

# Quantitative Trait Loci for Fusarium Head Blight Resistance in U.S. Hard Winter Wheat Cultivar Heyne

Xianghui Zhang, Guihua Bai\*, Willium Bockus, Xiaojia Ji, and Hongyu Pan\*

## ABSTRACT

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum* Schwabe, is a destructive disease that can significantly reduce grain yield and quality. Quantitative trait loci (QTL) for FHB type II resistance have been identified in many Chinese cultivars and other sources but have not been reported in U.S. hard winter wheat (HWW) (*Triticum aestivum* L.) to date. 'Heyne' is a Kansas HWW with moderate type II resistance to FHB. In this study, recombinant inbred lines (RILs) derived from 'Trego' × Heyne were evaluated for FHB resistance by single-floret inoculation in two field and three greenhouse experiments from 2009 to 2011. Percentage of symptomatic spikelets (PSS) in an inoculated spike was scored 18 d (in the greenhouse) and 21 d (in the field) postinoculation. A total of 209 polymorphic simple sequence repeats (SSRs) were used to screen the RILs. Three major QTL on chromosomes 3AS, 4DL, and 4AL were associated with FHB resistance. The QTL on 3AS was flanked by *Xgwm5* and *Xwmc428* and explained up to 17.9% of phenotypic variation. Another QTL on 4DL near *Xwmc720* explained 13.8 to 23.4% of phenotypic variation. The third QTL on 4AL was flanked by *Xwmc219* and *Xgwm160* and explained up to 18.1% of the phenotypic variation. Heyne contributed all resistance alleles of three QTL, and these QTL were designated as *Qfhb.hwwg-3AS*, *Qfhb.hwwg-4DL*, and *Qfhb.hwwg-4AL*. These QTL can be used for improving FHB resistance in U.S. HWW by pyramiding them with *Fhb1* or other major resistance QTL from Asian sources.

X. Zhang, College of Plant Science, Jilin University, Changchun, Jilin, China, 130062, and Department of Agronomy, Kansas State University, Manhattan, KS, 66506; G. Bai, Department of Agronomy, Kansas State University, Manhattan, KS, 66506 and USDA-ARS Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506; X. Ji, Department of Agronomy, Kansas State University, Manhattan, KS, 66506; W. Bockus, Department of Plant Pathology, Kansas State University, Manhattan, KS, 66506; H. Pan, College of Plant Science, Jilin University, Changchun, Jilin, China, 130062. Received 10 Aug. 2011. \*Corresponding author (guihua.bai@ars.usda.gov, panhongyu@jlu.edu.cn)

**Abbreviations:** DH, doubled haploid; DON, deoxynivalenol; FDK, *Fusarium* damaged kernels; FHB, Fusarium head blight; HWW, hard winter wheat; LOD, logarithm of odds; MAS, marker-assisted selection; PCR, polymerase chain reaction; PSS, percentage of symptomatic spikelets; QTL, quantitative trait locus/loci; RIL, recombinant inbred line; SSR, simple sequence repeat.

FUSARIUM HEAD BLIGHT (FHB), mainly caused by *Fusarium graminearum* Schwabe [telomorph = *Gibberella zeae* (Schw.) Petch], is one of the most destructive wheat (*Triticum aestivum* L.) diseases in warm and humid regions and seriously threatens wheat production worldwide (Bai and Shaner, 2004). Fusarium head blight causes premature plant death or blighting of infected spikes, which usually reduces grain yield and quality substantially (Bai and Shaner, 1994). Infected grain contaminated with mycotoxins is potentially harmful to human and animal health and therefore a safety concern in food or feed (Desjardins and Hohn, 1997). The most common toxin associated with FHB is deoxynivalenol (DON), and wheat grain contaminated with the toxin can significantly lower grain price (Wu, 2007).

Published in Crop Sci. 52:1187–1194 (2012).

doi: 10.2135/cropsci2011.08.0418

© Crop Science Society of America | 5585 Guilford Rd., Madison, WI 53711 USA

All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Permission for printing and for reprinting the material contained herein has been obtained by the publisher.

Although currently available cultural practices and fungicides can reduce disease damage to some extent, they are not completely effective at preventing disease epidemics. Growing resistant cultivars is the most economically effective and environmentally friendly approach to control this disease. In early 1980s, an extensive search for FHB-resistant germplasm was launched in China, and germplasm with high levels of resistance was identified from both Chinese landraces and elite breeding lines. Among them, 'Sumai 3' and its derivatives, such as 'Ning 7840', showed the best resistance to FHB spread within a spike (type II resistance) and have been used in breeding programs worldwide (Bai and Shaner, 2004). Later, FHB-resistant germplasm was reported from other regions including Europe and North America (Gervais et al., 2003; Klahr et al., 2007; Miedaner, 1997; Paillard et al., 2004; Rudd et al., 2001; Häberle et al., 2007). Several quantitative trait loci (QTL) were reported from European winter wheat cultivars, and QTL on 4DS associated with reduced plant height (*Rht2*) was identified in several populations (Draeger et al., 2007; Holzapfel et al., 2008). However, market classes of these resistant parents were not specified. In the United States, several soft winter wheat cultivars including Ernie and Truman were reported to show moderate resistance to FHB and were proposed to carry different resistance genes than Chinese sources. Quantitative trait loci mapping identified four QTL on 2B, 3B, 4BL, and 5A in Ernie that explained 43.3% of the phenotypic variation for type II resistance (Liu et al., 2007), 42% for *Fusarium* damaged kernels (FDK), and 31% for low DON (Abate et al., 2008) and indicated that the QTL on the short arm near the centromere region of the 3B chromosome was the major QTL for these traits and was different from those in Sumai 3 and other Asian sources. Although most U.S. hard winter wheat (HWW) cultivars remain highly susceptible to FHB, recent surveys identified several HWW cultivars with moderate resistance to FHB infection, including Heyne and 'Hondo' (Bockus et al., 2009). However, mapping work has not been reported from U.S. HWW, and whether the responsible genes or QTL for the resistance in these HWW are the same as those in U.S. soft wheat or Asian sources remains unknown.

With the aid of molecular markers, mapping of QTL has been successfully used to identify genetic factors responsible for FHB resistance. Many QTL have been identified from different sources (Anderson et al., 2001; Bai et al., 1999; Steiner et al., 2004), and some markers associated with these QTL have been used in marker-assisted selection (MAS). Several types of molecular markers have been used to map QTL for FHB resistance (Waldron et al., 1999; Bai et al., 1999; Anderson et al., 2001; Zhou et al., 2003). Among the markers, simple sequence repeats (SSRs) are highly polymorphic, chromosome specific, and suitable for automation (Roder et al.,

1998); therefore, SSRs have been used widely in dissection of QTL for FHB resistance.

To date, many QTL for FHB resistance have been reported from almost all 21 chromosomes (Bai et al., 1999; Steiner et al., 2004; Buerstmayr et al., 2002; Somers et al., 2003); however, most of them were from Chinese sources (Bai et al., 1999; Waldron et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2002; Zhou et al., 2002, 2003; Mardi et al., 2005), and only a few were from European and American sources (Gervais et al., 2003; Klahr et al., 2007; Paillard et al., 2004; Häberle et al., 2007). Profiling QTL from native U.S. sources may identify new QTL that are different from Chinese sources. The objectives of this study were to (i) determine whether the QTL for FHB resistance from the indigenous U.S. hard winter wheat cultivar Heyne are different from Asian sources, (ii) determine chromosome locations of these QTL, and (iii) identify molecular markers for MAS of these QTL in breeding.

## MATERIALS AND METHODS

### Plant Materials and Growth Conditions

A population of 94 recombinant inbred lines (RILs) was developed from the cross Trego × Heyne by single-seed descent. Heyne (PI 612577) is a moderately FHB-resistant winter wheat cultivar whereas Trego (PI 612576) is a highly FHB-susceptible winter wheat cultivar based on our previous unpublished FHB data (W. Bockus, unpublished data, 2005). Both are white wheat developed from Kansas State University breeding programs. After seedlings of both parents and RILs were vernalized for 7 wk at 4°C in a growth chamber, they were transplanted into 13.5-cm (5.25-in.) Dura-pots containing Metro-Mix 360 soil mix (Hummert International) and grown in a greenhouse with 12 h supplemental light.

### Fusarium Head Blight Evaluation

The RIL population was evaluated for spread of FHB symptoms within a spike in both field (2009 and 2011) and greenhouse (spring and fall 2009 and spring 2010) experiments at Kansas State University in Manhattan, KS. Production of *F. graminearum* (strain GZ3639 from Kansas) conidia and evaluation of FHB resistance followed Bai et al. (2000). In brief, 10 µL of conidial suspension (100 spores µL<sup>-1</sup>) was injected into a central floret of a spike with a syringe at anthesis (Feekes stage 10.51; Large, 1954). Inoculated plants were placed in a moist chamber at 20 to 25°C with 100% relative humidity for 48 h to promote initial infection. Visual symptoms ranging from dark-brown, water-soaked spots on the glumes to bleached spikelets were counted as symptomatic spikelets 18 d after inoculation. The greenhouse experiment was arranged in a random complete block design with two replications (pots) of five to six plants per replication; the experiments were repeated twice. The field plot was planted in a 1-m row per line with two replications. Disease inoculation was also conducted by single floret inoculation as described for the greenhouse experiments and was followed by misting plants with sprinklers for 3 min every hour from 2100 h through 0600 h daily until the hard dough

stage (Feekes stage 11.2; Large, 1954). Infected spikelets and total spikelets per spike were counted at 21 d after inoculation in field experiments (Bai and Shaner, 1994). Disease severity was calculated as the percentage of symptomatic spikelets (PSS) per inoculated spike. Mean PSS for each experiment and mean PSS over all experiments were used for QTL analysis. Phenotypic correlations among experiments were calculated using Excel (Microsoft, 2008).

### Simple Sequence Repeat Analysis

Wheat leaf tissue was collected and processed in 96 deepwell plates for DNA isolation before transplanting. Harvested tissue was dried in a freeze dryer (ThermoSavant) for 48 h and ground into fine powder using a Mixer Mill (MM 400; Retsch Inc.). A modified cetyltrimethylammonium bromide protocol was used to isolate genomic DNA (Saghai-Marooif et al., 1984). A total of 1306 pairs of SSR primers were screened for polymorphism between two parents, including the markers that were previously reported to link to QTL for FHB resistance. These primers are 22 DUP, 653 BARC, 226 WMC, 78 GDM, 148 GWM, 80 KSM, 65 CFD, and 34 CFA primers (Somers et al., 2004; Song et al., 2005), which include a core set of 384 highly informative SSRs. The core set of SSRs was carefully selected based on quality of polymerase chain reaction (PCR) amplification, polymorphism levels, and distribution across all chromosome arms from previous experience and reports. All primers that amplified at least one polymorphic band between parents were used to screen the RIL population. For SSR detection, all forward primers were added a tailed M-13 sequence (5'-ACGAC-GTTGTAAACGAC) at the 5'-end, and an additional primer with the same M-13 sequence labeled with different fluorescent dyes (FAM, VIC, NED, and PET) was used in each PCR for multiplex PCR detection. Simple sequence repeat markers were analyzed in a DNA Engine Tetrad Peltier thermal cycler (MJ Research). A 14  $\mu$ L PCR mixture contained 40 ng of template DNA, 0.1  $\mu$ M each primer, 0.2 mM each deoxyribonucleotide triphosphate (dNTP), 1x PCR buffer, 2.5 mM MgCl<sub>2</sub>, and 0.6 unit of *Taq* polymerase. A touchdown program was used for PCR amplification in which the reaction was incubated at 95°C for 5 min and 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. Polymerase chain reaction products were resolved in an ABI Prism 3730 Genetic Analyzer (Applied Biosystems). Data were analyzed using GeneMarker software (version 1.6; SoftGenetics, 2011) and manually checked twice to remove ambiguous scores.

### Data Analysis

Analysis of variance of the three seasons of greenhouse phenotype data was conducted using PROC ANOVA in SAS 9.1.2 (SAS Institute, 2004). Linkage maps were constructed using the regression mapping method and Kosambi function by JoinMap software version 3.0 (Van Ooijen and Voorrips, 2001). A minimum logarithm of odds (LOD) threshold value of 3 was used

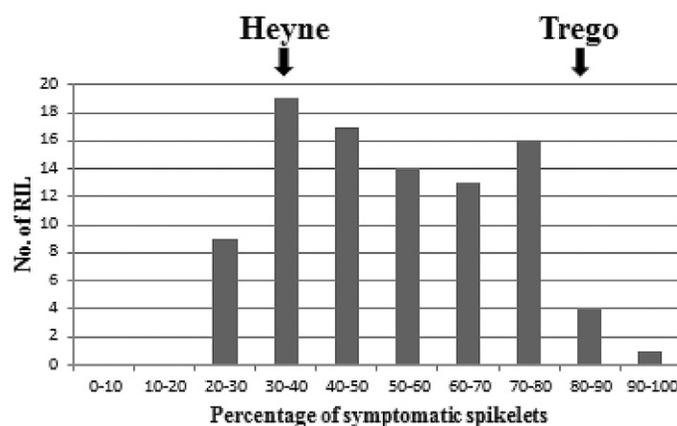


Figure 1. Frequency distribution of mean percentage of symptomatic spikelets (PSS) in recombinant inbred lines derived from the wheat cross Trego  $\times$  Heyne over five experiments. RIL, recombinant inbred line.

to determine linkage between markers. The composite interval mapping was performed using WinQTLCart 2.5 (Wang et al., 2006) using a 10.0 cM window size as background control with a walk speed of 2.0 cM. Quantitative trait loci were calculated based on the individual line means from each experiment and on the line means across all experiments. Permutation tests of 1000 times were performed to identify appropriate thresholds for claiming significant QTL (Doerge and Churchill, 1996). Based on 1000 times permutation calculations, a LOD threshold value was identified for claiming significant QTL. Quantitative trait loci were named according to standard nomenclature for QTL designation in wheat (McIntosh et al., 2008). *Qfhb.huwg* was designated as the QTL for FHB resistance reported from Hard Winter Wheat Genetics Research Unit, USDA-Agricultural Research Service.

## RESULTS

### Fusarium Head Blight Variation among Recombinant Inbred Lines and between Parents

Mean PSS over all five experiments was 80.0% for Trego and 38.4% for Heyne. Mean PSS of the RILs derived from Trego  $\times$  Heyne was 53.0%, ranging from 20.4 to 90.1%. Frequency distribution of mean PSS was continuous with a broad phenotypic variation across the RILs (Fig. 1). Correlations of PSS for RILs were highly significant among the five experiments, ranging from 0.35 to 0.74 ( $p < 0.0001$ ). Variance analysis on greenhouse data from three greenhouse experiments indicated that variations were significant for genotype, experiment, and genotype  $\times$  experiment interactions (Table 1).

### Linkage Analysis

Polymorphic level of SSRs was relatively low between the two parents (16%). Among 1306 SSRs screened, only 209 generated at least one polymorphic fragment between parents. These markers were used to screen all RILs. Among

**Table 1. Variation analysis of Fusarium head blight from three greenhouse experiments using recombinant inbred lines derived from the cross Trego × Heyne.**

Source	df	SS <sup>†</sup>	MS <sup>‡</sup>	F-value	Probability > F
Genotype	92	19.01	0.21	6.91	<0.0001
Experiment	2	16.86	8.43	282.08	<0.0001
Replicate	1	0.02	0.02	0.74	0.39
Genotype × experiment	184	7.64	0.04	1.39	0.0068
Replicate × experiment	2	0.06	0.03	1.05	0.3513
Error	276	8.25	0.03		

<sup>†</sup>SS, sum of squares.

<sup>‡</sup>MS, mean square.

209 polymorphic SSR markers, 179 were mapped in 31 linkage groups. The linkage map covered 784 cM of the wheat genome, with an average of 4.4 cM between markers. Chromosomes 5A, 6B, 5D, 3D, 3B, and 2A had better marker coverage than other chromosomes.

### Quantitative Trait Loci Analysis

Initially, single marker analysis was conducted to identify significant markers that were associated with FHB resistance. Based on map positions of these significant markers in previously reported maps (Somers et al., 2004; Song et al., 2005), four additional markers that are closely linked to these significant markers were added to the map. Composite interval mapping detected three major QTL for type II resistance in five experiments using PSS data from individual experiments and mean PSS from all five experiments (Table 2; Fig. 2). A QTL on 3AS, designated *Qfhb.hwwg-3AS*, was significant in two 2009 greenhouse

experiments and one 2010 greenhouse experiment and also in the 2011 field experiment and explained 13.4 to 17.9% of phenotypic variation for PSS, respectively. A QTL on 4DL, designated *Qfhb.hwwg-4DL*, was significant in the spring 2009 greenhouse and field experiments and the 2010 greenhouse experiment and explained 13.8 to 23.4% of phenotypic variation for PSS; the third QTL on 4AL, *Qfhb.hwwg-4AL*, was significant in all experiments and explained 8.1 to 18.1% of phenotypic variation for PSS (Table 2).

To evaluate the allelic substitution effect of the markers at the QTL regions, all three QTL were analyzed for marker allele substitution effect. The closest marker to QTL 3AS was *Xcfa2234*, the closest marker to 4DL was *Xwmc720*, and the closest marker to 4AL was *Xgwm160*. Three markers formed eight genotypes: RILs with all Heyne (AABBCC) or Trego alleles (aabbcc) at all three loci, RILs with Heyne alleles in two of the three loci (AABBcc, AAbbCC, and aaBBCC), and RILs with Heyne alleles in only one of the three loci (AAbbcc, aaB-Bcc, or aabbCC). The RILs with Heyne alleles at the three QTL showed the lowest mean PSS (33%) among the eight genotypes whereas the RILs with all Trego alleles had the highest mean PSS (84%) (Fig. 3). The RILs with two alleles from Heyne were the second, with mean PSS from 40 to 63%, and the RILs with one allele from Heyne were the third, with mean PSS from 56 to 75%. These results indicate that substitution of each Trego allele with a corresponding Heyne allele at any of the three loci can reduce the PSS.

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

Experiment	QTL	Interval	Chromosome	Length (cM)	LOD <sup>†</sup>	R <sup>2</sup> (%)
Spring 2009 greenhouse	<i>Qfhb.hwwg-3AS</i>	<i>Xbarc86-Xwmc428</i>	3AS	20.0	6.7	17.9
	<i>Qfhb.hwwg-4DL</i>	<i>Xwmc331-Xwmc720</i>	4DL	9.0	6.5	17.6
	<i>Qfhb.hwwg-4AL</i>	<i>Xwmc219-Xgwm160</i>	4AL	5.0	2.2	10.3
Spring 2010 greenhouse	<i>Qfhb.hwwg-3AS</i>	<i>Xbarc86-Xwmc428</i>	3AS	15.0	5.7	16.6
	<i>Qfhb.hwwg-4DL</i>	<i>Xwmc331-Xwmc720</i>	4DL	7.5	4.9	13.8
	<i>Qfhb.hwwg-4AL</i>	<i>Xwmc219-Xbarc78</i>	4AL	11.5	2.9	15.6
Fall 2009 greenhouse	<i>Qfhb.hwwg-3AS</i>	<i>Xbarc86-Xwmc428</i>	3AS	19.0	5.5	16.1
	<i>Qfhb.hwwg-4AL</i>	<i>Xwmc219-Xgwm160</i>	4AL	5.0	1.9	10.1
2009 field	<i>Qfhb.hwwg-4DL</i>	<i>Xwmc331-Xwmc720</i>	4DL	9.0	6.0	23.4
	<i>Qfhb.hwwg-4AL</i>	<i>Xwmc219-Xgwm160</i>	4AL	1.5	1.7	8.1
2011 field	<i>Qfhb.hwwg-3AS</i>	<i>Xbarc86-Xwmc428</i>	3AS	14.0	4.3	13.4
	<i>Qfhb.hwwg-4AL</i>	<i>Xwmc219-Xbarc78</i>	4AL	11.5	3.9	18.1
Mean PSS	<i>Qfhb.hwwg-3AS</i>	<i>Xbarc86-Xwmc428</i>	3AS	17.0	5.4	14.0
	<i>Qfhb.hwwg-4DL</i>	<i>Xwmc331-Xwmc720</i>	4DL	8.5	4.7	12.7
	<i>Qfhb.hwwg-4AL</i>	<i>Xwmc219-Xbarc78</i>	4AL	12.0	3.7	16.8

<sup>†</sup>LOD, logarithm of odds.

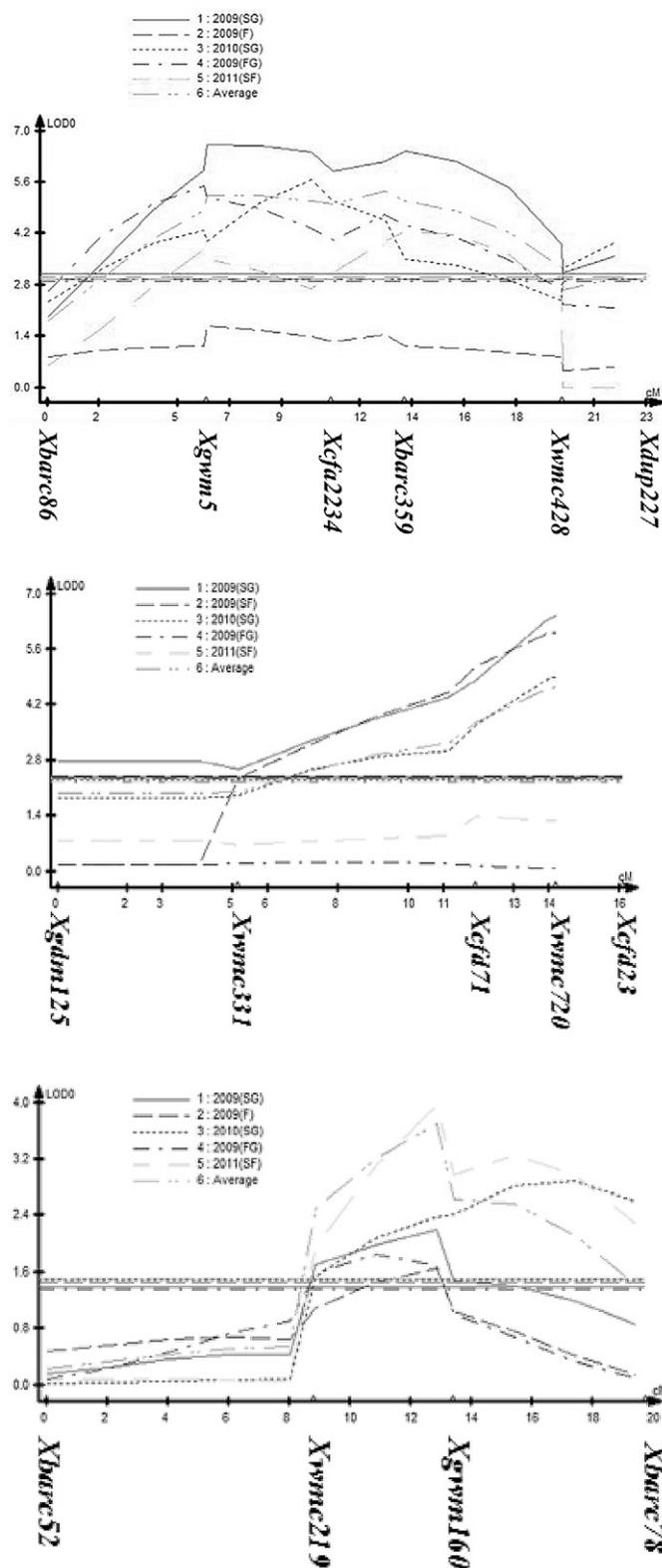


Figure 2. Maps of quantitative trait loci (QTL) for Fusarium head blight (FHB) type II resistance derived from a recombinant inbred line population (Trego × Heyne) based on percentage symptomatic spikelets after single-floret inoculation in three greenhouse and two field experiments. From top to bottom: linkage maps for QTL on 3A, 4DL, and 4AL. FG, fall greenhouse; SF, spring field; SG, spring greenhouse.

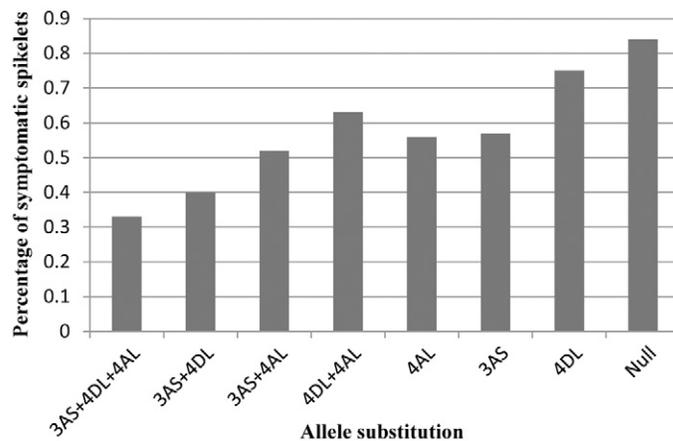


Figure 3. Allele substitution effect on percentage of symptomatic spikelets in the mapping population Trego × Heyne as evaluated in three greenhouse experiments and two field experiment at Kansas State University, Manhattan, KS. Null means recombinant inbred line (RIL) without Heyne alleles at the three quantitative trait loci, 3AS+4DL+4AL means RIL containing all three alleles from Heyne, 3AS+4DL means RIL containing 3AS and 4DL alleles from Heyne, 3AS+4AL means RIL containing 3AS and 4AL alleles from Heyne, 4DL+4AL means RIL containing 4DL and 4AL alleles from Heyne, 3AS means RIL containing only 3AS allele from Heyne, 4DL means RIL containing only 4DL allele from Heyne, and 4AL means RIL containing only the 4AL allele from Heyne.

## DISCUSSION

Wheat FHB epidemics have been severe in hard spring and soft winter wheat growing regions in the United States since the mid 1980s (Bai and Shaner, 1994). In the Great Plains, epidemics used to occur only in northern areas such as Minnesota, North and South Dakota, and part of Nebraska; however, FHB has recently spread to and become more frequent and severe in southern states of the region such as Kansas and Oklahoma. Hard winter wheat cultivars currently used for production in the region are mostly susceptible to FHB. Efforts to transfer *Fhb1* from Chinese sources into hard winter wheat have been made, but their unadapted agronomic traits make these sources difficult to use. Resistance from indigenous sources may reduce FHB damage more rapidly in the HWW region.

Several HWW cultivars from the southern Great Plains were reported to have better FHB resistance than others. These included Heyne, a hard white winter wheat cultivar from Kansas. However, QTL associated with FHB resistance in these cultivars have not been investigated. Because type II resistance is usually evaluated in controllable environments, data are more repeatable than other types of resistance, as proposed by Mesterhazy (1995). In this study, all materials were evaluated in both greenhouse and field experiments for type II resistance, and the correlations of PSS among experiments were highly significant, indicating that the data were reproducible and suitable for QTL analysis of type II resistance.

Significant interaction was observed between genotype and environment in greenhouse experiments, indicating some genotypes reacted to FHB inoculation differently in different experiments, especially these genotypes with moderate resistance. This could be due to variation in temperature among experiments during FHB inoculation and development and in plants growth conditions.

Composite interval mapping identified three putative QTL for FHB resistance in Heyne. Each of the QTL explained from 8.1 to 23.4% of the total phenotypic variation in the Trego × Heyne population. The first QTL was identified in the short arm of chromosome 3A (3AS) and had a significant effect in one field and three greenhouse experiments. This QTL is flanked by *Xbarc86* and *Xwmc428* and explained up to 17.9% of the total phenotypic variation for FHB severity over four experiments. Quantitative trait loci for FHB resistance have been reported on chromosome 3A in several populations. Chen et al. (2007) reported a major QTL, *Qfhs.ndsu-3AS*, near marker *Xgwm2* in a tetraploid durum line [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn. [syn. *Triticum turgidum* var. *durum* (Desf.) Bowden]] Langdon with chromosome 3A substituted by corresponding chromosome of *Triticum turgidum* subsp. *dicoccoides* (Körn. ex Asch. & Graebn.) Thell. [syn. *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf.] (LDN-Dic-3A). This QTL explained 55% of genetic variation for FHB resistance (Otto et al., 2002). Bourdoncle and Ohm (2003) identified a QTL with closest SSR marker *Xgwm5* on 3AS in a Chinese line ‘Huapei57-2’. This QTL explained 4.5 to 10% of phenotypic variation. Yu et al. (2008) mapped a QTL for type I resistance and the closest marker was *Xgwm674*. These QTL reported from different sources appear to be the same QTL identified in this study because the markers from different studies are closely linked (within 10 cM) (Somers et al., 2004). In addition, QTL on 3A were found in several other studies, but whether they are the same or different QTL remains unknown due to lack of common markers between these studies (Gervais et al., 2003; Anderson et al., 2001).

The second QTL was located on the long arm of chromosome 4D in three experiments. This QTL is close to *Xwmc720*. To date, two QTL for FHB resistance have been reported on 4D. One was mapped in the *Rht-D1* (*Rht2*) region in most European winter wheat, including ‘Spark’ (Srinivasachary et al., 2008), ‘Arina’ (Draeger et al., 2007), ‘Massey’, ‘History’, and ‘Apache’ (Holzapfel et al., 2008). This QTL centered on dwarf gene *Rht-D1* on 4DS in a doubled haploid (DH) population Arina × ‘Riband’ and explained about 24% of the phenotypic variance for type II resistance (Draeger et al., 2007). Another QTL was mapped on 4DL, and the marker close to the QTL was *Xwmc331* in ‘Chinese Spring’ (Ma et al., 2006), Arina (Draeger et al., 2007), and ‘DH181’ (Yang et al., 2005). The QTL in Chinese Spring in population CSSM3-7ADS

× Annonng 8455 was flanked by markers *Xcfd84* and *Xwmc331* and explained 9.2% of the phenotypic variation (Ma et al., 2006) whereas Yang et al. (2005) reported this QTL was responsible for type I resistance and low FDK in a DH spring wheat population of DH181 (resistant) × ‘AC Foremost’ (susceptible). In this study, the QTL on 4DL was also mapped near *Xwmc331*; therefore, it could be the same QTL as found in Chinese Spring and DH181. A much larger effect was observed in this study, which may be due to a different allele between the current and previous populations; however, it may also be due to the smaller population size used in this study than in previous studies (Ma et al., 2006).

The third QTL was mapped on 4AL and exhibited significant effects in all five experiments. Paillard et al. (2004) reported a minor QTL in Swiss winter wheat Arina. This QTL links to *Xgwm160* and explained 10% of the total phenotypic variation. Another QTL for type I resistance was flanked by markers *Xgwm165* and *Xgwm601* on the short arm of 4A in a ‘Hobbit Sib’ [*Triticum aestivum* subsp. *macha* (Dekapr. & A. M. Menabde) Mackey (syn. *Triticum macha* Dekapr. & A. M. Menabde)] single chromosome recombinant DH population (Steed et al., 2005). In this study, this QTL is flanked by *Xwmc219* and *Xgwm160* and explained 16.8% of the phenotype variation for mean PSS over five experiments. This QTL appears to be at the same location as the QTL reported in Arina but different from the one in Hobbit Sib.

In this study, 1306 primers were screened, and only 209 of them were polymorphic between the two parents, which was relatively low compared to other studies (Bourdoncle and Ohm, 2003). The screened 1306 primers included 384 primers that were carefully selected based on their high level of polymorphism in different populations (G. Bai, unpublished data, 2010) and on their even distribution over all 42 chromosome arms. Low level of polymorphism between Heyne and Trego is most likely due to their close relationship because they both are hard white winter wheat cultivars released from Kansas State University. The relatively low density of the map (748 cM) may also be due to high genetic similarity between parents. However, the markers that linked to previously reported QTL in other populations were also screened in the population, which significantly improved the coverage of genomic regions with potential QTL in this study. To date, sources of FHB resistance have been extensively explored in Asian spring wheat. A QTL on 3BS, also called *Fhb1*, has been found to be the major QTL with the largest effect on type II resistance in all sources of resistance identified (Bai and Shaner, 2004; Anderson et al., 2001). *Fhb1* has been found in most Asian sources of resistance (Bai and Shaner, 2004; Buerstmayr et al., 2002; Liu et al., 2009). Quantitative trait loci also have been reported in several European winter wheats (Häberle et

al., 2007; Miedaner et al., 2006; Gervais et al., 2003; Pailard et al., 2004). This study is the first report of the identification of QTL for type II resistance from a U.S. HWW cultivar. The result showed that Heyne has no major QTL on 3BS. The three QTL identified from Heyne have not been reported in Sumai 3 and its relatives, and although they are present in different sources of resistance including European winter wheat, they are different from QTL in Sumai 3 (Bai and Shaner, 2004; Anderson et al., 2001; Häberle et al., 2007). In addition, these QTL are different from those in the U.S. soft winter wheat cultivar Ernie (Liu et al., 2007). Although the size of mapping population used in this study was not very large, which may exaggerate effect of QTL, the experiments were conducted in three greenhouse and two field environments and the QTL were detected across several different experiments. A combination of the 3BS QTL from an Asian source with these QTL in Heyne may significantly improve FHB resistance in HWW in the United States.

### Acknowledgments

This project was partially supported by the National Research Initiative Competitive Grants CAP project 2011-68002-30029 from the USDA National Institute of Food and Agriculture and USDA Wheat and Barley Scab Initiative. The 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. This is contribution No. 11-220-J from the Kansas Agricultural Experiment Station, Manhattan, KS. China Scholarship Council funded First author.

### References

Abate, Z.A., S. Liu, and A.L. Mckendry. 2008. Quantitative trait loci associated with deoxynivalenol content and kernel quality in the soft red winter wheat 'Ernie'. *Crop Sci.* 48:1408–1418. doi:10.2135/cropsci2007.07.0411

Anderson, J.A, R.W. Stack, S. Liu, B.L. Waldron, A.D. Field, C. Coyne, B. Moreno-Sevilla, F.J. Mitchell, Q.J. Song, P.B. Cregan, and R.C. Frohberg. 2001. DNA markers for Fusarium head blight resistance QTL in two wheat populations. *Theor. Appl. Genet.* 102:1164–1168. doi:10.1007/s001220000509

Bai, G.H., and G. Shaner. 1994. Scab of wheat: Prospects for control. *Plant Dis.* 78:760–766. doi:10.1094/PD-78-0760

Bai, G.H., and G. Shaner. 2004. Management and resistance in wheat and barley to Fusarium head blight. *Annu. Rev. Phytopathol.* 42:135–61. doi:10.1146/annurev.phyto.42.040803.140340

Bai, G.H., F.L. Kolb, G. Shaner, and L.L. Domier. 1999. Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. *Phytopathology* 89:343–348. doi:10.1094/PHYTO.1999.89.4.343

Bai, G.H., G. Shaner, and H. Ohm. 2000. Inheritance of resistance to *Fusarium graminearum* in wheat. *Theor. Appl. Genet.* 100:1–8. doi:10.1007/PL00002902

Bockus, W.W., A.K. Fritz, and T.J. Martin. 2009. Reaction of the 2008 Kansas Intrastate Nursery to Fusarium head blight. *Plant Disease Management Reports*. Report 3:CF009. The American Phytopathological Society, St. Paul, MN. doi:10.1094/PDMR03

Bourdoncle, W., and H.W. Ohm. 2003. Quantitative trait loci for resistance to Fusarium head blight in recombinant inbred wheat lines from the cross Huapei 57-2/Patterson. *Euphytica* 131:131–136. doi:10.1023/A:1023056207513

Buerstmayr, H., M. Lemmens, L. Hartl, L. Doldi, B. Steiner, M. Stierschneider, and P. Ruckebauer. 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. doi:10.1007/s001220200009

Chen, X.F., J.D. Faris, J.G. Hu, R.W. Stack, T. Adhikari, E.M. Elias, S.F. Kianian, and X.W. Cai. 2007. Saturation and comparative mapping of a major Fusarium head blight resistance QTL in tetraploid wheat. *Mol. Breed.* 19:113–124. doi:10.1007/s11032-006-9049-7

Desjardins, A.E., and T.M. Hohn. 1997. Mycotoxins in plant pathogenesis. *Mol. Plant-Microbe Interact.* 10:147–152. doi:10.1094/MPMI.1997.10.2.147

Doerge, R.W., and G.A. Churchill. 1996. Permutation tests for multiple loci affecting a quantitative character. *Genetics* 142:285–294.

Draeger, R., N. Gosman, A. Steed, E. Chandler, M. Thomsett, N. Srinivasachary, J. Schondelmaier, H. Buerstmayr, M. Lemmens, M. Schmolke, A. Mesterhazy, and P. Nicholson. 2007. Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. *Theor. Appl. Genet.* 115:617–625. doi:10.1007/s00122-007-0592-3

Gervais, L., F. Dedryver, J.Y. Morlais, V. Bodusseau, S. Negre, M. Bilous, C. Groos, and M. Trotter. 2003. Mapping of quantitative trait loci for field resistance to Fusarium head blight in a European winter wheat. *Theor. Appl. Genet.* 106:961–970.

Häberle, J., M. Schmolke, G. Schweizer, V. Korzun, E. Ebmeyer, G. Zimmermann, and L. Hartl. 2007. Effects of two major Fusarium head blight resistance QTL verified in a winter wheat backcross population. *Crop Sci.* 47:1823–1831. doi:10.2135/cropsci2006.12.0800

Holzappel, J., H-H. Voss, T. Miedaner, V. Korzun, J. Häberle, G. Schweizer, V. Mohler, G. Zimmermann, and L. Hartl. 2008. Inheritance of resistance to Fusarium head blight in three European winter wheat populations. *Theor. Appl. Genet.* 117:1119–1128. doi:10.1007/s00122-008-0850-z

Klahr, A., G. Zimmermann, G. Wenzel, and V. Mohler. 2007. Effects of environment, disease progress, plant height and heading date on the detection of QTLs for resistance to Fusarium head blight in a European winter wheat cross. *Euphytica* 154:17–28. doi:10.1007/s10681-006-9264-7

Large, E.C. 1954. Growth stages in cereals: Illustration of the Feeke's scale. *Plant Path.* 3:128–129. doi:10.1111/j.1365-3059.1954.tb00716.x

Liu, S., Z.A. Abate, H. Lu, and A.L. Mckendry. 2007. QTL associated with Fusarium head blight resistance in the soft red winter wheat Ernie. *Theor. Appl. Genet.* 115:417–427. doi:10.1007/s00122-007-0577-2

Liu, S., M.D. Hall, C.A. Griffey, and A.L. Mckendry. 2009. Meta-analyses of Fusarium head blight resistance QTL in wheat. *Crop Sci.* 49:1955–1968. doi:10.2135/cropsci2009.03.0115

- Ma, H.X., G.H. Bai, X. Zhang, and W.Z. Lu. 2006. Main effects, epistasis, and environmental interactions of quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population. *Phytopathology* 96:534–541. doi:10.1094/PHYTO-96-0534
- Mardi, M., H. Buerstmayr, B. Ghareyazie, M. Lemmens, S.A. Mohammadi, R. Nolz, and P. Ruckebauer. 2005. QTL analysis of resistance to Fusarium head blight in wheat using a “Wangshuibai”-derived population. *Plant Breed.* 124:329–333. doi:10.1111/j.1439-0523.2005.01103.x
- McIntosh, R.A., Y. Yamazaki, J. Dubcovsky, J. Rogers, C. Morris, D.J. Somers, R. Appels, and K.M. Devos. 2008. Catalogue of gene symbols for wheat. USDA-ARS, Washington, DC. <http://wheat.pw.usda.gov/GG2/Triticum/wgc/2008/Catalogue2008.pdf> (accessed 20 Jan. 2012).
- Mesterhazy, A. 1995. Types and components of resistance to Fusarium head blight of wheat. *Plant Breed.* 114:377–386. doi:10.1111/j.1439-0523.1995.tb00816.x
- Microsoft. 2008. Microsoft Office 2008 for Mac. Microsoft, Redmond, WA.
- Miedaner, T. 1997. Breeding wheat and rye for resistance to *Fusarium* diseases. *Plant Breed.* 116:201–220. doi:10.1111/j.1439-0523.1997.tb00985.x
- Miedaner, T., F. Wilde, B. Steiner, H. Buerstmayr, V. Korzun, and E. Ebmeyer. 2006. Stacking quantitative trait loci (QTL) for Fusarium head blight resistance from non-adapted sources in an European elite spring wheat background and assessing their effects on deoxynivalenol (DON) content and disease severity. *Theor. Appl. Genet.* 112:562–569. doi:10.1007/s00122-005-0163-4
- Otto, C.D., S.F. Kianian, E.M. Elias, R.W. Stack, and L.R. Joppa. 2002. Genetic dissection of a major Fusarium head blight QTL in tetraploid wheat. *Plant Mol. Biol.* 48:625–632. doi:10.1023/A:1014821929830
- Paillard, S., T. Schnurbusch, R. Tiwari, M. Messmer, M. Winzeler, B. Keller, and G. Schachermayr. 2004. QTL analysis of resistance to Fusarium head blight in Swiss winter wheat (*Triticum aestivum* L.) *Theor. Appl. Genet.* 109:323–332. doi:10.1007/s00122-004-1628-6
- Rudd, J.C., R.D. Horsley, A.L. Mckendry, and E.M. Elias. 2001. Host plant resistance genes for Fusarium head blight: Sources, mechanisms, and utility in conventional breeding systems. *Crop Sci.* 41:620–627. doi:10.2135/cropsci2001.413620x
- Roder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy, and M.W. Ganal. 1998. A microsatellite map of wheat. *Genetics* 149:2007–2023.
- Saghai-Marouf, M.A., K.M. Solima, R.A. Jorgenson, and R.W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci. USA.* 81:8014–8018. doi:10.1073/pnas.81.24.8014
- SAS Institute. 2004. The SAS system for Windows. Release 9.1.2. SAS Inst., Cary, NC.
- SoftGenetics. 2011. GeneMarker 1.9. SoftGenetics, LLC, State College, PA.
- Somers, D.J., G. Fedak, and M. Savard. 2003. Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. *Genome.* 46:555–564. doi:10.1139/g03-033
- Somers, D.J., P. Isaac, and K. Edwards. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 109:1105–1114. doi:10.1007/s00122-004-1740-7
- Song, Q.J., J.R. Shi, S. Singh, E.W. Fickus, J.M. Costa, J. Lewis, B.S. Gill, R. Ward, and P.B. Cregan. 2005. Development and mapping of microsatellite (SSR) markers in wheat. *Theor. Appl. Genet.* 110:550–560. doi:10.1007/s00122-004-1871-x
- Srinivasachary, N., A. Gosman, and A. Steed. 2008. Susceptibility to Fusarium head blight is associated with the *Rht-D1b* semi-dwarfing allele in wheat. *Theor. Appl. Genet.* 116:1145–53. doi:10.1007/s00122-008-0742-2
- Steed, A., E. Chandler, M. Thomsett, N. Gosman, S. Faure, and P. Nicholson. 2005. Identification of type I resistance to Fusarium head blight controlled by a major gene located on chromosome 4A of *Triticum macba*. *Theor. Appl. Genet.* 111:521–529. doi:10.1007/s00122-005-2043-3
- Steiner, B., M. Griesser, U. Scholz, J. Schondelmaier, and H. Buerstmayr. 2004. Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. *Theor. Appl. Genet.* 109:215–224. doi:10.1007/s00122-004-1620-1
- Van Ooijen, J.W., and R.E. Voorrips. 2001. JoinMap 3.0: Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, The Netherlands.
- Waldron, B.L., B. Moreno-Sevilla, J.A. Anderson, R.W. Stack, and R.C. Frohberg. 1999. RFLP mapping of QTL for Fusarium head blight resistance in wheat. *Crop Sci.* 39:805–811. doi:10.2135/cropsci1999.0011183X003900030032x
- Wang, S., C.J. Basten, and Z.B. Zeng. 2006. Windows QTL Cartographer 2.5. Dep. of Statistics, North Carolina State Univ., Raleigh. <http://statgen.ncsu.edu/qlcart/WQTLCart.htm> (accessed 28 May 2008).
- Wu, F. 2007. Measuring the economic impacts of *Fusarium* toxins in animal feeds. *Ani. Feed Sci. & Tech.* 137:363–374. doi:10.1016/j.anifeedsci.2007.06.010
- Yang, J., G.H. Bai, and G.E. Shaner. 2005. Novel quantitative trait loci (QTL) for Fusarium head blight resistance in wheat cultivar Chokwang. *Theor. Appl. Genet.* 111:1571–1579. doi:10.1007/s00122-005-0087-z
- Yu, J.B., G.H. Bai, and W.C. Zhou. 2008. Quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population of Wangshuibai/Wheaton. *Phytopathology* 98:87–94. doi:10.1094/PHYTO-98-1-0087
- Zhou, W.C., F.L. Kolb, G.H. Bai, L.L. Domier, L.K. Boze, and N.J. Smith. 2003. Validation of a major QTL for scab resistance with SSR markers and use of marker-assisted selection in wheat. *Plant Breed.* 122:40–46. doi:10.1046/j.1439-0523.2003.00802.x
- Zhou, W.C., F.L. Kolb, G.H. Bai, G. Shaner, and L.L. Domier. 2002. Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. *Genome* 45:719–727. doi:10.1139/g02-034