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

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Abstract

The Chinese wheat landrace Huangfangzhu (HFZ) has a high level of resistance to Fusarium head blight (FHB). To identify chromosomal regions that are responsible for FHB resistance in HFZ, F_8 recombinant inbred lines (RIL) were developed from a cross between HFZ and Wheaton, a U.S. hard spring wheat. FHB was evaluated by single floret inoculation in both greenhouse and field environments. Two quantitative trait loci (QTL) with major effects were identified. One QTL was located on the short arm of chromosome 3B, and explained 35.4% of the phenotypic variation; the other QTL was assigned to 7AL and explained 18.0% of the phenotypic variation for FHB response. In addition, three minor QTL were detected on chromosomes 1AS, 1B and 5AS by single marker regression. HFZ contributed all favorable alleles. The RIL with HFZ alleles at the QTL on 3BS and 7AL displayed significantly lower percentages of infected spikelets (PIS) than RIL without these alleles in both greenhouse and field environments. HFZ combined several alleles from germplasm reported previously and is a promising alternative source for improving wheat FHB resistance.

Key words: Head scab

Introduction

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

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

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

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

Materials and methods

Plant materials and FHB evaluation

An SSD population of 106 F_8 RIL was developed from a cross between Wheaton, a susceptible U.S. wheat variety, and HFZ, a resistant wheat landrace from Jiangsu Province. The RIL were grown in 1.0 L Dura pots (Hummert International, St. Louis, MO, USA) filled with Metro-mix 360® growing medium (Hummert International) on a greenhouse bench at 17±2°C (night) and 22±5°C (day) with supplemental light for 12 h and evaluated for FHB response in three consecutive greenhouse (GH) experiments from 2007 to 2008 at Kansas State University (KSU), and one field experiment (2009) at KSU Rocky Ford FHB Nursery, Manhattan, KS. A *F. graminearum* conidia suspension was prepared following Bai et al. (1999). Wheat spikes were inoculated by delivering 10 uL of conidial suspension (100 conidia/uL)

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

DNA extraction and marker analysis

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

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

were used to screen two bulks with contrasting FHB responses. The resistant bulk was constructed by mixing equal amounts of DNA from 10 highly resistant RIL and the susceptible bulk was constructed by mixing equal amounts of DNA from 10 highly susceptible RIL. Primer pairs that detected polymorphism between the contrasting bulks were used to genotype the entire RIL population. For SSR analysis, each 10 uL PCR mixture contained 40 ng template DNA, 1 mM each of reverse and M13-tailed forward primers, 0.2 mM of each dNTP, 1×PCR buffer, 2.5 mM $MgCl_2$, and 0.6 U *Taq* polymerase. For PCR detection, 1 pmol of fluorescence-labeled M13 primer was added to each PCR. A touchdown PCR program was used for PCR amplification, in which the reaction mixture was incubated at 95℃ for 5 min, followed by five cycles of 45 s of denaturing at 95℃, 5 min of annealing at 68℃ with a decrease of 2℃ in each sequential cycle, and 1 min of extension at 72℃. For another five cycles, the annealing temperature started at 58℃ for 2 min with a decrease of 2℃ for each sequential cycle. PCR continued through an additional 25 cycles of 45 s at 94℃, 2 min at 50℃, and 1 min at 72℃ with a final extension at 72℃ for 5 min. The amplified PCR fragments were separated in an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). All marker data were scored using GeneMarker 1.6 (Softgenetics Inc. LLC), and visually checked twice to remove ambiguous data.

Genetic map construction and QTL analysis

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

markers with a maximum fraction of recombination at 0.4.

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

Statistical analysis

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

Results

FHB variation in RIL

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

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

QTL for type II resistance

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

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

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

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

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

Effects of QTL on type II resistance

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

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

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

Discussion

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

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

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

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

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

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

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

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

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Table 1 Analysis of variance (ANOVA) of percentage infected spikelets (PIS) for the RIL 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			

Table 2 Coefficients of determination (R^2), LOD values and additive effects of QTL regions detected by composite interval mapping based on mean FHB data for single greenhouse 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
Qfhb.uhgl-3BS	2007GHs[a]	*Xbarc147-Xumn10*	1.0	*Xumn10*	-12.6	4.45	0.234
	2007GHf	*Xbarc147-Xumn10*	1.0	*Xbarc147*	-14.1	7.17	0.281
	2008GHf	*Xbarc147-Xumn10*	1.0	*Xumn10*	-12.2	6.14	0.231
	Mean GH	*Xbarc147-Xumn10*	1.0	*Xumn10*	-14.1	9.74	0.356
	GH-FIELD combined	*Xbarc147-Xumn10*	1.0	*Xumn10*	-13.1	9.82	0.354
Qfhb.uhgl-7AL	2007GHs	*Xgwm276-Xbarc121*	4.0	*Xgwm276*	-12.2	3.56	0.182
	2007GHf	*Xgwm276-Xbarc121*	4.0	*Xbarc121*	-9.9	2.79	0.159
	Mean GH	*Xgwm276-Xbarc121*	4.0	*Xgwm276*	-9.3	3.44	0.177
	GH-FIELD combined	*Xgwm276-Xbarc121*	4.0	*Xbarc121*	-9.1	3.73	0.180

[a]s, spring; f, fall

Table 3. Coefficients of determination (R^2) of the closest markers associated with FHB resistance QTL identified by single marker analysis of data from single experiments or when combined

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

[a]s, spring; f, fall

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

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

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

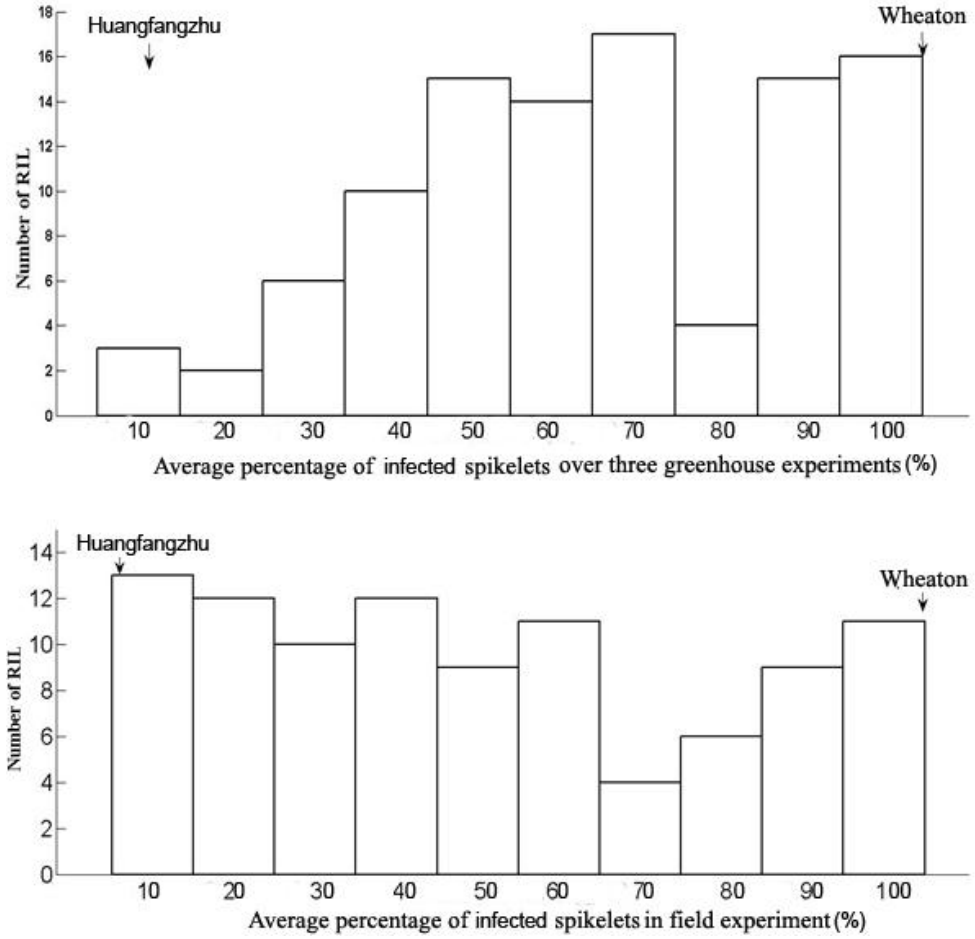
Huangfangzhu
Wheaton
Number of RIL
0
2
4
6
8
10
12
14
16
18
10
20
30
40
50
60
70
80
90
100
Average percentage of infected spikelets over three greenhouse experiments (%)
Huangfangzhu
Wheaton
Number of RIL
0
2
4
6
8
10
12
14
10
20
30
40
50
60
70
80
90
100
Average percentage of infected spikelets in field experiment (%)

Fig. 1

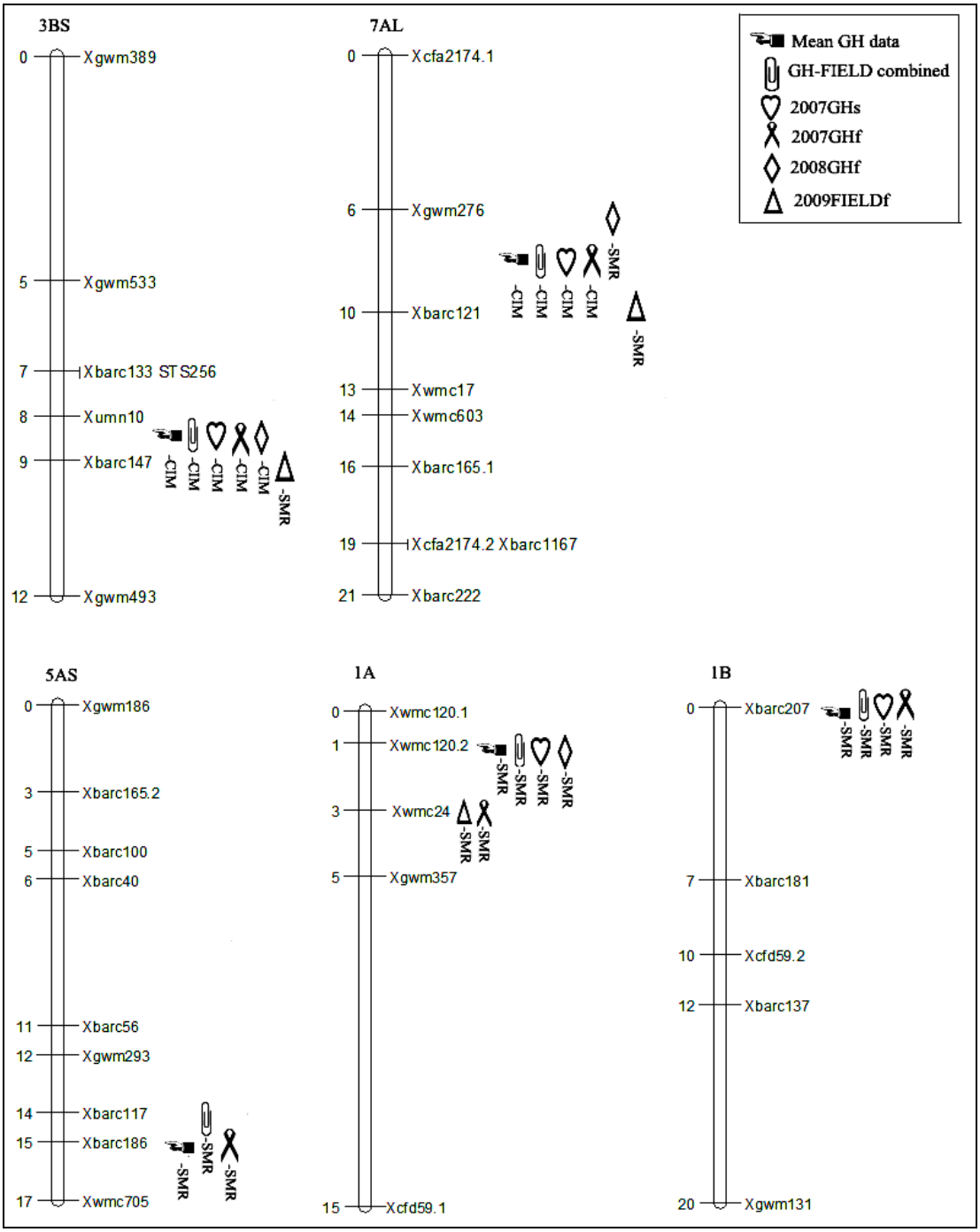
3BS
0 Xgwm389
5 Xgwm533
7 Xbarc133 STS256
8 Xumn10
9 Xbarc147
12 Xgwm493
-CIM
-SMR
7AL
0 Xcfa2174.1
6 Xgwm276
10 Xbarc121
13 Xwmc17
14 Xwmc603
16 Xbarc165.1
19 Xcfa2174.2 Xbarc1167
21 Xbarc222
Mean GH data
GH-FIELD combined
2007GHs
2007GHf
2008GHf
2009FIELDf
5AS
0 Xgwm186
3 Xbarc165.2
5 Xbarc100
6 Xbarc40
11 Xbarc56
12 Xgwm293
14 Xbarc117
15 Xbarc186
17 Xwmc705
1A
0 Xwmc120.1
1 Xwmc120.2
3 Xwmc24
5 Xgwm357
15 Xcfd59.1
1B
0 Xbarc207
7 Xbarc181
10 Xcfd59.2
12 Xbarc137
20 Xgwm131

Fig . 2

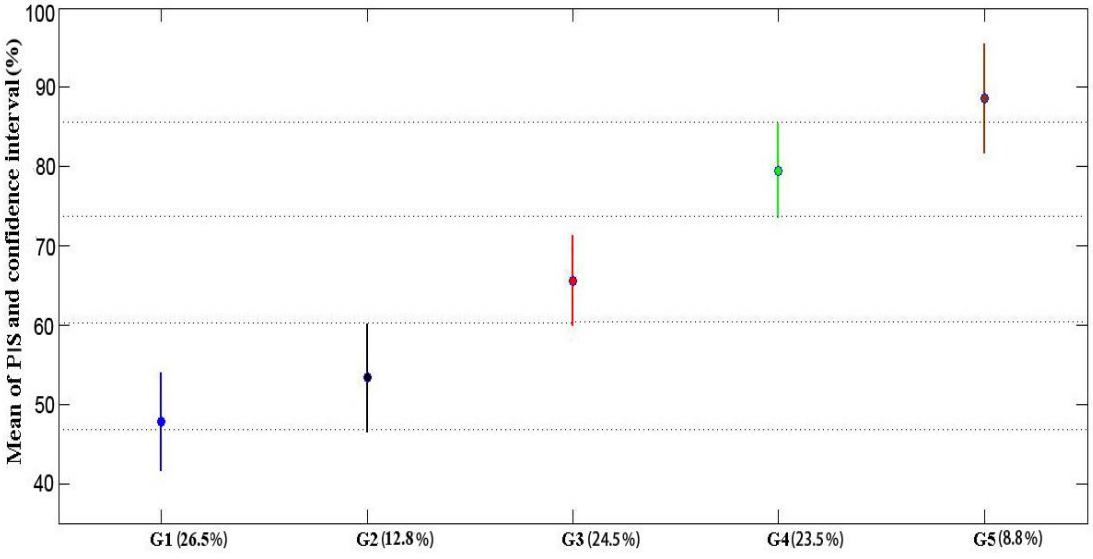
Mean of PIS and confidence interval (%)
100
90
80
70
60
50
40
G1 (26.5%)
G2 (12.8%)
G3 (24.5%)
G4 (23.5%)
G5 (8.8%)

Fig. 3