Crop Science Accepted paper, posted 05/07/2014. doi:10.2135/cropsci2013.12.0835

Quantitative trait loci for Fusarium head blight resistance in Huangcandou x 'Jagger' wheat population

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Abbreviations: FHB, Fusarium head blight; HCD, Huangcandou; QTL, quantitative trait locus; RIL, recombinant inbred line; PSS, percentage of symptomatic spikelet; DON, deoxynivalenol; SSR, simple-sequence repeat; 3BSd, the distal end of short arm of chromosome 3B; 3BSc, near centromere of short arm of chromosome 3B; type II resistance, resistance to FHB spread within a spike; R^2, determination of coefficient; PCR, polymerase chain reaction; LOD, logarithm of the odds.

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Abstract

Fusarium head blight (FHB) is a devastating disease in wheat (*Triticum aestivum*), and growing resistant cultivars is one of the most effective strategies to minimize its damage. Huangcandou (HCD) is a Chinese wheat landrace that shows a high level of resistance to FHB spread within a spike (type II). To identify quantitative trait loci (QTLs) for FHB resistance in HCD, 190 recombinant inbred lines (RILs) were developed from HCD x 'Jagger'. 'Jagger' is a susceptible hard winter wheat from Kansas. The population was evaluated for percentage of symptomatic spikelets (PSSs) per spike after single-floret inoculation in three greenhouse experiments. Initial marker screening identified 261 polymorphic simple-sequence repeats (SSRs) between the parents. Analysis of these markers in the RIL population identified five QTLs, three from HCD and two from 'Jagger' chromosomes. Two of the three QTLs from HCD were mapped on the short arms of chromosomes 3B, one in the distal end (3BSd) and another near centromere (3BSc); the third was on the short arm of 3A (3AS). The QTL on 3BSd coincides with the previously reported *Fhb1* and explained 26.1% of phenotypic variation. The QTL on 3AS explained up to 10.0% of phenotypic variation. The two QTLs from 'Jagger' on chromosomes 2D and 6D explained 9.5% and 6.7% of phenotypic variations, respectively. A combination of QTLs from HCD and 'Jagger' can enhance FHB type II resistance in wheat.

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Introduction

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a devastating disease in wheat (*Triticum aestivum*), especially in humid and semi-humid wheat growing regions worldwide (Bai and Shaner 2004). FHB epidemics have been reported in geographical regions including Asia, Europe, North America, and South America (Bai and Shaner 1994; Goswami and Kisler 2004). When high humidity coincides with anthesis and the early grain filling stage, susceptible wheat plants can easily become infected, and a severe epidemic may occur. FHB infection causes pre-matured and blighted spikes and shriveled kernels due to blockage of water and nutrient supplies and results in a significant reduction in grain yield and quality (Bai and Shaner 2004). FHB-infected grain also is contaminated with mycotoxins, especially deoxynivalenol (DON), which make the grains unsuitable for human and animal consumption (Windels, 2000). Although fungicide application can be used to reduce crops losses from FHB infection, they are not completely effective (Windels, 2000) therefore, growing FHB-resistant cultivars is a more environmentally friendly, effective, and economic approach to minimizing FHB damage.

FHB resistance is a complex trait with multiple components. Five types of resistance to FHB have been proposed: resistance to initial penetration of the pathogen (type I); resistance to disease spread within a spike (type II) (Schroeder and Christensen 1963); resistance to accumulation of DON in infected kernels (type III) (Miller et al. 1985); resistance to *Fusarium*-damaged kernels (type IV); and tolerance to FHB (type V) (Mesterházy et al. 1999). Although resistance types I, II and III are commonly accepted, type II resistance is characterized extensively and used in cultivar improvement due to its stability and robustness. FHB resistance is a quantitative trait that is usually controlled by a few major and several minor genes (Bai and Shaner 1994; Buerstmayr et al. 1999). Mapping of quantitative trait loci (QTLs) is widely used to determine the effect of QTLs underlining FHB resistance. To date, FHB associated QTLs have been reported from more than 50 wheat cultivars covering all 21 chromosomes (Buerstmayr et al., 2009; Liu et al. 2009). Among them, QTL on 3BS, designated

as *Fhb1* (Cuthbert et al. 2006; Liu et al., 2006), has the largest effect on type II and type III resistance (Bai et al. 1999; Anderson et al. 2001; Cuthbert et al. 2006). 'Sumai 3' and its derivatives such as 'Ning7840' carry *Fhb1* and are the most-used source of FHB resistance in breeding programs worldwide (Bai and Shaner 1996; Bai et al. 1999; Somers et al. 2003; Cuthbert et al. 2006; Buerstmayr et al. 2009). Several other QTLs were repeatedly mapped on chromosomes 5AS, 6BS, 3A, 4B, 2D, 1B, 7A, and 5B in more than two populations and are also considered stable QTLs (Liu et al. 2009). Five of these QTLs were formally named as *Fhb2* on chromosome 6B (Anderson et al. 2001; Cuthbert et al. 2007), *Fhb3* on 7AS from wheat-*Leymus* introgression (Qi et al. 2008), *Fhb4* on 4B (Lin et al. 2006; Xue et al. 2010), and *Fhb5* on 5A from Wangshuibai (Lin et al. 2004; Xue et al. 2011). To date, only 'Sumai 3'-derived *Fhb1* has been used extensively in breeding programs due to its stable major effect on type II resistance across different genetic backgrounds (Bai et al. 1999; Anderson et al. 2001); however, single-resistance QTL may not provide sufficient protection from severe epidemics, so exploring new sources of resistance will facilitate pyramiding of QTLs to enhance the level of resistance and diversity of resistance sources.

A Chinese landrace, 'Huangcandou' (HCD), showed a high level of type II resistance to FHB (Yu et al., 2008a), but the QTLs underlying the FHB resistance in HCD have not been characterized. In this study, F_5-derived recombinant inbred lines (RILs) developed from HCD x 'Jagger' were used to characterize QTLs associated with type II FHB resistance and to identify markers tightly linked to the QTLs for marker-assisted selection.

Materials and Methods

Plant materials and FHB evaluation

A population of 190 RILs was derived from HCD x 'Jagger' by single-seed descent. HCD is a resistant wheat landrace from China, and 'Jagger' (PI 593688) is an FHB-susceptible U.S. hard winter wheat from Kansas. $F_{5:6}$ and $F_{5:7}$ RILs were evaluated for FHB resistance at Kansas State University in Manhattan, KS. The experiments were conducted in the greenhouses of

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spring and fall 2010 and spring 2011 with two replications in each experiment. Seeds of the RILs and the parents were planted in plastic trays filled with Metro-mix 360 soil mix (Hummert International, Earth City, MO). After vernalization at 6°C in a cold room for 7 weeks, 5 seedlings per line were transplanted into each 13-cm^2 Dura pot containing Metro-mix 360 soil mix. The pots with plants were arranged on greenhouse benches in a randomized complete block design. The greenhouse was maintained at 15–20°C with 12 h supplemental daylight.

A Kansas strain of *F. graminearum* (GZ3639) was used as inocula, and conidia were prepared following Bai et al. (1999). The concentration of conidia was calculated using a hemocytometer under a light microscope. The final concentration of inocula was adjusted to 100,000 conidia per ml. At early anthesis, 10-µl conidial suspension (~1000 conidia/spike) was injected into a central spikelet of a spike using a syringe (Hamilton, Reno, NV). Five spikes per pot were inoculated and enclosed in a moist chamber at 100% relative humidity and 20–22°C to initiate fungal infection. After 48 h of incubation, the plants were moved to greenhouse benches at 17–25°C with 12 h supplemental daylight. Type II resistance (FHB symptoms spread within a spike) was evaluated by recording percentage of symptomatic spikelets (PSSs) in an inoculated spike 15 days after inoculation. Mean PSSs of the RILs for each experiment and across all three experiments were calculated for QTL analysis.

DNA extraction and genotyping

Leaf tissues were collected from $F_{5:6}$ RILs at the 3-leaf stage in 96-deepwell plates. Harvested tissues were dried in a freeze dryer (Thermo Savant, Holbrook, NY) for 48 h and ground in a Mixer Mill (MM 400, Retsch, Germany). Genomic DNA was isolated using a modified cetyltrimethyl ammonium bromide protocol (Saghai-Maroof et al. 1984). A core set of 384 pairs of SSR primers (http://wheat.pw.usda.gov) were used to screen the parents. This primer set was originally selected from 2,000 primer pairs according to previous studies conducted at the USDA Central Small Grain Genotyping Laboratory in Manhattan, KS. These markers are evenly distributed on all 21 chromosomes (Somers et al. 2004). Primer pairs that

detected polymorphism between parents were used to genotype a randomly selected subset of 96 RILs. Determination of coefficient (R^2) was calculated for each marker to determine significant markers associated with FHB type II resistance. A SSR linkage map constructed using these markers was used to map QTLs. After QTL regions had been identified, the SSR markers that linked to the QTLs in the linkage map were analyzed on the remainder of the RIL population. To increase the marker coverage in possible QTL regions, an additional 70 markers linked to known QTLs reported in previous studies were screened for polymorphism between the parents (Liu et al. 2009; Buerstmayr et al. 2009; http://wheat.pw.usda.gov), and segregating markers in the population were added to the linkage map for further QTL analysis.

Polymerase chain reaction (PCR) amplification was done in a DNA engine Tetrad Peltier thermal cycler (MJ Research, Waltham, MA). A 10-µl PCR master mix contained 1X ASB buffer, 2.5 mM of MgCl2, 200 µM of dNTP, 100 nM each of a fluorescent-dye-labeled M13 primer (5'- ACGACGTTGTAAAACGAC) and a forward primer with M13-tail added to 5'-end, and 200 nM of a reverse primer, 0.6 U of *Tag* polymerase, and 40 ng template genomic DNA. PCR amplification was done using a touchdown program. The PCR reaction mixture was incubated initially at 95^{o}C for 5 min, followed by five cycles of 96^{o}C for 1 min, annealing at 68^{o}C for 3 min with a decrease of 2^{o}C in each subsequent cycle, and extension at 72^{o}C for 1 min. For another five cycles, annealing temperature started from 58^{o}C for 2 min with a decrease of 2^{o}C in each subsequent cycle, then PCR went through an additional 25 cycles of 96^{o}C for 1 min, 50^{o}C for 1 min, and 72^{o}C for 1 min, ending with a final extension at 72^{o}C for 5 min. Amplified PCR products from four PCRs labeled with different florescent dyes (FAM, VIC, NED, and PET) were pooled and analyzed in an ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Data were scored using GeneMarker v1.75 (SoftGenetics LLC, State College, PA)

Data analysis

Broad sense heritability (*H*) of PSSs was calculated using $H=\sigma^2_G/[\sigma^2_G+(\sigma^2_{GE}/e)+(\sigma^2_\varepsilon/re)]$,

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where σ^2_G is an estimate of genetic variance, σ^2_{GE} is an estimate of genotype by environment variance, σ^2_ε is an estimate of residual error variance, r = number of replicates, and e = number of experiments. All variances were estimated by analysis of variance (ANOVA) using the PROC GLM function in SAS v. 9.1.2 (SAS Institute Inc., Cary, NC).

A linkage map was constructed by IciMapping v3.1 (Wang et al. 2011) using the Kosambi mapping function (Kosambi 1944) and a logarithm of odds (LOD) threshold of 3.0. QTLs for PSSs were analyzed by performing composite interval mapping (CIM) using WINQTL Cartographer version 2.5 with Model 6 (Wang et al. 2006). The permutation test was performed 1,000 times to determine the LOD threshold for claiming significant QTLs at $P < 0.05$ (Doerge and Churchill, 1996).

QTLs that explained larger than 5% of phenotypic variation of mean PSSs were selected to test the allele substitution effect. To compare the effect of these QTL combinations on FHB resistance, alleles for each QTL were represented by the alleles of the closest marker to the QTL. All RILs were separated into 2^n genotypic groups based on allele constitution of these QTLs (n) identified in the population. Multiple comparisons among the genotypic groups were conducted by the Tukey-Kramer method (Miller 1981).

Results

FHB in HCD / 'Jagger' population

The resistant parent HCD had a mean PSS of 16.7%, ranging from 9.6% to 27.2% across the three experiments, whereas 'Jagger', the susceptible parent, had a mean PSS of 84.4%, ranging from 69.9% to 98.3% (Fig. 1). The mean PSSs of RILs in each of the three experiments ranged from 4.7% to 100%. Different patterns of PSS frequency distributions were observed among the three experiments. In general, results showed continuous variation with two peaks, but with a major peak skewed toward HCD in spring and fall 2010 experiments and toward 'Jagger' in the spring 2011 experiment (Fig. 1). Mean PSSs over all RILs was 44.8%, ranging from 29.8% (fall 2010) to 55.0% (spring 2011); thus, disease pressure was highest in spring

2011 and lowest in fall 2010. Significant transgressive segregation was observed in all three experiments, suggesting that the susceptible parent might contribute resistance QTL in the population. The positive correlations of PSSs were highly significant among the three greenhouse experiments, ranging from 0.48 to 0.69 ($P < 0.001$). Analysis of variance indicated significant variations in genotypes, environments, and genotype by environment interactions (Table 1). Broad sense heritability for PSSs across three greenhouse experiments was high ($H = 0.80$).

Linkage map and QTLs for FHB resistance

After screening 454 selected SSR primers between parents, 261 primers were found to be polymorphic, indicating a high level of polymorphism (57.5%) for the set of primers. All polymorphic primers were used to screen a subset of 96 RILs. Among them, 242 markers were mapped to 43 linkage groups that covered 953.7 cM in genetic distance. Inclusive Composite Interval Mapping (ICIM) using the resulting map identified seven chromosome regions in 3A, 3B (2 QTLs), 4B, 5A, 2D, and 6D that were significantly associated with PSSs. All polymorphic markers from the six chromosomes were used to screen the rest of 94 RILs in the HCD x 'Jagger' population. The final linkage map was constructed using 190 RILs for final QTL detection; however, QTLs on 4B and 5A were not significant, so they were not analyzed further.

Five significant QTLs were identified by CIM in HCD x 'Jagger', with two from HCD mapped on the short arm of chromosome 3B (3BS) and one on 3AS. For two 3BS QTLs, one was mapped in the distal end (3BSd) of chromosome 3BS, and another was near the centromere (3BSc). The QTL on 3BSd showed a significant major effect on Type II resistance in all three experiments (Fig. 2), coinciding with previously reported *Fhb1* according to the haplotype of tightly linked markers *Xumn10* and *Xbarc133* (Liu et al., 2006). This QTL explained 11.0 to 26.1% of phenotypic variations across the three experiments (Table 2). The QTL on 3BSc, flanked by *Xwmc777* and *Xbarc139*, was significant only in the spring 2010

Crop Science Accepted paper, posted 05/07/2014. doi:10.2135/cropsci2013.12.0835

experiment and explained 6.6% of phenotypic variation (Table 2). The QTL on 3AS was flanked by *Xcfa2134* and *Xgwm2* and was significant in the fall 2010 experiment and mean PSSs over the three experiments. This QTL explained 7.5% to 10.0% of phenotypic variation (Table 2). The fourth QTL on 2D was tightly linked to the marker *Xgwm261* and was significant in spring 2010 and 2011 experiments and mean PSSs over the three experiments. This QTL explained 4.5 to 9.5% of phenotypic variations (Table 2). The fifth QTL was on the long arm of chromosome 6D (6DL) and was flanked by *Xcfd76* and *Xbarc175*, which was significant in the spring 2011 experiment and average PSSs of the three experiments and explained 3.8 to 6.7% of variation in PSSs. The QTLs on 3BS (*Fhb1* and 3BSc) and 3AS were from HCD, and QTLs on 2D and 6D were from 'Jagger'.

Effects of QTL on FHB resistance

To investigate the effect of individual QTL on type II resistance, RILs that carry different allele combinations at three (*Fhb1*, 3AS, and 2D) of the five QTLs were grouped and compared for their allele substitution effects. These three QTLs were selected because they were significant for mean PSSs over three greenhouse experiments. The QTLs on 3BSc and 6D were excluded from the analysis because they either were insignificant or showed minor effects (R^2-value < 5%) on mean PSSs over the three greenhouse experiments. Eight genotypes were derived from the eight possible allelic combinations of three QTLs: AABBCC, AABBcc, AAbbCC, AAbbcc, aaBBCC, aaBBcc, aabbCC, and aabbcc, where AA, BB, and CC represent resistance alleles at QTLs on 3BSd, 3AS, and 2D, respectively, whereas aa, bb, and cc represent corresponding opposite alleles, respectively. The closest markers to each of the three QTLs (*Xumn10* on 3BSd, *Xgwm674* on 3AS, and *Xgwm261* on 2D) were selected to represent these QTLs. Two contrasting alleles at each of the three SSR loci exhibited a 1:1 segregation ratio in the RIL population. Mean PSSs for the eight genotypic groups of 190 RILs ranged from 22.4% to 69.3%. The mean PSSs for the genotypic groups that had only one of the three resistance QTLs were 44.3% for *Fhb1*, 53.8% for QTL on 3AS, and 58.4% for QTL on 2D; in

contrast, the averaged PSS for the RIL group carrying none of the three alleles was 69.3% (null). The mean PSS for the groups with *Fhb1* or 3AS resistance allele alone were significantly lower than that of 'null' group. Meanwhile, the mean PSS of the RIL groups with resistance alleles at *Fhb1* plus an additional QTL (QTL on 3AS or 2D) were consistently lower than those with susceptible alleles at two or three QTLs. Thus, *Fhb1* had the largest effect on FHB resistance, and substitution of the susceptible allele at *Fhb1* by the resistant allele significantly increased PSSs.

Discussion

Although many Chinese wheat cultivars or landraces show a high level of FHB resistance (Yu et al. 2008ab; Li et al. 2011; Li et al. 2012; Yang et al. 2005ab), only a few, mainly Sumai 3 and its derivative Ning7840, have been well characterized as carrying QTLs for type II resistance (Bai and Shaner 2004; Rudd et al. 2001; Somer et al. 2003). QTLs in many other Chinese sources, especially landraces including HCD, are not well characterized. Characterization and utilization of QTLs in different sources of resistance will enhance genetic diversity of FHB resistance QTLs. In HCD x 'Jagger', the frequency distributions of mean PSSs from the three experiments showed two peaks of unequal sizes (Fig. 1), suggesting at least two QTLs segregating for FHB resistance in the population. Transgressive segregation in all three experiments suggested that 'Jagger' might also contribute QTL(s) to FHB resistance. QTL mapping using HCD x 'Jagger' RILs indicated that three QTLs on 3BS and 3AS mainly conditions FHB resistance in HCD and that 'Jagger' also contributes two minor QTLs for resistance. This result supports the observation from PSSs frequency distributions of the mapping population.

FHB resistance is a quantitative trait that is controlled by several genes/QTLs, and the environments in which plants are evaluated severely influence the resistance expression of these genes (Bai et al. 2000; Parry et al. 1995). Difference in FHB inoculation techniques and environmental conditions may contribute significantly to the differences in QTL detection.

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Thus, it is essential to evaluate FHB resistance repeatedly in different seasons and environments to improve the repeatability of QTL detection (Kolb et al. 2001). In this study, the RIL population was evaluated repeatedly in three greenhouse experiments. The correlation coefficients of PSSs for RILs were significant among experiments (r = 0.48 - 0.69, $P < 0.001$), with a high broad-sense heritability (0.80), suggesting that the phenotypic data were reliable for QTL analysis in this study. Variations in the patterns of frequency distributions and number of QTLs were observed for different experiments (Fig. 1), even though the study was conducted in greenhouses under environments with controlled temperature and moisture; thus, mean FHB data from three experiments may be more reliable for QTL determination. Initial QTL mapping starting with a small population of 96 RILs detected two additional QTLs, but they were not significant when 190 RILs were used for QTL analysis. Thus, increased size of population may reduce the number of false positive QTLs. The 190 RILs used in the current study is relatively large compared with previous mapping studies (Lemmens et al. 2005; Ma et al. 2006).

The QTL on 3BSd explained 11.0% to 26.1% of total phenotypic variation in the HCD x 'Jagger' population. *Xgwm493* and *Xgwm533* flanked this QTL with the peak at marker *Xumn10*, which coincides with a previously mapped *Fhb1* from 'Sumai 3' (Cuthbert et al. 2006; Waldron et al. 1999). This QTL has been reported in more than 30 studies in which 'Sumai 3' and its relatives were the major sources of the resistance. This QTL showed a stable major effect on FHB type II resistance across different genetic backgrounds (Buestmayr et al. 2009; Bai et al. 1999; Zhou et al. 2002, 2003; Buerstmayr et al. 2003; Shen et al. 2003a; Bourdoncle and Ohm 2003; Yang et al. 2005a; Chen et al. 2006; Jiang et al. 2007ab). It was also reported in Chinese and Japanese landraces that are not related to 'Sumai 3', such as 'Wangshuibai' (Lin et al. 2004; Zhou et al. 2004) and 'Nyu Bai' (Somers et al. 2003; Cuthbert et al. 2006), but with different levels of effects on type II resistance. Thus, the QTL on 3BS of HCD is more likely *Fhb1*.

The effect of *Fhb1* on type II resistance varied among studies, ranging from 6% in DH181 (Yang et al. 2005a) to 60% in 'Ning7840' (Bai et al. 1999). A wide range of R^2 values have

even been reported for the same source of resistance used in different studies (Waldron et al. 1999; Anderson et al. 2001; Zhou et al. 2002; Buerstmayr et al. 2002, 2003; Shen et al. 2003a; Bourdoncle and Ohm 2003; Somers et al. 2003; Chen et al. 2006; Jiang et al. 2007ab; Lin et al. 2004; Zhang et al. 2004; Ma et al. 2006; Yu et al. 2008b; Yang et al. 2005b). In the current study, the effect of *Fhb1* on type II resistance in HCD was highly significant in all three greenhouse experiments, with R^2 values up to 0.24 for mean PSSs across the three experiments; however, when individual experiments were examined, the phenotypic variations explained by *Fhb1* varied from 11.0% to 26.1%, indicating that QTL effects may vary significantly with environments even when the same population is used. This result may be due to differences in the seasons of inoculation in different experiments; inoculation during winter is usually under lower temperature conditions than in early summer, so higher levels of spread within a spike can be expected for early summer inoculation, especially for moderately resistant and susceptible genotypes. In field conditions, the situation is worse than in a greenhouse because the amount of inoculum available, flowering times, and temperature and moisture conditions during the infection period can differ dramatically among locations and years, which will lead to significant variations in QTL contributions from different studies. Discrepancies in the effects of *Fhb1* reported from different studies could therefore be due to differences in FHB evaluation environments, genetic backgrounds of different populations, population sizes, and inoculation methods. Different sources of resistance also may harbor different alleles of *Fhb1*. *Fhb1* in HCD is likely to be the same locus as in 'Sumai3'.

The second QTL on chromosome 3AS was significant in one greenhouse experiment and in means over three experiments. This QTL, closest to *Xgwm674,* was flanked by *Xgwm2* and *Xcfa2134* and explained 7.5 to 10.0% of the phenotypic variation. A QTL in a similar location was first reported on 'Huapei 57-2' centered at *Xgwm5*, which explained 8.1% of variation for type II resistance (Bourdoncle and Ohm 2003). In F201R, this QTL was flanked by *Xbarc76* and *Xgwm674* and explained 13.4% of type II resistance (Shen et al. 2003b). Meanwhile, a QTL from *Triticum dicoccoides* was mapped near *Xgwm2* and explained 37% of phenotypic

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variation (Chen et al. 2007). Because *Xgwm2* and *Xgwm5* are 2.8 cM apart (Somers et al. 2004), the QTL mapped in this study is most likely the same QTL reported from those studies. In this study, the effect of the QTL on 3AS was smaller than *Fhb1*; however, this QTL contributes the second-largest effect on type II resistance compared with other QTLs and was able to reduce FHB PSSs from 69.3% (Null) to 53.8% (Fig. 3). Thus, it can be a good candidate for pyramiding of different QTLs with major and stable effects to improve FHB type II resistance.

The third QTL detected in this study was also located on chromosome 3BS, but the QTL was near *Xgwm32* and was flanked by *Xwmc777* and *Xbarc139*. It explained 6.6% of phenotypic variation only in the spring 2010 greenhouse experiment. This QTL was first reported on 'Nyu Bai' centered by *Xgwm566*, which explained 4% of FHB severity variation (Somers et al. 2003), and then on 'Wangshuibai' (Zhou et al. 2004; Yu et al. 2008b) and 'Ernie' (Liu et al. 2007). One QTL reported on 'Wangshuibai' was close to *Xgwm376* and explained 8.1% variation for Type II resistance (Yu et al. 2008b). In U.S. wheat 'Ernie', this QTL was close to *Xgwm285* and explained 12.9% phenotypic variation (Liu et al. 2007). These reported QTLs from different studies seem to be the same QTL detected in this study, because the SSR markers linked to the QTL are all on the same chromosome region (Somers et al. 2004). In this study, the QTL on 3BSc was not as stable as *Fhb1* and was significant only in one of the three experiments; therefore, in wheat breeding programs, this QTL can be used in combination with other major, stable QTLs to improve FHB type II resistance.

The fourth QTL was identified on 2DS of 'Jagger'. This QTL was located in the same position as the QTL previously reported in a Japanese cultivar (Handa et al. 2008). Comparative analysis with the rice genome identified a candidate gene for FHB resistance, a gene for multidrug resistance-associated protein (MPR) in rice (Handa et al. 2008). In the current study, this QTL was close to *Xgwm261* in chromosome 2D and was flanked by *Xwmc112* and *Xwmc25*. It was significant in both spring 2010 and 2011 experiments and explained 4.5 to 9.5% of phenotypic variation. Previous studies revealed that SSR marker *Xgwm261* was linked to a reduced height locus, *Rht8* (Korzun et al. 1998). However,

association was not found between FHB resistance and plant height variation caused by *Rht8* in this study. The resistance QTL was also reported in a double haploid population from the cross Sumai3/Gamenya that explained 14% to 25% of phenotypic variations, with the resistance allele contributed by "susceptible parent" Gamenya (Xu et al. 2001). Other studies also detected the QTL from a moderate susceptible parent 'Alondra's', which explained 12.1% phenotypic variation (Shen et al. 2003a). In this study, the QTL on 2DS of 'Jagger' showed a minor effect on type II resistance, but it is more likely a real QTL because it is consistently detected in the same chromosome region of different genetic backgrounds. This QTL is often identified in susceptible parents, which suggests that some susceptible cultivars may carry resistance QTLs. When transferring a major QTL to U.S. wheat, using these adapted susceptible parents with minor resistance alleles can improve the level of resistance of selected new cultivars. These cultivars without any minor resistance allele should be avoided as parents for breeding crosses. In addition, this QTL was also found in several other studies where resistant parents contributed the resistance allele (Yang et al. 2005a; Jia et al. 2005).

QTL on the long arm of chromosome 6D of 'Jagger' also had a minor effect on type II resistance and was significant in only one greenhouse experiment (spring 2010) and the combined average over the three greenhouse experiments. This QTL explained 3.8 to 6.7% of the phenotypic variation, with a peak close to *Xbarc175*. Only one previous report indicated that the QTL on 6DL was associated with FHB severity and was detected in field experiments (Paillard et al. 2004). Paillard et al. (2004) also found that the FHB resistance QTL on 6D flanked by *Xcfd19a* and *Xpsr915* in 'Arina' overlapped with the QTLs for heading date and plant height on the same chromosome region. Although no overlapping marker was found between 6D QTL in 'Arina' and this study, they seem to be the same QTL because the markers linked to these QTLs from the two studies are mapped in the same chromosome region (Somers et al., 2004).

Acknowledgements

This is contribution number 14-009-J from the Kansas Agricultural Experiment Station. This

Crop Science Accepted paper, posted 05/07/2014. doi:10.2135/cropsci2013.12.0835

project is partly funded by the U.S. Wheat and Barley Scab Initiative and the National Research Initiative Competitive Grants CAP project 2011-68002-30029 from the USDA National Institute of Food and Agriculture. USDA is an equal opportunity provider and employer. 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 U.S. Department of Agriculture.

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Figure Legends

Fig. 1 Frequency distribution of mean percentage of symptomatic spikelets (PSSs) in a spike for the RIL population derived from cross HCD × 'Jagger' in three greenhouse experiments

Fig. 2 Maps of quantitative trait loci (QTL) for FHB type II resistance constructed from a recombinant inbred line (RIL) population derived from the cross HCD × 'Jagger' based on three greenhouse experiments.

Fig. 3 Effects of different combinations of QTLs for percentage of symptomatic spikelets (PSSs) of a spike in a RIL population derived from HCD × 'Jagger' based on FHB disease data collected from three greenhouse experiments. Group 1 carry resistance alleles from QTLs on 3BS, 3AS and 2D; Group 2 carry resistance alleles from QTLs on 3BS and 3AS; Group 3 carry resistance alleles from QTLs on 3BS, and 2D; Group 4 carry resistance allele from QTL on 3BS only; Group 5 carry resistance alleles from QTLs on 3AS and 2D; Group 6 carry resistance alleles from QTL on 3A only; Group7 carry resistance alleles from QTL on 2D only; Group 8 carry susceptible alleles from all three QTLs. The solid triabgles on the vertical lines are the mean PSS of each group, and lengths of the lines are 95% confidence intervals. If the confidence intervals are not overlapped to each other, then the two groups are significantly different at $LSD_{0.05}$.

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Table 1 Analysis of variance (ANOVA) of percentage of symptomatic spikelets (PSSs) data for the RIL based on three greenhouse experiments

Source	DF	Type III SS	Mean square	F value	Pr > F
Experiment	2	127782.50	63891.25	218.94	<.0001
Replication	3	1198.96	399.65	1.37	0.2512
Genotype	189	587702.09	3109.53	10.66	<.0001
Experiment*genotype	376	230449.33	612.89	2.10	<.0001
Error	548	159920.26	291.82		
Corrected total	1118	1119363.67			

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Table 2 Flanking markers, logarithm of the odds (LOD), coefficients of determination (R^2) of the significant QTL regions detected by composite interval mapping based on spring 2010, fall 2010, and spring 2011 greenhouse FHB data.

Locus	Resistance allele from	Flanking markers	Spring 2010		Fall 2010		Spring 2011		Combined mean	
			LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2
Fhb1	**HCD**	*Xgwm493~Xgwm533*	8.85	16.50%	6.34	11.00%	14.4	26.14%	14.93	23.80%
3BSc	**HCD**	*Xwmc777~ Xbarc139*	4.07	6.60%	-	-	-	-	-	-
3AS	**HCD**	*Xcfa2134~Xgwm2*			4.44	10.00%	-	-	5.11	7.50%
2D	**Jagger**	*Xwmc112~Xwmc25*	2.69	4.50%	-	-	5.06	9.50%	4.45	6.80%
6D	**Jagger**	*Xcfd76~Xbarc175*	3.73	6.74%	-	-	-	-	2.77	3.80%

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Fig. 1

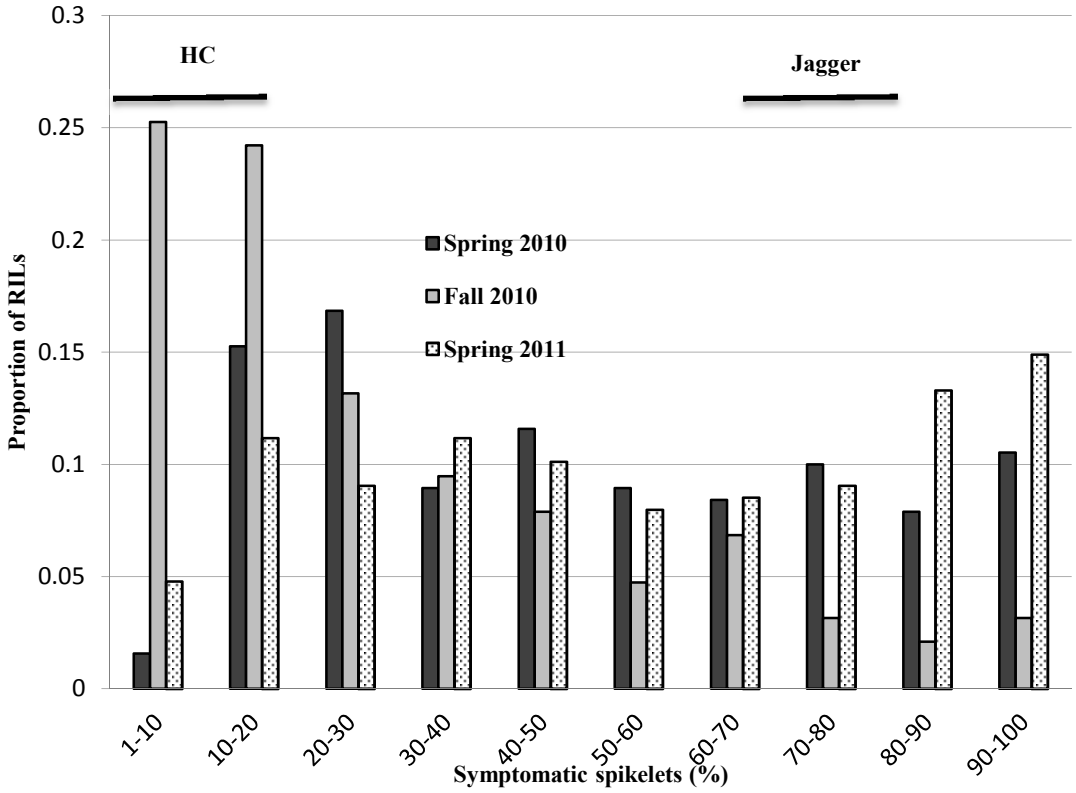

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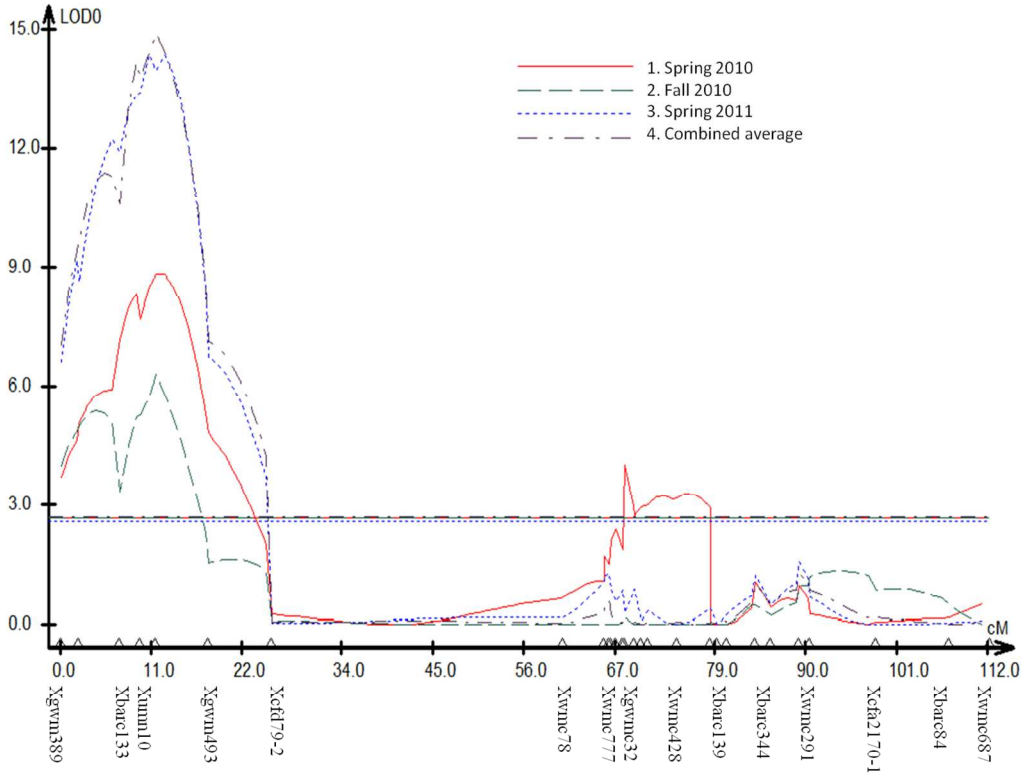
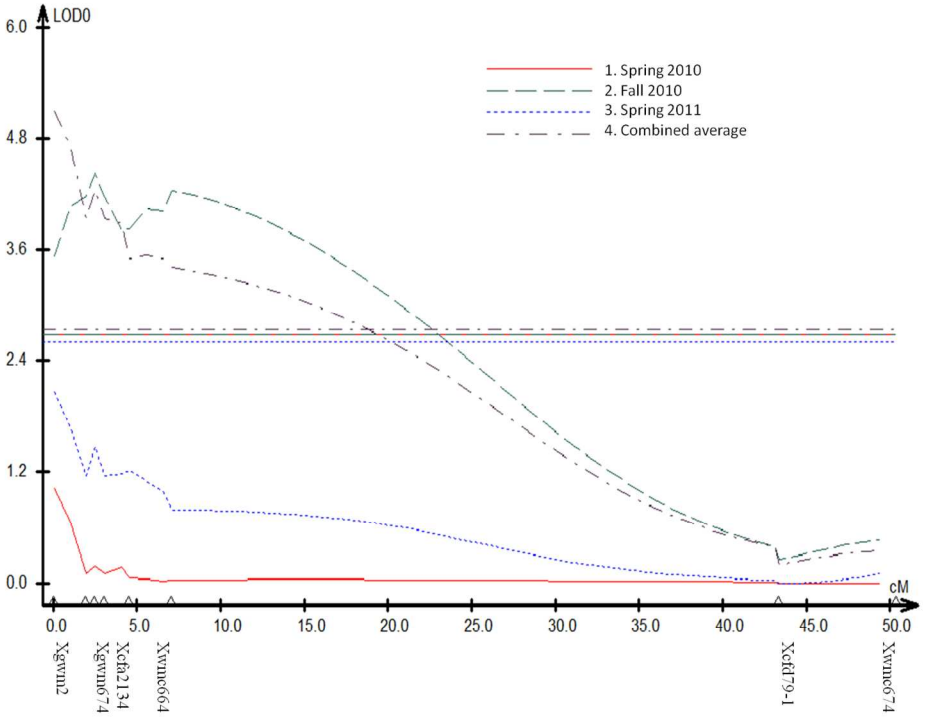
Fig. 2

A. 3BS (Left: *Fhb1*; right: 3BSc)



B. 3AS



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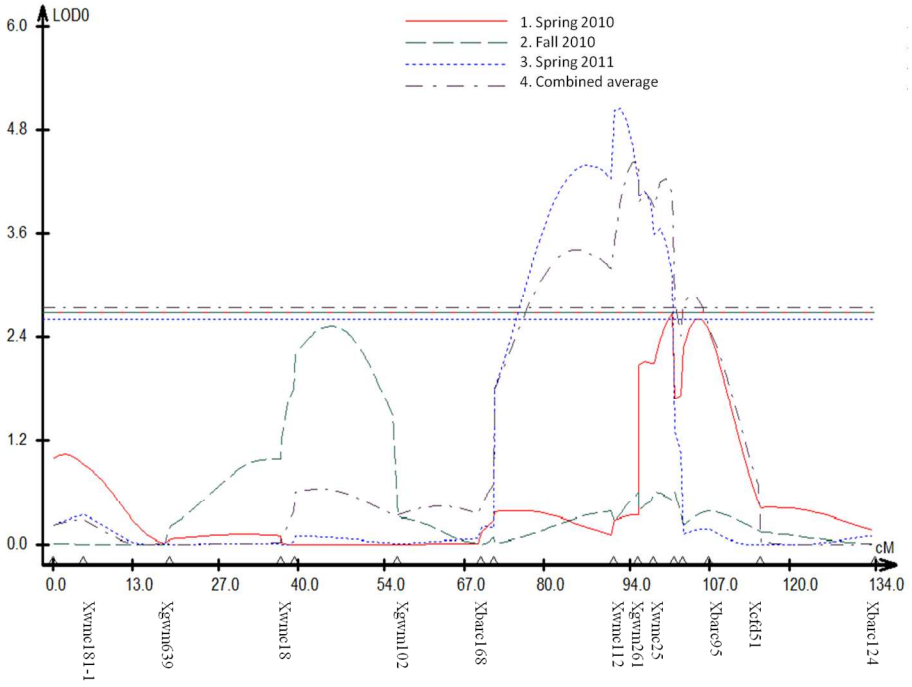
C. 2D



D. 6DL

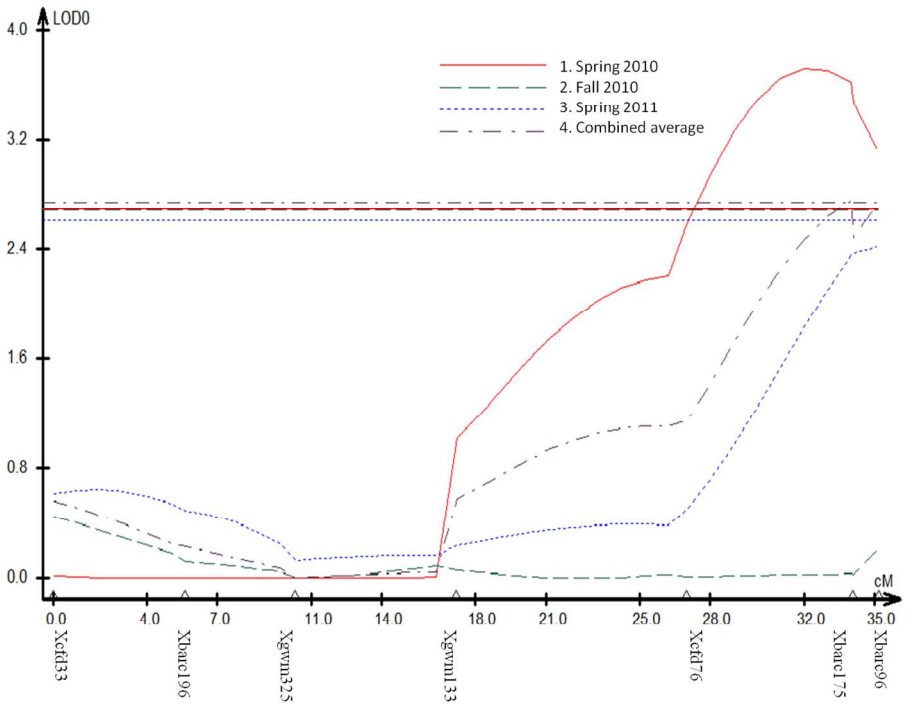


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Fig. 3