Theor Appl Genet (2010) 121:147–156
DOI 10.1007/s00122-010-1298-5

ORIGINAL PAPER

Fine mapping *Fhb4*, a major QTL conditioning resistance to Fusarium infection in bread wheat (*Triticum aestivum* L.)

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

Received: 27 November 2009 / Accepted: 5 February 2010 / Published online: 3 March 2010

Abstract *Qfhi.nau-4B* is a major quantitative trait locus (QTL) against *Fusarium graminearum* infection identified in the Fusarium head blight-resistant germplasm Wangshuibai. To fine map this QTL, a recombinant inbred line (RIL) population of 530 lines derived from Nanda2419 × Wangshuibai and the BC_3F_2 population derived from the cross of a *Qfhi.nau-4B* near isogenic line (NIL) with susceptible cultivar Mianyang 99-323 as the recurrent parent were screened for recombinants occurred between microsatellite markers *Xbarc20* and *Xwmc349* that flank *Qfhi.nau-4B*. A total of 95 recombinants were obtained, including 45 RIL recombinants obtained through reverse-selection of *Qfhi.nau-5A* and 50 NIL recombinants from the BC_3F_2 population. Genotyping these recombinant lines with 22 markers mapping to the *Xbarc20* and *Xwmc349* interval revealed fourteen genotypes of the RIL recombinants as well as of the NIL recombinants. Two-year field evaluation of their resistance to *Fusarium* infection showed that these lines could be clearly classified into two groups according to percentage of infected spikes. The more resistant class had over 60% less infection than the susceptible class and were common to have Wangshuibai chromatin in the 1.7-cM interval flanked by *Xhbg226* and *Xgwm149*. None of the susceptible recombinants had this Wangshuibai chromatin. *Qfhi.nau-4B* was thus confined between *Xhbg226* and *Xgwm149* and named *Fhb4*. The interval harboring *Fhb4* was mapped to 4BL5-0.86–1.00 bin using Chinese Spring deletion lines, a region with about 5.7 times higher recombination rate than the genome average. This study established the basis for map-based cloning of *Fhb4*.

S. L. Xue and G. Q. Li contributed equally to this article.

Communicated by F. Ordon.

S. Xue · G. Li · H. Jia · F. Xu · F. Lin · M. Tang · Y. Wang · X. An · H. Xu · L. Zhang · Z. Kong · Z. Ma
The Applied Plant Genomics Laboratory of Crop Genomics and Bioinformatics Centre, and National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, Jiangsu, China

Z. Ma (✉)
College of Agricultural Sciences, Nanjing Agricultural University, Nanjing 210095, Jiangsu, China
e-mail: zqm2@njau.edu.cn

Introduction

Fusarium head blight (FHB) or scab is a devastating wheat (*Triticum aestivum* L.) disease worldwide, caused mainly by *Fusarium graminearum* Schwabe. It reduces grain yield and causes deterioration of grain weight and quality (Gilbert and Tekauz 2000). FHB resistance in wheat can be classified into two major types, type I against initial penetration and type II against fungal spread within spikes (Schroeder and Christensen 1963). With the help of molecular genetic maps, more than 100 quantitative trait loci (QTLs) for FHB resistance, mostly for type II resistance, have been reported in Sumai No. 3 and its derivatives as well as in a few other resistant varieties using recombinant inbred lines (RILs) and doubled haploid lines (reviewed by Buerstmayr et al. 2009). Recently, an alien chromatin fragment from alien species *Leymus racemosus* was related to type II resistance and designated *Fhb3* (Qi et al. 2008). However, because of the limitation in population size and marker density of the genetic maps in most primary mapping studies, the identified QTLs often had large confidence intervals. Besides improving these two limiting factors, a better strategy to fine map QTLs is to use secondary populations

created with near-isogenic lines (NIL) or substitution lines of the target QTLs that have reduced genetic background noise. Currently, the QTLs on chromosomes 3BS and 6B for type II resistance in Sumai No. 3 have thus been fine mapped and named *Fhb1* and *Fhb2*, respectively (Liu et al. 2006; Cuthbert et al. 2007).

Type I resistance is the host's first barrier to infection by *Fusarium species* and is important for FHB resistance breeding. In a previous study in our lab (Lin et al. 2006), we detected two major type I resistance QTLs in the FHB-resistant germplasm Wangshuibai, including *Qfhi.nau-4B* on chromosome 4B and *Qfhi.nau-5A* on chromosome 5A, which consistently explained over 15% phenotypic variation in the RIL population. Evaluation of their NILs confirmed their contribution to type I resistance, and the presence of either of them could reduce the percentage of disease spikes by over 50% (Xue et al. 2009). These two intervals were also associated with FHB resistance in a few other FHB resistance germplasms, such as in Wuhan-1 (Somers et al. 2003), Chokwang (Yang et al. 2005a) and Ernie (Liu et al. 2007) for the 4B QTL interval, and in CM-82036 (Buerstmayr et al. 2003), DH181 (Yang et al. 2005b) and W14 (Chen et al. 2006) for the 5A QTL interval. Recently, Löffler et al. (2009) again detected these two QTLs through QTL meta-analysis.

Wheat is a self-fertilizing crop and low in polymorphism. The current DNA marker density in the *Qfhi.nau-4B* region is not enough for fine mapping (Lin et al. 2006; Liu et al. 2007), less to say for map-based cloning. However, resources are available to increase the marker density. Xue et al. (2008) developed and mapped STS (sequence tagged site) and SSR (simple sequence repeat) markers by taking advantage of the wheat ESTs (expressed sequence tags) deposited in http://www.ncbi.nlm.nih.gov. By using ditelosomic and nulli-tetrasomic lines or deletion stocks, Qi et al. (2004) mapped 16,000 wheat ESTs to individual chromosomal regions or bins. Some genomic SSR markers have also been placed to the chromosomal bins (Sourdille et al. 2004). In the 4BL5-0.86–1.00 bin probably corresponding to *Qfhi.nau-4B* interval according to the map in Sourdille et al. (2004), there were 182 wheat ESTs mapped (Miftahudin et al. 2004). Moreover, due to the collinearity between chromosomes of rice, *Brachipodium distachyon* and wheat (La Rota and Sorrells 2004; Bossolini et al. 2007), the genomic sequence information of these model species can be used for molecular mapping and gene isolation in wheat (Liu and Anderson 2003; Yu et al. 2009).

In this study, we report the fine mapping of *Qfhi.nau-4B* by screening and evaluating recombinants identified in the Nanda2419 × Wangshuibai (NW) RIL population and in a segregating population created with the *Qfhi.nau-4B* NIL (Xue et al. 2009).

Materials and methods

Plant materials

Five hundred and thirty RILs derived from Nanda2419 and Wangshuibai (Lin et al. 2004) were used in this study. In addition, a BC_3F_2 population of 66 plants was created for recombinant identification by selfing a heterozygous *Qfhi.nau-4B* NIL. This NIL, having the Wangshuibai *Qfhi.nau-4B* interval and 98.1% recipient genome composition, was developed by marker-assisted selection using Mianyang 99-323 as the recurrent parent (Xue et al. 2009).

Wheat cultivar 'Chinese Spring' (CS) and CS deletion lines 4BS-1, 4BS-4, 4BS-8, 4BL-1, 4BL-5 that miss different parts of chromosome 4B (Endo and Gill 1996) were used to assign markers to specific chromosomal bins.

Genotyping and mapping

All DNA markers mapping to the *Qfhi.nau-4B* interval flanked by *Xbarc20* and *Xwmc349* on chromosome 4B, including BARC markers (Song et al. 2005), CFD markers (Guyomarc'h et al. 2002), GWM markers (Röder et al. 1998), HBG (Torada et al. 2006), MAG markers (Xue et al. 2008) and WMC markers (Somers et al. 2004) were employed in genotyping. Markers flanking and closely linked to *Qfhi.nau-5A* were used in counter-selection of this QTL.

To saturate the *Qfhi.nau-4B* interval, EST-STS makers were developed and surveyed. Since the *Xbarc20–Xwmc349* interval is partially corresponding to deletion bin 4BL5-0.86–1.00, the 182 wheat ESTs mapped in this bin by Miftahudin et al. (2004) were ordered by comparing with rice pseudomolecule release 5 (ftp://ftp.tigr.org/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/pseudomolecules/version_5.0) and the 8X *Brachypodium distachyon* genome assemblies (http://www.brachybase.org/index.html) using cutoff parameters of *E*-value $\leq 1E^{-10}$, identity $\geq$80% and a minimum of 100 bp match length. Fifty-seven and 77 of them had homologs in rice and *Brachypodium*, respectively, which covered a 6,529,883 bp region in rice chromosome 3S and a 7,050,036 bp region in *Brachypodium* chromosome 1L. STS markers of 20 wheat ESTs whose homologs disperse along the rice and *Brachypodium* syntenic regions in certain intervals were first developed to delimit the boundaries covering the *Qfhi.nau-4B* interval. Then, another 24 STS markers were developed based on the wheat EST homologs of the predicted rice and *Brachypodium* genes present between the boundaries. All primers were designed with MacVector 9.5 (Accelrys, UK). The primer information for markers detecting polymorphism between the mapping parents is provided in Table 1.

Table 1 Newly developed markers that detected polymorphism between the parents

Marker	Forward primer	Reverse primer	Annealing temperature (°C)	Expected size (bp)
MAG4571	CATCCATGAATGCAAGCGATTC	CGTAGCGCGTCAATTATG	55	495
MAG4580	CACTCAGCAGTAACCCCGAGTAATC	TGATCTTTCTCCCCGTAGAGCG	50	181
MAG4742	TCCCATTATTCCCACCTGTC	TCCTTGTTGTCATGCAGCTC	55	136
MAG5417	CATGTCTTTAGCGTGCAAGG	ACTCTGGTCAGCAGCGATTT	50	166

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 or 39:1) at room temperature with 1× TBE buffer and visualized by silver staining (Bassam et al. 1991).

The 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).

FHB resistance evaluation

The FHB resistance evaluation of the RIL recombinants was performed in 2008 and 2009 in a field of Nanjing Agricultural University (NAU) Jiangpu experimental station in a randomized complete block design with two replicates. Each plot had two 1.5-m rows. The row spacing was 25 cm and the sowing-density was 20 seeds per row. Inoculation was carried out through scattering scabby wheat grains on the soil surface. Percentage of infected spikes (PIS) that mainly represent type I resistance was investigated. PIS of a plot was calculated by dividing the number of spikes with visible FHB symptom in at least one of their florets 15 days after the inoculation by the total number of spikes.

FHB resistance evaluation of the NIL recombinants was performed in 2008 in a field of the Weigang campus of NAU and in the field of the NAU Jiangpu experimental station in 2008 and 2009 using the experimental design described above. Inoculation was carried out through scattering scabby wheat grains on the soil surface except the Weigang trial. In the Weigang trial there was one 1.2-m row in each plot and inoculation was carried out through spraying mixed *F. graminearum* conidial suspension. PIS was investigated in all but the 2008 Jiangpu trial, in which percentage of diseased spikelets (PDS) was used to measure type I resistance. Here PDS of a plot was calculated by dividing the number of spikelets with visible disease symptom of 30 randomly chosen spikes 15 days after the inoculation by the total number of spikelets in these spikes. Although PDS could be affected by both type I and II resistances, the data collected at 15 days after the inoculation mainly reflected type I resistance in our experimental conditions, while the data collected at the later stage reflected both types of resistances (unpublished data). Since our previous studies (Lin et al. 2004, 2006; Xue et al. 2009) have demonstrated that *Qfhi.nau-4B* only conditions type I resistance, and the PIS and PDS data were equally effective to represent type I resistance in the 2008 trials, PDS was investigated only in 2008 Jiangpu trial to save labor.

Results

Marker saturation of the *Qfhi.nau-4B* interval

To construct a fine map of the *Qfhi.nau-4B* region, besides the 136 RILs from the NW population used in the primary map construction by Lin et al. (2004), another 394 lines from the same population were genotyped with the four markers mapped to the *Xbarc20–Xwmc349* interval by Lin et al. (2006). Moreover, 26 SSR markers mapped to regions surrounding this QTL interval and the 44 newly developed STS markers were surveyed for polymorphism between the mapping parents. Twelve of them detected polymorphism and were linked to the four previously mapped markers. With a total of 16 markers mapping to the *Xbarc20–Xwmc349* interval, a 20.1 cM map was constructed, which had an average marker density of one marker per 1.3 cM (Fig. 1).

Recombinant screening and genotyping

As the first step to fine map *Qfhi.nau-4B*, recombinants were identified from the RIL population and from a BC_3F_2 population derived from the *Qfhi.nau-4B* NIL with Mianyang 99-323 background. In the RIL population, there were 114 lines with recombination occurring in the *Xbarc20–Xwmc349* interval, and 45 of them do not have the Wangshuibai chromatin harboring *Qfhi.nau-5A*. These recombinants represented 14 genotypes of the examined interval, but no recombinants were found in the region flanked by *Xhbg225* and *Xgwm192* and in the region flanked by *Xmag4742* and *Xcfd22* (Table 2).

To identify markers for recombinant selection from the NIL-derived BC_3F_2 population, all markers used in the

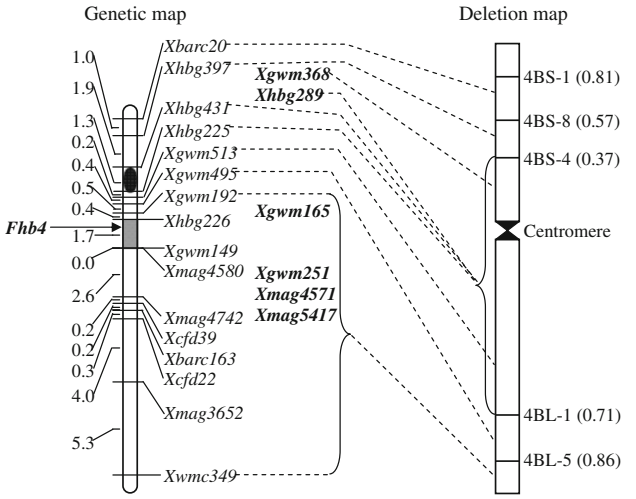

Fig. 1 Molecular marker map of the *Qfhi.nau-4B* region (*left*) and the corresponding positions of these markers in the deletion bin map (*right*) of chromosome 4B as shown with the *dashed line* connections. The short arm is at the *top*. The *black oval* indicates the approximate position of centromere. Numbers to the *left* of the genetic map are interval distances (cM). The *highlighted* markers were those mapped in the NIL-derived population and were placed on the map according to their positions relative to other markers. The breakpoints and fraction-lengths of the bins (within parentheses) are shown to the *right* of deletion bin map

survey of Wangshuibai and Nanda2419 were also surveyed in the NIL and Mianyang 99-323. Among the 16 markers mapped with the RIL population, HBG397 and CFD39 did not detect polymorphism between the two lines, but six additional polymorphic markers, including GWM165, GWM251, GWM368, HBG289, MAG4571 and MAG5417 were found.

Genotyping 66 BC_3F_2 plants with BARC20 and WMC349 identified 20 plants with recombination occurring between these two markers. Fifty homozygous recombinants, representing 14 genotypes of the examined interval, were obtained by surveying 10 to 15 BC_3F_3 progenies from each of these plants with the 20 polymorphic markers. According to the marker genotypes of these recombinants, the order of the polymorphic markers along the chromosome was determined (Table 3). This order ensured least crossovers between markers and was consistent with the linkage map constructed with the RIL population (Fig. 1). In this NIL-derived population, no recombination was obtained in five smaller intervals including the two that did not have recombination in the RIL population.

Fine mapping of *Qfhi.nau-4B* using the recombinants

To examine the type I resistance of the identified RIL and NIL recombinants, two and three repeated field trials were conducted from 2008 to 2009, respectively. In the RIL recombinant trials, two lines without the Wangshuibai chromatin harboring *Qfhi.nau-5A*, but with either the Wangshuibai chromatin or Nanda2419 chromatin in the *Xbarc20–Xwmc349* interval were used as controls. In the NIL recombinant trials, the *Qfhi.nau-4B* NIL and Mianyang 99-323 were employed as controls. As shown in Fig. 2, the phenotypic data displayed a discontinuous bimodal distribution for both kinds of recombinants, with the two areas of the distribution spaced by 6.6–13.6%, which were all significant at $P = 0.05$. Thus, the plants belonging to the area with lower PIS were classified as resistant, and those belonging to the area with higher PIS were classified as susceptible. For the RIL recombinants, the across-year average PIS of the resistant and susceptible classes ranged from 11.9 ± 2.3 to $22.3 \pm 1.7\%$ and from 32.5 ± 0.5 to $50.9 \pm 5.8\%$, respectively; that for the NIL recombinants ranged from 8.8 ± 2.4 to $21.2 \pm 0.8\%$ and from 33.4 ± 2.2 to $45.8 \pm 7.0\%$, respectively. The resistant class had over 60% less infection than the susceptible class. There was no phenotype class change in different trials for all the recombinants. The PDS data from the 2008 Jiangpu trial of the NIL recombinants produced similar result (data not shown).

Of the 14 RIL and 14 NIL recombinant genotypes, five and seven were resistant, respectively (Tables 2, 3). In addition, one of the two RIL recombinants of the type-5 genotype, one of the two RIL recombinants of the type-11 genotype, and one of the NIL recombinants of the type-3 genotype were also resistant (Tables 2, 3). Common to these resistant genotypes, the *Xhbg226–Xgwm149* interval or one of these two loci was derived from Wangshuibai. However, three of the recombinant lines having one of the two loci, which belonged to the types-5 and -11 RIL genotypes and to the type-3 NIL genotype, showed susceptible phenotypes. The *Xhbg226–Xgwm149* interval of the other susceptible phenotypes was all derived from Nanda2419 or Mianyang 99-323. It was thus concluded that *Qfhi.nau-4B* falls in the interval flanked by *Xhbg229* and *Xgwm149* that were 1.7 cM away from each other in the genetic map (Fig. 1).

Deletion bin assignment of the *Qfhi.nau-4B*

To determine the physical positions of *Qfhi.nau-4B*, all markers mapping to the *Xbarc20–Xwmc349* interval, but *Xbarc20*, *Xgwm368*, *Xgwm513* and *Xcfd22* that have been physically mapped by Sourdille et al. (2004), were mapped using the five deletion lines covering the 4B chromosome. As illustrated in Fig. 1, the *Xbarc20–Xwmc349* interval covered all the deletion bins but the top one. However, 14 of the 22 markers, distal to the centromere in the long arm were all placed to the bottom bin 4BL5-0.86–1.00. Thus, the *Xhbg226–Xgwm149* interval harboring *Qfhi.nau-4B* resides in this bin (Fig. 1).

Table 2 Genotypes and phenotypes of the selected RIL recombinants

Recombination types	Genotypes									No. of recombinants	Phenotypes		Resistance class (R/S)
	Xbarc20	*Xhbg397*	*Xhbg431*	*Xhbg225*, *Xgwm513*, *Xgwm495*, *Xgwm192*	*Xhbg226*	*Xgwm149*, *Xmag4580*	*Xmag4742*, *Xcfd39*, *Xbarc163*, *Xcfd22*	*Xmag3652*	*Xwmc349*		2008PIS (%)	2009PIS (%)	
1	**W**	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	2	42.2	35.8	S
2	**W**	**W**	*N*	*N*	*N*	*N*	*N*	*N*	*N*	1	47.8	34.9	S
3	**W**	**W**	**W**	*N*	*N*	*N*	*N*	*N*	*N*	4	50.2	40.6	S
4	**W**	**W**	**W**	**W**	*N*	*N*	*N*	*N*	*N*	1	40.1	40.0	S
5	**W**	**W**	**W**	**W**	**W**	*N*	*N*	*N*	*N*	2	42.9	37.7	S
	W	**W**	**W**	**W**	**W**	*N*	*N*	*N*	*N*	1	22.3	13.9	R
6	**W**	**W**	**W**	**W**	**W**	**W**	*N*	*N*	*N*	3	19.2	13.9	R
7	**W**	**W**	**W**	**W**	**W**	**W**	**W**	*N*	*N*	9	17.7	11.2	R
8	**W**	**W**	**W**	**W**	**W**	**W**	**W**	**W**	*N*	5	19.0	11.0	R
9	*N*	*N*	*N*	**W**	**W**	**W**	**W**	**W**	**W**	1	23.3	14.5	R
10	*N*	*N*	*N*	*N*	**W**	**W**	**W**	**W**	**W**	1	–	17.2	R
11	*N*	*N*	*N*	*N*	*N*	**W**	**W**	**W**	**W**	1	15.3	14.0	R
	N	*N*	*N*	*N*	*N*	**W**	**W**	**W**	**W**	2	39.1	37.1	S
12	*N*	*N*	*N*	*N*	*N*	*N*	**W**	**W**	**W**	3	47.7	37.9	S
13	*N*	*N*	*N*	*N*	*N*	*N*	*N*	**W**	**W**	3	41.2	35.9	S
14	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	**W**	6	44.3	36.1	S
C1	**W**	**W**	**W**	**W**	**W**	**W**	**W**	**W**	**W**		19.0	9.8	R
C2	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*		54.5	40.9	S

C1 Control 1 (NW98), *C2* Control 2 (NW9), *W* Wangshuibai allele, *N* Nanda2419 allele, *R* resistant, *S* susceptible

Table 3 Genotypes and phenotypes of the identified NIL recombinants

Recombination types	Genotypes										No. of recombinants	Phenotypes			Resistance class (R/S)
	Xbarc20, *Xgwm368*	*Xhbg289*	*Xhbg431*, *Xhbg225*, *Xgwm513*, *Xgwm495*	*Xgwm192*, *Xhbg226*, *Xgwm165*	*Xgwm149*, *Xmag4580*, *Xgwm251*	*Xmag4571*	*Xmag5417*	*Xmag4742*, *Xbarc163*, *Xcfd22*	*Xmag3652*	*Xwmc349*		2008PDS (%)	2008PIS (%)	2009PIS (%)	
1	**W**	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*	1	47.5	37.2	43.7	S
2	**W**	**W**	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*	2	43.9	34.2	43.3	S
3	**W**	**W**	**W**	**W**	*M*	*M*	*M*	*M*	*M*	*M*	1	49.1	35.2	41.1	S
	W	**W**	**W**	**W**	*M*	*M*	*M*	*M*	*M*	*M*	3	12.6	18.2	16.9	R
4	**W**	**W**	**W**	**W**	**W**	**W**	*M*	*M*	*M*	*M*	4	13.8	17.6	18.9	R
5	**W**	**W**	**W**	**W**	**W**	**W**	**W**	*M*	*M*	*M*	1	14.7	15.2	20.4	R
6	**W**	**W**	**W**	**W**	**W**	**W**	**W**	**W**	*M*	*M*	7	11.2	14.2	16.5	R
7	**W**	**W**	**W**	**W**	**W**	**W**	**W**	**W**	**W**	*M*	4	9.5	11.2	9.4	R
8	*M*	**W**	**W**	**W**	**W**	**W**	**W**	**W**	**W**	**W**	2	11.2	14.2	17.1	R
9	*M*	*M*	*M*	**W**	**W**	**W**	**W**	**W**	**W**	**W**	2	12.0	12.9	16.6	R
10	*M*	*M*	*M*	*M*	**W**	**W**	**W**	**W**	**W**	**W**	3	11.4	12.9	18.0	R
11	*M*	*M*	*M*	*M*	*M*	**W**	**W**	**W**	**W**	**W**	1	39.7	35.2	36.8	S
12	*M*	*M*	*M*	*M*	*M*	*M*	*M*	**W**	**W**	**W**	4	42.9	33.1	44.9	S
13	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*	**W**	**W**	10	41.5	33.0	41.3	S
14	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*	**W**	5	42.3	33.9	45.3	S
C1	**W**	**W**	**W**	**W**	**W**	**W**	**W**	**W**	**W**	**W**		11.2	9.3	11.6	R
C2	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*		44.7	41.6	45.8	S

C1 Control 1 (*Qfhi.nau-4B* NIL), *C2* Control 2 (Mianyang 99-323), *W* Wangshuibai allele, *M* Mianyang 99-323 allele, *R* resistant, *S* susceptible

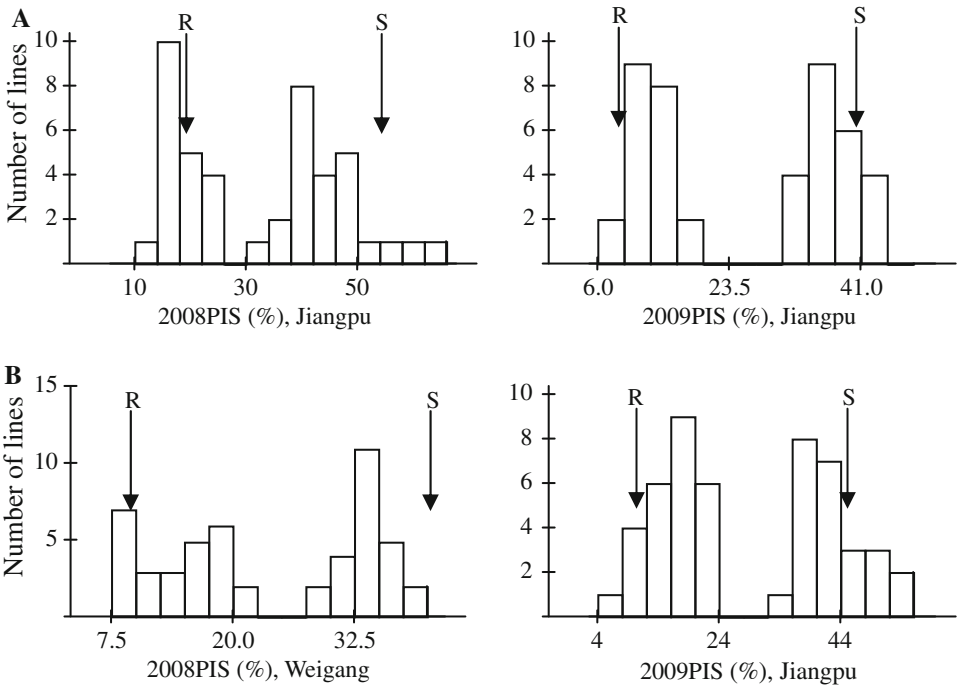

Fig. 2 Phenotypic distribution of 45 RIL recombinants (**a**) and 50 NIL recombinants (**b**) in the respective trials. *R* resistant control, *S* susceptible control

Discussion

In this study, we fine mapped the major type I FHB resistance QTL *Qfhi.nau-4B* in the germplasm Wangshuibai. By genotyping and phenotyping lines with recombination occurred in the *Xbarc20–Xwmc349* region, *Qfhi.nau-4B* was placed in a 1.7-cM interval flanked by *Xhbg226* and *Xgwm149* (Fig. 1). Using similar mapping strategies, Liu et al. (2006) and Cuthbert et al. (2007) fine mapped the two QTLs residing in chromosomes 3BS and 6B for type II resistance and named them as *Fhb1* and *Fhb2*, respectively. Here, we designated *Qfhi.nau-4B* as *Fhb4. Fhb3* was given to a *L. racemosus* chromatin transferred to wheat for FHB resistance (Qi et al. 2008).

Successful fine mapping of quantitative trait loci that resulted in isolation of the underlying genes have been reported in several plant species, for instance, *Gpc-B1* of wheat (Uauy et al. 2006), *Gw2* of rice (Song et al. 2007), and *CRY2* of *Arabidopsis* (El-Din El-Assal et al. 2001). All these studies used recombinants screened from secondary segregating populations derived from QTL NIL lines. We showed that this strategy is equally effective in fine mapping QTLs in wheat conferring resistance to *Fusarium* infection, whose evaluation is usually difficult since their effects are greatly influenced by environmental factors. In this study, the selected recombinants could be distinctly grouped based on the phenotypes and the phenotypes accorded with the genotypes (Table 3; Fig. 2). However, there were still over 5% PIS differences among different recombinants belonging to the same resistance group. We speculated that this was caused by some minor QTLs residing either at or out of the targeting region and/or by environmental errors in phenotyping.

Besides using the secondary segregating population, we also employed RIL population for recombinant screening. In the screening process, *Qfhi.nau-5A*, the non-targeting major QTL for type I resistance, was reversely selected based on the genotypes of the closely linked markers in order to avoid its influences on the resistance. With the 14 types of RIL recombinants identified (Table 2), the obtained result was in good agreement to that with the NIL recombinants, although the genetic background of the RIL recombinants might not be as uniform as the NIL recombinants. The same kind of success was achieved in fine mapping rice *qCTS4* QTL (Andaya and Tai 2007). Nonetheless, it should be kept in mind that resolution of QTL fine mapping using RIL populations depends on population size and marker density surrounding the target region in addition to the genetic background.

We noted that some types of the NIL recombinants or RIL recombinants had a small sample size and a few of them even had only one line. This could be a problem for getting accurate phenotype score due to the quantitative nature of the investigated trait and the influences of non-genetic factors such as genotype × environment interactions. However, with a total of five trials in two locations, there was no trial-wide discrepancy in the assignment of the individual recombinants to the respective resistance groups. This implies that the genetic variation caused by *Fhb4* is significantly larger than the variation caused by the environmental factors and experimental errors. Nevertheless, there were lines with the same marker genotypes showed

different phenotypes, no matter they were derived from the secondary segregating population or from the RIL population (Tables 2, 3). The recombination of all these lines occurred between the two boundary markers *Xhbg226* and *Xgwm149*. This phenomenon might indicate that there are different kinds of recombination in the respective recombinant types that all occurred between *Xhbg226* and *Xgwm149*. This is possible because there was still 1.7 cM genetic distance between the two boundary markers. The phenotype discrepancy was much less likely caused by experimental errors or environmental factors since these lines, no matter they were derived from the RIL or NIL populations, showed the same types of resistance in all the trials. Moreover, the type-5 RIL recombinants and the type-3 NIL recombinants apparently had the same kinds of recombination (Tables 2, 3); coincidently, there was a line with an off-type phenotype in both of them. Thus, non-genetic factors couldn't be the cause given the total types of recombinants surveyed in the experiments and the total number of trials conducted. Enriching the *Xhbg226* and *Xgwm149* interval with more markers would help clarifying this issue.

Although the 20.1 cM *Xbarc20–Xwmc349* interval physically covered almost the whole 4B chromosome, the inner interval harboring *Fhb4*, flanked by *Xgwm192* and *Xwmc349*, maps to 4BL5-0.86–1.00 bin (Fig. 1). Taking *Xgwm495* as the boundary marker, the distal portion (101.2 cM, Xue et al. 2008) corresponded to a physical size of 60 Mb according to Gill et al. (1991), assuming a wheat genome size of 5,000 cM and 16,800 Mb. Thus, the recombination rate of this portion is 1.7 cM/Mb, about 5.7 times higher than the genome average. This is consistent with the findings of Akhunov et al. (2003) that the distal chromosomal regions of wheat genome generally exhibit a high recombination rate. This feature makes it easier to isolate genes residing at these areas. The currently cloned wheat genes through map-based cloning, such as *Lr10* (Stein et al. 2000), *Q* (Faris et al. 2003), *VRN2* (Yan et al. 2004), *Pm3b* (Yahiaoui et al. 2004) and *Lr34* (Krattinger et al. 2009), mostly locate on recombination hotspots.

The *Xbarc20–Xwmc349* interval has been associated with plant height, thousand-grain weight (McCartney et al. 2005; Huang et al. 2006), ears per plant and grains per ear (Quarrie et al. 2005), in addition to with FHB resistance. It was found that the resistance conditioned by *Fhb4* of Wangshuibai were positively related to plant height and negatively related to thousand-grain weight (Xue et al. 2009; unpublished data). The fine map constructed in this study will facilitate investigation of the genetic association of these traits and reduce linkage drag through marker-assisted selection in breeding programs.

Rice and *Brachypodium distachyon* are two model species for cereal crops with full genome sequence information available (ftp://ftp.tigr.org/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/pseudomolecules/version_5.0; http://www.brachybase.org/index.html). The chromosome 4BL of wheat is collinear to rice chromosome 3S (La Rota and Sorrells 2004). We queried 182 wheat ESTs mapping to the chromosome 4BL against the *Brachypodium* genome sequence and found that 48.4% of these ESTs were homologous to *Brachypodium* chromosome 1L. Only 33.0% of these ESTs were homologous to rice chromosome 3S. Four STS makers derived from the wheat ESTs homologous to *Brachypodium* and rice genes revealed polymorphism between the mapping parents and showed closely linkage to *Fhb4*, although they were all distal to *Xgwm149* (Fig. 1). Their order in the genetic map is consistent with the order of the homologous genes in *Brachypodium,* while inversion disturbed the micro-collinearity between the compared regions of wheat and rice (data not shown). This is similar to results of Bossolini et al. (2007), who found a perfect collinearity between a 371-kb *Brachypodium* sequence and wheat and an approximately 220-kb inversion between the *Brachypodium* and rice sequences. The better collinearity between *Brachypodium* and wheat suggests that the *Brachypodium* genome sequence information is excellent resource for wheat genomics studies.

Acknowledgments This study was partially supported by '863' program (2006AA10A104), '973' program (2004CB117205), the National Natural Science Foundation of China programs (30430440, 30025030, 30671295, 30721140555), the Natural Science Foundation of Jiangsu Province of China program (BK2009305) and the '111' project B08025.

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