Theor Appl Genet (2011) 122:1189–1198
DOI 10.1007/s00122-010-1523-2

ORIGINAL PAPER

A novel quantitative trait locus for Fusarium head blight resistance in chromosome 7A of wheat

D. V. Jayatilake · G. H. Bai · Y. H. Dong

Received: 24 August 2010 / Accepted: 11 December 2010 / Published online: 8 January 2011
© Springer-Verlag (outside the USA) 2011

Abstract A Chinese Spring-Sumai 3 chromosome 7A disomic substitution line (CS-Sumai 3-7ADSL) was reported to have a high level of Fusarium head blight (FHB) resistance for symptom spread within a spike (Type II) and low deoxynivalenol accumulation in infected kernels (Type III), but a quantitative trait locus (QTL) on chromosome 7A has never been identified from this source. To characterize QTL on chromosome 7A, we developed 191 7A chromosome recombinant inbred lines (7ACRIL) from a cross between Chinese Spring and CS-Sumai 3-7ADSL and evaluated both types of resistance in three greenhouse experiments. Two major QTL with Sumai 3 origin, conditioning both Type II and III resistance, were mapped in the short arm of chromosomes 3B (3BS) and near the centromere of chromosome 7A (7AC). The 3BS QTL corresponds to previously reported *Fhb1* from Sumai 3, whereas 7AC QTL, designated as *Fhb7AC,* is a novel QTL identified from CS-Sumai 3-7ADSL in this study. *Fhb7AC* explains 22% phenotypic variation for Type II and 24% for Type III resistance. Marker *Xwmc17* is the closest marker to *Fhb7AC* for both types of resistance. *Fhb1* and *Fhb7AC* were additive, and together explained 56% variation for Type II and 41% for Type III resistance and resulted in 66% reduction in FHB severity and 84% reduction in deoxynivalenol (DON) content. Haplotype analysis of Sumai 3 parents revealed that *Fhb7AC* originated from Funo, an Italian cultivar. *Fhb7AC* has the potential to be used in improving wheat cultivars for both types of resistance.

Communicated by C. Feuillet.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-010-1523-2) contains supplementary material, which is available to authorized users.

D. V. Jayatilake · G. H. Bai (✉)
Department of Agronomy, Kansas State University,
Manhattan, KS, USA
e-mail: guihua.bai@ars.usda.gov

G. H. Bai
USDA-ARS Hard Winter Wheat Genetic Research Unit,
Manhattan, KS, USA

Y. H. Dong
Department of Plant Pathology, University of Minnesota,
St. Paul, MN, USA

Introduction

Fusarium head blight (FHB), caused by *Fusarium graminearum,* is an important wheat disease in humid and semi-humid wheat growing regions of the world. In a severe epidemic, FHB can drastically reduce grain yield and quality (Bai and Shaner 1994). Such major outbreaks have been reported in several countries including China, USA, Canada and several European countries, making FHB a global issue affecting wheat production worldwide (Parry et al. 1995). Apart from the yield losses, mycotoxins such as deoxynivalenol (DON) produced by *Fusarium* spp. make the grains unsuitable for human and animal consumption, thus degrade the grain quality (Parry et al. 1995; Korosteleva et al. 2007). Use of FHB resistant cultivars with low toxin content is one of the most efficient and economical strategies for FHB control. Therefore, improving wheat cultivars with a high level of FHB resistance has become one of the major objectives in many wheat-breeding programs worldwide over the last decade (Bai and Shaner 2004). Three types of resistance have been proposed for FHB: resistance to initial penetration by the pathogen (Type I), resistance to symptom spread (Type II) within a

spike (Schroeder and Christensen 1963) and low DON content (Type III) in infected seeds (Miller et al. 1985). Among these, Type II resistance is more stable and has been used as a major target in cultivar improvement. However, given the impact of mycotoxins on grain quality, Type III resistance has recently drawn great attention from breeders.

Resistance to FHB in wheat is a quantitative trait (Bai et al. 2000). Many quantitative trait loci (QTL) associated with FHB resistance have been reported. *Fhb1* on 3BS is a major QTL for both Type II (Anderson et al. 2001) and Type III FHB resistance (Lemmens et al. 2005). Other QTL from wheat include a QTL on chromosome 5A (Chen et al. 2006; Lin et al. 2006), *Fhb2* on chromosome 6B (Anderson et al. 2001; Cuthbert et al. 2007), *Fhb3* from *Leymus racemosus* (Qi et al. 2008), and *Fhb4* on chromosome 4B (Lin et al. 2006; Xue et al. 2010). However, to date only *Fhb1* showed a stable major effect on Type II and III resistance across different genetic backgrounds, while all other QTL have either minor or unstable effects in different genetic backgrounds (Anderson et al. 2007). To achieve expected levels of FHB resistance in a breeding line, pyramiding several such QTL with *Fhb1* is still a challenge even with marker-assisted selection (MAS). Additional QTL with a major effect on FHB resistance are urgently needed to improve resistance levels of breeding materials. Chinese germplasm is an important source for mining FHB resistance QTL (Bai and Shaner 2004). In a previous study, a Chinese Spring-Sumai 3-7A disomic substitution line (CS-Sumai 3-7ADSL) has been reported to show a very high level of resistance (Zhou et al. 2002). However in a mapping study using a population of Annong 8455/CS-Sumai 3-7ADSL, a QTL was not detected on chromosome 7A (Ma et al. 2006b). In this study, we developed a 7A chromosome recombinant inbred line (7ACRIL) population of Chinese Spring/CS-Sumai 3-7ADSL with the objectives to characterize QTL associated with FHB resistance Type II and III on chromosome 7A and to identify simple sequence repeat (SSR) markers associated with the QTL for MAS.

Materials and methods

Plant materials and disease inoculation

A population of 191 7ACRIL was derived from Chinese Spring (CS) and CS-Sumai 3-7ADSL by single seed descent. CS-Sumai 3-7ADSL is a highly resistant line derived by backcrossing of Chinese Spring 7A monosomic line with Sumai 3 four times (Ma et al. 2006b; Zhou et al. 2002). The CS parent is moderately susceptible to FHB. FHB data from $F_{4:5}$ and $F_{6:7}$ 7ACRIL were used for QTL discovery and $F_{6:8}$ were used for QTL confirmation.

$F_{4:5}$ and $F_{6:7}$ 7ACRIL were evaluated for FHB resistance in a greenhouse at Kansas State University, Manhattan, KS, USA, in spring and fall 2009, respectively, with three replications. A set of 89 $F_{6:8}$ 7ACRILs were randomly selected to represent four genotypes of two QTL combinations and tested for FHB in spring 2010 with four replications. Two parents were included as checks. About 15 seeds per entry were planted in Plug Flat trays (Hummert International, St. Louis, MO, USA) containing Metro-mix 360® growing medium (Hummert International, St. Louis, MO, USA). In the $F_{6:8}$ population, 20 seeds per entry were planted. Trays with geminated seeds were kept in a growth chamber at 4°C for 3 weeks of vernalization. Seedlings were transplanted into three ($F_{4:5}$ and $F_{6:7}$) and four ($F_{6:8}$) 1.0 L Dura pots (Hummert International, St. Louis, MO, USA) filled with Metro-mix 360® (Hummert International, St. Louis, MO, USA) with five plants per pot (replicate). The pots were placed on greenhouse benches in a randomized complete block design. The greenhouse was maintained at 20 ± 5°C with 12 h supplemental light. Plants were fertilized with Miracle-Gro® (The Scotts Miracle-Gro Company, Marysville, OH, USA) four times at a 2-week interval.

F. graminearum inoculum was prepared using a Kansas strain GZ3639. Inoculum was cultured in a mung bean liquid medium (Bai and Shaner 1996). The spore density was estimated by counting the spores using a hemocytometer under a light microscope. The inoculum was diluted to 100,000 conidia per milliliter. At anthesis, a central spikelet of a spike was injected with a 10-μl spore suspension using an inoculation syringe. In each pot, about five spikes at similar developmental stage were inoculated. Inoculated plants were placed in a humid chamber to facilitate disease development. After 48 h of incubation, the plants were moved back to the greenhouse benches at 22 ± 5°C with 12 h supplemental light.

Evaluation of Type II FHB resistance

The rate of FHB symptom spread within a spike (Type II) was evaluated on the 18th day after inoculation by counting the number of infected spikelets and total number of spikelets per inoculated spike. Percentage of symptomatic spikelets (PSS) in an inoculated spike was calculated to measure Type II resistance in all three experiments. Percentage of *Fusarium* damaged kernel (FDK) was calculated as an additional measurement of Type II resistance in two 2009 experiments by hand threshing all seeds from the inoculated spikes and calculating the ratio between *Fusarium* damaged seeds and total number of seeds per spike. Extra care was taken to prevent damaged kernels from getting blown away during threshing. Average PSS

and FDK (%) per spike was calculated for each 7ACRIL and parent in each experiment for QTL analysis.

Evaluation of Type III FHB resistance

Seeds from the inoculated spikes of each 7ACRIL and parents were individually weighed. DON content in infected spikes was determined at The University of Minnesota, St. Paul, MN, USA, using Gas Chromatography–Mass Spectrometry (GC-MS) as described by Mirocha et al. (1998). Line average for each 7ACRIL and parent was calculated for each experiment for QTL analysis.

Marker analysis

DNA was extracted from F_6 7ACRIL using a modified cetyltrimethylammonium bromide method (Saghai-Maroof et al. 1984). A genome-wide background screening was done using 84 locus-specific SSR markers representing 42 chromosome arms with two unlinked markers per arm, selected from GrainGenes 2.0 database (http://wheat.pw.usda.gov). The entire chromosome 7A and a small fragment of chromosome 3BS were found to be of Sumai 3 origin in CS-Sumai 3-7ADSL. Therefore, parents CS, CS-Sumai 3-7ADL and Sumai 3 were screened with markers that were mapped on chromosome 7A and 3BS. A total of 75 SSR markers from chromosome 7A and 30 SSR and 28 sequence tagged sites (STS) from the chromosome 3BS were screened between the parents. The markers included 23 BARC, 21 GWM, 7 CFD, 6 CFA, 47 WMC (Somers et al. 2004; Song et al. 2005) and 29 STS markers (Liu and Anderson 2003). Population screening was carried out using 33 polymorphic SSR markers from chromosome 7A and 3 SSR and 4 STS markers from chromosome 3BS. PCR amplification was done in Gene Amp® PCR System 9700 (Applied Biosystems Inc., Foster City, CA, USA) with a 14 µl PCR mix containing 1× ASB buffer, 2.5 mM of $MgCl_2$, 200 µM of dNTP, 100 nM of tailed forward primer, 200 nM of reverse primer, 100 nM of fluorescent-dye labeled M13 primer, 1 U of *Taq* DNA polymerase and 50 ng of template DNA. The touchdown program includes an initial denaturing step at 95°C for 5 min, 5 cycles of 96°C for 1 min, 68°C for 3 min with a decrease of 2°C in each following cycle and 72°C for 1 min, followed by 4 cycles of annealing temperature of 58°C for 2 min with a decrease of 2°C in each following cycle. The final step consisted of 40 cycles of 96°C for 20 s, 50°C for 20 s, 72°C for 30 s and ended with a final extension step of 72°C for 5 min. Pooled PCR products from four separate PCR labeled with different florescent dyes (FAM, VIC, NED and PET) were analyzed using an ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). GeneMarker v1.75 (SoftGenetics LLC. State College, PA, USA) was used to score polymorphic alleles.

Linkage maps were developed using JoinMap v3.0 (Van Ooijen and Voorrips 2001) and Haldane mapping function (Haldane 1919) with a LOD threshold set at 3.0. QTL for PSS, FDK and DON content in infected spikes were analyzed by single trait multiple-interval-mapping using Qgene v4.3.8 (Joehanes and Nelson 2008) and data from two individual 2009 experiments and combined averages. QTL was confirmed using spring 2010 PSS data from selected genotypes. A threshold to claim a significant QTL was determined by 1000 permutation at $p < 0.05$. PROC REG function of SAS v9.1 (SAS Institute Inc. Cary, NC, USA) was used to calculate R^2-values for QTL effects and to determine the interactions between QTL.

Data analysis

Statistical analysis was performed using SAS v9.1 (SAS Institute Inc. Cary, NC, USA). PROC CORR function was used to calculate the correlation among PSS, FDK and DON content in infected spikes. Using PSS from two 2009 experiments, an ANOVA was conducted using PROC GLM function. Because PSS data from spring 2010 were derived from only selected genotypes, they were not included in this analysis. Broad sense heritability (H^2) was calculated for trait PSS based on the ANOVA results using the formula $H^2 = \sigma^2_G/\sigma^2_G + (\sigma^2_{GE}/e) + (\sigma^2_\varepsilon/re)$, where σ^2_G = genotypic variance, σ^2_ε = residual error variance, σ^2_{GE} = genotype × environment variance, r = number of replicates (pots) and e = number of experiments (seasons). Trait averages were calculated for genotypes AABB, AAbb, aaBB and aabb with AB alleles from Sumai 3 and ab alleles from CS for 7AC and 3BS QTL, respectively. The percentage disease/ toxin reduction due to substitution of CS alleles (a/b) by Sumai 3 alleles (A/B) at the two QTL was calculated.

Origin of 7AC QTL

To trace the origin of chromosome 7AC QTL, a haplotype comparison of marker alleles in the 7AC QTL region (*Xwmc17* and *Xwmc9*) was conducted between parents of Sumai 3 (Funo and Taiwan Wheat). Forty-one Funo-related accessions from China were evaluated for PSS under greenhouse conditions to calculate the average disease reduction contributed by the QTL at chromosomes 3BS and 7AC individually and in a combination (Supplemental Table S1).

Results

FHB symptom spread and DON content in 7A CRIL population

The frequency distribution of PSS, FDK and DON content showed a continuous variation in the 7ACRIL population (Fig. 1). Means for PSS, FDK and DON content of infected spikes were 13%, 5% and 1.3 ppm, respectively, for CS-Sumai 3-7ADSL and 60%, 32% and 11.1 ppm, respectively, for CS. Therefore, the three measurements of FHB damage were significantly lower in CS-Sumai 3-7ADSL than in CS. The correlation for PSS of 7ACRIL was significant between two experiments ($r = 0.62$, $P < 0.01$). A significant correlation ($P < 0.01$) was also observed between all three traits with 0.84 between PSS and FDK, 0.83 between PSS and DON content and 0.91 between FDK and DON content. The correlations between experiments were 0.61 for FDK and 0.63 for DON content. ANOVA indicated significant effects ($P < 0.01$) of genotype, environment and genotype × environment for the trait PSS. The mean PSS in spring 2009 was significantly higher than that of fall 2009. Broad sense heritability for PSS was 0.71 across the seasons.

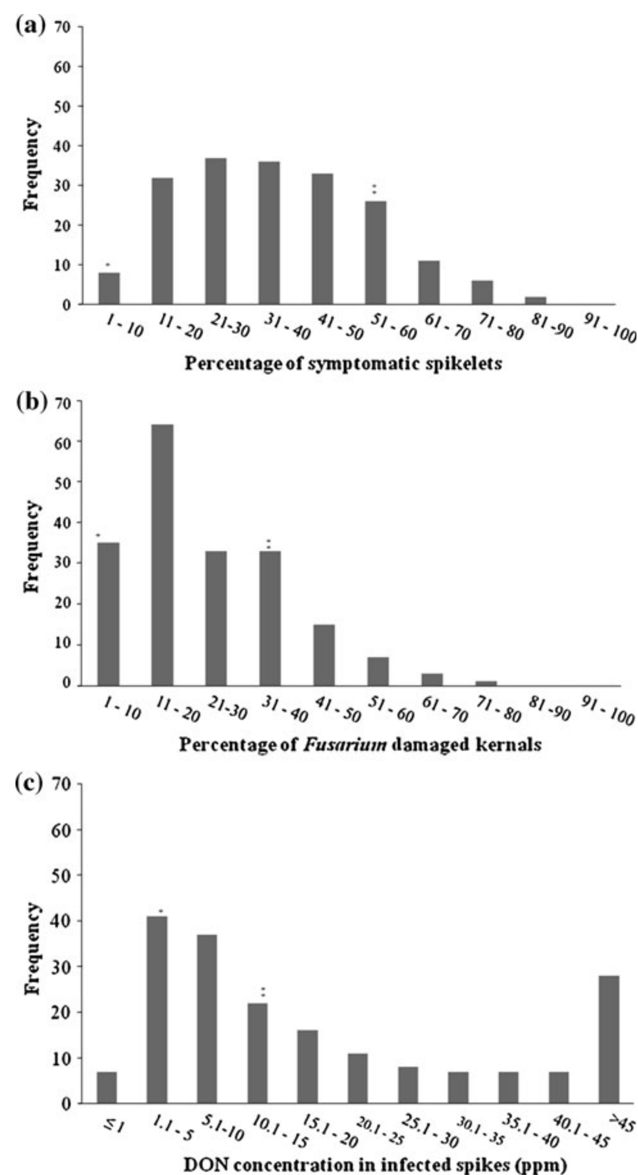

Fig. 1 Frequency distribution of recombinant inbred lines of a CS/CS-Sumai 3-7ADSL mapping population for **a** combined average of percentage of symptomatic spikelet, **b** combined average of percentage of *Fusarium* infected kernel and **c** combined average of deoxynivalenol (DON) content in ppm

Marker analysis and QTL mapping

The genome-wide background check using evenly distributed SSR markers across three wheat genomes confirmed that CS-Sumai 3-7ADSL carried Sumai 3 alleles in a 2.1 cM fragment between *Xumn10* and *Xsts66* in the short arm of chromosome 3B (3BS) and entire chromosome 7A (Fig. 2). The chromosome 7A consisted of three linkage groups that covered 23.8, 113.5, and 39.7 cM in genetic distance (Fig. 2).

Two major QTL were detected in the population with significant effects on PSS ($P < 0.05$) in chromosomes 3BS and 7A (7AC) close proximity to the centromere (Fig. 3 and Supplemental Fig. S1). The QTL on 3BS was most likely the same QTL as previously reported *Fhb1* because the closest marker for the QTL was *Xumn10*. The flanking markers for QTL 7AC were *Xbarc174* and *Xwmc9* (Fig. 3) with *Xwmc17* as the closest marker to the QTL. The QTL 7AC and *Fhb1* were consistently detected across three experiments (Supplemental Fig. S1). The variations explained by individual QTL (R^2) were 22% for 7AC QTL and 35% for *Fhb1* and by both QTL together was 56% of the total phenotypic variation of PSS (Table 1). In the confirmation study using the selected genotypes, *Fhb1* was mapped at the same chromosomal position. The 7AC QTL was mapped in a similar position as two other experiments but with a slight shift. Detected QTL explained 18% (7AC) to 19% (*Fhb1*) of the FDK variation and together they accounted for 36% of the FDK variation (Table 1). The same set of QTL was significant ($P < 0.05$) for low DON content (Fig. 3, Supplemental Fig. S1) and explained 18% (*Fhb1*) and 24% (7AC) of variation for DON content individually and 41% both together (Table 1).

QTL effect on reduction of FHB and DON content

In the mapping population, individuals with Sumai 3 marker alleles at the two QTL regions showed lower PSS, FDK and DON content in infected spikes compared to

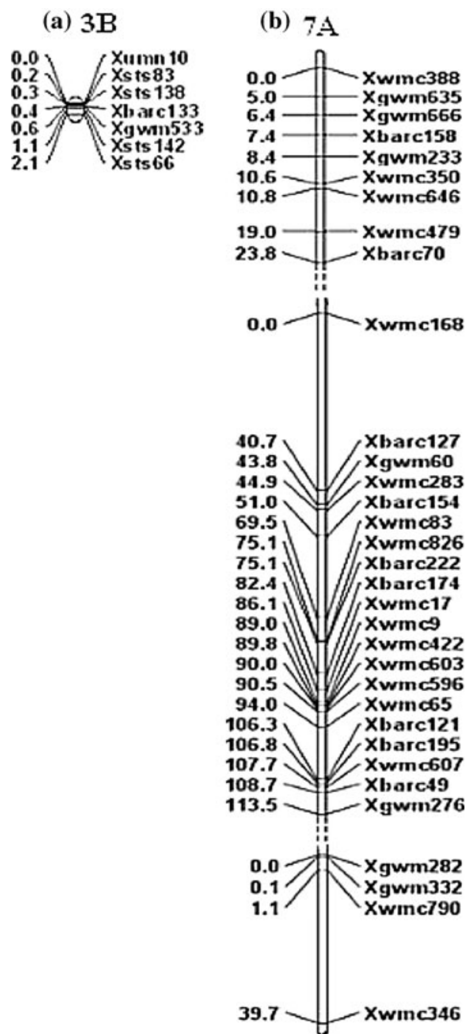

Fig. 2 Linkage map of **a** short arm of chromosome 3B and **b** chromosome 7A. Relative marker position in centimorgan (cM) distance is shown to the right and the maker name shown to the left in each linkage map

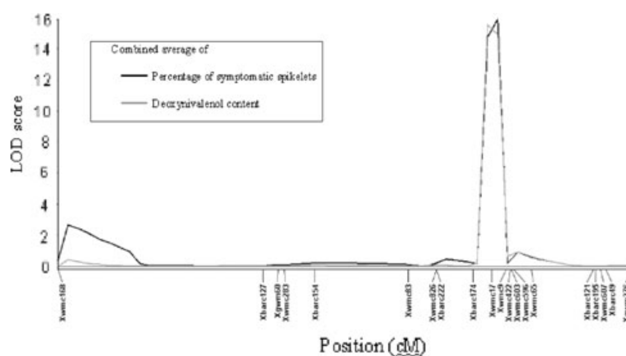

Fig. 3 Single trait multiple interval mapping (SMIM) of quantitative trait loci associated with resistance to Fusarium head blight symptom spread and lower DON accumulation using combined average of percentage of symptomatic spikelets PSS and deoxynivalenol (DON) content in ppm in CS/CS-Sumai 3-7ADSL mapping population on chromosome 7A

those with CS alleles (Table 2). Replacement of CS alleles by Sumai 3 alleles led to a significant reduction in PSS, FDK and DON content in infected spikes (Table 2). Reduction in PSS and FDK was higher in the lines having only *Fhb1* than those with 7AC QTL alone. However, 7AC QTL alone contributed to slightly lower DON content than that of *Fhb1*. Both 3BS and 7AC QTL together reduced PSS by 66%, FDK by 55% and DON content by 84% (Table 2).

Origin of QTL in CS- Sumai 3-7ADSL

To investigate the origin of 7AC QTL, alleles from two markers, *Xwmc17* and *Xwmc9* were compared among the parents of CS-Sumai 3-7ADSL, the parents of Sumai 3, and two CS-Sumai 3-3B disomic substitution lines. The alleles for the two markers amplified by CS-Sumai 3-7ADSL parent were the same as those by Sumai 3, but different from those by CS, which demonstrates that 7AC QTL originated from Sumai 3, not from CS. Two independent CS-Sumai 3-3B disomic substitution lines (CS-Sumai 3-3BDSL10 and CS-Sumai 3-3BDSL31), carrying the 3B chromosome of Sumai 3 in a CS background, had the same haplotype as in CS parent for the two markers from 7A and Sumai 3 alleles at *Xumn10* locus linked to *Fhb1*. Interestingly, CS-Sumai 3-7ADSL also carried the Sumai 3 allele of the *Xumn10* marker linked to *Fhb1*, suggesting that both 3BS and 7AC QTL in CS-Sumai 3-7ADSL originated from Sumai 3.

Sumai 3 is a transgressive segregant with the best FHB resistance selected from a cross between a Chinese landrace Taiwan Wheat and an Italian wheat cultivar Funo. Fingerprinting of both parents of Sumai 3 with the markers that flanked 7AC QTL revealed the same haplotype between Sumai 3 and Funo, but a different haplotype between Sumai 3 and Taiwan Wheat. Chinese accessions with the target marker alleles and Funo ancestry in its pedigree had various levels of resistance to FHB (Supplemental Table S1). Fourteen accessions that only carried 7AC QTL showed a 26% PSS reduction and 11 accessions that carried both *Fhb1* and 7AC QTL showed a 51% reduction in PSS as compared to 16 accessions without any of the two QTL.

Discussion

Evaluation of FHB resistance in the mapping population

FHB is a complex disease and its occurrence and development are often heavily influenced by environments (Jia et al. 2005). Accuracy in phenotyping significantly affects

Table 1 Summary of multiple interval mapping (MIM) analysis of quantitative trait loci for Fusarium head blight resistance in a CS/CS-Sumai 3-7ADSL mapping population using percentage of symptomatic spikelet (PSS), percentage of *Fusarium* infected kernel (FDK) and deoxynivalenol (DON) content of infected kernels in ppm

Trait	Chromosome	Marker interval	Spring 2009 ($F_{4:5}$)		Fall 2009 ($F_{6:7}$)		Combined average	
			LOD	R^2	LODtwo	R^2	LOD	R^2
PSS	3BS	*Xumn10–Xsts83*	16.66	0.28	17.6	0.28	23.87	0.35
	7AC	*Xbarc174–Xwmc9*	9.83	0.17	12.45	0.18	15.88	0.22
	Combined		–	0.44	–	0.46	–	0.56
FDK	3BS	*Xumn10*	9.43	0.16	12.42	0.21	14.87	0.19
	7AC	*Xbarc174–Xwmc17*	9.44	0.17	8.17	0.14	12.69	0.18
	Combined		–	0.33	–	0.34	–	0.36
DON	3BS	*Xumn10*	9.59	0.16	8.57	0.16	11.76	0.18
	7AC	*Xbarc174–Xwmc17*	12.13	0.20	10.94	0.20	15.51	0.24
	Combined		–	0.35	–	0.35	–	0.41

Table 2 Average and disease/toxin reduction in percentage of symptomatic spikelets (PSS), percentage of *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) content with the replacement of Chinese Spring (CS) alleles by Sumai 3 (SM 3) at 3BS and 7AC quantitative trait loci in CS/CS-Sumai 3-7ADSL mapping population

Genotype[A]	Average PSS	Average FDK (%)	Average DON content
aabb (No 3BS/7AC)	56	38	44.2
aaBB (3BS only)	33 (41)	22 (42)	19.6 (54%)
AAbb (7A only)	37 (34)	23 (39)	17.5 (61%)
AABB (3BS and 7A)	19 (66)	17 (55)	6.9 (84%)

Values in parenthesis are percentage of reduction (%) for three FHB measurements

[A] a, CS allele at chromosome 7AC; A, SM 3 allele at chromosome 7AC; b, CS allele at chromosome 3BS; B, SM 3 allele at chromosome 3BS

the power of QTL detection (Kolb et al. 2001). Thus, evaluation of the disease in a controlled greenhouse environment with multiple replications may improve quality of the phenotypic data (Bai et al. 1999). An experiment may need to be repeated over multiple seasons/locations under field conditions to minimize environmental variation. Further, a large size population can be used to improve the precision in determining the QTL effect and location (Cuthbert et al. 2006). In the present study, a relatively large population of 191 individuals was evaluated for FHB resistance in comparison with some other studies (Lemmens et al. 2005; Ma et al. 2006b). The mapping population was evaluated in three experiments over 2 years in a greenhouse at Kansas State University, and two QTL with a major effect on FHB resistance were mapped in similar chromosome locations in three experiments, indicating the data were reliable for QTL mapping.

In this study, PSS and FDK were separately measured to reflect FHB symptom spread within a spike and DON content in infected spikes was quantified to reflect DON content across experiments. All FHB damage measurements were based on point inoculation, which avoids differences in Type I resistance between lines and targets only Type II resistance. As this provides uniform inoculation among the plants, differentiation of genotypes based on Type II is less complicated (Bai et al. 1999). Therefore, FHB data obtained in this study should be reproducible. In addition, FHB scoring needs to be precisely timed, so that plants show the highest level of phenotypic differences among resistant and susceptible genotypes at the time of scoring (Bai et al. 1999). In the current study FHB scoring was done when susceptible control reached 95% PSS, which provided clear separation between resistant and susceptible genotypes.

FDK was quantified by classifying the seeds into damaged and normal seeds categories based on visual inspection as previously reported (Bai et al. 2001; Verges et al. 2006). However, only 200 random seeds per sample were selected for FDK counting in these studies. In the current study, we carefully harvested and manually threshed all inoculated spikes to minimize highly infected and shriveled seeds from getting blown away (Ma et al. 2006a; Yu et al. 2008a), which not only significantly improved the accuracy of FDK data, but also prevented dilution of DON levels in

harvested kernels. Further DON content quantified by GC-MS provided a more accurate measurement than enzyme-linked immunosorbent assay (Mirocha et al. 1998).

The continuous distribution of all three traits PSS, FDK and DON content in the mapping population derived from CS/CS-Sumai 3-7ADSL (Fig. 1) supported previous reports that FHB resistance showed quantitative inheritance (Ma et al. 2006c; Yang et al. 2003). A higher broad-sense heritability was reported for PSS in the current study ($H^2 = 0.71$), which agrees with several previous reports (Lin et al. 2006; Ma et al. 2006b; c). A high heritability indicates consistency and repeatability of PSS data (Bai et al. 1999). Therefore, PSS in conjunction with single point inoculation can be effectively used for screening resistant lines in a breeding program.

Relationship between Type II and Type III FHB resistance

PSS is a reliable parameter to estimate Type II FHB resistance in a large-scale experiment (Bai and Shaner 1994). Recently, DON content in infected spikes has drawn great attention from FHB researchers due to its impacts on quality of wheat products (Verges et al. 2006). However, DON content in infected kernels is an expensive trait to measure and is not feasible for routine breeding selection. Although the association between PSS and DON content is still debatable, a significantly high correlation ($r = 0.83$, $P < 0.01$) observed between PSS and DON content in this study indicates that PSS is a reasonable alternative measurement to predict DON content in breeding programs. Such a high correlation was also observed in some previous studies (Bai et al. 2001; Lemmens et al. 2005; Yu et al. 2008a), but not in the others (Ma et al. 2006c; Mesterházy et al. 1999). The discrepancy among reports can be caused by overestimating PSS and underestimating DON content because of mishandling. Accuracy in measuring DON content in a cultivar can be affected by plant growth stage in which the infection occurs, methods used for threshing and DON measurement. Under greenhouse conditions, visual FHB symptoms start spreading to uninoculated spikelets in susceptible genotypes in 6–8 days after inoculation and spikes can be blighted within about 12 days after inoculation (Bai and Shaner 1996). By the time an inoculated spike gets bleached, it may still be in early stages of seed development. Although such seeds contain high content of DON, they may be too small to be collected, thus get blown away easily during threshing, which may significantly reduce DON content in harvested samples. In addition, under such a condition it may be difficult to distinguish scabby seeds from uninfected shriveled seeds. These factors may significantly increase the variation of FDK across experiments. However given that the above constraints can be minimized by careful harvesting and threshing, FDK can be a reasonable alternative for quantifying Type II resistance, because a high correlation between FDK and PSS was observed in this and other studies (Bai et al. 2001; Verges et al. 2006).

A novel quantitative trait locus in chromosome 7A

In this study, two major FHB resistances QTL were mapped on chromosome 3BS and 7AC for Type II and Type III resistance in CS/ CS-Sumai 3-7ADSL population (Table 1). These QTL were consistent across all experiments. Zhou et al. (2002) reported a very high level of Type II and Type III resistance in CS-Sumai 3-7ADSL compared to original CS. This study confirmed the report and further identified that the high level of resistance in CS-Sumai 3-7ADSL was due to CS-Sumai 3-7ADSL carrying two major QTL from Sumai 3 on chromosomes 7AC and 3BS. Haplotype analysis confirmed that the 7AC QTL was not mapped in the previous study (Ma et al. 2006b) due to lack of marker polymorphism between Annong 8455 and CS-Sumai 3-7ADSL because Annong 8455 showed the same alleles as those of Sumai 3 at marker loci that linked to the 7AC QTL. Therefore, polymorphic markers between CS and CS-Sumai 3-7ADSL in the QTL region were critical for detection of *Fhb7AC* QTL in this study. The novel 7AC QTL showed a major effect on both Type II and Type III resistance and was designated as *Fhb7AC*. *Fhb7AC* was flanked by *Xbarc174* and *Xwmc9* with *Xwmc17* as the closest linked marker. These markers should be useful for pyramiding *Fhb7AC* with other QTL.

Several QTL have been reported on chromosome 7A previously (Jia et al. 2005; Mardi et al. 2006; Semagn et al. 2007; Yu et al. 2008b; Zhou et al. 2004). However, chromosome locations of these reported QTL were different from *Fhb7AC*. A QTL on the short arm of chromosome 7A was mapped at marker interval *Xe77m47-22* – *Xgwm233* from Frontana (Mardi et al. 2006). This 7AS QTL was positioned distal to *Fhb7AC* and therefore is a different QTL. Semagn et al. (2007) mapped a FHB severity QTL from NK93604 at the marker interval *Xgwm276* and *XDuPw226*. A QTL for FHB severity was mapped proximal to marker *Xgwm282* (Jia et al. 2005) in Wangshuibai. Zhou et al. (2004) and Yu et al. (2008b) also identified a QTL proximal to centromere of chromosome 7A for FHB Type II resistance in Wangshuibai. This QTL was tightly linked to *Xwms1083*, which is closer to marker *Xgwm276* in our linkage map (Fig. 2) and different from the QTL reported in this study because it is about 30 cM away from *Fhb7AC*. In addition, *Fhb7AC* showed a major effect on Type II resistance and previously reported QTL had only minor effects. The alleles of flanking markers for *Fhb7AC* were different between Wangshuibai and Sumai 3.

Therefore, we believe that *Fhb7AC* is a major QTL different from that of previously reported on chromosome 7A.

Relationship between *Fhb1* and *Fhb7AC*

Based on the closely linked marker *Xumn10,* QTL on chromosome 3BS is most likely the same QTL as *Fhb1* (Liu et al. 2008). *Fhb1* is the single most important QTL mapped so far for both Type II and Type III FHB resistance (Anderson et al. 2007; Lemmens et al. 2005). The contribution of *Fhb1* in this study for Type II FHB resistance was greater than that of *Fhb7AC*. Therefore, *Fhb1* remains the highest contributor to FHB Type II resistance so far. In this study, the phenotypic variation for lower DON content explained by the *Fhb7AC* was greater than that of *Fhb1* across experiments (Table 1), which disagrees with Lemmens et al. (2005). The differences in R^2-values reported in different studies could be due to differences in population, test condition, phenotypic evaluation method and interaction of QTL (Yang et al. 2003). Even though marker *Xwmc17* was the closest linked marker for *Fhb7AC* (Supplemental Fig. S1), a slight shift in the QTL position for PSS was observed among experiments, which could be due to environmental variations or population size change in the 2010 confirmation experiment.

Multiple regression analysis on PSS, FDK and DON content of infected kernels in 7ACRILs did not detect any significant ($P < 0.01$) interaction between *Fhb1* and *Fhb7AC* in the mapping experiments, suggesting that an additive effect contributed mostly to FHB resistance. This result agrees with several previous reports that concluded additive effects to be the main effect for FHB resistance (Bai et al. 2000; Jia et al. 2005). In this study, *Fhb1* and *Fhb7AC* together explained 56% of PSS, 36% of FDK and 41% of DON content in infected spikes (Table 1). The unexplained variation of phenotype could be due to environmental effects and QTL that have not been detected in this study. A QTL could go unnoticed if it has minor effects, poor marker density around it or lack of marker polymorphism between two parents (Bai et al. 1999). Presence of *Fhb7AC* provided an additional 34% reduction in PSS besides a 41% reduction provided by *Fhb1* and together reduces PSS by 66% (Table 2). A similar trend was observed among Funo-related accessions, where presence of *Fhb7AC* alone reduced PSS by 31% and together by 54%. In terms of reducing DON content in infected spikes *Fhb7AC* alone contributed to a 61% reduction, whereas *Fhb1* contributed to a 54% reduction and together an 84% reduction in DON content in the mapping population (Table 2). *Fhb1* and *Fhb7AC* QTL showed additive effects and can be effectively pyramided on to a cultivar to build up a high level of resistance to FHB. In this study, the QTL for Type II and Type III resistance were mapped to the same locations in both chromosomes 3BS and 7AC (Fig. 3), suggesting existence of pleiotropy for Type II and Type III resistance (Lemmens et al. 2005; Semagn et al. 2007). Such tight association between Type II and Type III resistance suggested that selecting both *Fhb1* and *Fhb7AC* for Type II resistance could significantly enhance Type III resistance when Sumai 3 is used as the FHB resistance source.

Origin of chromosome 7A QTL

Many Chinese cultivars are known to have a high level of Type II and Type III resistance from Sumai 3 or its derivatives (Bai et al. 2001). *Fhb1* was identified from Sumai 3 and donated by Taiwan wheat (Yu et al. 2006), which was confirmed in this study. Furthermore, we found that *Fhb7AC* QTL also has a Sumai 3 origin and it was contributed by Funo parent. Funo is an old cultivar from Italy, and has been widely used as a popular breeding parent in Chinese breeding programs in 1960s. An extensive survey of Chinese germplasm with Funo in their lineage using closely linked marker to *Fhb7AC* confirmed that *Fhb7AC* was present in most of these Chinese accessions that showed some levels of FHB resistance. It further confirms that the *Fhb7AC* originated from Funo. To date *Fhb1* is the only QTL with a major effect that has been successfully used in breeding programs with MAS (Anderson et al. 2007). Given the additive effects between *Fhb1* and *Fhb7AC*, they can be pyramided to enhance the resistance levels of US wheat cultivars.

Acknowledgments US Wheat and Barley Scab Initiative provided part of funding for this project. Dr. Hongxiang Ma from Jiangsu Academy of Agricultural Science, Nanjing China provided Funo-related materials and Dr. Dadong Zhang from Kansas State University provided FHB data of Funo-related materials. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. Contribution 11-050-J from the Kansas Agricultural Experiment Station, Manhattan, Kansas, USA.

References

Anderson JA, Chao S, Liu S (2007) Molecular breeding using a major QTL for Fusarium head blight resistance in wheat. Crop Sci 47(S3):S112–S119

Anderson JA, Stack RW, Liu S, Waldron BL, Fjeld AD, Coyne C, Moreno-Sevilla B, Fetch JM, Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor Appl Genet 102:1164–1168

Bai GH, Plattner R, Desjardins A, Kolb F (2001) Resistance to Fusarium head blight and deoxynivalenol accumulation in wheat. Plant Breed 120:1–6

Bai GH, Shaner G (1996) Variation in *Fusarium graminearum* and cultivar resistance to wheat scab. Plant Dis 80:975–979

Bai GH, Shaner G, Ohm H (2000) Inheritance of resistance to Fusarium graminearum in wheat. Theor Appl Genet 100:1–8

Bai GH, Shaner GE (1994) Scab of wheat: perspective and control. Plant Dis 78:760–766

Bai GH, Kolb FL, Shaner G, Domier LL (1999) Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89:343–348

Bai GH, Shaner G (2004) Management and resistance in wheat and barley to Fusarium head blight. Annu Rev Phytopathol 42:135–161

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (Type II resistance). Theor Appl Genet 104:84–91

Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (2003) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor Appl Genet 107:503–508

Chen J, Griffey CA, Saghai-Maroof MA, Stromberg EL, Biyashev RM, Zhao W, Chappell MR, Pridgen TH, Dong Y, Zeng Z (2006) Validation of two major quantitative trait loci for Fusarium head blight resistance in chinese wheat line W14. Plant Breed 125:99–101

Cuthbert PA, Somers DJ, Thomas J, Brulé-Babel A (2007) Mapping of *Fhb2* on chromosome 6BS: a gene controlling Fusarium head blight field resistance in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 114:429–437

Cuthbert PA, Somers DJ, Thomas J, Cloutier S, Brulé-Babel A (2006) Fine mapping *Fhb1*. a major gene controlling Fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 112:1465–1472

Haldane JBS (1919) The combination of linkage values, and the calculation of distance between the loci of linked factors. J Genet 8:299–309

Jia G, Chen P, Qin G, Bai G, Wang X, Wang S, Zhou B, Zhang S, Liu D (2005) QTLs for Fusarium head blight response in a wheat DH population of Wangshuibai/Alondra's. Euphytica 146:183–191

Joehanes R, Nelson JC (2008) QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 24:2788–2789

Kolb FL, Bai GH, Muehlbauer GJ, Anderson JA, Smith KP, Fedak G (2001) Host plant resistance genes for Fusarium head blight: mapping and manipulation with molecular markers. Crop Sci 41:611–619

Korosteleva SN, Smith TK, Boermans HJ (2007) Effects of feed borne Fusarium mycotoxins on the performance, metabolism, and immunity of dairy cows. J Dairy Sci 90:3867–3873

Lemmens M, Scholz U, Berthiller F, Dall'Asta C, Koutnik A, Schuhmacher R, Adam G, Buerstmayr H, Mesterhāzy Ã, Krska R, Ruckenbauer P (2005) The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for Fusarium head blight resistance in wheat. Mol Plant Microbe Interact 18:1318–1324

Lin F, Xue SL, Zhang ZZ, Zhang CQ, Kong ZX, Yao GQ, Tian DG, Zhu HL, Li CJ, Cao Y, Wei JB, Luo QY, Ma ZQ (2006) Mapping QTL associated with resistance to Fusarium head blight in the Nanda2419 × Wangshuibai population. II: type I resistance. Theor Appl Genet 112:528–535

Liu S, Anderson JA (2003) Targeted molecular mapping of a major wheat QTL for Fusarium head blight resistance using wheat ESTs and synteny with rice. Genome 46:817–823

Liu S, Pumphrey MO, Gill BS, Trick HN, Zhang JX, Dolezel J, Chalhoub B, Anderson JA (2008) Toward positional cloning of *Fhb1*, a major QTL for Fusarium head blight resistance in wheat. Cereal Res Commun 36:195–201

Ma H-X, Bai G-H, Gill BS, Hart LP (2006a) Deletion of a chromosome arm altered wheat resistance to Fusarium head blight and deoxynivalenol accumulation in Chinese Spring. Plant Dis 90:1545–1549

Ma HX, Bai GH, Zhang X, Lu WZ (2006b) Main effects, epistasis, and environmental interactions of quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population. Phytopathology 96:534–541

Ma HX, Zhang KM, Gao L, Bai GH, Chen HG, Cai ZX, Lu WZ (2006c) Quantitative trait loci for resistance to Fusarium head blight and deoxynivalenol accumulation in Wangshuibai wheat under field conditions. Plant Pathol 55:739–745

Mardi M, Buerstmayr H, Ghareyazie B, Lemmens M, Mohammadi SA, Nolz R, Ruckenbauer P (2005) QTL analysis of resistance to Fusarium head blight in wheat using a 'Wangshuibai'-derived population. Plant Breed 124:329–333

Mardi M, Pazouki L, Delavar H, Kazemi MB, Ghareyazie B, Steiner B, Nolz R, Lemmens M, Buerstmayr H (2006) QTL analysis of resistance to Fusarium head blight in wheat using a 'Frontana'-derived population. Plant Breed 125:313–317

Mesterházy Á, Bartók T, Mirocha CG, Komoróczy R (1999) Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. Plant Breed 118:97–110

Miller JD, Young JC, Sampson DR (1985) Deoxynivalenol and Fusarium head blight resistance in spring cereals. Phytopathol Z 113:359–367

Mirocha CJ, Kolaczkowski E, Xie W, Yu H, Jelen H (1998) Analysis of deoxynivalenol and its derivatives (batch and single kernel) using gas chromatography/mass spectrometry. J Agric Food Chem 46:1414–1418

Parry DW, Jenkinson P, McLeod L (1995) Fusarium ear blight (scab) in small grain cereals—a review. Plant Pathol 44:207–238

Qi LL, Pumphrey MO, Friebe B, Chen PD, Gill BS (2008) Molecular cytogenetic characterization of alien introgressions with gene *Fhb3* for resistance to Fusarium head blight disease of wheat. Theor Appl Genet 117:1155–1166

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Schroeder HW, Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by Gibberella zeae. Phytopathology 53:831–838

Semagn K, Skinnes H, Bjornstad A, Maroy AG, Tarkegne Y (2007) Quantitative trait loci controlling Fusarium head blight resistance and low deoxynivalenol content in hexaploid wheat population from 'Arina' and NK93604. Crop Sci 47:294–303

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Van Ooijen JW, Voorrips RE (2001) JoinMap 3.0: Software for the calculation of genetic linkage maps. 3.0 edn. Plant Res Int, Wageningen

Verges VL, Sanford DV, Brown-Guedira G (2006) Heritability estimates and response to Selection for Fusarium head blight resistance in soft red winter wheat. Crop Sci 46:1587–1594

Xue S, Li G, Jia H, Xu F, Lin F, Tang M, Wang Y, An X, Xu H, Zhang L, Kong Z, Ma Z (2010) Fine mapping *Fhb4*. a major QTL conditioning resistance to Fusarium infection in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 121:147–156

Yang ZP, Gilbert J, Somers DJ, Fedak G, Procunier JD, McKenzie IH (2003) Marker assisted selection of Fusarium head blight resistance genes in two doubled haploid populations of wheat. Mol Breed 12:309–317

Yu JB, Bai GH, Cai SB, Ban T (2006) Marker-assisted characterization of Asian wheat lines for resistance to Fusarium head blight. Theor Appl Genet 113:308–320

Yu JB, Bai GH, Cai SB, Dong YH, Ban T (2008a) New Fusarium head blight-resistant sources from Asian wheat germplasm. Crop Sci 48:1090–1097

Yu JB, Bai GH, Zhou WC, Dong YH, Kolb FL (2008b) Quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population of Wangshuibai/Wheaton. Phytopathology 98:87–94

Zhou WC, Kolb FL, Bai GH, Domier LL, Yao JB (2002) Effect of individual Sumai 3 chromosomes on resistance to scab spread within spikes and deoxynivalenol accumulation within kernels in wheat. Hereditas 137:81–89

Zhou W, Kolb FL, Yu J, Bai G, Boze LK, Domier LL (2004) Molecular characterization of Fusarium head blight resistance in Wangshuibai with simple sequence repeat and amplified fragment length polymorphism markers. Genome 47:1137–1143