

This is the author's final, peer-reviewed manuscript as accepted for publication. The publisher-formatted version may be available through the publisher's web site or your institution's library.

Quantitative trait loci for resistance to Fusarium head blight in the Chinese wheat landrace Huangfangzhu

Tao Li, Guihua Bai, Shuangye Wu, Shiliang Gu

How to cite this manuscript

If you make reference to this version of the manuscript, use the following information:

Li, Tao, Bai, Guihua, Wu, Shuangye, & Gu, Shiliang. (2012). Quantitative trait loci for resistance to Fusarium head blight in the Chinese wheat landrace Huangfangzhu. Retrieved from <http://krex/ksu/edu>

Published Version Information

Citation: Li, Tao, Bai, Guihua, Wu, Shuangye, & Gu, Shiliang. (2012). Quantitative trait loci for resistance to Fusarium head blight in the Chinese wheat landrace Huangfangzhu. *Euphytica*, 185(1), 93-102.

Copyright: © Springer, Part of Springer Science+Business Media

Digital Object Identifier (DOI): doi:10.1007/s10681-012-0631-2

Publisher's Link: <http://www.springerlink.com/content/u22646v7270g4873/>

This item was retrieved from the K-State Research Exchange (K-REx), the institutional repository of Kansas State University. K-REx is available at <http://krex.ksu.edu>

1 **Quantitative trait loci for resistance to Fusarium head blight in the**
2 **Chinese wheat landrace Huangfangzhu**

3
4 Tao Li^{1,2*}, Guihua Bai^{3,4*}, Shuangye Wu³, Shiliang Gu¹

5 ¹Jiangsu Provincial Key Laboratory of Crop Genetics and Physiology; Key
6 Laboratory of Plant Functional Genomics of Ministry of Education, Yangzhou

7 University, Yangzhou 225009, China; Email: taoli@yzu.edu.cn; Tel:

8 +86-514-87979358; Fax: +86-514-87998967

9 ²Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA

10 ³Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA

11 ⁴USDA-ARS Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506,

12 USA; Email: guihua.bai@ars.usda.gov; Tel: +01-785-532-1124; Fax:

13 +01-785-532-6167

14 *Corresponding authors

15

16

1 **Abstract**

2 The Chinese wheat landrace Huangfangzhu (HFZ) has a high level of resistance to
3 Fusarium head blight (FHB). To identify chromosomal regions that are responsible
4 for FHB resistance in HFZ, F₈ recombinant inbred lines (RIL) were developed from a
5 cross between HFZ and Wheaton, a U.S. hard spring wheat. FHB was evaluated by
6 single floret inoculation in both greenhouse and field environments. Two quantitative
7 trait loci (QTL) with major effects were identified. One QTL was located on the short
8 arm of chromosome 3B, and explained 35.4% of the phenotypic variation; the other
9 QTL was assigned to 7AL and explained 18.0% of the phenotypic variation for FHB
10 response. In addition, three minor QTL were detected on chromosomes 1AS, 1B and
11 5AS by single marker regression. HFZ contributed all favorable alleles. The RIL with
12 HFZ alleles at the QTL on 3BS and 7AL displayed significantly lower percentages of
13 infected spikelets (PIS) than RIL without these alleles in both greenhouse and field
14 environments. HFZ combined several alleles from germplasm reported previously and
15 is a promising alternative source for improving wheat FHB resistance.

16

17 **Key words:** Head scab

18

1 **Introduction**

2 Fusarium head blight caused by *Fusarium graminearum* is a destructive disease in
3 wheat (*Triticum aestivum* L.) worldwide (Bai and Shaner 2004). It not only reduces
4 grain yield and quality but also contaminates wheat grain with mycotoxins such as
5 deoxynivalenol (DON), rendering the grain unsuitable for human or animal
6 consumption (Trail 2009). FHB resistance in common wheat is a quantitative trait and
7 controlled by a few major genes and some modifier genes (Liu et al. 2009). More than
8 200 quantitative trait loci (QTL) have been reported on all 21 chromosomes of
9 hexaploid wheat after 46 wheat accessions were studied worldwide, and 19 loci have
10 been identified in multiple mapping populations (Buerstmayr et al. 2009).

11 Growing resistant cultivars within an integrated cultural system is the most
12 economic, effective and environmentally safe approach to reducing losses caused by
13 this disease. Because environments significantly affect FHB response, large-scale
14 phenotypic selection for resistance is difficult and requires costly and laborious field
15 evaluations with poor repeatability among testing seasons and locations.
16 Marker-assisted selection (MAS) may greatly facilitate selection efficiency. To date,
17 progress has been made in breeding for resistance to FHB; some resistant varieties
18 have been released for commercial production. Sumai 3 and its derivatives have been
19 the major sources of resistance used in breeding programs worldwide (Bai and Shaner
20 2004). However, only a limited number of resistant sources have been genetically
21 dissected to date, and these provide wheat breeders with only limited choices to

1 enhance FHB resistance. Further sources of resistance therefore need to be genetically
2 analyzed to identify major-effect QTL for gene pyramiding in wheat breeding
3 programs by MAS.

4 Several Chinese landraces show high levels of resistance (Yu et al. 2006, 2008a).
5 The QTL for FHB resistance in these landraces have not been investigated.
6 Huangfangzhu (HFZ) is a Chinese spring wheat landrace with superior resistance to
7 FHB (Yu et al. 2006, 2008a). The objectives of this study were to investigate QTL for
8 type II resistance in HFZ and to quantify their effects using recombinant inbred lines
9 (RIL) of HFZ/Wheaton.

10 **Materials and methods**

11 **Plant materials and FHB evaluation**

12 An SSD population of 106 F₈ RIL was developed from a cross between Wheaton, a
13 susceptible U.S. wheat variety, and HFZ, a resistant wheat landrace from Jiangsu
14 Province. The RIL were grown in 1.0 L Dura pots (Hummert International, St. Louis,
15 MO, USA) filled with Metro-mix 360[®] growing medium (Hummert International) on
16 a greenhouse bench at 17±2°C (night) and 22±5°C (day) with supplemental light for
17 12 h and evaluated for FHB response in three consecutive greenhouse (GH)
18 experiments from 2007 to 2008 at Kansas State University (KSU), and one field
19 experiment (2009) at KSU Rocky Ford FHB Nursery, Manhattan, KS. A *F.*
20 *graminearum* conidia suspension was prepared following Bai et al. (1999). Wheat
21 spikes were inoculated by delivering 10 uL of conidial suspension (100 conidia/uL)

1 into the floral cavity between the lemma and palea of one floret of a middle spikelet
2 per spike using a syringe. Five spikes per RIL in each pot were inoculated. Following
3 exposure to 100% relative humidity for 48 h in a mist chamber, the pots were returned
4 to a greenhouse bench for further FHB development. Experiments were arranged in a
5 randomized complete block design with two replicates (pots) of 5 plants per pot. In
6 the field experiment, the RIL population and both parents were arranged in a
7 randomized complete block design with two replications (blocks), with about 50 seeds
8 per entry sown in a one-row plot in each replication. At anthesis, five spikes per row
9 were inoculated by single-floret injection as described for the greenhouse experiments.
10 Between heading and the late dough stage, plants in the FHB nursery were misted for
11 10 min every hour using sprinklers. In both field and greenhouse experiments, the
12 total number of spikelets and the number of infected spikelets were counted for each
13 inoculated spike at 21 d after inoculation. The percentage of infected spikelets (PIS)
14 per spike was calculated.

15 **DNA extraction and marker analysis**

16 Genomic DNA was isolated from 2-week-old wheat leaves of each RIL using a
17 modified CTAB method (Maguire et al. 1994). The harvested wheat leaves were dried
18 in a freeze dryer (ThermoSavant, Holbrook, NY) for 48 h and ground using a Mixer
19 Mill (MM 300, Retsch, Germany) before DNA extraction.

20 A total of 1,125 SSR primer pairs including primer sets with BARC, WMC,
21 GWM, KSM, CFA, CFD and DUP (<http://wheat.pw.usda.gov>) designations were used
22 to screen the parents. Primer pairs that detected polymorphism between the parents

1 were used to screen two bulks with contrasting FHB responses. The resistant bulk was
2 constructed by mixing equal amounts of DNA from 10 highly resistant RIL and the
3 susceptible bulk was constructed by mixing equal amounts of DNA from 10 highly
4 susceptible RIL. Primer pairs that detected polymorphism between the contrasting
5 bulks were used to genotype the entire RIL population. For SSR analysis, each 10 μ L
6 PCR mixture contained 40 ng template DNA, 1 mM each of reverse and M13-tailed
7 forward primers, 0.2 mM of each dNTP, 1 \times PCR buffer, 2.5 mM MgCl₂, and 0.6 U
8 *Taq* polymerase. For PCR detection, 1 pmol of fluorescence-labeled M13 primer was
9 added to each PCR. A touchdown PCR program was used for PCR amplification, in
10 which the reaction mixture was incubated at 95°C for 5 min, followed by five cycles
11 of 45 s of denaturing at 95°C, 5 min of annealing at 68°C with a decrease of 2°C in
12 each sequential cycle, and 1 min of extension at 72°C. For another five cycles, the
13 annealing temperature started at 58°C for 2 min with a decrease of 2°C for each
14 sequential cycle. PCR continued through an additional 25 cycles of 45 s at 94°C, 2
15 min at 50°C, and 1 min at 72°C with a final extension at 72°C for 5 min. The
16 amplified PCR fragments were separated in an ABI 3730 DNA Analyzer (Applied
17 Biosystems, Foster City, CA). All marker data were scored using GeneMarker 1.6
18 (Softgenetics Inc. LLC), and visually checked twice to remove ambiguous data.

19 **Genetic map construction and QTL analysis**

20 Genetic linkage maps were constructed with SSR markers using JoinMap version 3.0
21 (Van Ooijen and Voorrips 2001) and the Kosambi function (Kosambi 1944). The
22 threshold for LOD (logarithm of odds) value was set at 3.0 to claim linkage between

1 markers with a maximum fraction of recombination at 0.4.

2 For QTL analysis, composite interval mapping (CIM) was performed using
3 WINQTL Cartographer version 2.5 (Wang et al. 2007) Model 6. Five markers were
4 used as cofactors with a window size of 10 cM. QTL were analyzed using line means
5 from individual experiments and from combined line means across all experiments.
6 The LOD threshold for declaring a significant QTL was determined by 1,000
7 permutations. Single marker regression (SMR) was used to reveal marker-phenotype
8 associations when a QTL was not significant either using CIM or simple interval
9 mapping (SIM).

10 **Statistical analysis**

11

12 Broad sense heritability (H^2) was calculated for trait PIS based on ANOVA results
13 using the formula $H^2 = \sigma_G^2 / (\sigma_G^2 + (\sigma_{GE/e}^2) + (\sigma_{re}^2))$, where σ_G^2 = genotypic variance,
14 σ_e^2 = residual error variance, σ_{GE}^2 = genotype \times environment variance, r = number of
15 replicates (pots) and e = number of experiments (seasons) following Jayatilake et al.
16 (2011). Multiple comparisons of PIS among groups of RIL harboring different
17 numbers of QTL were conducted using the Least Significant Difference (LSD)
18 method at $\alpha = 0.05$. Statistical analyses were performed using Matlab software
19 (MathWorks Inc., Natick, MA, USA, 2007).

20 **Results**

21 **FHB variation in RIL**

1 In the greenhouse experiments, PIS for the resistant parent (HFZ) averaged 15.3%,
2 ranging from 11.5 to 22.1%, and 100% for the susceptible parent (Wheaton). The
3 frequency distributions of PIS among RIL were continuous with an average PIS of
4 64.7%, ranging from 9.6 to 100% (Fig. 1). The most resistant RIL showed PIS similar
5 to that of the resistant parent (HFZ), but most RIL means were distributed toward the
6 susceptible parent, with about 75% of RIL having an average PIS higher than 50%.

7 In the field experiment, PIS ranged from 5.8 to 14.6% for HFZ with an average of
8 9.3%, and from 92.7 to 100% for Wheaton with an average of 97.0%. The frequency
9 distributions of PIS among RIL were continuous with an average PIS of 49.1%,
10 ranging from 6.5 to 100% in the field experiment (Fig. 1). The disease levels on RIL
11 were less severe than in the greenhouse experiments and half of them had PIS less
12 than 50%. The chi-squared test of homogeneity demonstrated that the data from
13 individual greenhouse and field experiments were not significantly different ($\chi^2 = 3.36$,
14 $P_{\chi^2, df} = 0.34$), and thus could be combined. The PIS differences among RIL,
15 environment, and genotype \times environment interaction were highly significant (Table
16 1). Significant correlations were observed among the three greenhouse experiments
17 ($r > 0.42$, $P < 0.0001$) and between greenhouse mean FHB data and field FHB data (r
18 $= 0.43$, $P < 0.0001$). The mean heritability of PIS for RIL was 0.90 over
19 three-greenhouse experiments and was 0.80 over the combined greenhouse and field
20 experiments.

21 **QTL for type II resistance**

22 Among 1,125 primer pairs screened, 318 markers were polymorphic between the

1 parents. Among them, 27 from five chromosomes were polymorphic between the
2 contrasting bulks. Polymorphic markers from all five chromosomes were genotyped
3 for all RIL and five linkage groups were constructed, covering 85.0 cM in genetic
4 distance. CIM detected two QTL with major effects on type II resistance in HFZ. One
5 QTL on chromosome 3BS was detected in all individual greenhouse experiments and
6 the combined field-greenhouse data. SSR marker *Xbarc147* and STS marker *Xumn10*
7 flanked this QTL which coincided with *Fhb1* and explained 23.0 to 28.0% of the
8 phenotypic variation in individual greenhouse experiments, 35.6% for mean
9 greenhouse data and 35.4% for combined greenhouse-field data (Table 2, Fig. 2). It
10 was not detected in the field experiment alone when CIM was conducted; however,
11 single marker analysis showed that *Xbarc147* on 3BS accounted for 12.9% of PIS
12 variation (Table 3, Fig. 2).

13 A second major effect QTL on 7AL was flanked by SSR markers *Xgwm276* and
14 *Xbarc121*. This QTL was detected in the two 2007 greenhouse experiments, mean
15 greenhouse data and combined greenhouse-field data (Table 2, Fig. 2), but not in the
16 2008 greenhouse and 2009 field experiments when CIM was used, although SSR
17 markers *Xgwm276* and *Xbarc121* were significantly associated with the PIS in single
18 marker regression analyses (Table 3, Fig. 2).

19 Single marker regression detected five additional markers on 1AS, 1B and 5AS
20 associated with FHB resistance (Table 3, Fig. 2), each with R^2 values smaller than
21 0.12. Markers *Xwmc120.2* on 1AS, *Xbarc207* on 1B and *Xbarc186/Xbarc117* on 5AS
22 were significantly associated with mean greenhouse data and combined

1 greenhouse-field data, whereas marker *Xwmc24* on 1AS associated with FHB
2 resistance only in the field experiment.

3 **Effects of QTL on type II resistance**

4 The segregations of contrasting alleles at each SSR locus closely linked to QTL
5 exhibited 1:1 ratios. In the greenhouse experiments, the average PIS for RIL carrying
6 HFZ alleles at *Xumn10* on 3BS and *Xgwm276* on 7AL were 50.0 and 56.0%,
7 respectively, while the average PIS of RIL carrying the Wheaton alleles were 78.0 and
8 74.0%, respectively. In the field experiment, the PIS of RIL with HFZ alleles at
9 *Xumn10* and *Xgwm276* were 38.0 and 43.0%, respectively, and those with Wheaton
10 alleles were 58.0 and 55.0%, respectively. For the other three markers on 1AS, 1B and
11 5AS, the average PIS of RIL with HFZ alleles in greenhouse experiments ranged from
12 58.0 to 59.0%, compared with 71.0 to 74.0% for those with the corresponding
13 Wheaton-alleles. In the field experiment, the average PIS of the RIL with HFZ alleles
14 ranged from 44.0 to 47.0% compared with 51.0 to 54.0% for those with the Wheaton
15 alleles. The lower average PIS of RIL with HFZ alleles and the negative effects of all
16 five Wheaton alleles confirmed that all favorable alleles for FHB resistance were
17 contributed by HFZ. The 3BS QTL contributed the largest effect on FHB resistance
18 and the 7AL QTL was next.

19 To elucidate the effect of single and combined QTL on FHB response, the RIL
20 were divided into five groups: group 1 contained the HFZ alleles at QTL on 3BS and
21 7AL ignoring the effects of the minor QTL; group 2 carried the HFZ allele on 3BS but
22 not the HFZ allele on 7AL; group 3 carried only the HFZ allele on 7AL; group 4

1 contained only HFZ minor alleles (1-3); and group 5 carried only Wheaton alleles at
2 all five loci. Frequencies of lines within the five groups ranged from 8.8 to 26.5%. In
3 the greenhouse experiments, the mean PIS of groups 1 and 2 were significantly lower
4 (*LSD*, $\alpha= 0.05$) than those of groups 3, 4 and 5 (Fig. 3). Group 3 had significantly
5 lower PIS than groups 4 and 5. In the field experiment, group 1 showed lower PIS
6 than the other four groups, and groups 2, 3 and 4 had almost the same PIS but all were
7 lower than group 5. However, differences were significant only between groups 1 and
8 5 (*LSD*, $\alpha=0.05$).

9 **Discussion**

10 Five putative QTL for type II resistance to FHB were identified on chromosomes 3BS,
11 7AL, 5AS, 1AS and 1B of Chinese landrace HFZ. The QTL on 3BS was first reported
12 in Sumai 3, designated as *Qfhs.ndsu-3BS* (Waldron et al. 1999) and in Ning 7840 (Bai
13 et al. 1999). This QTL has been detected in at least 26 different studies and shows a
14 stable major effect on type II resistance (resistance to fungal spread within spikes)
15 (Buerstmayr et al. 2009; Liu et al. 2009). In addition to Sumai 3 and its derivatives,
16 including Ning7840 (Bai et al. 1999; Zhou et al. 2002), Ning 894037 (Shen et al.
17 2003), CM-82036 (Buerstmayr et al. 2002), W14 (Chen et al. 2006), CJ 9306 (Jiang et
18 al. 2007a, b) and Huapei 57-2 (Bourdoncle and Ohm 2003; Shen et al. 2003), this
19 QTL was also reported in materials not related to Sumai 3, such as Wangshuibai (Lin
20 et al. 2004; Zhang et al. 2004; Zhou et al. 2004; Mardi et al. 2005; Yu et al. 2008b)
21 and Nyu Bai (McCartney et al. 2007). Because of its large effect on FHB response,

1 this QTL was fine mapped as a single Mendelian gene within a 1.2 cM interval, and
2 renamed as *Fhb1* (Cuthbert et al. 2006; Liu et al. 2006). *Xumn10* was proposed as the
3 best marker for prediction of *Fhb1* (Liu et al. 2008). *Xumn10* was also the closest
4 marker in the present study indicating the QTL is most likely *Fhb1*. The
5 non-significance of the QTL in CIM analysis of the field experiment may be due to
6 confounding effects of further infections. In the field experiment, plants were infected
7 by both single floret injection and naturally. Thus disease rating reflects not only
8 disease spread from the artificially inoculated site but also from natural infections at
9 other positions in the spike. Single marker analysis showed that flanking markers
10 *Xumn10* and *Xbarc147* were significantly associated with PIS in the field experiment.
11 Another problem could be the large differences in flowering time across the RIL
12 population leading to non-uniform conditions for FHB development between early
13 and late flowering lines.

14 A QTL flanked by *Xgwm276* and *Xbarc121* was identified on 7AL of HFZ. Like
15 the 3BS QTL, this QTL was also non-significant in CIM analysis of the field
16 experiment, but was significant in single marker regression of *Xbarc121*. A QTL on
17 7AL was also reported in Wangshuibai (Zhou et al. 2004; Jia et al. 2005), NK93604
18 (Semagn et al. 2007) and Ritmo (Klahr et al. 2007). *Xgwm276* was the most closely
19 linked marker to the QTL in Wangshuibai (Jia et al. 2005) and NK93604. In another
20 study, a QTL on *T. dicoccoides* 7AL (Kumar et al. 2007), was tightly associated with
21 *Xbarc121*. This result suggests that the 7AL QTL may be the same QTL as previously
22 reported in these various lines.

1 Three QTL on 5AS, 1AS and 1B showed only minor effects on type II resistance
2 and were detected only by single marker regression. QTL from several sources were
3 reported on chromosome 5AS. These were associated with either type I or type II
4 resistance and explained 4 to 26% of the phenotypic variation in different experiments
5 (Buerstmayr et al. 2002, 2003; Steiner et al. 2004; Yang et al. 2005; Chen et al. 2006;
6 Jiang et al. 2007a, b; Liu et al. 2007; McCartney et al. 2007). In our study, markers
7 *Xbarc117* and *Xbarc186* on 5AS were associated with mean PIS in the three
8 greenhouse experiments, but not the field experiment, suggesting that a QTL with a
9 minor effect on type II resistance might be present in HFZ. According to the linked
10 common marker location, it may be the same QTL as described by Chen et al. (2006).

11 CJ 9306 carried a QTL for FHB resistance on 1AS (*QFhs.nau-1AS*), which
12 reduced PIS by 11.7 to 21.2%. The QTL detected on 1AS in our study also enhanced
13 type II resistance. Marker *Xwmc120.2* was the closest marker for the QTL in HFZ.

14 The QTL on chromosome 1B was significantly associated with SSRs *Xbarc207*
15 and *Xbarc181*. In previous reports, a QTL from Arina was detected on 1BL (Semagn
16 et al. 2007). Twelve QTL for type II resistance reported on 1BL fell into three
17 different regions when subjected to a meta-analysis (Liu et al. 2009). Because
18 common markers were not found between this study and others, the relationship of the
19 present QTL on 1B to others remains unknown.

20 In summary, FHB resistance in HFZ investigated in this study was contributed by
21 a combination of five QTL that were probably reported previously in different
22 germplasms. The QTL on chromosomes 3BS and 7AL contributing major effects on

1 type II resistance and consistently detected in multiple experiments in this and other
2 studies should be used together to improve FHB resistance in breeding. Three other
3 QTL showing minor effects and detected in only some experiments need further
4 validation before they are used in breeding. Thus with a unique combination of QTL
5 compared to other resistance sources, HFZ can be used as a valuable alternative
6 source for improvement of FHB resistance.

7 **Acknowledgements**

8 This project is partly funded by NSFC (Grant no. 31171537), the Priority Academic
9 Program Development of Jiangsu Higher Education Institution, Jiangsu Provincial
10 Natural Science Foundation of China (Grant no. BK2010312), and National Research
11 Initiative Competitive Grants CAP project 2011-68002-30029 from the USDA
12 National Institute of Food and Agriculture. Mention of trade names or commercial
13 products in this article is solely for the purpose of providing specific information and
14 does not imply recommendation or endorsement by the U.S. Department of
15 Agriculture. This is contribution no. 11-207-J from the Kansas Agricultural
16 Experiment Station, Manhattan, Kansas, USA.

17 **References**

- 18 Bai G, Kolb FL, Shaner G, Domier LL (1999) Amplified fragment length
19 polymorphism markers linked to a major quantitative trait locus controlling scab
20 resistance in wheat. *Phytopathology* 89:343-348
- 21 Bai G, Shaner G (2004) Management and resistance in wheat and barley to fusarium

1 head blight. *Annu Rev Phytopathol* 42:135-161

2 Bourdoncle W, Ohm HW (2003) Quantitative trait loci for resistance to *Fusarium*

3 head blight in recombinant inbred wheat lines from the cross Huapei

4 57-2/Patterson. *Euphytica* 131:131-136

5 Buerstmayr H, Ban T, Anderson J (2009) QTL mapping and marker-assisted selection

6 for *Fusarium* head blight resistance in wheat: a review. *Plant Breeding* 128:1-26

7 Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M,

8 Ruckebauer P (2002) Molecular mapping of QTLs for *Fusarium* head blight

9 resistance in spring wheat. I. Resistance to fungal spread (Type II resistance).

10 *Theor Appl Genet* 104:84-91

11 Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T,

12 Schneider B, Lemmens M (2003) Molecular mapping of QTLs for *Fusarium*

13 head blight resistance in spring wheat. II. Resistance to fungal penetration and

14 spread. *Theor Appl Genet* 107:503-508

15 Chen J, Griffey CA, Maroof MAS, Stromberg EL, Biyashev RM, Zhao W, Chappell

16 MR, Pridgen TH, Dong Y, Zeng Z (2006) Validation of two major quantitative

17 trait loci for *Fusarium* head blight resistance in Chinese wheat line W14. *Plant*

18 *Breeding* 125:99-101

19 Cuthbert PA, Somers DJ, Thomas J, Cloutier S, Brule-Babel A (2006) Fine mapping

20 *Fhb1*, a major gene controlling *Fusarium* head blight resistance in bread wheat

21 (*Triticum aestivum* L.). *Theor Appl Genet* 112:1465-1472

22 Jayatilake DV, Bai GH, Dong YH (2011) A novel quantitative trait locus for *Fusarium*

23 head blight resistance in chromosome 7A of wheat. *Theor Appl Genet*

24 122:1189-1198

25 Jia G, Chen PD, Qin GJ, Bai GH, Wang X, Wang SL, Zhou B, Zhang SH, Liu DJ

26 (2005) QTLs for *Fusarium* head blight response in a wheat DH population of

27 Wangshuibai/Alondra's'. *Euphytica* 146:183-191

28 Jiang GL, Dong Y, Shi J, Ward RW (2007a) QTL analysis of resistance to *Fusarium*

29 head blight in the novel wheat germplasm CJ 9306. II. Resistance to

30 deoxynivalenol accumulation and grain yield loss. *Theor Appl Genet*

1 115:1043-1052

2 Jiang GL, Shi JR, Ward RW (2007b) QTL analysis of resistance to Fusarium head
3 blight in the novel wheat germplasm CJ 9306. I. Resistance to fungal spread.
4 Theor Appl Genet 116:3-13

5 Klahr A, Zimmermann G, Wenzel G, Mohler V (2007) Effects of environment, disease
6 progress, plant height and heading date on the detection of QTLs for resistance
7 to Fusarium head blight in an European winter wheat cross. Euphytica
8 154:17-28

9 Kosambi DD (1944) The estimation of map distance from recombination values. Ann
10 Eugen:172-175

11 Kumar S, Stack RW, Friesen TL, Faris JD (2007) Identification of a novel Fusarium
12 head blight resistance quantitative trait locus on chromosome 7A in tetraploid
13 wheat. Phytopathology 97:592-597

14 Lin F, Kong ZX, Zhu HL, Xue SL, Wu JZ, Tian DG, Wei JB, Zhang CQ, Ma ZQ
15 (2004) Mapping QTL associated with resistance to Fusarium head blight in the
16 Nanda2419 x Wangshuibai population. I. Type II resistance. Theor Appl Genet
17 109:1504-1511

18 Liu S, Abate ZA, Lu H, Musket T, Davis GL, McKendry AL (2007) QTL associated
19 with Fusarium head blight resistance in the soft red winter wheat Ernie. Theor
20 Appl Genet 115:417-427

21 Liu S, Hall MD, Griffey CA, McKendry AL (2009) Meta-analysis of QTL associated
22 with Fusarium head blight resistance in wheat. Crop Sci 49:1955-1968

23 Liu S, Zhang X, Pumphrey MO, Stack RW, Gill BS, Anderson JA (2006) Complex
24 microcolinearity among wheat, rice, and barley revealed by fine mapping of the
25 genomic region harboring a major QTL for resistance to Fusarium head blight in
26 wheat. Funct Integr Genomics 6:83-89

27 Liu SX, Pumphrey MO, Gill BS, Trick HN, Zhang JX, Dolezel J, Chalhoub B,
28 Anderson JA (2008) Toward positional cloning of *Fhb1*, a major QTL for
29 Fusarium head blight resistance in wheat. In: 3rd Int. FHB Symposium, Szeged,
30 Hungary. Cereal Res Comm, Suppl. B 36:195-201

1 Maguire TL, Collins GG, Sedgley M (1994) A modified CTAB DNA extraction
2 procedure for plants belonging to the family proteaceae. *Plant Mol Biol Repr*
3 12:106-109

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

7 McCartney CA, Somers DJ, Fedak G, DePauw RM, Thomas J, Fox SL, Humphreys
8 DG, Lukow O, Savard ME, McCallum BD, Gilbert J, Cao W (2007) The
9 evaluation of FHB resistance QTLs introgressed into elite Canadian spring
10 wheat germplasm. *Mol Breeding* 20:209-221

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

14 Shen X, Zhou M, Lu W, Ohm H (2003) Detection of *Fusarium* head blight resistance
15 QTL in a wheat population using bulked segregant analysis. *Theor Appl Genet*
16 106:1041-1047

17 Steiner B, Lemmens M, Griesser M, Scholz U, Schondelmaier J, Buerstmayr H (2004)
18 Molecular mapping of resistance to *Fusarium* head blight in the spring wheat
19 cultivar Frontana. *Theor Appl Genet* 109:215-224

20 Trail F (2009) For blighted waves of grain: *Fusarium graminearum* in the
21 postgenomics era. *Plant Physiol* 149:103-110

22 Van Ooijen J, Voorrips R (2001) JoinMap® 3.0, Software for the calculation of
23 genetic linkage maps. Plant Research International, Wageningen, the
24 Netherlands

25 Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Froberg RC (1999) RFLP
26 mapping of QTL for *Fusarium* head blight resistance in wheat. *Crop Sci*
27 39:805-811

28 Wang S, Basten C, Zeng Z-B (2007) Windows QTL Cartographer 2.5. Department of
29 Statistics, North Carolina State University, Raleigh, NC
30 (<http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>)

- 1 Yang Z, Gilbert J, Fedak G, Somers DJ (2005) Genetic characterization of QTL
2 associated with resistance to Fusarium head blight in a doubled-haploid spring
3 wheat population. *Genome* 48:187-196
- 4 Yu JB, Bai GH, Cai SB, Ban T (2006) Marker-assisted characterization of Asian
5 wheat lines for resistance to Fusarium head blight. *Theor Appl Genet*
6 113:308-320
- 7 Yu JB, Bai GH, Cai SB, Dong YH, Ban T (2008a) New Fusarium head blight-resistant
8 sources from Asian wheat germplasm. *Crop Sci* 48:1090-1097
- 9 Yu JB, Bai GH, Zhou WC, Dong YH, Kolb FL (2008b) Quantitative trait loci for
10 Fusarium head blight resistance in a recombinant inbred population of
11 Wangshuibai/Wheaton. *Phytopathology* 98:87-94
- 12 Zhang X, Zhou MP, Ren LJ, Bai GH, Ma HX, Scholten OE, Guo PG, Lu WZ (2004)
13 Molecular characterization of Fusarium head blight resistance from wheat
14 variety Wangshuibai. *Euphytica* 139:59-64
- 15 Zhou WC, Kolb FL, Bai GH, Shaner G, Domier LL (2002) Genetic analysis of scab
16 resistance QTL in wheat with microsatellite and AFLP markers. *Genome*
17 45:719-727
- 18 Zhou WC, Kolb FL, Yu JB, Bai GH, Boze LK, Domier LL (2004) Molecular
19 characterization of Fusarium head blight resistance in Wangshuibai with simple
20 sequence repeat and amplified fragment length polymorphism markers. *Genome*
21 47:1137-1143
- 22
- 23

1 **Table 1** Analysis of variance (ANOVA) of percentage infected spikelets (PIS) for the RIL
2 population over experiments and blocks

Source of variation	df	SS	MS	F-value	p-value
Experiments	3	6.739	2.246	59.646	<0.0001
Genotypes	105	37.830	0.360	9.567	<0.0001
Blocks	4	0.149	0.037	0.990	0.413
Experiment×Genotype	315	22.267	0.071	1.877	<0.0001
Error	420	15.817	0.038		
Total	847	82.801			

3

4

1 **Table 2** Coefficients of determination (R^2), LOD values and additive effects of QTL regions
 2 detected by composite interval mapping based on mean FHB data for single greenhouse
 3 experiments, mean GH data (Mean GH) and the combined GH-FIELD data

QTL	Experiment	QTL interval	cM distance	Closest marker	Additive effect (%)	LOD	R^2
<i>Qfhb.uhgl-3BS</i>	2007GHs ^a	<i>Xbarc147-Xumn10</i>	1.0	<i>Xumn10</i>	-12.6	4.45	0.234
	2007GHf	<i>Xbarc147-Xumn10</i>	1.0	<i>Xbarc147</i>	-14.1	7.17	0.281
	2008GHf	<i>Xbarc147-Xumn10</i>	1.0	<i>Xumn10</i>	-12.2	6.14	0.231
	Mean GH	<i>Xbarc147-Xumn10</i>	1.0	<i>Xumn10</i>	-14.1	9.74	0.356
	GH-FIELD combined	<i>Xbarc147-Xumn10</i>	1.0	<i>Xumn10</i>	-13.1	9.82	0.354
<i>Qfhb.uhgl-7AL</i>	2007GHs	<i>Xgwm276-Xbarc121</i>	4.0	<i>Xgwm276</i>	-12.2	3.56	0.182
	2007GHf	<i>Xgwm276-Xbarc121</i>	4.0	<i>Xbarc121</i>	-9.9	2.79	0.159
	Mean GH	<i>Xgwm276-Xbarc121</i>	4.0	<i>Xgwm276</i>	-9.3	3.44	0.177
	GH-FIELD combined	<i>Xgwm276-Xbarc121</i>	4.0	<i>Xbarc121</i>	-9.1	3.73	0.180

4 ^as, spring; f, fall

1 **Table 3.** Coefficients of determination (R^2) of the closest markers associated with FHB resistance

2 QTL identified by single marker analysis of data from single experiments or when combined

Experiment	Closest marker	Chr.	Additive effect (%)	<i>p</i> -value	R^2
2007GHs ^a	<i>Xbarc207</i>	1B	-9.1	0.002	0.099
	<i>Xwmc120.2</i>	1AS	-8.9	0.003	0.093
2007GHf	<i>Xbarc207</i>	1B	-7.2	0.007	0.071
	<i>Xbarc186</i>	5AS	-7.6	0.005	0.069
	<i>Xwmc24</i>	1AS	-5.4	0.048	0.037
2008GHf	<i>Xgwm276</i>	7AL	-6.4	0.017	0.078
	<i>Xwmc120.2</i>	1AS	-6.6	0.009	0.072
2009FIELDs	<i>Xbarc147</i>	3BS	-10.4	0.0003	0.129
	<i>Xwmc24</i>	1AS	-7.8	0.008	0.070
	<i>Xbarc121</i>	7AL	-7.6	0.015	0.061
Mean GH	<i>Xwmc120.2</i>	1AS	-7.0	0.002	0.111
	<i>Xbarc207</i>	1B	-6.8	0.002	0.091
	<i>Xbarc186</i>	5AS	-6.3	0.006	0.073
GH-FIELD combined	<i>Xwmc120.2</i>	1AS	-6.7	0.001	0.102
	<i>Xbarc117</i>	5AS	-5.9	0.006	0.073
	<i>Xbarc207</i>	1B	-5.6	0.006	0.071

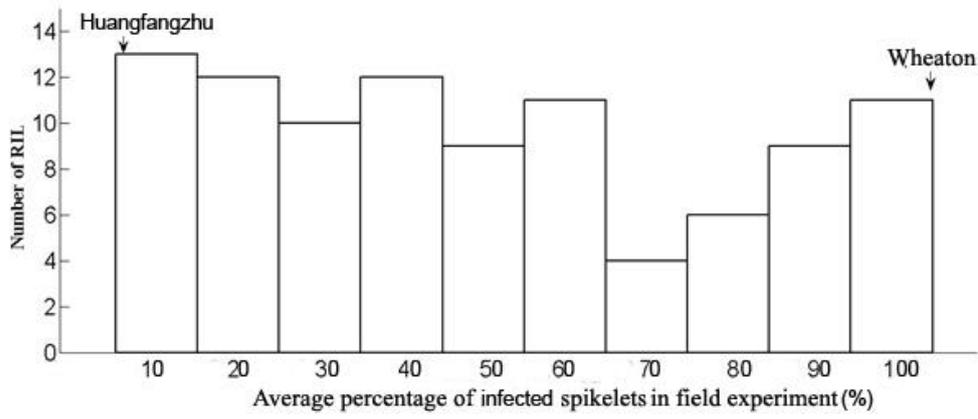
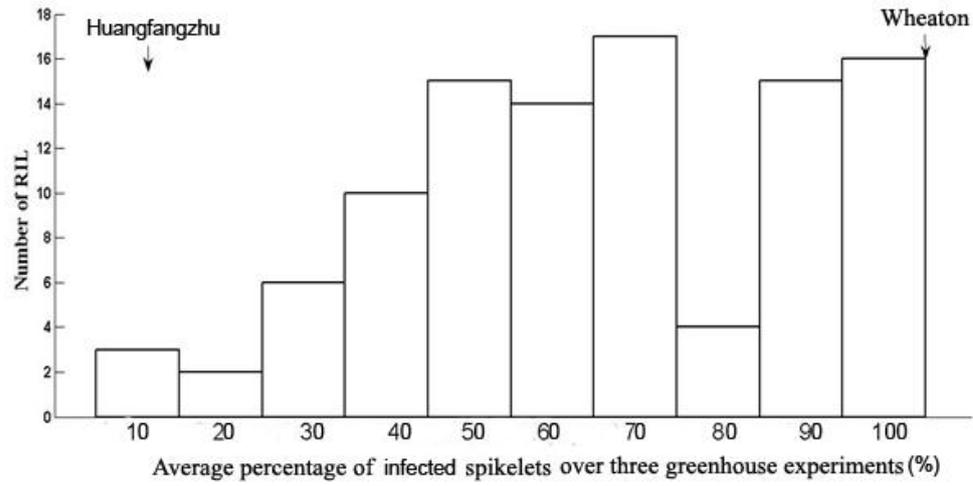
3 ^as, spring; f, fall

4

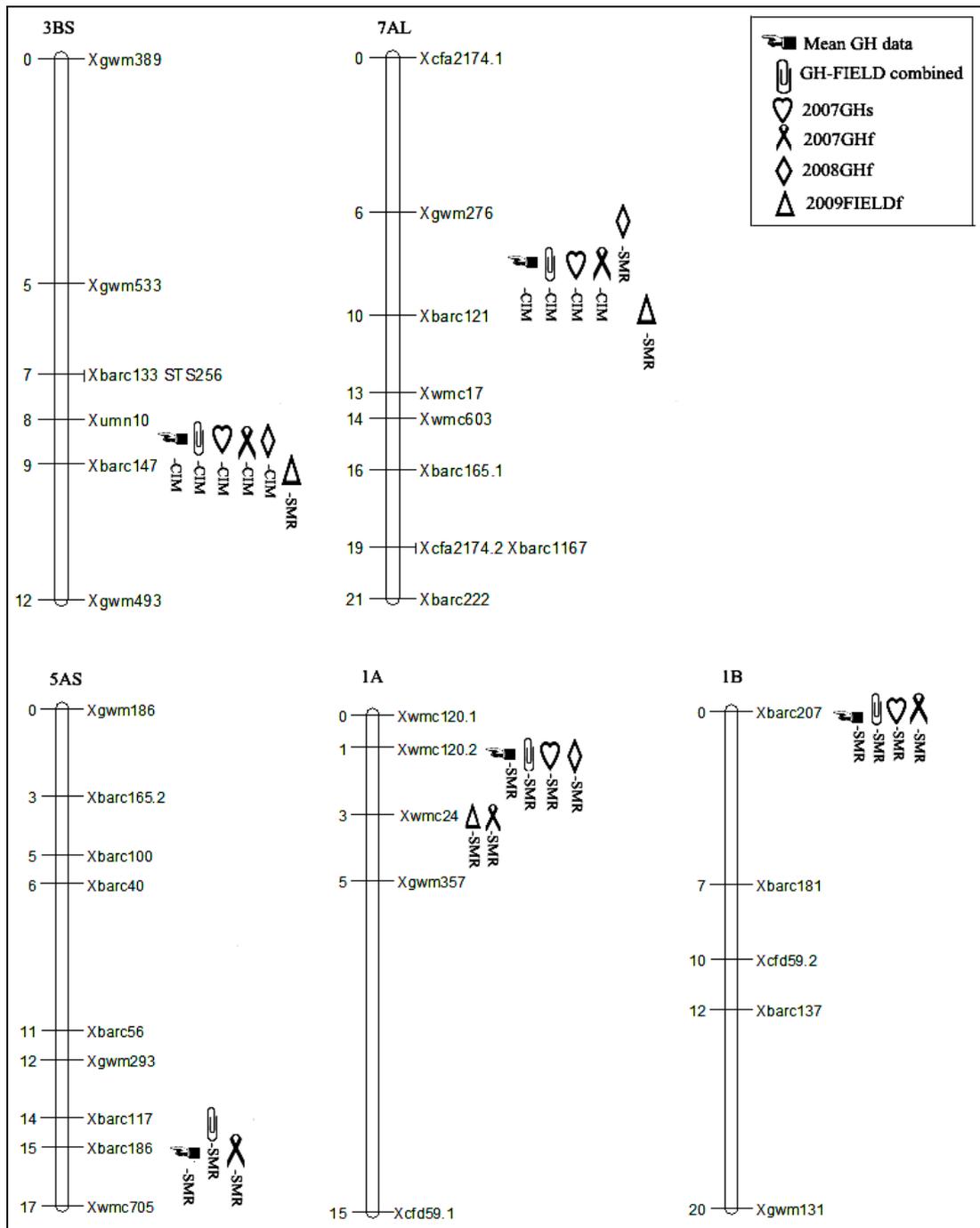
1 **Fig. 1** Frequency distributions of percentage infected spikelets (PIS) per spike for recombinant
2 inbred lines in greenhouse (upper) and field (lower) experiments

3 **Fig. 2** QTL map based on four individual experiments (2007GHs, 2007GHf, 2008GHf and
4 2009FIELDs), mean greenhouse data and combined greenhouse-field data

5 **Fig. 3** Comparisons of percentage infected spikelets (PIS) among genotypes with different QTL
6 combinations based on FHB data in greenhouse experiments. G1=*Qfhb.uhgl-3BS* + *Qfhb.uhgl-7AL*
7 + 0-3 minor QTL; G2 = *Qfhb.uhgl-3BS* + 0-3 minor QTL; G3 = *Qfhb.uhgl-7AL* + 0-3 minor QTL;
8 G4 = 1-3 minor QTL; G5 = no identified QTL. The solid circle on the vertical line is the mean PIS
9 of each group and the length of the line represents the confidence interval. Two groups not sharing
10 a horizontal dashed line are significantly different at $LSD_{.05}$. Numbers in parentheses on the
11 horizontal axis are frequencies of RIL in each group

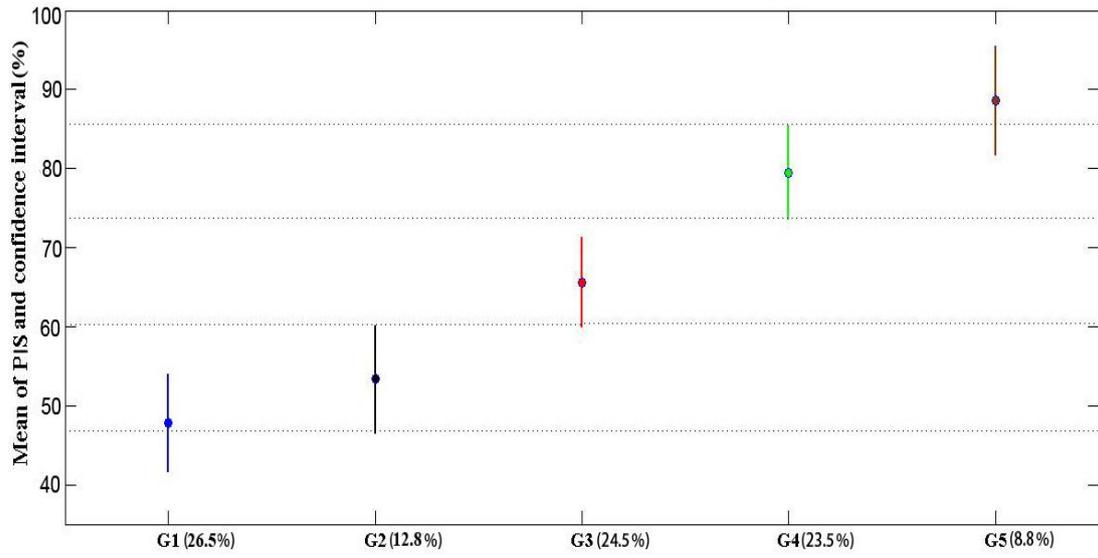


1
2 **Fig. 1**
3



1
2
3

Fig. 2



1
2
3

Fig. 3