Theor Appl Genet (2011) 123:1055–1063
DOI 10.1007/s00122-011-1647-z

ORIGINAL PAPER

Precise mapping *Fhb5*, a major QTL conditioning resistance to *Fusarium* infection in bread wheat (*Triticum aestivum* L.)

Shulin Xue · Feng Xu · Mingzhi Tang · Yan Zhou · Guoqiang Li · Xia An · Feng Lin · Haibin Xu · Haiyan Jia · Lixia Zhang · Zhongxin Kong · Zhengqiang Ma

Received: 14 March 2011 / Accepted: 22 June 2011 / Published online: 8 July 2011

Abstract *Qfhi.nau-5A* is a major quantitative trait locus (QTL) against *Fusarium graminearum* infection in the resistant wheat germplasm Wangshuibai. Genetic analysis using BC_3F_2 and BC_4F_2 populations, derived from selfing two near-isogenic lines (NIL) heterozygous at *Qfhi.nau-5A* that were developed, respectively, with Mianyang 99-323 and PH691 as the recurrent parent, showed that *Qfhi.nau-5A* inherited like a single dominant gene. This QTL was thus designated as *Fhb5*. To fine map it, these two backcross populations and a recombinant inbred line (RIL) population derived from Nanda2419 × Wangshuibai were screened for recombinants occurring between its two flanking markers *Xbarc56* and *Xbarc100*. Nineteen NIL recombinants were identified from the two backcross populations and nine from the RIL population. In the RIL recombinant selection process, selection against *Fhb4* present in the RIL population was incorporated. Genotyping these recombinant lines with ten markers mapping to the *Xbarc56–Xbarc100* interval revealed four types of Mianyang 99-323-derived NIL recombinants, three types of PH691-derived NIL recombinants, and four types of RIL recombinants. In different field trials, the percentage of infected spikes of these lines displayed a distinct two-peak distribution. The more resistant class had over 55% less infection than the susceptible class. Common to these resistant genotypes, the 0.3-cM interval flanked by *Xgwm304* and *Xgwm415* or one of these two loci was derived from Wangshuibai, while none of the susceptible recombinants had Wangshuibai chromatin in this interval. This interval harboring *Fhb5* was mapped to the pericentromeric C–5AS3-0.75 bin through deletion bin mapping. The precise localization of *Fhb5* will facilitate its utilization in marker-assisted wheat breeding programs.

S. L. Xue and F. Xu contributed equally to this article.

Communicated by F. Ordon.

S. Xue · F. Xu · M. Tang · Y. Zhou · G. Li · X. An · F. Lin · H. Xu · H. Jia · L. Zhang · Z. Kong · Z. Ma (✉)
Crop Genomics and Bioinformatics Centre and National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, Jiangsu, China
e-mail: zqm2@njau.edu.cn

Introduction

Fusarium head blight (FHB) or scab is a devastating wheat disease worldwide, leading to yield reduction and poor grain quality. FHB resistance is a typical quantitative trait and controlled by multiple genes. Even though chemical and biological measures could be employed to control this disease, the deployment of disease-resistant varieties is the most effective strategy. There are two major FHB resistance types in wheat, with type I against initial penetration and type II against fungal spread within spikes (Schroeder and Christensen 1963). They were governed by different genetic factors (Lin et al. 2004, 2006). Usually, the single floret or point inoculation method is used for evaluation of type II resistance. To evaluate type I resistance, inoculation is usually carried out by spraying *Fusarium graminearum* conidiospores at anthesis or by planting in disease nurseries and percentage of infected spikes (PIS) and percentage of diseased spikelets (PDS) were used as the resistance indexes. Although PDS could be affected by both type I and type II resistances, it represents mainly type I resistance if the data are collected at the suitable time after the infection

(Xue et al. 2010b). In breeding practice, much attention has been paid to type II resistance due to lack of germplasm with excellent type I resistance and a more tedious task in measuring type I resistance.

The availability of molecular markers and genetic maps makes quantitative trait loci (QTLs) mapping a routine strategy for the discovery of genes involved in complex quantitative traits in wheat (Liu et al. 2005; Quarrie et al. 2005). So far, more than 100 QTLs for FHB resistance have been identified in Sumai No. 3 and its derivatives as well as in a few other resistant germplasms using recombinant inbred lines (RILs) and doubled haploid lines (Buerstmayr et al. 2009; Löffler et al. 2009). Most of these QTLs are type II resistance related. In the indigenous germplasm Wangshuibai originating in Jiangsu, China, we have identified two chromosomal intervals, one on chromosome 4B and one on chromosome 5A, showing major effects on type I resistance (Lin et al. 2006).

With primary mapping populations, QTL is usually placed to a large confidence interval due to the limitation in population size and marker density of the genetic maps. Thus, secondary populations created with near-isogenic lines (NIL) or substitution lines of the target QTLs that have reduced genetic background noise are often used in QTL fine mapping and isolation (Zhang et al. 2006; Shan et al. 2009). A number of QTLs of agronomical importance have accordingly been isolated in several plant species, for instance, *Gpc-B1* of wheat (*Triticum aestivum* L.) (Uauy et al. 2006), *Gw2* of rice (*Oryza sativa* L.) (Song et al. 2007), and *fw2.2* of tomato (Frary et al. 2000). QTL fine mapping is not only the prerequisite for QTL isolation but also beneficial for marker-assisted breeding. In marker-assisted selection (MAS), selection for large intervals often means cotransfer of deleterious genes or unfavorable traits together with the target QTLs due to linkage drag (Gervais et al. 2003; Somers et al. 2003; Draeger et al. 2007; McCartney et al. 2007; Xue et al. 2010a). Currently, the FHB resistance QTLs that have been fine mapped using a similar strategy included *Fhb1*, *Fhb2*, and *Fhb4* (Cuthbert et al. 2006, 2007; Liu et al. 2006; Xue et al. 2010b). *Fhb4* is for type I resistance.

A previous study using multiple-year trials has revealed that the type I resistance QTL on chromosome 5A of Wangshuibai, *Qfhi.nau-5A*, consistently explained 16.6–27.0% phenotypic variation in the RIL population (Lin et al. 2006). This QTL interval has also been associated with type I resistance in a few other germplasms, such as CM-82036 (Buerstmayr et al. 2003), DH181 (Yang et al. 2005), and W14 (Chen et al. 2006). Evaluation of the *Qfhi.nau-5A* NIL in susceptible background demonstrated that *Qfhi.nau-5A* only conditioned type I resistance and its presence reduced PIS by over 60% (Xue et al. 2010a). A recent meta-analysis for FHB resistance using 30 mapping populations again detected this QTL interval (Löffler et al. 2009).

In this study, we report the precise mapping of *Qfhi.nau-5A* by screening and evaluation of recombinants identified in two segregating populations created with the *Qfhi.nau-5A* NILs and in the Nanda2419 × Wangshuibai (NW) RIL population.

Materials and methods

Plant materials

A BC_3F_2 population of 94 plants and a BC_4F_2 population of 71 plants were created for recombinant identification by selfing two different *Qfhi.nau-5A* NILs heterozygous at *Qfhi.nau-5A*. These two NILs were developed, respectively, by using susceptible cultivars Mianyang 99-323 and PH691 as the recurrent parent and confirmed with marker genotypes. They had over 97% recipient genome composition (Xue et al. 2010a; unpublished data). In addition, a RIL population of 530 lines derived from Nanda2419 × Wangshuibai (Lin et al. 2004) was also used for recombinant screening. A F_2 population of 491 plants developed by crossing a homologous *Qfhi.nau-5A* NIL with its recurrent parent Mianyang 99-323 was used for genetic map construction of the *Qfhi.nau-5A* region.

Wheat cultivar "Chinese Spring" (CS), its ditelosomic lines, as well as a set of deletion lines of chromosome 5A (Endo and Gill 1996) were used for chromosomal arm and bin assignments of molecular markers.

Genotyping, mapping, and recombinant screening

Genomic DNA was extracted from young leaves according to Ma and Sorrells (1995). Polymerase chain reaction (PCR) was performed following the procedure of Ma et al. (1996). The PCR products were separated in 8% non-denaturing polyacrylamide gels (Acr:Bis = 19:1) at room temperature with 1 × TBE buffer and visualized by silver staining (Bassam et al. 1991).

For map construction, all DNA markers mapping to the *Qfhi.nau-5A* interval flanked by *Xbarc56* and *Xbarc100* on chromosome 5A (Röder et al. 1998; Somers et al. 2004; Song et al. 2005; Lin et al. 2006; Torada et al. 2006; Xue et al. 2008) were employed in genotyping. Linkage map was constructed with MAPMAKER/EXP version 3.0 (Lincoln et al. 1992) using the Kosambi mapping function (Kosambi 1944) and drawn with MapDraw version 2.1 (Liu and Meng 2003).

Plants with recombination occurring within the *Qfhi.nau-5A* interval were identified in the above-mentioned BC_3F_2 population with the Mianyang 99-323

background using flanking markers *Xbarc303* and *Xbarc180*, and in the BC_4F_2 population with the PH691 background using flanking markers *Xbarc56* and *Xbarc100*. The selfed progenies of the identified plants were then genotyped for recombinant screening with all the markers mapped in the QTL interval and polymorphic in the respective populations. The identified homozygous recombinants were selfed for seed production for resistance evaluation.

In the RIL population, lines with recombination occurring within the QTL interval and without the Wangshuibai chromatin corresponding to *Fhb4* were selected based on the marker genotypes within the respective regions. Markers closely linked to *Fhb4* were used in selection against *Fhb4* (Xue et al. 2010b).

FHB resistance evaluation

Evaluation of the BC_3F_2 population and the BC_4F_2 population was carried out in 2006 and 2008 seasons, respectively, in a field of Nanjing Agricultural University (NAU) Jiangpu (JP) experimental station. Single-space planted plants were evaluated in these experiments. At planting, fifteen seeds were evenly distributed in each 1.5-m row. The row spacing was 50 cm. Inoculation was carried out 1 week before anthesis through scattering on the soil surface wheat grains fully infected with four locally isolated highly virulent strains F4, F15, F34, and F7136, courtesy of Plant Protection Institute of Jiangsu Academy of Agricultural Sciences. Fifteen days after the anthesis, the total number of spikelets and the number of diseased spikelets (with visible disease symptom) of each spike were counted and the percentage of diseased spikelets (PDS) was then calculated. The PDS mean (from 3 to 5 spikes) of each F_2 plant was used here as the index for type I resistance. In our experiments, the PDS data collected at 15 days after anthesis mainly reflected type I resistance in our experimental conditions (Xue et al. 2010b).

Evaluation of the RIL and NIL homozygous recombinant lines was made in 2009 in a field of the Weigang (WG) campus of NAU, in 2010 in a field of the Pailou (PL) experimental station of NAU, and in 2009 and 2010 in a field of the JP experimental station, all using a randomized complete block design with two replicates. Each line was planted in one 1.4-m row in the WG and PL trials and two 1.5-m rows in the JP trials. The row spacing was 25 cm, and 20 seeds were planted per row. Inoculation was carried out at anthesis through spraying mixed *F. graminearum* conidial suspension prepared with the four virulent strains with a concentration of 5×10^4 conidia/ml in the WG and PL trials as described in Lin et al. (2006) and through scattering the scabby wheat grains on the soil surface in the JP trial as described above. Percentage of infected spikes (PIS) in a sample size of about 100 spikes was investigated in all but the 2010 JP trial. PIS indicates the number of spikes with at least one floret with visible FHB symptom 15 days after anthesis divided by the total number of spikes of each line. In the 2010 JP trial, PDS was investigated in the NIL recombinants due to heavy infection, for which about 30 randomly chosen spikes of each line were scored. Under the condition of heavy infection or small spike sample size as for the F_2 plants, PDS is more suitable as the type I resistance index than PIS.

In these trials, the *Qfhi.nau-5A* NILs and RIL NW127 that has Wangshuibai chromatin in the *Xbarc56–Xbarc100* interval and has Nanda2419 chromatin in the *Fhb4* region were used as the resistant control; Nanda2419, Mianyang 99-323, and PH691 were used as the susceptible control.

Statistical analysis

Statistical analysis was performed using software Data Desk v. 5.0 (Data Description, Inc., Ithaca, NY, USA). The effects of genotypes and environments on PIS were determined according to the fixed-effects model of two-factor analysis of variance (ANOVA). In this study, each site-year combination was treated as an environment. Dunnett's test was used for recombinant-versus-control comparisons, and Tukey's HSD test was applied for multiple mean comparisons of the BC_3F_2 and BC_4F_2 genotypes.

Results

Inheritance of *Qfhi.nau-5A*

The mode of inheritance of *Qfhi.nau-5A* was analyzed using the PDS data of 94 BC_3F_2 plants derived from the *Qfhi.nau-5A* NIL heterozygous at *Qfhi.nau-5A* with the Mianyang 99-323 background and of 71 BC_4F_2 plants derived from the *Qfhi.nau-5A* NIL heterozygous at *Qfhi.nau-5A* with the PH691 background. PDS in these two populations all displayed a two-peak frequency distribution with the valley at 25% (Fig. 1). Divided at the valley, the resistant group or group with smaller PDS and the group with larger PDS fit the 3:1 ratio in each of these two populations ($\chi^2 = 0.13$ and 0.38, respectively), suggesting that *Qfhi.nau-5A* was inherited like a single dominant gene. The means of the resistant and susceptible groups of the BC_3F_2 plants were $17.7 \pm 0.5\%$ and $37.7 \pm 1.0\%$, and those of the BC_4F_2 plants were $13.8 \pm 0.5\%$ and $32.1 \pm 0.8\%$, respectively. The differences between the group means were all significant at $P \leq 0.0001$.

To prove that *Qfhi.nau-5A* represents a single dominant gene, the PDS data were classified into three classes based on the marker genotype at *Xgwm415* that was tightly linked to *Qfhi.nau-5A* (Lin et al. 2006; Xue et al. 2010a), including

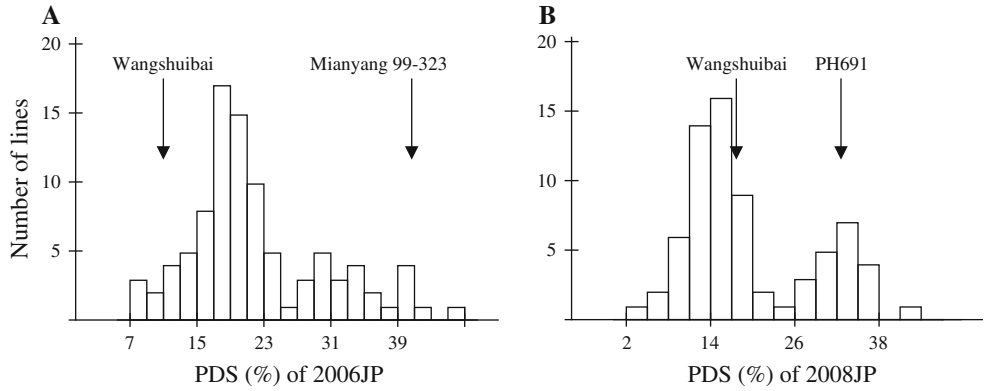

Fig. 1 Frequency distribution of percentage of diseased spikelets in the BC_3F_2 population with Mianyang 99-323 background (**a**) and the BC_4F_2 population with PH691 background (**b**)

Table 1 Average percentage of diseased spikelets (PDS) of plants with the same marker genotypes in the BC_3F_2 and BC_4F_2 populations

Population	Genotype[a]	Number of plants	Average PDS ± SE (%)	Tukey test[b]
(Wangshuibai × Mianyang 99-323) BC_3F_2	WW	27	16.5 ± 0.9	a
	WM	48	20.6 ± 0.8	a
	MM	19	33.4 ± 1.8	b
(Wangshuibai × PH691) BC_4F_2	WW	14	13.6 ± 0.8	a
	WP	34	13.8 ± 0.7	a
	PP	23	29.9 ± 1.4	b

[a] *WW, MM, and PP* Wangshuibai, Mianyang 99-323, and PH691 genotypes; *WM and WP* heterozygous genotypes of Wangshuibai with Mianyang 99-323 or PH691

[b] Genotypes with the same letter were not significantly different from each other at $P = 0.01$

Wangshuibai genotype (WW), Mianyang 99-323 (MM) or PH691 genotype (PP), and heterozygous genotypes of Wangshuibai with Mianyang 99-323 (WM) or PH691 (WP). As shown in Table 1, the PDS means of the MM or PP classes were all significantly larger than those of the WW class and the WM or WP classes, while the difference between the PDS means of the WW class and the WM or WP classes did not reach the significant threshold. These results showed that plants with *Qfhi.nau-5A* at either homozygous or heterozygous state contributed equally to type I resistance.

Marker saturation of the *Qfhi.nau-5A* interval

Qfhi.nau-5A was previously mapped to a 12.6-cM confidence interval flanked by *Xbarc56* and *Xbarc100* (Lin et al. 2006). To construct a fine map of this region, a F_2 population of 491 plants developed from the cross of a *Qfhi.nau-5A* NIL × Mianyang 99-323 was used. A total of 25 SSR and STS markers mapping to the *Qfhi.nau-5A* interval were surveyed for polymorphism between the Mianyang 99-323-derived *Qfhi.nau-5A* NIL and Mianyang 99-323. The flanking markers *Xbarc56* and *Xbarc100* were monomorphic between the two mapping parents. Ten markers detected polymorphism and were used in genotyping of the F_2 plants. With these marker data, a 4.2-cM map bordered by *Xbarc303* and *Xmag3794* was constructed (Fig. 2), which had an average marker density of one marker per 0.4 cM.

Recombinant identification of the *Qfhi.nau-5A* interval

Recombinants were identified from the mentioned BC_3F_2 and BC_4F_2 populations and from the Nanda2419 × Wangshuibai RIL population.

Genotyping the 94 BC_3F_2 plants with the Mianyang 99-323 background at *Xbarc303* and *Xbarc180* led to identification of four plants with recombination occurring between these two markers. Twelve homozygous recombinants were then obtained by surveying 10–15 BC_3F_3 plants derived from each of these plants with the ten mapped polymorphic markers. These recombinants represented four kinds of genotypes at the *Qfhi.nau-5A* interval (Fig. 3a). No recombinants were found between *Xbarc303* and *Xgwm304* and between *Xbarc180* and *Xmag3794*.

Based on the genotypes at *Xbarc56* and *Xbarc100*, three heterozygous recombinants were identified among the 71

BC_4F_2 plants with the PH691 background. By genotyping their BC_4F_3 plants with the 12 markers shown in Fig. 2, which all detected polymorphism between the PH691-derived *Qfhi.nau-5A* NIL and PH691 as well as between Wangshuibai and Nanda2419, seven homozygous recombinants, representing three kinds of genotypes at the *Qfhi.nau-5A* interval, were obtained (Fig. 3b). Among the 530 RILs derived from Nanda2419 × Wangshuibai, nine lines without *Fhb4* were identified with recombination occurring within the *Xbarc56–Xbarc100* interval. These RIL recombinants represented four kinds of genotypes at the *Qfhi.nau-5A* interval (Fig. 3c). No recombinants were obtained in the *Xbarc56–Xbarc180* interval in these two populations (Fig. 3b, c). The marker order determined using the identified recombinants fits well with the genetic map (Fig. 2).

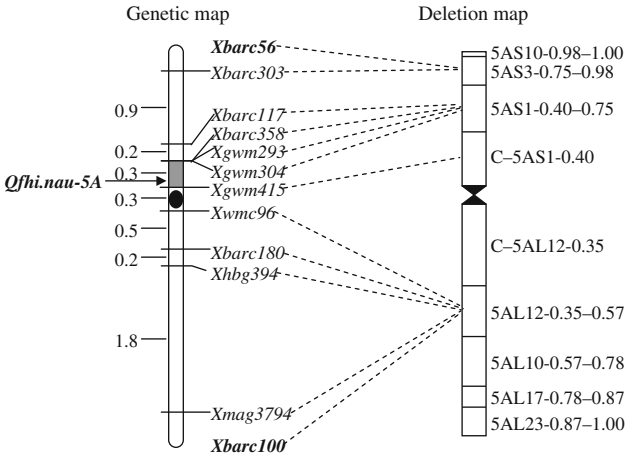

Fig. 2 Molecular marker map of the *Qfhi.nau-5A* region (*left*) constructed using the 491 F_2 plants derived from a *Qfhi.nau-5A* NIL × Mianyang 99-323, with the markers aligned to the deletion bin map (*right*) of chromosome 5A. The short arm is at the *top*. The *black oval* indicates the approximate position of centromere. Numbers to the *left* of the genetic map are interval distances (cM). The highlighted markers *Xbarc56* and *Xbarc100* were placed on the map according to Xue et al. (2008). The bin assignments delineated by neighboring deletion breakpoints are shown to the *right* of deletion bin map

Precise mapping of *Qfhi.nau-5A*

To precisely map *Qfhi.nau-5A*, the recombinant lines as well as the controls were surveyed for resistance with two to four field trials. Analysis of variance for PIS of the Mianyang 99-323 NIL recombinants and RIL recombinants across the environments revealed highly significant effects for both genotypes and environments, but not for the replications and genotypes × environments (Table 2). Moreover, the mean squares for genotypes were much larger than those for the genotype-by-environment interactions. The between-environment correlation coefficients of the genotype variable for the NIL and RIL recombinants ranged from 0.78 to 0.87 and from 0.91 to 0.98, respec-

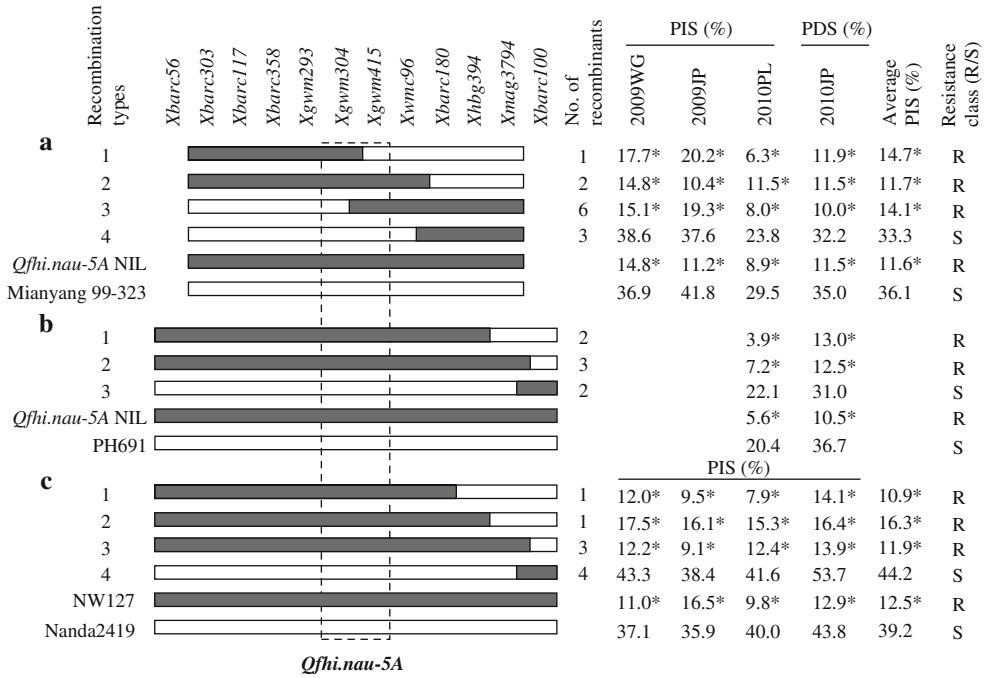

Fig. 3 Graphical illustrations of the recombinant genotype types in the *Qfhi.nau-5A* interval and their phenotypes in multiple trials. **a** Mianyang 99-323 background recombinants; **b** PH691 background recombinants; **c** Nanda2419 × Wangshuibai RIL recombinants. The *black rectangles* indicate Wangshuibai chromatin, the *open rectangles* indicate Mianyang 99-323, PH691, or Nanda2419 chromatin. The *broken rectangle* defines the interval harboring *Qfhi.nau-5A*. *R*, resistant and *S*, susceptible. *Asterisk* significantly different from Mianyang 99-323, PH691, or Nanda2419 at $P \leq 0.05$ according to Dunnett's test

Table 2 Results of two-way analysis of variance for percentage of infected spikes (%) of NIL and RIL recombinants

Variables	DF	Mean square	*F* value	Probability
Mianyang 99-323 NIL recombinant experiment[a]				
Genotype	13	540.9	29.0	<0.0001
Environment	2	702.0	37.7	<0.0001
Genotype × environment	25	37.1	2.0	0.03
RIL recombinant experiment[b]				
Genotype	10	1941.7	79.4	<0.0001
Environment	3	214.4	8.8	0.0001
Genotype × environment	30	31.5	1.3	0.22

[a] Genotypes included NIL recombinants, a *Qfhi.nau-5A* NIL and Mianyang 99-323

[b] Genotypes included RIL recombinants, RIL NW127 and Nanda2419

tively, all significant at $P \leq 0.0001$. The 2-year data of the PH691 NIL recombinants, one in PIS and one in PDS, were highly correlated ($r = 0.96$, $P \leq 0.0001$). Similarly, the PDS data of the Mianyang 99-323 NIL recombinants were also highly correlated with the three sets of PIS data ($r = 0.89$–0.94, $P \leq 0.0001$). A discontinuous bimodal distribution showed up for the PIS data of all three kinds of recombinants (Fig. 4), with the two areas of the distribution spaced by 13.8–23.7%, which were significant at $P \leq 0.001$. This made it possible to classify the recombinant lines into resistant group (R, falling in the area with lower PIS) and susceptible group (S, falling in the area with higher PIS). The classification of the recombinants did not change in trials. For the PH691 NIL recombinants, the PIS means of the R and S groups ranged from 3.2 to 7.5% and from 21.4 to 22.9%. For the Mianyang 99-323 NIL recombinants, the PIS means of the R and S groups over all the experiments ranged from 9.7 ± 0.4 to 17.2 ± 5.1% and from 31.5 ± 3.7 to 34.6 ± 6.4%. Those for the RIL recombinants ranged from 8.9 ± 1.1 to 16.3 ± 0.5% and from 40.1 ± 2.0 to 49.3 ± 5.7%. On the whole, the R group had over 55% less infection than the S group.

Of the four Mianyang 99-323 NIL recombinant genotypes, three were resistant and one was susceptible (Fig. 3a). Common to these resistant genotypes, the *Xgwm304–Xgwm415* interval or one of these two loci was derived from Wangshuibai. Both *Xgwm304* and *Xgwm415* loci in the susceptible genotype were derived from Mianyang 99-323. Among the PH691-derived NIL and RIL recombinants, the resistant genotypes had the *Xgwm304–Xgwm415* interval derived from Wangshuibai and the susceptible genotypes had this interval derived from PH691 or Nanda2419 (Fig. 3b, c). These results indicated that *Qfhi.nau-5A* fallen in the 0.3-cM interval flanked by *Xgwm304* and *Xgwm415*.

Physical mapping of *Qfhi.nau-5A*

To determine the physical position of *Qfhi.nau-5A*, all markers mapping to the *Xbarc56–Xbarc100* interval were mapped using the CS chromosome 5A ditelosomic and deletion lines. As shown in Fig. 2, the *Xbarc56–Xbarc100* interval covered nearly the whole short arm and about one-half of the long arm of chromosome 5A. However, *Xgwm304*, as well as the cosegregating *Xbarc358* and *Xgwm293*, was placed to the 5AS1-0.40–0.75 bin, and *Xgwm415* was placed to the C–5AS1-0.40 bin, all on the short arm. Thus, *Qfhi.nau-5A* resides in the C–5AS3-0.75 bin (Fig. 2). Since *Xbarc117* was the locus closest to the distal boundary of the 5AS1-0.40–0.75 bin and *Xgwm415* was the locus closest to the centromere, the 0.5-cM interval of *Xbarc117–Xgwm415* was corresponding to 219 Mb at the most, estimated based on the physical lengths of chromosome 5AS and the entire genome (Smith and Flavell 1975; Gill et al. 1991).

Discussion

A major type I FHB resistance QTL on chromosome 5A has been reported in several germplasms (Buerstmayr et al. 2003; Yang et al. 2005; Chen et al. 2006). *Qfhi.nau-5A* in scab resistance germplasm Wangshuibai explained over 16% phenotypic variation of type I resistance in a RIL population (Lin et al. 2006). In this study, we conducted precise mapping of this QTL. Using the genotype and

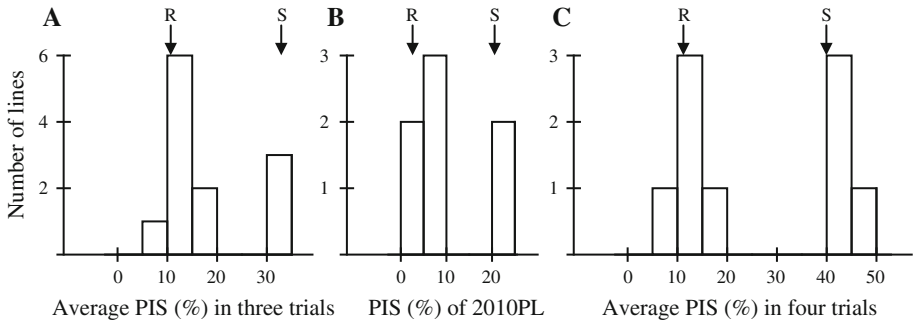

Fig. 4 Distribution of percentage of infected spikes (PIS) of the twelve Mianyang 99-323-derived NIL recombinants (**a**), seven PH691-derived NIL recombinants (**b**), and nine RIL recombinants (**c**). *R*-resistant control and *S*-susceptible control

phenotype data of the identified lines with recombination occurring within the *Xbarc56–Xbarc100* interval, *Qfhi.nau-5A* was placed in the 0.3-cM region flanked by *Xgwm304* and *Xgwm415* (Fig. 2).

The *Qfhi.nau-5A* NILs used in this study showed a stable and significantly decreased PIS and PDS compared with their susceptible recurrent parents Mianyang 99-323 and PH691 (Fig. 3). Resistance in the BCF_2 populations derived from these NILs segregated in the 3:1 ratio, suggesting that *Qfhi.nau-5A* inherits like a single Mendelian factor. It was thus designated as *Fhb5*. Although FHB resistance is a quantitative trait, the use of NILs has significantly reduced the background noises and makes it possible to precisely map it. Since there was no statistical difference in resistance performance between plants with homozygous Wangshuibai alleles of this QTL and plants in its heterozygous state (Table 1), *Fhb5* functions dominantly.

Fine mapping and cloning QTLs using recombinants identified from the NIL-derived secondary segregating populations or RIL populations have been effective in plant species (Uauy et al. 2006; Andaya and Tai 2007; Chen et al. 2008). Using this strategy, QTLs for wheat FHB resistance, including *Fhb1*, *Fhb2* and *Fhb4*, have been fine mapped (Cuthbert et al. 2006, 2007; Liu et al. 2006; Xue et al. 2010b). In this study, the 28 homozygous recombinants for the *Fhb5* interval were clearly classified into two groups based on their resistance levels (Fig. 4). The categorization of the individual lines did not change in the trials conducted in difference locations or years. This implies that the genetic variation caused by *Fhb5* is significantly larger than the variation caused by genotype × environment interactions and experimental errors, thus allowing us precisely determine the location of *Fhb5* relative to the molecular markers.

The C–5AS3-0.75 bin harboring *Fhb5* has an approximate physical distance versus genetic distance ratio of 438 Mb/cM. Assuming a wheat genome size of 5,000 cM and 16,800 Mb, the recombination rate of this interval (0.002 cM/Mb) is about 130 times smaller than the genome average. However, this could be a severe underestimate, since both *Xbarc117* and *Xgwm415* could be far from the bin boundaries. In wheat the pericentromeric regions usually have lower recombination rate (Akhunov et al. 2003). This low recombination makes it difficult to conduct map-based cloning of *Fhb5*. Qi and Gill (2001) obtained similar findings in their studies of the male-sterile gene *Ms3* that maps to the C–5AS1-0.40 bin. Most of the wheat genes isolated by map-based cloning so far, such as *Q* (Faris et al. 2003), *VRN2* (Yan et al. 2004), *Pm3b* (Yahiaoui et al. 2004), and *Gpc-B1* (Uauy et al. 2006), reside in regions with a recombination rate of more than 0.5 cM/Mb. However, since the two flanking markers *Xgwm304* and *Xgwm415* map to two adjacent bins (Fig. 1) that cover 75% of the 5AS physical length, more markers mapping between *Xgwm304* and *Xgwm415* must be developed and more recombinants of this interval must be identified to get accurate estimate of the recombination rate near *Fhb5*. This is possible. It was shown that in this study, by using a population of 491 F_2 plants derived from the *Fhb5* NIL × Mianyang 99-323, two recombinants were identified for the *Xbarc117–Xgwm304* interval and three were identified for *Xgwm304–Xgwm415* interval, while these three loci cosegregated in the NW map constructed with 136 RILs (Xue et al. 2008) and in the CSSQ1 map constructed with 96 doubled haploid lines (Quarrie et al. 2005). Moreover, the recombination rate near a particular locus could differ in different crosses (Bentolila and Hanson 2001; Imai et al. 2003; Dadkhodaie et al. 2011). Thus, development of multiple populations using different crossing parents could increase the chance to obtain the desired recombinants.

Fhb5 in Wangshuibai is one of the most effective type I resistance QTLs (Lin et al. 2006; Xue et al. 2010a). However, its 12.6-cM *Xbarc56–Xbarc100* interval has been associated with flag leaf width, thousand-grain weight, and spike compactness, with the Wangshuibai allele negatively affecting these agronomical traits (Ma et al. 2007, 2008; Xue et al. 2010a). Through precise mapping in this study, *Fhb5* is now confined to a 0.3-cM interval. This will facilitate investigation into the genetic association of *Fhb5* with these agronomical traits and reduce linkage drag in marker-assisted selection of *Fhb5* in breeding programs.

Acknowledgments This study was partially supported by "973" program (2010CB125902), the National Natural Science Foundation of China programs (31000535, 31030054, 30721140555), funds for transgenic plants (2008ZX08002-001 and 2009ZX08009-049B), the Natural Science Foundation of Jiangsu Province of China program (BK2009305), the Research Fund for the Doctoral Program of Higher Education of China program (20100097120042), the "111" project B08025, and the PAPD project of Jiangsu Higher Education Institutions.

References

Akhunov ED, Goodyear AW, Geng S, Qi LL, Echalier B, Gill BS, Miftahudin, Gustafson JP, Lazo G, Chao S, Anderson OD, Linkiewicz AM, Dubcovsky J, La Rota M, Sorrells ME, Zhang D, Nguyen HT, Kalavacharla V, Hossain K, Kianian SF, Peng J, Lapitan NL, Gonzalez-Hernandez JL, Anderson JA, Choi DW, Close TJ, Dilbirligi M, Gill KS, Walker-Simmons MK, Steber C, McGuire PE, Qualset CO, Dvorak J (2003) The organization and rate of evolution of wheat genomes are correlated with recombination rates along chromosome arms. Genome Res 13:753–763

Andaya VC, Tai TH (2007) Fine mapping of the *qCTS4* locus associated with seedling cold tolerance in rice (*Oryza sativa* L.). Mol Breed 20:349–358

Bassam BJ, Gaetano-Anollé G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196:80–83

Bentolila S, Hanson MR (2001) Identification of a BIBAC clone that co-segregates with the petunia restorer of fertility (*Rf*) gene. Mol Genet Genomics 266:223–230

Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (2003) Molecular mapping of QTL for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor Appl Genet 107:503–508

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breed 128:1–26

Chen J, Griffey CA, Maroof MAS, Stromberg EL, Biyashev RM, Zhao W, Chappell MR, Pridgen TH, Dong Y, Zeng Z (2006) Validation of two major quantitative trait loci for fusarium head blight resistance in Chinese wheat line W14. Plant Breed 125:99–101

Chen YS, Chao Q, Tan GQ, Zhao J, Zhang MJ, Ji Q, Xu ML (2008) Identification and fine-mapping of a major QTL conferring resistance against head smut in maize. Theor Appl Genet 117:1241–1252

Cuthbert PA, Somers DJ, Thomas J, Cloutier S, Brulé-Babel A (2006) Fine mapping *Fhb1*, a major gene controlling fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 112:1465–1472

Cuthbert PA, Somers DJ, Brulé-Babel A (2007) Mapping of *Fhb2* on chromosome 6BS: a gene controlling Fusarium head blight field resistance in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 114:429–437

Dadkhodaie NA, Karaoglou H, Wellings CR, Park RF (2011) Mapping genes *Lr53* and *Yr35* on the short arm of chromosome 6B of common wheat with microsatellite markers and studies of their association with *Lr36*. Theor Appl Genet 122:479–487

Draeger R, Gosman N, Steed A, Chandler E, Thomsett M, Srinivasachary, Schondelmaier J, Buerstmayr H, Lemmens M, Schmolke M, Mesterhazy A, Nicholson P (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

Endo TR, Gill BS (1996) The deletion stocks of common wheat. J Hered 87:295–307

Faris JD, Fellers JP, Brooks SA, Gill BS (2003) A bacterial artificial chromosome contig spanning the major domestication locus *Q* in wheat and identification of a candidate gene. Genetics 164:311–321

Frary A, Nesbitt TC, Frary A, Grandillo S, Knaap EVD, Cong B, Liu JP, Meller J, Elber R, Alpert KB, Tanksley SD (2000) *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit size. Science 289:85–88

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

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). Genome 34:830–839

Imai R, Koizuka N, Fujimoto H, Hayakawa T, Sakai T, Imamura J (2003) Delimitation of the fertility restorer locus *Rfk1* to a 43-kb contig in Kosena radish (*Raphanus sativus* L.). Mol Genet Genomics 269:388–394

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

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

Lin F, Xue SL, Zhang ZZ, Zhang CQ, Kong ZX, Yao GQ, Tian DG, Zhu HL, Li CJ, Cao Y, Wei JB, Luo QY, Ma ZQ (2006) Mapping QTL associated with resistance to Fusarium head blight in the Nanda2419 × Wangshuibai population. II: Type I resistance. Theor Appl Genet 112:528–535

Lincoln SE, Daly MJ, Lander ES (1992) Constructing genetic maps with MAPMAKER/EXP version 3.0. Technical report, 3rd edn. Whitehead Institute, Cambridge

Liu RH, Meng JL (2003) MapDraw: a Microsoft Excel macro for drawing genetic linkage maps based on given genetic linkage data. Heraditas 25:317–321

Liu ZH, Anderson JA, Hu J, Friesen TL, Rasmussen JB, Faris JD (2005) A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. Theor Appl Genet 111:782–794

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

Löffler M, Schön CC, Miedaner T (2009) Revealing the genetic architecture of FHB resistance in hexaploid wheat (*Triticum aestivum* L.) by QTL meta-analysis. Mol Breed 23:473–488

Ma ZQ, Sorrells ME (1995) Genetic analysis of fertility restoration in wheat using restriction fragment length polymorphisms. Crop Sci 35:1137–1143

Ma ZQ, Röder MS, Sorrells ME (1996) Frequencies and sequence characteristics of di-, tri-, and tetra-nucleotide microsatellites in wheat. Genome 39:123–130

Ma ZQ, Zhao DM, Zhang CQ, Zhang ZZ, Xue SL, Lin F, Kong ZX, Tian DG, Luo QY (2007) Molecular genetic analysis of five spike-related traits in wheat using the RIL and immortalized F_2 populations. Mol Genet Genomics 277:31–42

Ma ZQ, Xue SL, Lin F, Yang SH, Li GQ, Tang MZ, Kong ZX, Cao Y, Zhao DM, Jia HY, Zhang ZZ, Zhang LX (2008) Mapping and validation of scab resistance QTLs in the Nanda2419 × Wangshuibai population. Cereal Res Commun 36(Suppl B):245–251

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

Qi LL, Gill BS (2001) High-density physical maps reveal that the dominant male-sterile gene *Ms3* is located in a genomic region of low recombination in wheat and is not amenable to map-based cloning. Theor Appl Genet 103:998–1006

Quarrie SA, Steed A, Calestani C, Semikhidskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusic D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti MC, Hollington PA, Aragues R, Royo A, Dodig D (2005) A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring × SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor Appl Genet 110:865–880

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixer MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Schroeder HW, Christensen JJ (1963) Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopath 53:831–838

Shan JX, Zhu MZ, Shi M, Gao JP, Lin HX (2009) Fine mapping and candidate gene analysis of *spd6* responsible for small panicle and dwarfness in wild rice (*Oryza rufipogon* Griff.). Theor Appl Genet 119:827–836

Smith DB, Flavell RB (1975) Characterisation of the wheat genome by renaturation kinetics. Chromosoma 50:223–242

Somers DJ, Fedak G, Savard M (2003) Molecular mapping of novel genes controlling *Fusarium* head blight resistance and deoxynivalenol accumulation in spring wheat. Genome 46:555–564

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110:550–560

Song XJ, Huang W, Shi M, Zhu MZ, Lin HX (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet 39:623–630

Torada A, Koike M, Mochida K, Ogihara Y (2006) SSR-based linkage map with new markers using an intraspecific population of common wheat. Theor Appl Genet 112:1042–1051

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science 314:1298–1301

Xue SL, Zhang ZZ, Lin F, Kong ZX, Cao Y, Li CJ, Yi HY, Mei MF, Zhao DM, Zhu HL, Xu HB, Wu JZ, Tian DG, Zhang CQ, Ma ZQ (2008) A high-density intervarietal map of the wheat genome enriched with markers derived from expressed sequence tags. Theor Appl Genet 117:181–189

Xue SL, Li GQ, Jia HY, Lin F, Cao Y, Xu F, Tang MZ, Wang Y, Wu XY, Zhang ZZ, Zhang LX, Kong ZX, Ma ZQ (2010a) Marker-assisted development and evaluation of near-isogenic lines for scab resistance QTLs of wheat. Mol Breed 25:397–405

Xue SL, Li GQ, Jia HY, Xu F, Lin F, Tang MZ, Wang Y, An X, Xu HB, Zhang LX, Kong ZX, Ma ZQ (2010b) Fine mapping *Fhb4* a major QTL conditioning resistance to Fusarium infection in bread wheat (*Triticum aestivum* L.). Theor Appl Genet 121:147–156

Yahiaoui N, Srichumpa P, Dudler R, Keller B (2004) Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. Plant J 37:528–538

Yan L, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J (2004) The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. Science 303:1640–1644

Yang ZP, Gilbert J, Fedak G, Somers DJ (2005) Genetic characterization of QTL associated with resistance to Fusarium head blight in a doubled-haploid spring wheat population. Genome 48:187–196

Zhang YS, Luo LJ, Xu CG, Zhang QF, Xing YZ (2006) Quantitative trait loci for panicle size, heading date and plant height co-segregating in trait-performance derived near-isogenic lines of rice (*Oryza sativa*). Theor Appl Genet 113:361–368