in nature (Somers et al., 2003). The second factor limiting progress using phenotypic selection is the effect of environment on both initiation and development of FHB epidemics that provide consistent and reliable phenotypic data. An effective selection system relies on successful establishment of consistent and optimal infection levels, which require a rather specific combination of both humidity and temperature regimes. While establishment of high humidity can be achieved using a fine-mist irrigation system in field screening nurseries, little can be done to alter temperature other than test site selection, which in itself is not highly effective in most geographic regions nor feasible. The third factor is genotype x environment

interaction, which confounds genotypic effects and adversely influences phenotypic selection especially in early generations.

Marker assisted selection (MAS) for FHB resistance QTL is extremely attractive to breeders in developing cultivars and/or adapted germplasm with effective FHB resistance as over eighteen wheat chromosome regions have been reported having association with FHB resistance (Table 1 in Chapter I in this dissertation). Two QTL on chromosome 3BS and 5AS having a large and stable effect reported in several known resistance sources (Anderson et al., 2001; Liu et al., 2003; Zhou et al., 2002; Buerstmayr et al., 2003; Zhou et al., 2004; Zhang et al., 2004; Chen et al., 2005). However, little is known about the effectiveness of MAS of the two QTL in adapted wheat backgrounds.

The goal of this research study was to evaluate and validate presence of the two QTL on chromosomes 3BS and 5AS in elite wheat backgrounds. Allelic and QTL effects were assessed on the basis of comparisons of marker haplotypes for the two QTL regions. Results of this study are also discussed with respect to strategy and feasibility in implementing MAS for these two QTL in variety development programs.

MATERIALS AND METHODS

I. Plant Materials

Three known type II resistance sources, Sumai 3, Ning7840, and W14, and one known type I resistance source Frontana were used as controls in haplotyping of 70 wheat lines. QTL for FHB resistance in Sumai 3, Ning7840, W14, and Frontana have been characterized in previous papers (Anderson et al., 2001; Zhou et al., 2002; Chen et al., 2005; Steiner et al., 2004).

A total of 70 wheat lines were evaluated in the current study including four check cultivars and eight recurrent parents (RCPs). The four checks included two known FHB susceptible cultivars Coker9835 and PioneerBrand26R46, and two known native resistance sources Neuse and Tribute. The remaining 58 elite lines were derived using diverse breeding methods including top-crossing, backcrossing and doubled haploid development via wheat by maize hybridization to incorporate FHB resistance from seven exotic sources (W14, Futai8944, Futai8946, Shaan85, Ning7840, Ning9016, and VR95B717) into adapted SRW wheat backgrounds such as the cultivars Jackson, Renwood3260, PioneerBrand2684, Roane, Sisson sib, Madison, AgriproMason, and Ernie (Table 1). The exotic sources are all Sumai 3 derivatives except for the French line VR95B717.

Topcross-derived lines were developed using the following procedures: phenotypic mass selection for FHB resistance and plant type was applied in F_2 to F_4 generations and single spike selection was applied in F_4 to F_6 generations in a mist-irrigated nursery inoculated with Fusarium-colonized maize kernels. Resistant spikes with no more than three colonized spikelets

were selected. The selected spikes from F_4 to F_6 were threshed separately and grown and evaluated in 1.5 m long progeny rows. Pure lines were derived from progeny rows selected for plant type, adaptation and resistance to prevalent diseases other than FHB. Selected lines were then evaluated in single 4.2 m^2 plots in yield tests called observation tests at two locations and for FHB resistance in non-replicated 1.5 long two-row plots in a mist-irrigated nursery inoculated twice using spray-inoculation (Chen et al., 2000). The elite lines with FHB resistance and/or the best performance compared to commercial check cultivars were selected and used in the current study. Backcross-derived lines were developed using a similar procedure as topcross-derived lines except that the initial backcrosses were made in the greenhouse.

II. Phenotypic Evaluation of FHB Resistance in Greenhouse and Field Experiments

1. Type II resistance evaluated in greenhouse experiment

Three vernalized plants of all 70 lines were planted in the greenhouse during winter 2004. Five to eight plants of each known resistance source (spring type) also were planted as checks with the elite lines for evaluation only in greenhouse experiment. One to three spikes per plant were evaluated for type II resistance using a single floret inoculation method (50,000 spores/ml, Chen et al. 2000) during spring 2005. The inoculated plants received overhead mist-irrigation for 3 days at an interval of 45 seconds per half hour. FHB severity of one to three inoculated spikes per plant was evaluated on the 21st day after inoculation and calculated using the formula: (number of colonized spikelets/total number of spikelets) x 100. FHB severity for each line was based on a grand mean of all inoculated spikes and used for evaluation of type II resistance.

2. Type I and II resistance evaluated in field experiment

The field experiment was conducted during the 2004-2005 season. The 70 wheat lines were planted in 1.5 m long two-row plots with three replications arranged in a randomized complete block design at Blacksburg, VA. Each line was spray-inoculated twice, once at 100 percent spike emergence and again at 50 percent anthesis, with 160 ml conidial suspension (50,000 spores/ml) using a CO_2 pressurized sprayer (R and D Sprayers, Opelousas, LA 70570, USA). Overhead mist-irrigation was applied at 1-hour intervals from 8:00 to 9:00 A.M. and again from 16:00 to 17:00 P.M. daily for three weeks when natural precipitation was absent. Ten spikes of each line were evaluated for FHB incidence on the 21st day after the second inoculation. FHB incidence for each line was calculated as (number of infected spikes/10) x 100 and used for evaluation of type I resistance. FHB severity for each line was based on a grand mean of 10 infected spikes per line. Because the spray-inoculation method was used, FHB severity evaluated in field is a combination of type I and II resistance.

3. Yield and agronomic performance evaluated in field experiments at two locations

Evaluations of the 70 wheat lines for yield and agronomic performance were conducted in conventional yield nurseries at two locations, Warsaw and Blacksburg, VA during the 2004-05 season. All lines were planted in 4.2 m^2 plots with three replications arranged in a randomized complete block design. In addition to yield performance, heading date, and plant height were evaluated. Reaction to prevalent diseases, such as powdery mildew, barley yellow dwarf, leaf rust, and stripe rust were also evaluated under natural infection but data is not presented herein.

III. Haplotyping of 70 Wheat Lines for two FHB QTL/Markers

Molecular markers used in the current study were those validated in Chinese wheat line W14 (Fig.1.). A total of nine markers were used in the haplotyping study and included six in the 3BS QTL region (Xgwm533, Xbarc133, XSTS-66, XSTS-142, Xgwm493, and Xcfd79) and three in the 5AS QTL region (Xbarc117, Xbarc56, and Xbarc186). Marker haplotypes were determined and designated on the basis of the presence ('+') or absence ('-') of W14 marker alleles. QTL haplotypes of wheat lines were classified on the basis of marker combinations of 3BS and 5AS QTL. Leaf tissue for DNA extraction was collected from plants grown in greenhouse experiments. DNA extraction was conducted according to Clarke et al. (1989) with some modifications. Leaf tissue from wheat plants at the two leaf stage was collected into 2.0 ml microcentrifuge tubes and stored at -70°C prior to DNA extraction. The leaf tissue was ground with a glass rod in liquid nitrogen then the ground sample was mixed with 600 ul of extraction buffer. After 20 to 40 minutes of incubation at 65°C, 800 ul of 24:1 (v/v) chloroform/isoamyl alcohol solution was added and the tube contents were mixed vigorously for 20 minutes on a shaker. Centrifugation was performed at 10, 000 rpm for 10 minutes, and 600 ul of the upper phase solution was transferred to another 1.5 ul microcentrifuge tube. The DNA was precipitated with 800 ul of cold (-20°C) isopropanal alcohol and rinsed with 1 ml of 76% ethanol/10 mM NH_4AC. The air dried DNA was dissolved in 100 ul TE buffer. PCR amplification and SSR assays were conducted as previously described by Saghai Maroof et al. (1994).

IV. Data Analysis

Analyses of variance (ANOVA), correlation and regression analyses were conducted for all entries evaluated in greenhouse and field experiments using AGROBASE software (version 1999, http://Agronomix.mb.ca). Allelic effect was analyzed by F-test and displayed by Box Plot using SPSS 12.0 software (http://SPSS.com). The skeletal box plot was constructed by drawing a box between the lower and upper quartiles with a solid line drawn across the box to locate the median. A straight line is then drawn connecting the box to the largest value; a second line is drawn from the box to the smallest value. These straight lines are called whiskers and the entire graph is called a box-and-whiskers plot (Lyman Ott, 1992). The entry means for field incidence (type I) and greenhouse severity (type II) were used in homogeneous analysis (Duncan test) of different haplotypes of elite lines using SPSS software. The haplotype differences were considered significant at $p \leq 0.05$. The ideal haplotypes, favorable marker alleles, and desirable lines were identified upon analysis of all data.

RESULTS

FHB Resistance of Elite Lines

Significant variation ($p \leq 0.05$) was observed among wheat lines for FHB resistance (Fig. 2) and a significant ($p \leq 0.05$) Entry x Replication interaction was observed in field tests (ANOVA table not presented). The elite lines showed continuous variation for FHB severity evaluated in the greenhouse experiment, while a bimodal distribution of variation was observed for field incidence and field severity (Fig.2). A high correlation coefficient was obtained between field

incidence and severity (r^2 = 0.72), but weak correlation was obtained between greenhouse and field severity (r^2 = 0.28).

Verification of the Two QTL on 3BS and 5AS in Elite Wheat Backgrounds

Oneway ANOVA regression indicated that eight of the nine marker loci were significantly ($p \leq$ 0.01) associated with FHB resistance on the basis of significance of one to three FHB assessment parameters. Markers XSTS3B142 and XSTS3B66 co-segregate; therefore only seven marker loci are presented in Table 2. Markers Xbarc133 and XSTS3B142 in the 3BS QTL region had higher R^2 values than the other two distal markers Xgwm533A and Xgwm493. Markers Xbarc117 and Xbarc056 in the 5AS QTL region also had high R^2 values that are comparable to those of two best 3BS markers, especially for field resistance. The 5AS QTL marker Xbarc186 had the lowest R^2 value and only was significant for field incidence.

Haplotypes of Marker Loci at the 3BS and 5AS QTL Regions in Known Type II, Type I and Native FHB Resistance Sources

The three known type II resistance sources (Sumai 3, Ning7840, and W14) have the same haplotype for three markers in the 5AS QTL region (Table 3). These sources also have the same haplotype for most markers in the 3BS QTL region, except for marker Xgwm533A. The Xgwm533A marker allele in W14 is two base pairs larger than the one in the other two sources as quantified by capillary method (Bai and Brown-Guedira, personal communication). Both marker alleles are associated with FHB resistance (Anderson et al., 2001; Chen et al., 2005). Frontana, a known type I resistance source, has only one marker allele (Xbarc186) that is the same as the type II sources. Haplotypes among five native FHB resistance sources were

different; however, four of them have the Xgwm493 marker target allele in common and this allele was the same or similar to that of the type II sources. Ernie and Neuse have more FHB QTL target marker alleles that are the same or similar to those of the type II sources than the other native sources.

Haplotypes for Marker Loci at the Two QTL Regions in Elite Wheat Lines and Their Effect on FHB Resistance

Allelic effect of seven marker loci in Table 2 on three FHB assessment parameters were analyzed by F-test (Table 4) and six of them are displayed via Boxplot analysis of the 70 wheat lines (Fig. 3). Presence of target alleles for markers Xbarc133, XSTS3B142, Xbarc117, and Xbarc056 in elite lines significantly ($p \leq 0.05$) decreased FHB field incidence and severity, as well as greenhouse severity (Table 4). In contrast, presence of target marker allele Xgwm493 in elite wheat lines significantly increased field incidence and severity. Presence of target marker allele Xgwm 533A only significantly decreased FHB field severity; whereas marker allele Xbarc186 only significantly decreased field incidence (Table 4).

A total of six haplotype groups were characterized on the basis of allelic composition for seven marker loci at the 3BS and 5AS QTL regions in the 70 wheat lines. Field and greenhouse FHB data and marker data for five haplotype groups are presented in Table 5. Haplotype 6 only included one elite line having the target alleles for two markers at the 5AS QTL region, but lacking the target alleles for all 3BS markers (Table 7), therefore, this line was not included in Table 5 or Table 6.

Elite lines in haplotype group 1 have none of the target marker alleles at the two QTL regions. Three haplotype groups (2, 3, and 4) were comprised of wheat lines having different combinations of 3BS target marker alleles but lacking the target alleles for the 5AS markers. Haplotype 2 was comprised of wheat lines having only the Xgwm533A target allele; haplotype 3 lines had only the Xgwm493 target allele, and haplotype group 4 was comprised of lines having two to four target marker alleles at the 3BS QTL region (Table 5). Haplotype group 5 was comprised of lines having different combinations of target alleles for both 3BS and 5AS FHB QTL markers.

Comparison of lines in haplotype groups 1 to 5 for mean differences in three FHB assessment parameters are presented in Table 6 and Fig. 4. Wheat lines in haplotype group 5 were significantly different from those in the other haplotype groups for all three FHB assessment parameters. Elite lines in haplotype group 5 had significantly ($p \leq 0.05$) higher levels of overall FHB resistance (type I plus type II) compared to mean FHB resistance of lines in the other haplotype groups. For type II resistance evaluated as severity in the greenhouse experiment, wheat lines in haplotype groups 5, 4 and 3 had lower mean severities than those in haplotype groups 2 and 1. For field resistance assessed as incidence and severity, lines in haplotype groups 5, 4 and 2 had lower mean FHB incidence and severity than those in haplotype groups 1 and 3. Elite lines in haplotype group 3 had significantly higher FHB incidence and severity than those in haplotype groups 2, 4, and 5. Rank of the five haplotype groups for best type II resistance is: $5 \geq 4 = 3 \geq 2 \geq 1$ and rank for best type I resistance is: $5 > 4 = 2 \geq 1 \geq 3$.

DISCUSSION

Genetic Mechanism of FHB Resistance for the Two Major QTL on Chromosomes 3BS and 5AS

The FHB QTL on chromosomes 3BS and 5AS are two of the major ones reported in several mapping studies. The 3BS QTL, having association with type II resistance, has been identified and verified in Sumai 3 (Waldron et al., 1999; Anderson et al., 2001) and Sumai 3-derived resistance sources, including Ning7840 (Zhou et al., 2002), CM-82036 (Buerstmayr et al., 2002 and 2003), Ning894037 (Shen et al., 2003), DH181 (Yang et al., 2005), Wangshuibai (Zhang et al., 2004), and W14 (Chen et al., 2005). This QTL also has been identified in two genotypes that are not related to Sumai 3 including one Japanese line NyuBai (Somers et al., 2003) and a SRW wheat cultivar Ernie, which was identified in the current study via haplotyping of the known 3BS QTL markers. In addition, the 3BS QTL also was reported to be associated with type I resistance in DH181 (Yang et al., 2005) and in W14 (Chen et al., 2005), and type III (DON) resistance in Nyubai (Somers et al., 2003) and in W14 (Chen et al., 2005).

The 5AS QTL was reported to have association with type I, type II, and type III resistance in diverse sources, such as Sumai3-related sources CM-82036, DH181, and W14, Brazilian cultivar Frontana, Japanese cultivar Nyubai, and SRW wheat cultivar Ernie (Buerstmayr et al., 2003, Yang et al., 2005; Chen et al., 2005; Steiner et al., 2004; Somers et al., 2003; McKendry et al., 2004).

The current study confirmed results reported in previous studies wherein the 3BS and 5AS QTL have effects on both type I and type II resistance in elite wheat line backgrounds. However, in the current study, the effect of the 3BS QTL on type I FHB resistance was comparable to its effect on type II resistance (Table 2 and Table 4). This is in disagreement with two previous reports where the effect of 3BS on type II resistance was greater than that on type I resistance (Yang et al., 2005; Chen et al., 2005). This may be because two markers (Xbarc133 and XSTS3B142) tightly linked to the 3BS QTL were used in the current study; whereas two distal markers were used in the previous studies. The effect of the 5AS QTL on type I resistance was greater than that on type II resistance. This is in agreement with a previous report (Chen et al., 2005). Three haplotype groups were characterized on the basis of allelic differences of four markers in the 3BS QTL region. This supports results of the previous mapping study (Chapter II) in that the 3BS QTL region may be comprised of multiple loci or a gene cluster governing FHB resistance.

Marker Assisted Selection of Favorable Marker Alleles at the 3BS and 5AS QTL Regions

Results of this study indicate that presence of the 3BS target marker alleles at Xbarc133 and XSTS3B142 loci are consistently associated with FHB resistance, whereas presence of 3BS target marker alleles for Xgwm493 and Xgwm533A are not consistently associated with FHB resistance. Therefore, MAS of the 3BS QTL should be based on markers Xbarc133 and XSTS3B142 or markers tightly linked or co-segregated with these two. This study also indicates that target alleles for markers Xbarc056 and Xbarc117 in the 5AS QTL region have a significant and positive effect on both type II and field (type I and type II) resistance (Tables 2 and 4), especially, when combined with favorable marker alleles in 3BS QTL region (Tables 4, 5, and 6;

Figs. 3 and 4). Wheat lines in haplotype group 5 possess two to six target marker alleles in both QTL regions, and had significantly ($p \leq 0.05$) higher levels of both type II and field resistance (Tables 5 and 6). This finding verifies that combing the 3BS and 5AS QTL via MAS is the best strategy for developing FHB resistant cultivars. Some elite lines having desirable haplotypes include VA04W-389, VA04W-433, VA04W470, and all of the Ernie backcross-derived lines.

Results from this study also suggest that a combination of limited backcrossing (BC_1 or BC_2) with subsequent top-crossing to elite genotypes may be the most effective breeding strategy for developing viable cultivars having good FHB resistance derived from non-adapted sources combined with desirable agronomic traits such as yield performance. Genetic gains are severely limited in traditional backcrossing programs as most progeny resemble their recurrent parents. Entries 62 to 70 (Table 5) are Ernie backcross-derived lines, and while these lines have a desirable marker haplotype for FHB 3BS and 5AS QTL and the best FHB resistance, they have low yield potential similar to Ernie. The last parent of a top cross also significantly affects the potential performance of the progeny. VA04W-389, a top-cross derived line, and VA04W-470, a doubled haploid line derived from a three-way cross, both have Ernie as the last parent. These lines also have good FHB resistance but low grain yield. In contrast, the topcross-derived line VA04W-433 and the doubled haploid line VA04W-474 have good FHB resistance and high yield potential (Table 5). Both of these lines were derived from 3-way crosses in which the last parent was an adapted elite line or released cultivar that was crossed to an F_1 comprised of an exotic FHB resistant parent W14 or Ning7840 and another adapted cultivar. These observations confirm that initial incorporation of FHB resistance from non-adapted sources into desirable elite lines is difficult; however, subsequent progeny derived from crosses with these initial FHB

resistant lines likely will have improved grain yields. Roane backcross-derived lines in haplotype group 3 had the highest yield potential in the current study. FHB resistance of these lines can be further improved by incorporating and selecting favorable alleles for markers, such as XSTS3B142, Xbarc133, Xbarc117, and Xbarc056 (Table 5).

Genetic background of recurrent parents also had a significant effect on expression of introgressed FHB QTL (Table 7). Incorporation of the 3BS and 5AS QTL into the Pioneer 2684 background did not significantly improve FHB resistance even in lines such as VA04W-521 that has target marker alleles for both the 3BS and 5AS QTL. This suggests that use of diverse sources of FHB resistance as well as diverse backgrounds are both important, and that the presence of epistatic genes in some backgrounds may inhibit or suppress expression of introgressed resistance genes.

Haplotyping in the current study was based only on available markers in the 3BS and 5AS QTL regions; and associations between haplotypes and FHB resistance were based on data collected from a single year in both greenhouse and field experiments. Therefore, multiple year experiments and additional QTL/marker haplotyping are being conducted to verify the results of the current study.

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Table 1. Pedigrees of elite wheat lines, recurrent parents and check cultivars used in current study.

ENTRY	LINE	PEDIGREE
Top-cross derived lines		
1	VA00W-38	VA91-54-343 /VA91-54-222, F13
2	VA01W-99	FFR525/VA93-52-55(MSY*3/BALKAN//SAL), F10
3	Neuse	NC96-13156=Coker86-29//STELLA /CHD75680/3/Coker9907
4	Tribute	VA98W-593=VA92-51-39 / AL870365(COKER747*2/AMIGO)
5	Pion26R46	Susceptible Check
6	Coker9835	Susceptible Check
7	VA04W-360	VR95B717 / Roane//VA96W-391, F6
8	VA04W-389	Ernie/3/P92823A1-1-2-3-5(CLARK*4/NING7840)//Roane/Pion2643,F7
9	VA04W-396	SHAAN85-2/MADISON//PION2580 AND PION2684//SHAAN85-2/VA96-52-71,F7
10	VA04W-404	ERNIE/3/P92823A1-1-2-3-5(CLARK*4/NING7840)//VA94-52-68
11	VA04W-433	NING 7840/PION2684//VA96-54-244(Coker9803/FREEDOM),F8
12	VA04W-439	NING 7840/PION2691//Roane, F8
13	VA04W-465	WUHAN1//90-52-82/Coker9835/3/Coker9803,F10
Doubled haploid lines		
14	VA04W-470	ROANE/FREEDOM//ERNIE,H4
15	VA04W-472	ROANE//W14/Coker9134,H4
16	VA04W-474	ROANE//W14/Coker9134,H4
17	VA04W-478	Renwood 3260(VA96W-326)//FREEDOM/ERNIE,H4
18	VA04W-486	ERNIE//INW 9824(CLARK*4/NING7840)/McCORMIC,H4
19	VA04W-491	ROANE//IL89-6489 /AGS 2000,H4
20	VA04W-493	ROANE//IL89-6489 /AGS 2000),H4
21	VA04W-495	ROANE//INW 9824/AGS 2000,H4
22	VA04W-498	ROANE/AGS 2000//AGRIPRO GIBSON,H4

Table 1, continue,

ENTRY	LINE	PEDIGREE
Backcross derived lines		
23	Jackson	Recurrent Parent
24	VA04W-503	Jackson/3/Jackson//Shaan85-2/Jackson,BC2F6
25	Renwood 3260	Recurrent Parent
26	VA04W-508	Renwood 3260//W14/Renwood 3260,BC1F6
27	VA04W-513	Renwood 3260*2//W14/Renwood 3260/3/2*Renwood 3260,BC4F4
28	VA04W-514	Renwood 3260/4/Renwood 3260*2//W14/Renwood 3260/3/Renwood 3260,BC4F4
29	VA04W-515	Renwood 3260/4/Renwood 3260*2//W14/Renwood 3260/3/Renwood 3260,BC4F4
30	VA04W-517	Renwood 3260*2//W14/Renwood 3260/3/2*Renwood 3260,BC4F4
31	Pion2684	Recurrent Parent
32	VA04W-520	Pion2684*2//Ning9016/Pion2684,BC2F6
33	VA04W-521	Pion2684*2//Ning9016/Pion2684,BC2F6
34	VA04W-522	Pion2684*2//Ning9016/Pion2684,BC2F6
35	VA04W-535	Pion2684//Yumai7/Pion2684,BC1F6
36	VA04W-536	Pion2684*2//Shaan85-2/Pion2684,BC2F5
37	VA04W-538	Pion2684*2//Shaan85-2/Pion2684,BC2F5
38	VA04W-547	Pion2684*2//W14/Pion2684,BC2F5
39	VA04W-554	Pion2684*2//VR95B717/Pion2684,BC2F5
40	VA04W-555	Pion2684*2//VR95B717/Pion2684,BC2F5
41	VA04W-557	Pion2684*2//VR95B717/Pion2684,BC2F5
42	VA04W-558	Pion2684*2//VR95B717/Pion2684,BC2F5
43	Roane	Recurrent Parent
44	VA04W-561	Roane*2//Futai8944/Roane,BC2F5
45	VA04W-563	Roane//Futai 8946/Roane,BC1F6
46	VA04W-568	Roane*2//W14/Roane/3/2*Roane,BC4F4
47	VA04W-569	Roane*2//VR95B717/Roane,BC2F5
48	VA04W-570	Roane*2//VR95B717/Roane,BC2F5
49	VA04W-571	Roane*2//VR95B717/Roane,BC2F5
50	VA96W-234	Recurrent Parent
51	VA04W-574	VA96W-234//Ning9016/VA96W-234,BC1F6
52	VA04W-575	VA96W-234//Ning9016/VA96W-234,BC1F6

Table 1, continue,

ENTRY	LINE	PEDIGREE
53	Madison	Recurrent Parent
54	VA04W-581	Madison/4/Madison//Futai8944/Madison/3/Madison/ 5/Madison,BC4F4
55	VA04W-584	Madison/4/Madison//Futai8944/Madison/3/Madison/ 5/Madison,BC4F4
56	AgriproMason	Recurrent Parent
57	VA04W-587	AgriPro Mason/4/ AgriPro Mason //Shaan85-15/ AgriPro Mason /3/ AgriPro Mason /5/2* AgriPro Mason, BC5F4
58	VA04W-589	AgriPro Mason /4/AgriPro Mason //Shaan85-15/AgriPro Mason /3/ AgriPro Mason /5/2* AgriPro Mason, BC5F4
59	GA891283LE18	Recurrent Parent
60	VA04W-592	GA891283LE18//Er-Mai 9/GA891283LE18,BC1F6
61	Ernie	Recurrent Parent
62	VA04W-607	Ernie*2//Shaan85-2/Ernie/3/2*Ernie, BC4F4
63	VA04W-608	Ernie*2//VR95B717/Ernie,BC2F5
64	VA04W-611	Ernie*2//W14/Ernie,BC2F5
65	VA04W-621	Ernie*2//Futai8944/Ernie,BC2F5
66	VA04W-626	Ernie*2//Futai8944/Ernie/3/2*Ernie,BC4F4
67	VA04W-628	Ernie//Ning7840/Ernie,BC1F6
68	VA04W-629	Ernie//Ning9016/Ernie,BC1F6
69	VA04W-631	Ernie//Ning9016/Ernie,BC1F6
70	VA04W-632	Ernie//Ning9016/Ernie,BC1F6

Table 2. Coefficients of determination (R^2 x 100) and *p* values for markers significantly associated with FHB resistance in 70 elite wheat lines.

Chromosome/ QTL-markers	Greenhouse Severity		Field Severity		Field Incidence	
	R^2 (%)	*p*	R^2 (%)	*p*	R^2 (%)	*p*
3BS						
Xgwm533A	-	-	7	0.028	-	-
Xbarc133	21	< 0.0001	24	< 0.0001	21	< 0.0001
XSTS3B142	15	0.0011	18	0.0003	26	< 0.0001
Xgwm493	-	-	15	0.0011	8	0.0174
5AS						
Xbarc117	15	0.0011	23	< 0.0001	22	< 0.0001
Xbarc056	17	0.0004	24	< 0.0001	23	< 0.0001
Xbarc186	-	-	-	-	7	0.0227

Table 3. Haplotypes for marker loci at chromosome 3BS and 5AS QTL regions in known type II, type I and native FHB resistant wheat sources. "+" represents the presence of resistant genotype allele and "-" represents the absence of resistant genotype allele of a given marker locus. '*+' allele has 2 bp difference from '+' allele.

Line and type of resistance	Haplotypes at two QTL regions						
	3BS				5AS		
	Xgwm 533A	*Xbarc 133*	*STS3B 142*	*Xgwm 493*	*Xbarc 117*	*Xbarc 056*	*Xbarc 186*
Type II							
W14	*+	+	+	+	+	+	+
Sumai3	+	+	+	+	+	+	+
Ning7840	+	+	+	+	+	+	+
Type I							
Frontana	-	-	-	-	-	-	+
Native							
Ernie	-	+	+	-	+	+	-
Neuse	-	-	-	*+	+	+	-
Roane	-	-	-	+	-	-	-
McCormick	-	-	-	+	-	-	-
Tribute	-	-	-	*+	-	-	-

Table 4. F-test of significance for mean FHB disease data for lines with alternative genotypes ("+" or "-") for seven marker loci in the 3BS and 5AS QTL regions in 70 elite wheat lines.

QTL	Marker	No. of Lines		Greenhouse Severity		Field Incidence		Field Severity	
Region	Loci	+	-	+	-	+	-	+	-
3BS	*Xgwm553A*	28	42	21.1	18.5	70.2	72.6	14.6*	17.7
3BS	*Xbarc133*	18	52	12.9**	21.8	58.6**	76.2	11.6**	18.1
3BS	*XSTS3B142*	18	52	14.0**	21.5	56.9**	76.7	12.3**	17.9
3BS	*Xgwm493*	30	40	18.7	20.1	77.2*	67.5	19.0**	14.5
5AS	*Xbarc117*	18	52	14.0**	21.5	58.3**	76.3	11.7**	18.1
5AS	*Xbarc056*	23	47	14.5**	22.0	60.0**	77.3	12.4**	18.4
5AS	*Xbarc186*	6	64	14.2	20.0	56.7*	73.0	13.3	16.8

"+" = genotype with target marker allele and "-" = genotype lacking target marker allele. "*" and "" indicate that mean disease data for lines with target marker allele was significantly different from lines lacking target marker allele at $p \leq 0.05$ and $p \leq 0.01$, respectively.**

Table 5. Marker haplotypes of wheat lines for chromosomes 3BS and 5AS QTL regions and their effect on FHB resistance.

Haplotype Group	Entry	Line	Mean data, 2005				Haplotypes at two QTL regions						
			Field			GH	3BS				5AS		
			Yield (bu/A)	Incidence (%)	Severity (%)	Severity (%)	Xgwm 533	Xbarc 133	XSTS 142	Xgwm 493	Xbarc 117	Xbarc 56	Xbarc 186
5		Sumai3				9.6	+	+	+	+	+	+	+
5		Ning7840				12.8	+	+	+	+	+	+	+
5		W14				10.9	*+	+	+	+	+	+	+
5	61	Ernie	68.2	55	8.9	8.6	-	+	+	-	+	+	-
5	62	VA04W-607	68.8	45	11.8	10.6	-	+	+	-	+	+	-
5	63	VA04W-608	65.7	60	10.0	12.2	-	+	+	-	+	+	-
5	64	VA04W-611	71.0	60	11.8	7.8	-	+	+	-	+	+	-
5	65	VA04W-621	67.9	35	8.9	12.8	-	+	+	-	+	+	-
5	66	VA04W-626	65.7	50	10.0	10.8	-	+	+	-	+	+	-
5	67	VA04W-628	68.9	60	11.1	8.5	-	+	+	-	+	+	-
5	68	VA04W-629	67.7	60	8.9	7.7	-	+	+	-	+	+	-
5	69	VA04W-631	69.9	60	11.8	14.7	-	+	+	-	+	+	-
5	70	VA04W-632	70.3	35	5.9	12.3	-	+	+	-	+	+	-
5	8	VA04W-389	73.3	45	8.9	15.8	-	+	+	-	+	+	-
5	14	VA04W-470	64.2	45	8.9	12.4	+	+	+	-	+	+	+
5	11	VA04W-433	77.0	55	14.7	10.0	-	+	+	+	-	+	+
5	33	VA04W-521	70.0	85	20.6	24.2	+	-	+	+	+	+	-
5	52	VA04W-575	62.3	50	17.7	20.9	+	-	+	+	-	+	+
5	3	Neuse	68.2	80	17.6	18.8	-	-	-	+	+	+	-
5	1	VA00W-38	87.7	65	14.7	12.8	-	-	-	+	+	+	-
5	10	VA04W-404	61.5	70	11.8	15.9	+	-	-	-	+	+	-
5	16	VA04W-474	76.8	50	11.8	12.5	-	-	-	+	+	+	+
5	42	VA04W-558	66.1	85	14.7	21.5	+	-	-	-	-	+	-
5	50	VA96W-234	77.5	85	14.7	16.5	+	-	-	-	-	+	+
5	51	VA04W-574	71.0	55	11.8	12.8	+	-	-	+	-	+	+

Table 5, continue

Haplotype Group	Entry	Line	Mean data, 2005				Haplotypes at two QTL regions						
			Field			GH	3BS				5AS		
			Yield (bu/A)	Incidence (%)	Severity (%)	Severity (%)	Xgwm 533	Xbarc 133	XSTS 142	Xgwm 493	Xbarc 117	Xbarc 56	Xbarc 186
4	45	VA04W-563	82.8	80	17.6	21.6	+	+	+	+	-	-	-
4	18	VA04W-486	59.5	70	14.7	9.9	-	+	+	-	-	-	-
4	34	VA04W-522	70.4	75	20.0	30.3	+	-	+	+	-	-	-
4	36	VA04W-536	64.1	70	13.4	19.5	+	+	-	+	-	-	-
4	38	VA04W-547	78.7	90	20.0	13.9	+	+	-	+	-	-	-
4	57	VA04W-587	74.2	80	11.8	22.3	+	+	-	+	-	-	-
4	56	Ag.Mason	73.5	55	8.9	37.7	+	-	-	+	-	-	-
4	58	VA04W-589	75.0	70	8.9	13.1	+	-	-	+	-	-	-
4	59	GA891283LE18	71.9	55	17.7	14.9	+	-	-	+	-	-	-
4	60	VA04W-592	84.6	70	16.7	11.1	+	-	-	+	-	-	-
3	4	Tribute	78.2	80	18.8	14.3	-	-	-	+	-	-	-
3	43	Roane	85.0	90	29.4	11.2	-	-	-	+	-	-	-
3	2	VA01W-99	75.0	75	17.6	10.0	-	-	-	+	-	-	-
3	7	VA04W-360	79.7	65	20.6	43.8	-	-	-	+	-	-	-
3	19	VA04W-491	77.7	95	20.6	14.1	-	-	-	+	-	-	-
3	20	VA04W-493	85.4	95	17.6	6.0	-	-	-	+	-	-	-
3	37	VA04W-538	70.8	80	21.9	26.7	-	-	-	+	-	-	-
3	39	VA04W-554	71.0	85	20.6	31.4	-	-	-	+	-	-	-
3	40	VA04W-555	68.0	85	20.6	19.4	-	-	-	+	-	-	-
3	41	VA04W-557	70.7	80	14.7	17.2	-	-	-	+	-	-	-
3	44	VA04W-561	85.3	95	35.3	20.6	-	-	-	+	-	-	-
3	46	VA04W-568	84.7	95	29.4	17.6	-	-	-	+	-	-	-
3	47	VA04W-569	86.1	100	26.5	17.5	-	-	-	+	-	-	-
3	48	VA04W-570	86.5	95	26.5	19.0	-	-	-	+	-	-	-
3	49	VA04W-571	85.9	95	23.5	15.5	-	-	-	+	-	-	-

Table 5, continue

Haplotype Group	Entry	Line	Mean data, 2005				Haplotypes at two QTL regions						
			Field			GH	3BS				5AS		
			Yield (bu/A)	Incidence (%)	Severity (%)	Severity (%)	Xgwm 533	Xbarc 133	XSTS 142	Xgwm 493	Xbarc 117	Xbarc 56	Xbarc 186
2	23	Jackson	<u>80.7</u>	85	14.7	23.4	+	-	-	-	-	-	-
2	13	VA04W-465	<u>79.9</u>	80	<u>11.1</u>	23.6	+	-	-	-	-	-	-
2	15	VA04W-472	66.7	85	18.2	23.4	+	-	-	-	-	-	-
2	21	VA04W-495	<u>79.1</u>	90	17.6	18.3	+	-	-	-	-	-	-
2	22	VA04W-498	72.8	75	<u>11.8</u>	13.9	+	-	-	-	-	-	-
2	24	VA04W-503	<u>78.1</u>	90	17.6	20.7	+	-	-	-	-	-	-
2	25	Renwood 3260	<u>78.7</u>	<u>50</u>	17.7	31.9	+	-	-	-	-	-	-
2	27	VA04W-513	74.3	<u>60</u>	<u>11.8</u>	<u>16.2</u>	+	-	-	-	-	-	-
2	28	VA04W-514	<u>77.0</u>	<u>35</u>	<u>8.9</u>	21.9	+	-	-	-	-	-	-
2	29	VA04W-515	<u>79.3</u>	<u>60</u>	<u>11.8</u>	39.5	+	-	-	-	-	-	-
2	30	VA04W-517	71.2	<u>55</u>	14.7	29.2	+	-	-	-	-	-	-
1	6	Coker9835	<u>77.2</u>	85	25.1	33.2	-	-	-	-	-	-	-
1	5	Pion26R46	69.6	90	26.5	27.4	-	-	-	-	-	-	-
1	31	Pion2684	74.2	90	20.6	20.4	-	-	-	-	-	-	-
1	53	Madison	72.7	75	20.6	29.6	-	-	-	-	-	-	-
1	9	VA04W-396	72.3	80	21.9	35.2	-	-	-	-	-	-	-
1	12	VA04W-439	<u>92.9</u>	85	17.6	21.1	-	-	-	-	-	-	-
1	17	VA04W-478	75.8	75	17.6	<u>10.6</u>	-	-	-	-	-	-	-
1	26	VA04W-508	66.8	<u>55</u>	14.7	31.0	-	-	-	-	-	-	-
1	35	VA04W-535	71.7	70	17.6	22.3	-	-	-	-	-	-	-
1	54	VA04W-581	74.2	80	14.7	27.8	-	-	-	-	-	-	-
1	55	VA04W-584	74.1	60	20.6	34.6	-	-	-	-	-	-	-

GH = greenhouse. The underlined numbers are below the mean disease data of elite lines with haplotype 1 minus one standard deviation (STDEV) for the three FHB assessment parameters, or greater than the mean yield of elite lines plus one STDEV for grain yield. "+" = genotype with target marker allele and "-" = genotype lacking target marker allele. '*+' allele has 2 bp difference from '+' allele.

Table 6. Homogeneous analysis of means for type I and type II resistance among five haplotype groups (1-5) for chromosomes 3BS and 5AS QTL regions over 69 elite wheat lines. A value in the same column followed by the same letter is not significantly different ($p \leq 0.05$) on the basis of Duncan's Multiple Range Test.

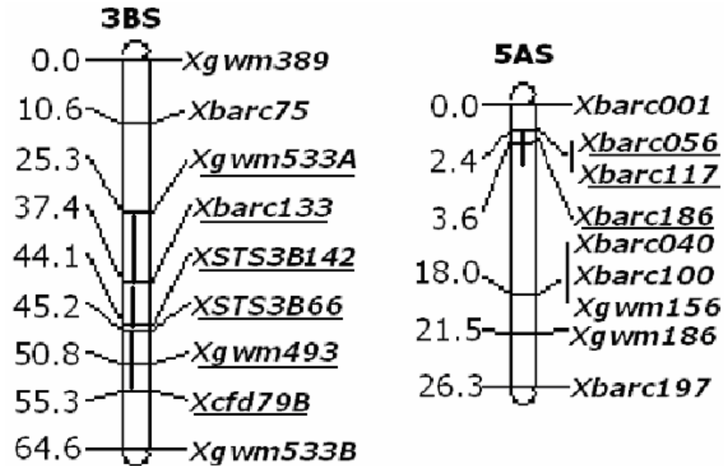
Haplotype Group	No. of Lines	Type II Greenhouse Severity (%)	Type I Field Incidence (%)	Type I & II Field Severity (%)
1	11	26.7 a	77 ab	19.8 a
2	11	23.8 ab	70 b	14.2 b
3	15	19.0 bc	87 a	22.9 a
4	10	19.4 bc	72 b	15.0 b
5	22	13.6 c	59 c	12.1 b

Table 7. Genetic background effect on FHB resistance in Pioneer 2684 backcross-derived lines in which the 3BS and/or 5AS QTL were introgressed.

Line	Mean data, 2005			Marker haplotypes at two QTL regions					
	Field		Greenhouse	3BS				5AS	
	Incidence (%)	Severity (%)	Severity (%)	Xgwm 533	Xbarc 133	XSTS 142	Xgwm 493	Xbarc 117	Xbarc 56
Pion2684	90	20.6	20.4	-	-	-	-	-	-
VA04W-520	90	17.9	32.7	-	-	-	-	+	+
VA04W-521	85	20.6	24.2	+	-	+	+	+	+
VA04W-522	75	20.0	30.3	+	-	+	+	-	-
VA04W-547	90	20.0	13.9	+	+	-	+	-	-
VA04W-536	70	13.4	19.5	+	+	-	+	-	-
VA04W-538	80	21.9	26.7	-	-	-	+	-	-
VA04W-554	85	20.6	31.4	-	-	-	+	-	-
VA04W-555	85	20.6	19.4	-	-	-	+	-	-
VA04W-557	80	14.7	17.2	-	-	-	+	-	-

"+" = genotype with target marker allele and "-" = genotype lacking target marker allele.

Fig. 1. Linkage maps of two major FHB QTL generated using a DH population of W14 x Pion2684. Numbers at each marker represent the distance in cM. Dark area indicates the QTL positions based on the composite interval mapping method (Chen et al., 2005). The underlined markers were used in the current study.



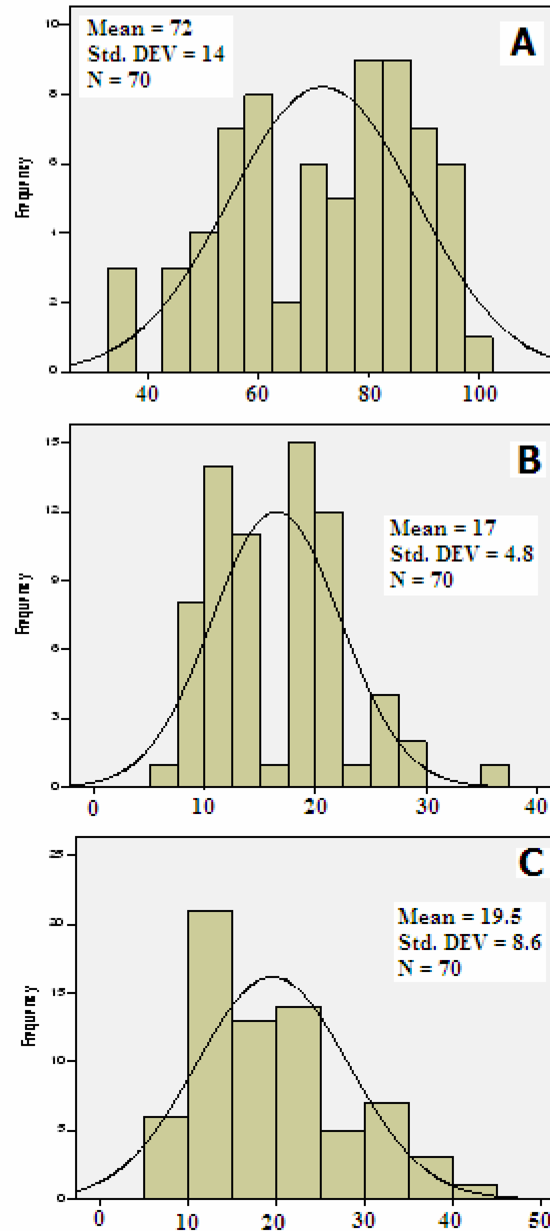
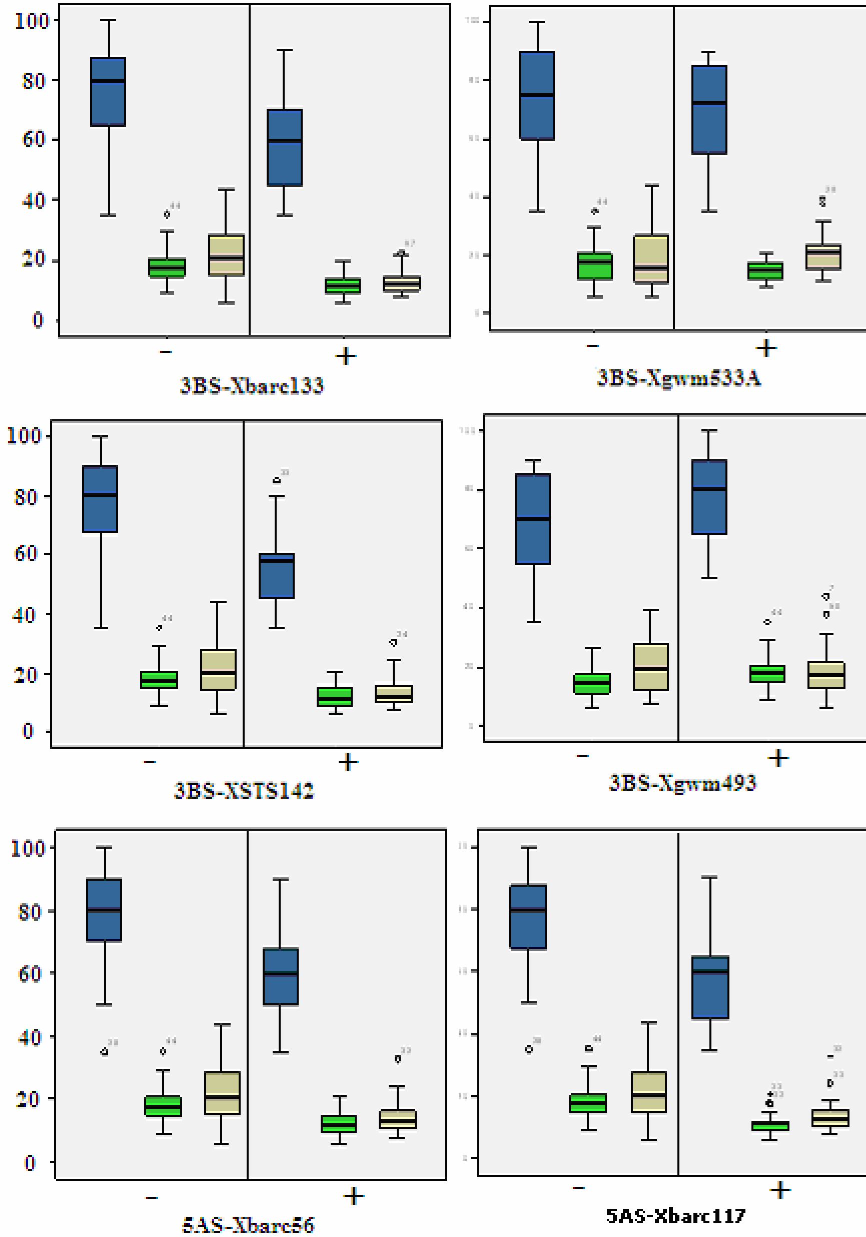


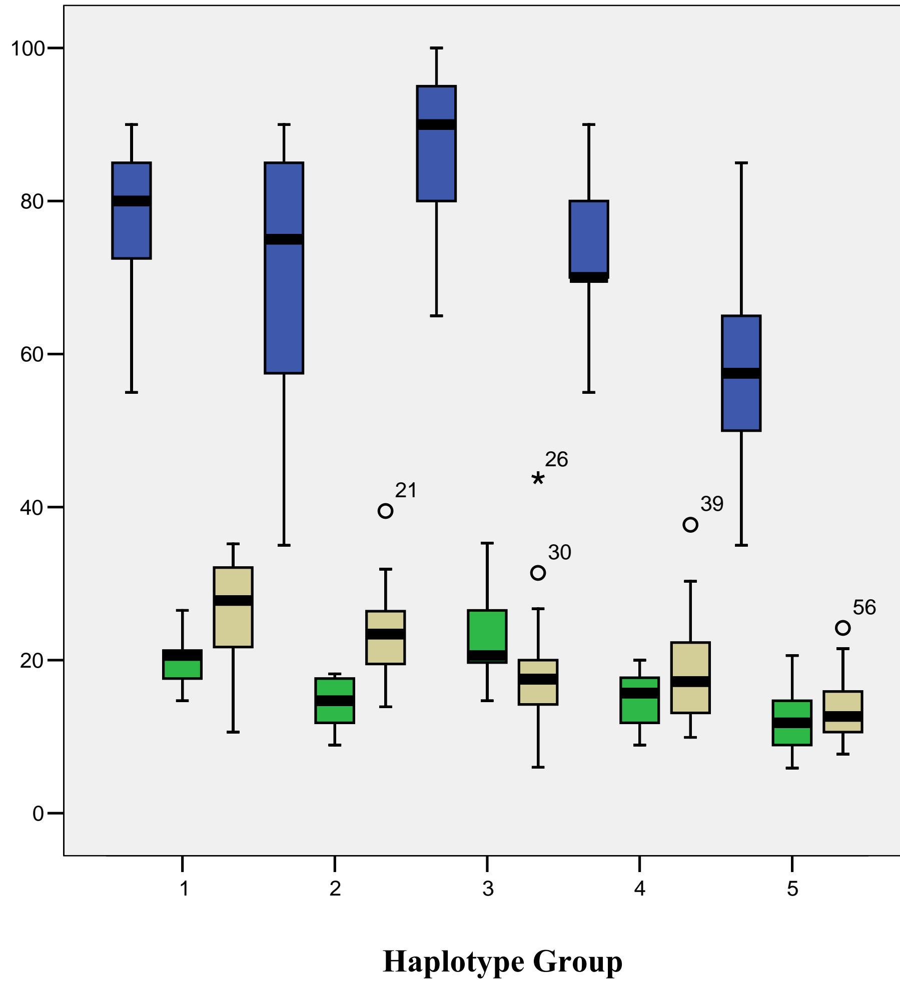
Fig. 2. Frequency histograms of 70 wheat genotypes for field FHB incidence % (Diagram A), field FHB severity % (Diagram B), and greenhouse FHB severity % (Diagram C).

Fig. 3. Boxplot showing mean FHB disease data on Y axis for lines with alternative marker allele genotype (“+” or “-”on X axis) for six marker loci in the 3BS and 5AS QTL regions in 70 wheat lines*.



***Blue = field incidence (type I), green = field severity (type I & II), and yellow = greenhouse severity (type II). “+” = genotype with target marker allele, “-” = genotype lacking target marker allele.**

Fig. 4. Haplotype difference and their effect on mean FHB disease data in 69 adapted wheat lines. Blue = field incidence, Green = field severity, and Yellow = greenhouse severity. Designations of haplotype 1 to 5 on the X-axis are corresponding to Table 4. The Y-axis represents the percentage of disease data for the three FHB resistance parameters.



Haplotype Group

APPENDIX A

Chen, J., C.A. Griffey, M.A. Saghai Maroof, E.L. Stromberg, R.M. Biyashev, W. Zhao, M. R. Chappell, T.H. Pridgen, Y. Dong, and Z. Zeng. 2005. Validation of two major quantitative trait loci for fusarium head blight resistance in Chinese wheat line W14. Plant Breeding (in press).

Validation of Two Major Quantitative Trait Loci for *Fusarium H*ead Blight Resistance in Chinese Wheat Line W14

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ABSTRACT

Identity of QTL governing resistance to Fusarium Head Blight (FHB) initial infection (type I), spread (type II), kernel infection, and deoxynivalenol (DON) accumulation were characterized in Chinese wheat (*Triticum aestivum* L.) line W14. Ninety-six doubled haploid lines derived from a cross of W14 x Pioneer2684 were evaluated for FHB resistance in two greenhouse and one field experiments. Two known major QTL were validated on chromosomes 3BS and 5AS in W14 using the composite interval mapping method. The 3BS QTL had a larger effect on resistance than the 5AS QTL in the greenhouse experiments, whereas, the 5AS QTL had a larger effect in the field experiment. These two QTL together explained 33%, 35%, and 31% of the total phenotypic variation for disease spread, kernel infection, and DON concentration in the greenhouse experiments, respectively. In the field experiment, the two QTL explained 34% and 26% of the total phenotypic variation for FHB incidence and severity, respectively. W14 has both QTL, which confer reduced initial infection, disease spread, kernel infection, and DON accumulation. Therefore, marker-assisted selection (MAS) for both QTL should be implemented in incorporating W14 resistance into adapted backgrounds. Flanking markers *Xbarc*133 and *Xgwm*493 on 3BS and *Xbarc*117 and *Xbarc*56 on 5AS are suggested for MAS.

Keywords: *Triticum aestivum* - Fusarium head blight - Microsatellite - QTL mapping

Fusarium head blight (FHB), commonly called scab, is one of the most destructive diseases of wheat (*Triticum aestivum* L.), and causes significant reductions in grain yield and quality. Mapping of QTL associated with FHB resistance and application of marker-assisted selection (MAS) can be used to accelerate the development of FHB resistant cultivars. Mapping efforts to

date have identified a major QTL on wheat chromosome 3BS for resistance to disease spread in most Chinese and Japanese resistance sources, such as ‘Sumai3’ and related sources (Anderson et al. 2001, Zhou et al. 2002, Buerstmayr et al. 2003, and Somers et al. 2003). In addition, a QTL on chromosome 5AS has been identified for resistance to initial infection in a Sumai 3-derived line (Buerstmayr et al. 2003) and in a Brazilian source Frontana (Steiner et al. 2004). Objectives of the current study were to characterize FHB resistance in Chinese wheat line W14 in comparison to QTL identified in previous studies and to determine the extent of allelic variation at the two known FHB QTL for resistance to initial infection (type I), spread (type II), kernel infection, and DON accumulation.

MATERIALS AND METHODS

Mapping population and FHB assessment: One double haploid (DH) population of wheat, *Triticum aestivum* L., comprised of 96 lines was developed using the wheat by maize hybridization method (Laurie and Bennett 1988) from a cross between W14 and Pioneer Brand ‘2684’ (Pion2684). W14 was derived from a recurrent selection population comprised of 20 parents, including 15 FHB resistant cultivars, such as Sumai3, Ning7840, Zhen7495, Wangshuibai, Fanshanxiaomai, Shinchunaga, Frontana, Yangmai 4, etc. (Jiang, 1997, and personal communication).   This line has improved FHB resistance compared to Sumai3 on the basis of lower observed disease spread, kernel infection and deoxynivalenol (DON) production (Chen et al. 2000, Buerstmayr, personal communication). Pion2684 is a FHB susceptible soft red winter (SRW) wheat cultivar.

The DH lines and parents were evaluated in two greenhouse (2001 and 2002) and one field (2004) experiments using the single floret inoculation and spray-inoculation methods (Chen et al. 2000), respectively. In the greenhouse experiments, four inoculated plants per line received overhead mist-irrigation for 3 days at an interval of 45 seconds per half hour. FHB severity of one to three inoculated spikes per plant was evaluated on the 21st day after inoculation and was calculated using the formula: (number of infected spikelets/total number of spikelets) x 100. FHB severity for each line was based on a grand mean of all inoculated spikes. Percentage of fusarium damaged kernels (FDK) for each line was determined as the number of infected kernels subdivided by the total number of kernels in the inoculated and hand-threshed spikes. These seeds were then assessed for DON concentration (ppm) using a Shimadzu QP2010 GC/MS system (Shimadzu Corporation, Kyoto, Japan, Mirocha et al., 1998). Severity data were collected from two independent experiments (2001 and 2002), whereas FDK and DON content were only evaluated in the 2001 experiment. In the field experiment, the two parental and 96 doubled haploid lines were planted in single 1.5 m long rows with two replications arranged in a completely randomized design. Each row was spray-inoculated twice, once at 100 percent spike emergence and again at 50 percent anthesis, with an 80 ml conidial suspension (50,000 spores/ml) using a CO_2 pressurized sprayer (R and D Sprayers, Opelousas, LA 70570, USA). Overhead mist-irrigation was applied at 1-hour intervals from 8:00 to 9:00 A.M. and again from 16:00 to 17:00 P.M. daily for three weeks when natural precipitation was absent. Ten spikes of each parental and DH line were evaluated on the 21st day after the second inoculation. FHB incidence for each line was calculated as (number of infected spikes/10) x 100. FHB severity for each infected spike was calculated as (number of infected spikelets/total number of spikelets) x 100. FHB severity for each line was based on a grand mean of 10 infected spikes per line.

Genetic mapping and QTL analysis: Four sets of SSR primers were used in the current study: 1) Gwm primers from Röder et al. (1998); 2) BARC primers provided by P. Cregan, USDA-ARS, Beltsville, MD; 3) WMC primers from published sequences (Gupta et al. 2002); and 4) PSP primers (Bryan et al. 1997) provided by M. Gale, John Innes Centre, Norwich, UK. A total of 308 SSRs were screened for DNA polymorphism among parents (W14 and Pion2684) and two extreme bulks using BSA analysis (Michelmore et al. 1991). Polymorphic primer pairs were used to characterize the DH population of 96 lines. Marker segregation data were used to construct linkage maps using MAPMAKER 3.0b (Lander et al., 1987). A logarithm of odd (LOD) threshold of 3.0 was used for grouping. The most-likely marker orders were determined using the MAPMAKER 'ripple' command. DNA extraction, PCR amplification and SSR assays were conducted as previously described by Saghai Maroof et al. (1994).

Analyses of variance of field and greenhouse data, and correlation and regression analysis were conducted using AGROBASE software (http://Agronomix.mb.ca). QTL analysis was performed using averages from individual experiments for FHB assessment data as well as a grand mean over experiments. FHB incidence (%), FHB severity (%), FDK, and DON concentration (ppm) were tested separately in the QTL analyses. Composite interval mapping (CIM, Zeng, 1994) was applied using QTL Cartographer 2.0 software (Basten et al., 2002). Significance threshold level was determined by a permutation test with 1000 permutated samples (Doerge and Churchill 1996). A genome-wide significance level $P \leq 0.01$ for the type I error with a logarithm of odd (LOD) threshold of 1.8 was used as a criterion to indicate putative QTL positions. Genetic variance (R^2) explained by QTL was calculated by the software.

RESULTS

Phenotypic analysis of parental lines and their DH progeny

The parents, W14 and Pion2684, consistently displayed significant differences in response to *F. graminearum* infection for all traits evaluated. Except for FHB incidence, distribution of DH lines was skewed towards the resistant parent W14 for all traits evaluated (Data not presented). High correlation coefficients were obtained for FHB severity between the two greenhouse experiments, and among FHB severity, FDK and DON in the 2001 greenhouse experiment (r = 0.69 – 0.91, $p < 0.0001$). A high coefficient of correlation also was observed between FHB incidence and FHB severity in the field experiment (r = 0.78, $p < 0.0001$); however, weaker correlations were obtained between disease data from single floret inoculations (greenhouse) and spray inoculations (field) (r = 0.27 – 0.37, $p < 0.01$ to $p < 0.001$).

Bulked segregant analysis and genetic maps

Out of 308 primer pairs screened, 18 pairs detected polymorphism between parents and bulks in the DH population. When these SSRs were assessed in the entire population, sixteen retained significance at $p \leq 0.05$. Five chromosomal regions (1B, 2B, 3B, 5A, and 7A) showed association with FHB resistance on the basis of single marker analysis. Additional markers flanking these 16 SSRs were used to construct the genetic maps of these linkage groups. A total of 39 SSRs were mapped in the DH population. Genetic maps of the linkage groups were constructed, and chromosomal identities were determined via comparison with the SSR maps of wheat published by Röder et al. (1998) and Shi et al. (2002). Genetic maps of 3BS and 5AS, which contain the major FHB QTL, are presented in Fig. 1.

QTL in W14 for FHB resistance identified in greenhouse experiments

QTL analyses indicated that one major QTL on chromosome 3BS was significantly associated with resistance to disease spread, kernel infection, and DON content, and another minor QTL on chromosome 5AS was significantly associated with resistance to kernel infection and DON content. Chromosomal map positions of these two QTL were the same or in the flanking regions for all three disease assessment parameters (Table 1 and Fig.1). This explains the highly significant correlation coefficients among FHB severity, FDK and DON content (r = 0.79 – 0.88). QTL estimates (Table 1) indicate that the 3BS QTL has a greater effect than the 5AS QTL, and the two QTL together explained 33%, 35% and 31% of the total phenotypic variation for disease spread, kernel infection, and DON concentration, respectively.

QTL in W14 for FHB resistance identified in field experiment: Two QTL on chromosomes 3BS and 5AS were also detected for FHB incidence and severity in the field experiment (Table 1). On the basis of QTL estimates provided by CIM, the 5AS QTL had a greater effect than 3BS, especially for FHB incidence, type I resistance. These two QTL together explained 34% and 26% of the total phenotypic variation for FHB incidence and severity, respectively (Table 1).

DISCUSSION

Mapping efforts to date have targeted several QTL on wheat chromosomes 2D, 3A, 3B, 4B, 5A, and 6B (Anderson et al., 2001, Zhou et al., 2002, Buerstmayr et al., 2003, Shen et al, 2003, Somers, et al., 2003, and Steiner et al., 2004). The current study confirmed only the presence of major FHB resistance QTL on chromosomes 3BS and 5AS. Furthermore, an additive by additive

epistasis between these two QTL was detected in the greenhouse experiments, but not in the field (Data not presented).

The two W14 QTL were mapped to similar chromosomal positions on 3BS and 5AS in both greenhouse and field experiments. However, the 3BS QTL explained a greater proportion of the phenotypic variance for resistance to fungal spread in the greenhouse experiments than for FHB resistance in the spray inoculated field experiment, while the opposite was observed for the 5AS QTL in the greenhouse versus field experiments. This suggests that genetic control of type I resistance is likely different from that of type II resistance. It is also consistent with the moderate to low correlation values observed between FHB resistance data from single floret- and spray-inoculated experiments (r = 0.27 – 0.39).

This study confirms previous reports that FHB resistance in many Chinese lines, such as W14, is governed by two major QTL on chromosomes 3BS and 5AS. The 3BS QTL was confirmed to have a major effect on type II resistance (Anderson et al. 2001, Zhou et al. 2002, and Buerstmayr et al. 2003) and DON accumulation (Somers et al. 2003); whereas the 5AS QTL was confirmed to have major effect on type I resistance (Buerstmayr et al. 2003; Steiner et al. 2004) and DON accumulation (Somers et al. 2003). Therefore, marker-assisted selection for both QTL should be implemented to facilitate the development of cultivars having more overall effective FHB resistance via reduction of initial infection, FHB spread, kernel infection, and DON production.

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REFERENCE

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Fig.1. Linkage maps of two major FHB QTL generated using a DH population of W14 x Pion2684. Numbers at each marker represent the distance in cM. Dark area indicate the QTL positions based on the CIM method.

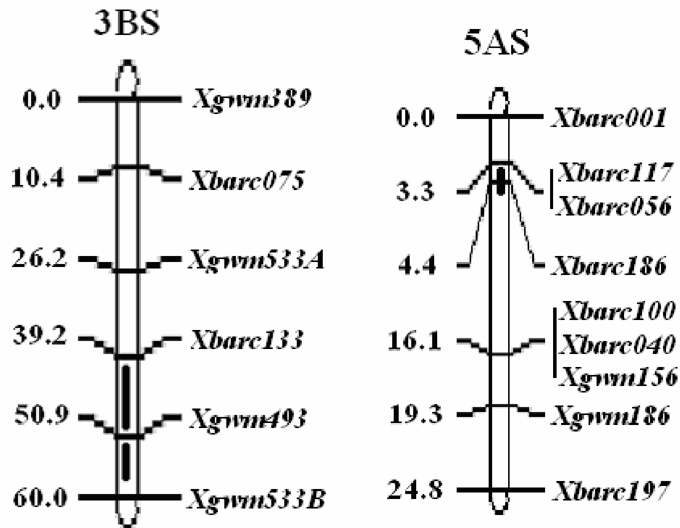

Table 1. QTL estimates for various FHB parameters in greenhouse and field experiments in a DH population of W14 x Pion2684. QTL analysis was done by composite interval mapping, and was declared significant at LOD ≥ 1.8 determined by a permutation test.

Map Interval	Position (left marker, cM)	Chromosome	LOD	R^2(%)
Mean FHB Severity (greenhouse 2001&02)				
Xgwm493-Xgwm533B	52.9	3BS	8.4	33
FDK (greenhouse 2001)				
Xbarc133-Xgwm493	45.2	3BS	7.7	29
Xbarc117-Xbarc186	3.3	5AS	1.9	6
DON (greenhouse 2001)				
Xbarc133-Xgwm493	45.2	3BS	6.1	23
Xbarc117-Xbarc186	3.6	5AS	2.7	8
FHB Incidence (field 2004)				
Xbarc133- Xgwm493	41.3	3BS	2.8	10
Xbarc117- Xbarc186	3.6	5AS	6.5	24
FHB Severity (field 2004)				
Xgwm493-Xgwm533B	54.7	3BS	2.9	10
Xbarc117- Xbarc186	3.6	5AS	4.4	16

APPENDIX B

Curriculum Vitae

CURRICULUM VITAE

JIANLI CHEN

Current Address
2008 Scott Drive
Blacksburg, VA 24060

EDUCATION

- Ph.D. in Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, December 2005
- M.S. in Agronomy, Northwest Agricultural University, Shaanxi, P.R. China, October 1988
- B.S. in Agronomy, Northwest Agricultural College, Shaanxi, P.R. China, July 1983

PROFESSIONAL EXPERIENCE

Research Associate and Co-Principal Investigator, Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA. January 1997 to present.

- Overall coordination of wheat and barley FHB research program and projects
- Mapping and MAS for FHB resistance
- Coordinate implementation of special research oriented greenhouse and field projects
- Breeding, selection, and development of FHB resistant wheat and barley cultivars
- Prepare and write and present FHB grant proposals and progress reports
- Acting as a technical consultant in the region on FHB pathogen production, germplasm ultilization, and novel breeding method (wheat x maize hybridization and MAS)

Visiting Scientist, USDA-ARS Forage and Range Research Laboratory, Utah State University, Logan, Utah, USA. April 1996 to December 1996

- Conduct a collaborative research project on wheat apomoxsis using molecular marker and genomic in situ hybridization
- Development of molecular markers specific for apomictic genes from *Elymus* rectisetus, a wild species possessing apomixes that can be transferred to wheat to fix hertorosis

Adjunct Associate Professor and Principal Investigator, Shaanxi Academy of Agricultural Sciences, Shaanxi, P.R. China. Febaury 1994 to April 1996.

- Breeding, selection, and development of wheat cultivars/germplasm with high yielding, high glutenin subunits, and resistance to stripe rust, powdery mildew, and barley yellow drarf using chromosome engineering and molecular marker assisted selection

Visiting Scholar, USDA-ARS Forage and Range Research Laboratory, Utah State University, Logan, Utah, USA. April 1993 to Febrauy 1994

- Development of wheat chromosome specific RAPD markers

Adjunct Assistant Professor, Shaanxi Academy of Agricultural Sciences. Shaanxi, Yangling 712100, P. R. China. January 1990 to April 1993

- Breeding, selection, and development of wheat cultivars/germplasm with high yielding, high glutenin subunits, and resistance to stripe rust, powdery mildew, and barley yellow drarf using chromosome engineering

RESEARCH INTERESTS AND ACTIVITIES

- Identifying molecular markers associated with resistance genes in wheat and other small grain crops and developing resistant cultivars/germplasm using molecular marker assisted selection.
- Breeding, selection, and development of resistant cultivars using a combination of top-crossing, backcrossing, and doubled haploid methods.
- Incorporation of resistance genes from wheat relatives, exotic source into adapted backgrounds using wild hybridization, tissue culture, wheat by maize hybridization, and MAS.

PRODUCTIVITY

- Oversee, coordinate and implement all major research activities of the FHB Breeding Program in the Virginia Tech Small Grain Breeding Program.
- Elite breeding lines having FHB resistance derived from adapted sources or transferred from un-adapted sources have been developed and currently are being evaluated for potential release as cultivars and/or germplasm.
- Taking leading role in successfully preparing and obtaining funding for grants submitted to the Virginia Agricultural Council, Virginia Small Grain Board, and U.S. Wheat and Barley Scab Initiative. Prepare and presents grants and annual progress reports to these groups. Obtained over 500,000 in competitive grants from 1999 to 2005.
- Present research findings at local field day, state, regional, national, and international meetings.
- Cooperate and coordinate research activities with colleagues in the region and U.S.
- Serve as mentor and advisor to other research associates, research specialists, and students.

HONORS AND AWARDS

- 1983. Outstanding Junior in Agronomy, Northwest Agricultural College, Yangling, Shaanxi, P.R.China.
- 1985. Outstanding Teacher, Professional School in Shangnan, Shangnan, Shaanxi, P.R.China.
- 1988. Outstanding Senior in Plant Genetics and Breeding, Northwest Agricultural University, Yangling, Shaanxi, P.R.China.
- 1992. The First Progressive Prize of Sciences and Technology, Shaanxi Committee of Sciences and Technology.
- 1995. Outstanding Fellow from Abroad, Shaanxi Committee of Sciences and Technology.
- 1995. 21st Century Scientist, Shaanxi Academy of Agricultural Sciencs, Yangling, Shaanxi,

P.R.China.

- 1998. The Third Prize of National Invention, Chinese Academy of Sciences.
- 1998. Excellent performance prize, China Ministry of Agriculture, China National Plan Committee, China National Sciences and Technology Committee.

MEMBERSHIP IN PROFESSIONAL SOCIETY

- Genetic Society of China
- Crop Science Society of China
- Crop Science Society of America
- The American Phytopathological Society
- The American New York Academy of Sciences

RESEARCH EXPERIENCE

Leadership

- Writing competitive proposals
- Implementation of national and provincial grants in both China and America
- Supervision and training of graduate students and research associates

Molecular Genetics

- Molecular mapping of QTLs for Fusarium head blight resistance in wheat
- Marker assisted selection for Fusarium head blight resistance in wheat
- Cloning of PCR-based marker products
- Genomic in situ hybridization (GISH and FISH)
- Plant and plasmid DNA extraction, electrophoresis, and purification
- RAPDs, Microsatellite, RFLPs, Southern hybridization
- QTL analysis: Mapmaker, Window QTL Cartographer software
- Bioinformatics Methods: Rasmol, DOGMA, GOAT, BLAST, Primer3

Plant Breeding

- Design for field and greenhouse trials on breeding of wheat disease resistance (scab, stripe rust and powdery mildew) and quality (HMW and LMW Glu analysis)
- Selection and evaluation of progeny developed by pedigree and modified bulk selection methods
- Data analysis using Excel, SAS, SPSS, Agrobase

Cytogenetics and Chromosome Engineering

- Chromosome banding
- Development of monosomic, nullisomic lines
- Development of addition, substitution, and translocation wheat lines for wheat-H.vilosa, wheat-rye, wheat-Th.intermedium, wheat-E. *rectisetus*

Tissue culture

- Callus induction, organogenesis, somatic embryogenesis, and plant regeneration

- Anther culture, embryo culture
- Wheat x Maize hybridization

Plant pathology

- Isolation, identification, and purification of fungal diseases, especially on *Fusarium graminerum* Schw

PUBLICATIONS

Chen, J., C. A. Griffey, M. A. Saghai Maroof, E. L. Stromberg, R. M. Biyashev, W. Zhao, M. R. Chappell, T. H. Pridgen, Y. Dong, and Z. Zeng. 2005. Validation of Two Major QTL for *Fusarium* Head Blight Resistance in Chinese Wheat Line W14. Plant Breeding (In press).

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Wang, RR-C., J. Chen and L.R. Joppa. 1995. Production and identification of chromosome specific RAPD markers for Langdon durum wheat disomic substitution lines. Crop Science 35: 886-888.

ORAL PRESENTATION

Chen, J., C.A. Griffey, M.A. Saghai Maroof, J.A. Wilson, D. Nabati, and R.M.Biyashev. 2003. Molecular mapping and marker assisted selection for Fusarium head blight resistance in a Chinese wheat line W14. *P*. 7-9. In: 2003 National Fusarium Head Blight Forum Proceedings. Dec.13-15, 2003. Bloomington, MN, USA.

Chen, J., C.A. Griffey, J. Wilson, and M.A. Saghai Maroof. 2003. QTL Mapping and marker assisted selection of Fusarium head blight resistance in a Chinese wheat line W14. *P*. 342-345. *In*: Proceeding of Tenth International Wheat Genetics Symposium. Sep.1-6, 2003. *Paestum*, Italy. Edited by Norberto E. Pogna, Maurizio Romano, Edgar A. Pogna, and Giovanni Galterio. Printed in Rome by S.I.M.I., Via N. Nisco 3/A – 00179 Roma, Italy.

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POSTER PRESENTATION

Chappell M., C. Griffey, J. Chen, T. Pridgen, D. Nabati, W. Zhao, and M. Vaughn. 2000. Assessment and Reaction of *Triticum aestivum* Genotypes to *Fusarium graminearum* and Its Effects on Traits Related to Grain Yield and Quality. *P*.246-250. In: Proceedings of 2000 National Fusarium Head Blight Forum. December 10-12. Holiday Inn Cincinnati-Airport, Erlanger, KY, USA.

Chen, J., C. A. Griffey, M.A. Saghai Maroof, J. Fanelli, J. Wilson, T. Pridgen, J. Paling, D. Nabati, and W. Brooks. 2005a. Haplotype Selection of Two Major QTL Conditioning Fusarium Head Blight Resistance in Wheat. *P*. 23. *In:* Proceedings of the 2005 National Fusarium Head blight Forum. Dec. 11-13, 2005. Hilton Milwaukee City Center, Milwaukee, Wisconsin. USA. Compiled by Susan M. Candy, Timothy Boring, Jerri Wardwell, Lee Siler, and Richard W. Ward. Office Max Print and Document Service, Okemos, MI.

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Chen, J., X.Z. Xue, X.Z. Wang and X. Xu. 1995. RAPD markers for alien chromatin linked to wheat powdery mildew and yellow rust resistance genes. Proceedings of 9th Cereal Rusts and Powdery Mildew Conference, Wangeningen, The Netherlands, 1996.9.

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Wilson, J.A., C.A. Griffey, J. Chen, D. Nabati, and T. Pridgen. 2003. Success of alternative breeding methods in transferring Fusarium head blight resistance to soft red winter wheat. *P*. 295-299. In: 2003 National Fusarium Head Blight Forum Proceedings. Dec. 13-15, 2003. Bloomington, MN, USA.