

Quantitative trait loci responsible for *Fusarium* head blight resistance in Chinese landrace Baishanyuehuang

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Abstract *Fusarium* head blight (FHB), mainly caused by *Fusarium graminearum*, is a destructive disease that can significantly reduce grain yield and quality. Deployment of quantitative trait loci (QTLs) for FHB resistance in commercial cultivars has been the most effective approach for minimizing the disease losses. ‘Baishanyuehuang’ is a highly FHB-resistant landrace from China. Recombinant inbred lines (RILs) developed from a cross of ‘Baishanyuehuang’ and ‘Jagger’ were evaluated for FHB resistance in three greenhouse experiments in 2010 and 2011 by single-floret inoculation. Percentage of symptomatic spikelets in an inoculated spike was recorded 18 days post-inoculation. The RIL population was screened with 251 polymorphic simple sequence repeats. Four QTLs were associated with FHB resistance and mapped on three chromosomes. Two QTLs were located on the short arm of chromosome 3B (3BS) with

one in distal of 3BS and another near centromere (3BSc), designated as *Qfhb.hwwg-3BSc*. The QTL in the distal of 3BS is flanked by *Xgwm533* and *Xgwm493*, thus corresponds to *Fhb1*. This QTL explained up to 15.7 % of phenotypic variation. *Qfhb.hwwg-3BSc* flanked by *Xwmc307* and *Xgwm566* showed a smaller effect than *Fhb1* and explained up to 8.5 % of phenotypic variation. The other two QTLs were located on 3A, designated as *Qfhb.hwwg-3A*, and 5A, designated as *Qfhb.hwwg-5A*. *Qfhb.hwwg-3A* was flanked by *Xwmc651* and *Xbarc356* and explained 4.8–7.5 % phenotypic variation, and *Qfhb.hwwg-5A* was flanked by markers *Xgwm186* and *Xbarc141*, detected in only one experiment, and explained 4.5 % phenotypic variation for FHB resistance. ‘Baishanyuehuang’ carried all resistance alleles of the four QTL. *Qfhb.hwwg-3BSc* and *Qfhb.hwwg-3A* were new QTLs in ‘Baishanyuehuang’. ‘Baishanyuehuang’ carries a combination of QTLs from different sources and can be a new source of parent to pyramid FHB-resistant QTLs for improving FHB resistance in wheat.

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Introduction

Fusarium head blight (FHB), also known as scab, is mainly caused by *Fusarium graminearum* and is one of the most destructive diseases of wheat (*Triticum aestivum*) when warm and wet weather coincides with anthesis. FHB epidemics not only cause significant yield losses, but also reduce grain quality due to shriveled seeds and deoxynivalenol (DON) and other mycotoxins produced by the pathogen during disease development (Bai and Shaner 1994). Infected grain contaminated with these mycotoxins is harmful to human and animal health and has been a serious safety concern in food and feed

(Desjardins and Hohn 1997). In the past three decades, FHB has received great attention due to its spread (Bai and Shaner 2004); outbreaks have been more frequent and severe than before, and have become a threat to wheat production worldwide.

Although currently available cultural practices and fungicides can reduce disease damage to some extent, host-plant resistance has long been considered the most practical and effective means to reduce the disease losses. In wheat, three types of FHB resistance have been reported: resistance to initial infection (type I), resistance to fungal spread within a spike (type II), and degradation of mycotoxin (type III) (Schroeder and Christensen 1963; Mesterhazy 1995). Of the three resistance types, type II has been extensively studied because it is the most stable type of resistance and more robust for disease evaluation. Inheritance of FHB type II resistance is conditioned by multiple quantitative trait loci (QTLs) (Windels 2000; Bai and Shaner 2004). The identification of QTLs from different sources of resistance and incorporation of these QTLs into adapted wheat varieties can accelerate the development of wheat varieties with improved FHB resistance.

In the early 1980s, an extensive nationwide search for FHB-resistant germplasm was launched in China, and germplasm lines with high levels of resistance were identified from both Chinese landraces and breeding lines. More recently, molecular marker technologies have been used successfully to identify QTLs for FHB resistance from Chinese sources, mainly ‘Sumai 3,’ ‘Wangshuibai,’ and ‘Ning 7840’ (Bai et al. 1999; Waldron et al. 1999; Zhou et al. 2004; Anderson et al. 2001; Buerstmayr et al. 2002). Among several types of molecular markers used, simple sequence repeat (SSR) marker is highly polymorphic, chromosome-specific, and suitable for automation (Roder et al. 1998; Waldron et al. 1999; Bai et al. 1999; Anderson et al. 2001; Zhou et al. 2003), thus has been widely used in dissection of QTLs for FHB resistance.

Among QTL identified to date, one QTL on the short arm of chromosome 3B (*Fhb1*) has been reported to have a major effect on type II resistance and to be stable in various genetic backgrounds and environments (Bai et al. 1999; Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002), and therefore has been extensively used in wheat breeding programs worldwide (Pumphrey et al. 2007; Snijders 1994; McCartney et al. 2007). Other QTLs have not been as successful due to their unstable effects in different genetic backgrounds (Salameh et al. 2008; Anderson and Liu 2007); however, extensive use of single resistance source may increase selection pressure on the pathogens to wear away the effectiveness of the resistance QTL. New FHB-resistant germplasm lines are still urgently needed to broaden the genetic diversity of FHB-resistant sources and improve the level of wheat FHB resistance

(Ruckenbauer et al. 2001; Gervais et al. 2003). Identification of new QTLs from diverse sources would be of great importance to effective introgression and pyramiding of resistance genes into adapted wheat cultivars. More recently, several Chinese landraces have been found to have a level of resistance similarly high to ‘Sumai 3,’ but the QTLs underlining the resistance in these accessions were unknown (Yu et al. 2008a). The objective of the present research was to characterize QTLs for type II resistance to FHB from ‘Baishanyuehuang’, a highly FHB-resistant Chinese wheat landrace.

Materials and methods

Plant materials and growth conditions

A population of 188 recombinant inbred lines (RILs) was developed from the cross ‘Baishanyuehuang’/‘Jagger’ by single-seed descent. ‘Baishanyuehuang’ is a highly FHB-resistant wheat landrace from China whereas ‘Jagger’ is a highly FHB-susceptible wheat cultivar from Kansas. After seedlings of both parents and $F_{6,7}$ RILs were vernalized at 4 °C in a growth chamber for 7 weeks, they were transplanted into 13-cm Dura pots containing Metro-Mix 360 soil mix (Hummert International, Earth City, MO) and grown in a greenhouse with 12 h supplemental light.

FHB evaluation

The RIL population was evaluated for spread of FHB symptoms within a spike in three greenhouse experiments (spring and fall 2010, and spring 2011) at Kansas State University in Manhattan, KS. Production of *F. graminearum* conidia and evaluation of FHB symptoms in RIL population followed Bai et al. (2000). In brief, 10 µL of conidial suspension (100 spores/µL) was injected into a central floret of a spike with a syringe at anthesis. Inoculated plants were placed in a moist chamber at 20–25 °C with 100 % relative humidity for 48 h to promote initial infection. Infected spikelets and total spikelets per spike were counted 18 days after inoculation. All greenhouse experiments were arranged in a randomized complete block design with two replications (pots) of five to six plants per replication. The experiments were repeated twice. Disease severity was calculated as percentage of symptomatic spikelets (PSS) per inoculated spike. Mean PSS for each experiment and mean over all three experiments were calculated for QTL analysis.

SSR marker analysis

Wheat leaf tissue was collected in 96-deepwell plates before transplanting for DNA isolation. Harvested tissue

was dried in a freeze dryer (ThermoSavant, Holbrook, NY) for 48 h and ground into fine powder using a Mixer Mill (MM 400, Retsch Inc., Germany). A modified cetyltrimethylammonium bromide (CTAB) protocol was used to isolate genomic DNA (Saghai-Maroo et al. 1984). Initially a core set of 384 pairs of SSR primers were screened between two parents. These markers were selected from about 2,000 primers by the USDA Small Grain Genotyping Lab at Manhattan, KS, based on their high levels of polymorphism as detected in several previous studies. In addition, these primers were well distributed across 21 chromosomes of wheat (Somers et al. 2004; Song et al. 2005). Primers that amplified at least one polymorphic band between parents were used to screen a subset of 94 RILs. In addition, 27 SSR markers that were previously reported to link to QTLs for FHB resistance in other populations were also screened for polymorphism (Anderson et al. 2001; Zhou et al. 2002; Buerstmayr et al. 2002). Single marker analysis was conducted for the subset of 94 RILs to identify possible markers linked to FHB resistance. After significant markers were identified, additional markers close to these markers associated with FHB resistance were screened in the subpopulation and added to the linkage map for further QTL analysis. After QTL regions were identified by composite interval mapping (CIM), SSR around QTL regions were analyzed for an additional 94 lines for further QTL analysis.

For SSR detection, a tailed M-13 sequence (5'-ACGACGTTGTAACGAC) was added to 5'-end of all forward primers, and an additional primer with the same M-13 sequence labeled with different fluorescent dyes (FAM, VIC, NED and PET) was added in each PCR (polymerase chain reaction) for multiplex PCR detection. SSR were analyzed in a DNA Engine Tetrad Peltier thermal cycler (MJ Research, Waltham, MA). A 14- μ L PCR mixture contained 40 ng of template DNA, 0.1 μ M each primer, 0.2 mM each dNTP, 1 \times PCR buffer, 2.5 mM MgCl₂, and 0.6 U of *Taq* polymerase. A touchdown program was used for PCR amplification, in which the reaction was incubated at 95 °C for 5 min, then continued for five cycles of 45 s of denaturing at 95 °C and 5 min of annealing at 68 °C, with a decrease of 2 °C in each subsequent cycle and 1 min of extension at 72 °C. In another five cycles, the annealing temperature started at 58 °C for 2 min, with a decrease of 2 °C for subsequent cycles. Then, PCR went through an additional 25 cycles of 45 s at 94 °C, 2 min at 50 °C, and 1 min at 72 °C, with a final extension at 72 °C for 5 min. PCR products were resolved in an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA). Data were scored using GeneMarker software (Version 1.6, Applied Biosystem) and manually checked twice to eliminate ambiguous data.

Statistical analysis

Analysis of variance (ANOVA) of the three seasons of greenhouse FHB data was conducted using PROC ANOVA in SAS 9.1.2 (SAS Institute Inc., Cary, NC). Correlations between experiments were calculated using Excel. A linkage map was constructed using QTL IciMapping (available from <http://www.isbreeding.net>). A minimum logarithm of odds (LOD) threshold of three was used to identify linkage groups. CIM was performed using WinQTLCart 2.5 (Wang et al. 2006). QTLs were calculated based on the line means from each experiment and line means across all experiments. Permutation tests of 1,000 times were performed to identify appropriate significant threshold values for claiming significant QTLs (Doerge and Churchill 1996). QTL was named using QTL nomenclature convention. For example, in *Qfhb.hwwg-3BSc*, *Qfhb* represents QTL for FHB resistance, *hwwg* represents USDA Hard Winter Wheat Genetics Research Unit, in Manhattan *3BSc* represents QTL on chromosome 3BS near the centromere.

Results

FHB variation among RILs and between parents

Mean PSS over all three experiments was 84.0 % for 'Jagger' and 6.5 % for 'Baishanyuehuang,' indicating great contrast in type II resistance between parents and that 'Baishanyuehuang' is highly resistant. Mean PSS across the RILs was 25.0 %, ranging from 5.9 to 85.4 %, indicating significant genetic variation in type II resistance among RILs. Frequency distribution of mean PSS over three experiments was continuous with a bias toward resistant parent 'Baishanyuehuang' (Fig. 1), suggesting more than one QTL controlling FHB resistance in the population. Correlations of PSS of RILs were highly significant among the three experiments, ranging from 0.59 to 0.66 ($P < 0.0001$). Variance analysis indicated that variations were significant for genotypes, experiments, and genotype \times experiment (Table 1).

Linkage analysis

The level of polymorphism between the two parents was very high for the core set of primers. Among 384 primers screened, 251 amplified polymorphic fragments (65 %) between two parents, and were used to screen the subpopulation of 94 RILs. A total of 184 SSRs were mapped in 35 linkage groups. The linkage map covered 803 cM of the wheat genome, with an average of 4.36 cM between markers. Chromosomes 3B, 4B, 4D, 5A, and 4A had a better

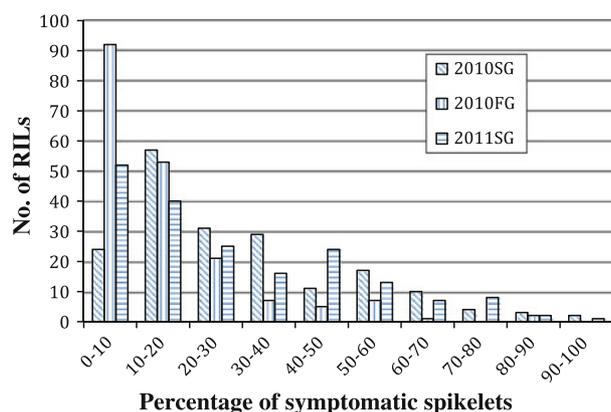


Fig. 1 Frequency distributions of percentage of symptomatic spikelets (PSS) for the recombinant inbred lines (RILs) developed from ‘Baishanyuehuang’/‘Jagger’ evaluated in three greenhouse experiments at Kansas State University, Manhattan, KS

Table 1 Variation analysis of *Fusarium* head blight (FHB) from three greenhouse experiments using recombinant inbred lines derived from the cross ‘Baishanyuehuang’/‘Jagger’

Source	DF	SS	MS	F value	Pr > F
Genotype	187	28.93	0.15	11.49	<.0001
Experiment	2	4.39	2.19	163.05	<.0001
Replicate	1	0.05	0.02	3.59	0.0587
Genotype × experiment	374	10.98	0.03	2.18	<.0001
Replicate × experiment	2	0.03	0.01	0.95	0.3859
Error	561	7.55	0.01		

marker coverage than other chromosomes. The resulting map was used for initial QTL analysis.

QTLs for FHB resistance

Initially, all polymorphic markers between parents were used to identify markers that were significantly associated

with FHB resistance in single marker analysis using 94 RILs. Twenty-five markers on chromosomes 3B, 2A, 3A, 5A, 5B, 6D, and 7D showed a correlation with PSS data in at least one experiment, so linkage groups containing these markers were used for the first round of CIM; however, CIM detected only four significant regions in three chromosomes—3B (2 QTLs), 3A (1 QTL), and 5A (1 QTL)—that showed a significant effect on PSS (Table 2; Fig. 2). Other regions with significant markers identified in single marker analysis were not significant in CIM, and thus were not analyzed further. All polymorphic markers from the three linkage groups were used to screen an additional 94 RILs. In addition, 12 SSRs on 3A and 30 SSRs and 3 SNP markers on 3B (Somers et al. 2004; Song et al. 2005; Bernardo et al. 2011) were screened between two parents, and 8 SSRs and 2 SNPs that are closely linked to the QTL were added to the map. The final map was constructed using 188 RILs that were used for final QTL analysis with CIM.

The first QTL was located on the distal end of the short arm of chromosome 3B, and was significant in all three experiments. This QTL is tightly linked to *Xumn10*, a diagnostic marker for *Fhb1*, thus this QTL is most likely *Fhb1* previously reported from Sumai 3 (Anderson et al. 2001; Somers et al. 2003). This QTL explained up to 15.7 % of phenotypic variation for PSS, and demonstrated the largest effect on type II resistance among the four QTLs detected. The second QTL was also significant in all three experiments and located on 3BS, but near the centromere region (3BSc). This QTL, designated *Qfhb.hwwg-3BSc*, explained 5.2–8.6 % of phenotypic variation for PSS. The third QTL was located on 3A, was designated as *Qfhb.hwwg-3A*, and was significant in two 2010 experiments (spring and fall 2010), and explained 4.8–7.5 % of phenotypic variation for PSS. The last QTL was located on 5A, designated as *Qfhb.hwwg-5A*, and explained 4.5 % of phenotypic variation (Table 2), but was significant only in 2010 fall experiment.

Table 2 Quantitative trait loci (QTL) for *Fusarium* head blight type II resistance detected by composite interval mapping (CIM) using mean percentage of symptomatic spikelets (mean PSS) of recombinant inbred lines (RIL) over three greenhouse experiments conducted in spring (2010SG) and fall 2010 (2009FG) and spring 2011 (2010SG)

Experiment	QTL	Interval	Chromosome	LOD	R ² (%)
2010SG	<i>Qfhb.hwwg-3BS</i>	<i>Xgwm533-Xgwm493</i>	3BS	5.4	13.7
	<i>Qfhb.hwwg-3BSc</i>	<i>Xwmc307-Xgwm566</i>	3BSc	4.4	8.5
	<i>Qfhb.hwwg-3A</i>	<i>Xwmc651-Xbarc356</i>	3A	2.3	4.8
2010FG	<i>Qfhb.hwwg-3BS</i>	<i>Xgwm533-Xgwm493</i>	3BS	2.1	4.0
	<i>Qfhb.hwwg-3BSc</i>	<i>Xbarc73-Xgwm566</i>	3BSc	1.9	3.8
	<i>Qfhb.hwwg-3A</i>	<i>Xwmc651-Xbarc356</i>	3A	3.8	7.5
2011SG	<i>Qfhb.hwwg-5A</i>	<i>Xgwm304-Xbarc141</i>	5A	2.3	4.5
	<i>Qfhb.hwwg-3BS</i>	<i>Xgwm533-Xgwm493</i>	3BS	8.7	15.7
	<i>Qfhb.hwwg-3BSc</i>	<i>Xwmc307-Xgwm566</i>	3BSc	4.5	7.6
Mean PSS	<i>Qfhb.hwwg-3BS</i>	<i>Xgwm533-Xgwm493</i>	3BS	8.3	14.2
	<i>Qfhb.hwwg-3BSc</i>	<i>Xwmc307-Xgwm566</i>	3BSc	4.6	8.1
	<i>Qfhb.hwwg-3A</i>	<i>Xwmc651-Xbarc356</i>	3A	2.8	4.8

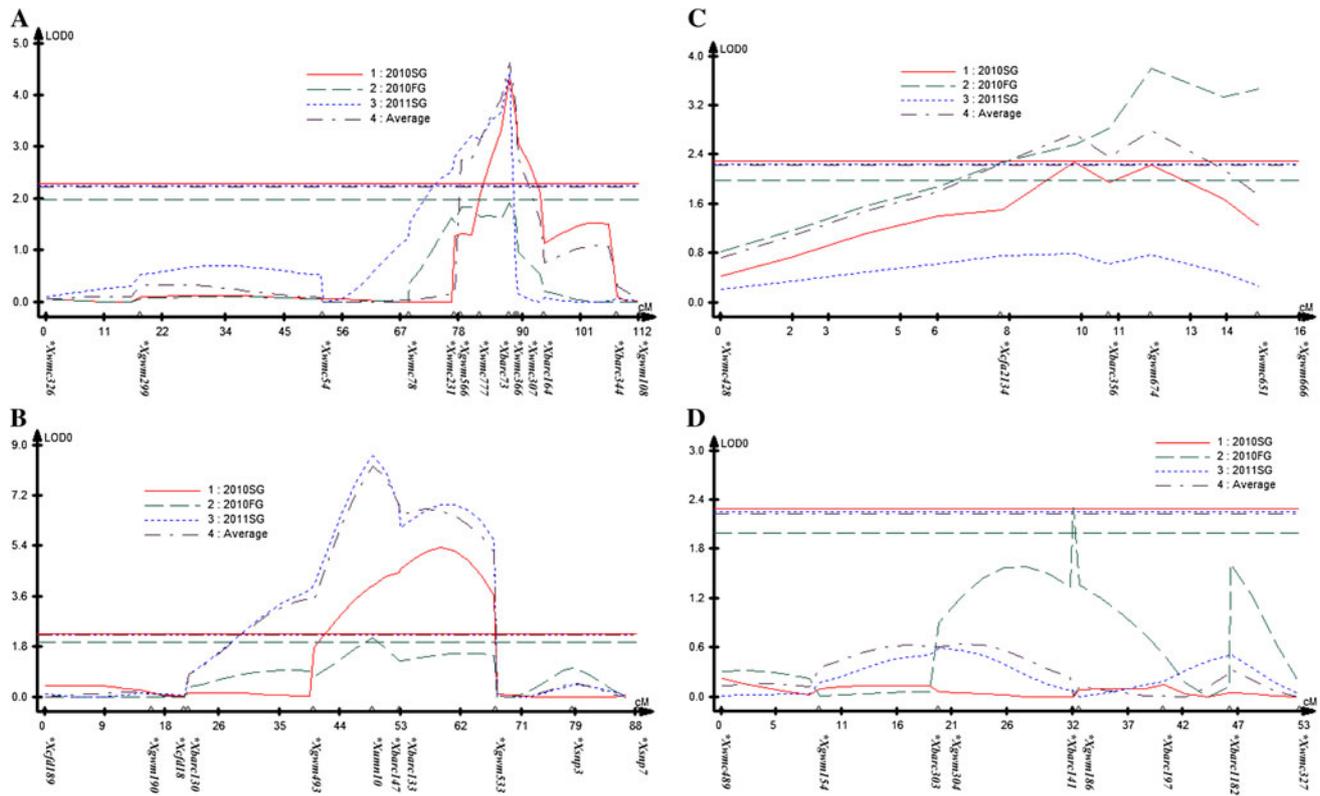


Fig. 2 Maps of quantitative trait loci (QTL) for *Fusarium* head blight (FHB) type II resistance based on percentage symptomatic spikelets (PSS) after single-floret inoculation of a recombinant inbred line (RIL) population ('Baishanyuehuang'/'Jagger') conducted in three green-

house experiments at Kansas State University, Manhattan, KS. **a** QTL on distal end of 3BS, **b** QTL near the centromere of 3BS, **c** QTL on 3A and **d** QTL on 5A

Allelic substitution effect of QTLs on FHB resistance

To evaluate the allelic substitution effect of markers at QTL regions, three QTLs that were significantly associated with FHB resistance in at least two experiments were selected for analysis. The closest marker to *Qfhb.hwwg-3BS* was *Xwmc366* (designated as genotype AA), the closest marker to *Fhb1* was *Xumn10* (designated as genotype BB), and the closest marker to *Qfhb.hwwg-3A* was *Xgwm674* (designated as genotype CC). All the alleles linked to resistance came from 'Baishanyuehuang' (designated as genotype AABBCC), and all the three alleles from 'Jagger' were linked to susceptibility (designated as genotype aabbcc). Three markers formed a total of eight genotypes in the RILs. The RILs with all three 'Baishanyuehuang' alleles (AABBCC) showed the lowest mean PSS (12.7 %) among the eight genotypes, whereas the RILs with all three 'Jagger' alleles (aabbcc) had the highest mean PSS (45.3 %, Fig. 3). RILs with one or two alleles from 'Baishanyuehuang' were in between, with mean PSS of 14.3 % for AABbCc, 17.9 % for AAbbCC, 19.4 % for aaBBCC, 29.6 % for AAbbcc, 30.0 % for aabbCC, and 32.7 % for aabbCC. These results indicate that substitution of each or a combination of two or three alleles from 'Jagger' with corre-

sponding allele(s) from 'Baishanyuehuang' can significantly reduce the PSS.

Discussion

Several cultivars from China, Europe, and the United States have been reported to show a high level of FHB resistance, including 'Sumai 3,' 'Ning7840,' 'Wangshuibai,' and 'Ernie,' and QTLs in these cultivars have been well characterized (Anderson et al. 2001; Bai et al. 1999; Zhou et al. 2004; Liu et al. 2007). 'Baishanyuehuang' is a Chinese landrace and was reported to have a similar level of resistance to 'Sumai 3' (Yu et al. 2008a); however, QTLs underlining the resistance in this accession have not been characterized previously. In this study, a total of four QTLs were identified from 'Baishanyuehuang,' and two of them were on 3BS that showed consistent type II resistance in all three experiments. This result indicates that these two QTLs are stable for type II FHB resistance, and both QTLs together led to a significant reduction in FHB level compared with one QTL alone (Fig. 3). Thus, transferring both 3BS together into one cultivar may provide much better protection from FHB damage than *Fhb1* alone. The QTL

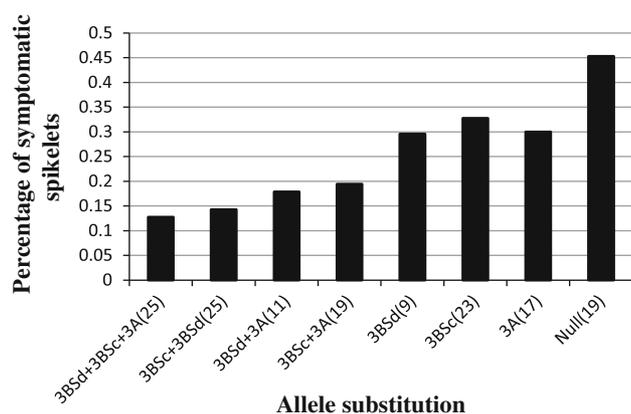


Fig. 3 Allele substitution effect on percentage of symptomatic spikelets (PSS) in the recombinant inbred line (RIL) derived from ‘Baishanyuehuang’/‘Jagger’ as evaluated in three greenhouse experiments at Kansas State University, Manhattan, KS. Null means recombinant inbred lines (RILs) without ‘Baishanyuehuang’ alleles at the three QTL, 3BS + 3BSc + 3A means RIL containing all three alleles from ‘Baishanyuehuang’, 3BSc + 3BS means RIL containing 3BSc and 3BS alleles from ‘Baishanyuehuang’, 3BS + 3A means RIL containing 3BS and 3A alleles from ‘Baishanyuehuang’, 3BSc + 3A means RIL containing 3BSc and 3A alleles from ‘Baishanyuehuang’, 3BS means RIL containing only 3BS allele from ‘Baishanyuehuang’, 3BSc means RIL containing only 3BSc allele from ‘Baishanyuehuang’ and, 3A means RIL containing only the 3A allele from ‘Baishanyuehuang’

on 3A was significant for FHB data from two individual experiments and mean across all three experiments, so it is also a real QTL, but with a smaller effect than the two on 3BS. The QTL on 5A was detected in only one environment and is likely an unstable QTL. In addition, the QTLs were calculated based on 188 RILs evaluated for FHB resistance in the three greenhouse experiments at different seasons. FHB ratings were significantly correlated among experiments; therefore, the disease data were highly reproducible and suitable for QTL analysis of type II resistance.

In this study, ‘Baishanyuehuang’ carries all four putative QTLs for FHB resistance that were previously reported from different sources, thus ‘Baishanyuehuang’ is a good parent for introducing several QTL of different sources into locally adapted cultivars to enhance type II FHB resistance. Each of the QTLs explained from 3.8 to 15.7 % of the total phenotypic variation in the ‘Baishanyuehuang’/‘Jagger’ population. The QTL in the distal end of 3BS showed a major effect, with the largest R^2 value (up to 15.7 %) among four QTLs. This QTL is located between *Xgwm533* and *Xgwm493* and close to *Xumn10*, a diagnostic marker for *Fhb1*. Also *Umn10* amplified same size of band (260 bp) in ‘Baishanyuehuang’, and other *Fhb1* carrying cultivars including ‘Sumai 3’, ‘Ning7840’, and ‘Wangshuibai’, but a smaller size of band (258 bp) in ‘Jagger’, ‘Ernie’ and ‘Chinese Spring’, etc. that do not have *Fhb1* locus. Thus, this QTL is at the same location as *Fhb1* as reported previously (Anderson et al. 2001; Somers et al. 2003; Waldron et al.

1999). Significant variations in the effect of the QTL were observed across various studies using different Chinese cultivars or their derivatives (Buerstmayr et al. 2002; Shen et al. 2003; Bourdoncle and Ohm 2003; Somers et al. 2003). This QTL explained up to 60 % phenotypic variation in a Chinese cultivar ‘Ning7840’ (Bai et al. 1999; Zhou et al. 2002), 41.6 % in ‘Sumai3’ (Anderson et al. 2001), and from 6 to 37.3 % in ‘Wangshuibai’ (Zhou et al. 2004; Yu et al. 2008b; Ma et al. 2006). All these cultivars demonstrated a stable and high level of type II resistance despite of variation in R^2 for *Fhb1*; thus, *Fhb1* is a critical QTL responsible for FHB resistance in most Chinese sources. The significant variation in the QTL effect among studies can be due to difference in sizes of populations used for mapping, in effects of genetic backgrounds in different populations, and in disease evaluation methods used in different studies. Different sources also may carry different alleles of *Fhb1*. In any case, this QTL is the most important QTL in Asian sources and has been proven to be the best QTL for improving type II FHB resistance in breeding (Bai and Shaner 2004; Anderson et al. 2001).

QTL *Qfhb.hwwg-3BSc* was located in the short arm of chromosome 3B near centromere with *Xwmc307* and *Xgwm566* as flanking markers. This QTL explained 3.8–8.5 % of phenotypic variation for FHB severity in three experiments. QTL at similar positions have been reported in several populations (Somers et al. 2003; Zhou et al. 2004). Somers et al. (2003) identified a QTL on 3BSc that was close to SSR marker *Xgwm566* in a Chinese line ‘Wuhan-1’. Zhou et al. (2004) and Yu et al. (2008b) mapped a QTL for type II resistance close to marker *Xbarc344* in ‘Wangshuibai,’ and this QTL explained 3.4–7.4 % of phenotypic variation. Liu et al. (2007) reported a QTL on 3BSc in soft red winter wheat ‘Ernie’; this QTL was close to marker *Xgwm285* and explained 12.9 % phenotypic variation. These reported QTLs from different sources appear to be the same QTL as identified in this study because the markers linked to these QTL from all these studies are in the same chromosome region (Somers et al. 2004). This QTL is also likely a stable QTL for type II resistance in different Chinese germplasm and some US germplasm lines, but with much smaller effect than *Fhb1*. The QTL appears to have an additive effect with *Fhb1*; together they can significantly reduce FHB rating from 30 % (AAbbcc or aaBBcc) to 14.3 % (AABBcc) in an inoculated spike (Fig. 3). RILs with both alleles of ‘Baishanyuehuang’ from the two QTLs had a similar PSS (14.3 %) to that for three ‘Baishanyuehuang’ alleles (AABBCC, 12.7 %). Thus, in wheat breeding programs, simultaneously transferring the two QTLs on 3BS may provide sufficient type II resistance in a moderate epidemic.

QTL *Qfhb.hwwg-3A* located on 3AS was significant in two greenhouse experiments. This QTL explained 4.8–

7.5 % phenotypic variation, and was flanked by *Xwmc307* and *Xbarc356*. Several QTLs have been found on 3A. Bourdoncle and Ohm (2003) reported a QTL on 3AS in Chinese cultivar ‘Huapei 57-2’ with *Xgwm5* as the closest marker, and the QTL explained 8.1 % phenotypic variation. Shen et al. (2003) identified a similar QTL in ‘F201R’ flanked by *Xbarc76* and *Xgwm674*. Chen et al. (2007) identified a QTL for FHB resistance on 3A from *T. dicoccoides*, a wild relative of durum wheat with the closest marker *Xgwm2* about 8 cM from *Xgwm5* (Somers et al. 2004). In this study, marker *Xwmc674* was mapped in the region of the 3A QTL, which is about 1 cM away from *Xwmc5* (Somers et al. 2004), thus we believe that QTL identified in this study is the same as identified in other studies. In addition, QTL on 3A were also reported in spring wheat ‘DH181’ (Yang et al. 2005a) and Swiss winter wheat cultivar ‘Arina’ (Paillard et al. 2004), but they were located on the long arm of 3A and linked to *Xwmc264*, and thus are different QTL from the one reported in this study (3AS). For the 3A QTL in a Brazilian spring wheat cultivar ‘Frontana’ (Steiner et al. 2004), whether it is the same QTL is unknown due to lack of common markers between the two maps.

QTL *Qfhb.hwwg-5A* showed a minor effect on type II resistance and was significant in only one experiment. This QTL explained 4.5 % of the phenotypic variation with the peak close to *Xbarc141*, so it may be similar to the one in ‘Wangshuibai’ (Lin et al. 2006). Several previous reports indicated that the QTLs on 5A were often associated with type I resistance and most often detected in field experiments (Yu et al. 2008b; Buerstmayr et al. 2003; Yang et al. 2005a; Steiner et al. 2004). Buerstmayr et al. (2003) detected a QTL on 5A for type I resistance in ‘CM-82036’ under field conditions. Another QTL on 5A for type I resistance was found in ‘DH181’ (Yang et al. 2005a); however, this minor QTL was also detected for other types of resistance in ‘Wangshuibai’ (Yu et al. 2008b), ‘F201R’ (Shen et al. 2003), and ‘CM-82036’ (Buerstmayr et al. 2002). One QTL was found on 5A in Brazilian cultivar ‘Frontana’ and was flanked by *Xgwm129* and *Xbarc197*, so this QTL should be the same as the one in this study because they share the markers *Xgwm304* and *Xbarc197* (Steiner et al. 2004).

In this study, a core set of 384 SSR primers were selected for parent screening and 65 % of them were polymorphic between two parents. Such a high level of polymorphism has never been reported before. Polymorphic rate of SSR were usually low, about 10–30 % (Liu et al. 2007; Zhou et al. 2002; Yang et al. 2005b), and 40 % was the highest in a rare case (Bourdoncle and Ohm 2003). In this study, we evaluated over 2,000 SSR primers and carefully selected the set of 384 primers (Supplemental Fig. 1) based on quality of amplification, chromosome distribution, and polymorphism levels in different populations. The core

set of 384 primers distributed over all arms of 21 chromosomes easily amplified good-quality PCR products in most wheat accessions with very high levels of polymorphism across different genetic accessions. These primers were arranged in two 384-well plates and readily for semiautomatic PCR setup. Thus, this set of primers is ideal for initial screening of linkage mapping population and for structure analysis in association mapping.

Wheat resistance to FHB is a complex trait, and evaluation of FHB resistance is time-consuming and labor-intensive. Identification of FHB resistance QTLs and closely linked markers could facilitate marker-assisted selection of FHB resistance in breeding programs. In this study, four QTLs were found on three different chromosomes. Among them, *Fhb1*, a key QTL to provide a high level of resistance, has been reported from almost all Chinese accessions and their derivatives. The second QTL *Qfhb.hwwg-3BSc* has been reported in a few studies and with a smaller effect than *Fhb1*, but it is also a very important QTL because it was stable and showed additive effect with *Fhb1*. Interestingly, this QTL was also detected in US soft wheat. The two stable QTLs on 3BS are good candidates for marker-assisted improvement of FHB resistance in breeding programs.

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