Reevaluation of Two Quantitative Trait Loci for Type II Resistance to Fusarium Head Blight in Wheat Germplasm PI 672538

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Accepted for publication 20 August 2016.

ABSTRACT

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum*, is a destructive disease in wheat. A population consisting of 229 F_2 and $F_{2:3}$ plants derived from the cross PI 672538 $\times$ L661 was used to evaluate the reactions to FHB. The FHB resistance data distribution in the F_2 population indicates that some quantitative trait loci (QTLs) were controlling the FHB resistance in PI 672538. We further detected two major QTLs (*Qfhs-2B*, *Qfhs-3B*) from analysis of the resistance data and the PCR-amplified results using WinQTLCart 2.5 software. *Qfhs-2B*, flanked by *Xbarc55-2B* and *Xbarc1155-2B*, explained more than 11.6% of the phenotypic variation of the percentage of diseased spikelets (PDS), and

Qfhs-3B, flanked by *Xwmc54-3B* and *Xgwm566-3B*, explained more than 10% of the PDS phenotypic variation in the $F_{2:3}$ population. In addition, *Qfhs-3B* was different from *Fhb1* in terms of the pedigree, inheritance, resistance response, chromosomal location, and marker diagnosis. We also detected QTLs for other disease resistance indices, including the percentage of damaged kernels and 1,000-grain weight, in similar chromosomal regions. Therefore, the FHB resistance of PI 672538 was mainly controlled by two major QTLs, mapped on 2B (*FhbL693a*) and 3B (*FhbL693b*). PI 672538 could be a useful germplasm for improving wheat FHB resistance.

Fusarium head blight (FHB), caused mostly by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schw.) Petch), is a destructive disease in wheat, especially in humid climates. This disease not only severely reduces yields due to shriveled seeds but also produces deoxynivalenol (DON), which is a danger to human health (Bai and Shaner 2004). Chemical control and agronomic measures are alternatives used to prevent this disease, but the development of FHB-resistant cultivars has been the most effective strategy for controlling the disease (Bai and Shaner 2004). Unfortunately, to date, wheat FHB resistance has not significantly improved due to the severe shortage of FHB-resistant germplasms, as well as the diversity of exhibited resistance and the complexity of genetic behavior (Liu et al. 2015; Mesterhazy 1995).

To develop resistant cultivars, the identification of new wheat FHB-resistant germplasms and the further utilization of new FHB resistance genes or quantitative trait loci (QTLs) are important tasks in breeding programs (Mesterhazy 1995). In the past, studies on the improvement of FHB resistance in hexaploid wheat have mainly focused on well-known resistant germplasms, such as Wangshuibai (Ma et al. 2008; Xiao et al. 2013; Xue et al. 2010, 2011; Zhou et al. 2004) and Sumai 3 (Cai and Bai 2014; Kolb et al. 2001; Waldron et al. 1999).

Genetic research has revealed that FHB resistance is usually a quantitative trait and controlled by multiple genes (Zhang et al. 2010; reviewed by Buerstmayr et al. 2009). To date, only seven major FHB resistance QTLs have been reported: *Fhb1* (Cuthbert et al. 2006; Liu et al. 2006), *Fhb2* (Cuthbert et al. 2007), *Fhb3* (Qi et al. 2008), *Fhb4* (Xue et al. 2010), *Fhb5* (Xue et al. 2011), *Fhb6* (Cainong et al. 2015), and *Fhb7* (Guo et al. 2015). In addition, numerous QTLs are reportedly associated with FHB resistance (reviewed by Buerstmayr et al. 2009). In fact, only few FHB resistance QTLs have been applied for wheat FHB resistance improvement due to their weak resistance, poor adaptation, and complex genetic backgrounds. Globally, wheat breeding heavily relies on the FHB resistance QTLs from Sumai 3 and its derivatives (Rudd et al. 2001). Therefore, it is very important to identify new resistance sources in wheat breeding programs.

Alien gene transfer is an important means of developing new resistant germplasms and increasing the genetic diversity of wheat FHB resistance. Of the seven permanently designated *Fhb* loci, three loci were transferred from wild relatives: *Fhb3* from *Leymus racemosus* (Qi et al. 2008), *Fhb6* from *Elymus tsukushiensis* (Cainong et al. 2015), and *Fhb7* from *Thinopyrum ponticum* (Guo et al. 2015). *Thinopyrum intermedium* (Host) (Barkworth and D. R. Dewey) (2n = 6x = 42; JJJ*J*SS) (syn. *Elytrigia intermedia* (Host) Nevski) is a useful source of resistance to FHB (Fedak and Han 2005; Liu et al. 2015), as well as against stripe rust and powdery mildew (Huang et al. 2014; Liu et al. 2013; Luo et al. 2009a, b; Shen et al. 2015).

The line L693 (Reg. No. GP-972, PI 672538) derived from the wild cross between common wheat and *Thinopyrum intermedium* (Liu et al. 2015) exhibits strong FHB resistance, which has been repeatedly evaluated by point- and spray-inoculation in multiple years and locations (Liu et al. 2015; Yang et al. 2016). This line also shows high resistance to stripe rust (Huang et al. 2014; Luo et al.

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*The e-Xtra logo stands for “electronic extra” and indicates that seven supplementary figures and two supplementary tables are published online.

2009a; Li et al. 2015) and powdery mildew (Luo et al. 2009b; Ma et al. 2015). Genomic in situ hybridization demonstrated the absence of alien chromosomal fragments in PI 672538 (Li et al. 2015). Moreover, PI 672538 has suitable agronomic and morphological traits (Liu et al. 2015). All data imply that PI 672538 had genes that were resistant to multiple diseases, especially FHB resistance. Therefore, characterizing the genetic behavior of FHB resistance and mapping the resistance QTLs in this line are valuable to wheat breeding.

To identify the genes affecting FHB resistance as quantitative traits, we first map the QTLs affecting the trait using early segregating populations, such as the F_2 and $F_{2:3}$ populations (Tamburic-Illincic et al. 2002; Zhang et al. 2010). Next, we focus on each QTL region and further narrow the genomic intervals that carry the gene or genes affecting this resistance with a secondary mapping population. For example, the chromosomal intervals of *Fhb2* (Cuthbert et al. 2007), *Fhb4* (Xue et al. 2010), *Fhb5* (Xue et al. 2010), and *Fhb7* (Guo et al. 2015) were narrowed by recombinant inbred lines (RILs). Additionally, the chromosomal intervals of *Fhb1* (Cuthbert et al. 2006), *Fhb4* (Xue et al. 2010) and *Fhb5* (Xue et al. 2010) were also narrowed by near isogenic lines (NILs), while some FHB resistance QTLs were narrowed by doubled haploid (DH) lines (Somers et al. 2006). Finally, we can pinpoint and clone the genes by chromosome walking or homogenous cloning. The early generation mapping populations are critical to the study of the uncertain genetic components in novel FHB resistance germplasm. First, the primary mapping F_2 and $F_{2:3}$ populations play a key role in elucidating how many genes control FHB resistance and how the genes interact to cause resistance. Second, the primary mapping F_2 and $F_{2:3}$ populations are essential in determining the original chromosomal locations of the FHB resistance QTLs, although the genetic mapping of the resistance QTLs with these populations has large confidence intervals due to the limitation of population size and marker density (Xue et al. 2010). Third, using the F_2 and $F_{2:3}$ populations shortens the time required to construct the RIL population, which is useful for narrowing down the gene mapping on a finer scale. Moreover, a recent study suggested that F_2 populations could be effective indicators of the expression levels of FHB resistance QTLs prior to the secondary mapping population (Dvorjak 2014).

To accelerate the application of wheat resistance germplasm PI 672538 in wheat breeding, the objectives of this research were to study the inheritance of FHB resistance in PI 672538 and to construct a genetic map of the genes with simple sequence repeat (SSR) markers to determine the chromosomal locations of the genes. We also screened some closely linked markers to aid the molecular marker-assisted selection of FHB resistance.

MATERIALS AND METHODS

Plant materials. A total of 229 F_2 plants from the cross L661 $\times$ PI 672538 and the corresponding $F_{2:3}$ families were used to map the QTLs. The wheat line PI 672538 was resistant to FHB (Liu et al. 2015; Zhang et al. 2011), whereas L661 was susceptible to FHB. The parents PI 672538 and L661 are sister lines with similar genetic backgrounds (Huang et al. 2014; Li et al. 2015) and were used to study the inheritance of FHB resistance. In addition, Chinese Spring nulli-tetrasomic (NT) lines were used to identify the chromosomal location of the resistance QTLs and the linked markers.

Evaluation of the reaction to FHB. The parental lines L661 and PI 672538 and the 229 F_2 plants were grown spaced out individually in Ya'an (lat. 29°59'N, long. 102°58'E) in 2011. The L661 and PI 672538 lines and 229 $F_{2:3}$ plants were grown in three locations, Ya'an (lat. 29°59'N, long. 102°58'E), Qionglai (lat. 30°24'N, long. 103°32'E), and Wenjiang (lat. 30°43'N, long. 103°52'E), to evaluate the resistance to FHB in field trials in 2012. A completely random block design was used in every replication at each location. Each plot consisted of 3-m long rows, and the space

between rows was 0.25 m with 0.15 m of plant space. To determine FHB resistance, 5 μ l of a macroconidial suspension (200 macroconidia per μ l) of the spore-derived isolate of *F. graminearum* No. 4 (provided by Z. Ma at Nanjing Agricultural University, Nanjing, Jiangsu Province, China) was injected into two opposite florets in a spikelet at early anthesis; four spikes of each F_2 individual were inoculated in 2011, and the phenotype of the given F_2 plant is presented as the average of the four spikes. For each $F_{2:3}$ population in 2012, 10 plants were selected at each location, and three spikes of each plant were inoculated. The average of three locations represents the phenotypic value of the corresponding $F_{2:3}$ plant for mapping.

The inoculated spikes were covered with plastic bags for 3 days to maintain the relative humidity. The percentage of visibly diseased spikelets (PDS), including the inoculated spikelets, was recorded at 21 days after inoculation. At the maturity stage, the inoculated spikes were carefully harvested by hand and then dried at 35°C. The number of kernels per spike and the number of damaged kernels were counted after threshing, and the total grain weight per spikes was evaluated. The data were used to calculate the percentage of damaged kernels (PDK) and the 1,000-grain weight (KGW).

Molecular marker screening. Genomic DNA was extracted from 5-week-old seedling leaves using the CTAB method

Fig. 1. Wheat reaction to *Fusarium graminearum* A, PI 672538 spike, B, L661 spike, C, PI672538 seed, and D, L661 seed. The photos of the spikes were taken 21 days after *F. graminearum* inoculation.

previously described (Tai and Tanksley 1990). Forty-one polymorphic primers out of 781 microsatellites (from the *gwm* and *wmc* series) between L661 and PI 672538 (Huang et al. 2014) were directly applied to screen the whole $F_{2:3}$ mapping population. PCR amplification was performed according to a previously described method with minor modifications (Röder et al. 1998a, b). Each PCR mixture contained each SSR primer at a concentration of 200 nmol/liter, 0.2 mmol/liter dNTPs, 1.5 mmol/liter $MgCl_2$, 1 unit of *Taq* polymerase, and 50 ng of template DNA. PCR was performed following a previously described program (Luo et al. 2008). Each PCR product was mixed with 3 μ l of loading buffer (98% formamide, 10 mM EDTA [pH 8.0], 0.25% bromophenol blue, and 0.25 mg/ml xylene cyanol), denatured at 95°C for 5 min, and chilled on ice. Subsequently, a 6- μ l aliquot of each sample was loaded onto a 6% polyacrylamide gel (19:1 acrylamide-bis-acrylamide) with 8 M urea and 1 $\times$ TBE (90 mM tris-borate [pH 8.3], 2 mM EDTA) prior to separation at 80 W for approximately 1.5 h and visualization by silver staining (Bassam et al. 1991).

Based on the original linkage analysis, we determined that two primers, *Xwmc441-2B* and *Xgwm566-3B*, out of the 30 polymorphic primers from the *gwm* and *wmc* series (Huang et al. 2014) were linked with the FHB resistance QTLs in PI 672538 to some degree. Therefore, to verify the linkage relationship between the FHB resistance QTLs and markers, we further employed the other microsatellite primers, including the *gwm* (Röder et al. 1998b), *wmc* (Gupta et al. 2002; Somers et al. 2004), *barc* (Song et al. 2002), *gpw* (Sourdille et al. 2004), *cfb* (Guyomarc'h et al. 2002), *cni* (Ma et al. 1996), *mag* (Xue et al. 2008), and *psp* (Bryan et al. 1997; Stephenson et al. 1998) series and the EST primer on the 2B and 3B chromosome, in PCR amplification. Nineteen published SSR primers on the 3B chromosome were linked with the FHB resistance QTLs, and 14 published SSR primers and 1 published EST primer, BF478477 (Shen et al. 2015), on the 2B chromosome were also linked with the FHB resistance QTLs in PI 672538. After linkage analysis, we further saturated 2B by developing 40 new SSR primers according to the contig sequences carrying the markers that mapped to chromosome 2B using the previously described methods (Shen et al. 2015; Supplementary Table S1).

Statistical analysis. Significant differences among the averages of the populations L661 and PI 672538 were determined by independent sample *t* test using SPSS 19 (IBM SPSS Statistics for Windows, Version 19.0. IBM Corp., Armonk, NY), and the correlation analysis was determined with the same software using a bivariate method.

Linkage map construction and QTL analysis. The phenotype data were first quality controlled using QTmodel software (Yang et al. 2007) at a *t* value threshold of 3.0. The genetic map was constructed with JoinMap 4.0 (Van Ooijen 2006) using the regression mapping algorithm and Kosambi (1943) mapping function. The logarithm of an LOD value of 3.0 was set as the threshold for grouping. The markers associated with FHB resistance were detected by simple regression analysis using SPSS software. The QTL analysis was carried out through composite interval mapping (CIM) for linkage groups using WinQTLCart 2.5 (Wang et al. 2007) and interval mapping (IM) for unlinked markers using MapQTL 5.0 (Van Ooijen 2004). The threshold of the LOD score for determining the significance of a QTL was determined according to a permutation test with 1,000 runs. The LOD peaks of the significant QTLs were considered the QTL locations on the linkage map.

Chromosomal assignment of the FHB resistance QTLs. The linked SSR markers were assigned to specific chromosomes (Röder et al. 1998 a, b; Somers et al. 2004), but microsatellite markers are not always chromosome-specific because of shared partial homology (Plaschke et al. 1996). Therefore, to further ensure the accurate chromosomal location of the linked microsatellite markers, the following lines were used: Chinese Spring nullisomic 2A tetrasomic 2B (N2AT2B), nullisomic 2A tetrasomic 2D (N2AT2D), nullisomic 2B tetrasomic 2A (N2BT2A), nullisomic 2B tetrasomic 2D (N2BT2D),

nullisomic 2D tetrasomic 2A (N2DT2A), nullisomic 2D tetrasomic 2B (N2DT2B), nullisomic 3A tetrasomic 3B (N3AT3B), nullisomic 3A tetrasomic 3D (N3AT3D), nullisomic 3B tetrasomic 3A (N3BT3A), nullisomic 3B tetrasomic 3D (N3BT3D), nullisomic 3D tetrasomic 3A (N3DT3A), and nullisomic 3D tetrasomic 3B (N3DT3B). All lines were provided by D. C. Liu, Triticeae Research Institute, Sichuan Agricultural University, Chengdu, Sichuan.

RESULTS

FHB reaction in the F_2 and $F_{2:3}$ populations. The FHB resistance test revealed that PI 672538 was resistant to FHB; L661 was susceptible to FHB (Fig. 1); and their respective PDS, PDK, and KGW values were different at $P = 0.05$ (Table 1) (Liu et al. 2015). The data in Table 1 and Supplementary Table S2 show that all three resistance parameters exhibited continuous variation to some degree. The distribution of the resistance parameters is shown in Figure 2. The F_2 population in 2011 exhibited two peaks in the PDS, whereas the $F_{2:3}$ population in 2012 exhibited only one peak; the average PDS in the $F_{2:3}$ population is lower than that in the F_2 population (Fig. 2A). The PDK and KGW showed only one peak in both populations (Fig. 2B and C). The data in Table 1 and the distribution of the resistance parameters in Figure 2 imply that FHB resistance is controlled by more than one gene.

The correlation analysis between the F_2 and $F_{2:3}$ populations revealed a significant relevance of the PDS and PDK at $P = 0.05$ (Table 2); only the relationship between the KGW of the $F_{2:3}$ population and the three parameters of the F_2 population was not significant, whereas all other relationships both within populations and among populations were significant at the $P = 0.05$ level.

Linkage molecular marker screening. We originally found that two SSR markers, *Xwmc441* on chromosome 2B and *Xgwm566* on chromosome 3B, out of the 41 primary polymorphic SSR markers from the *gwm* and *wmc* series were associated with FHB resistance. Following this, we further screened all available markers on 2B and 3B and found that 22 markers covering 135.51 cM with an average distance of 6.15 cM between the two markers and 19 markers on 3B covering 96.77 cM with an average distance of 5.38 cM were linked with FHB resistance. The order of the markers on the chromosomes corresponds well with the reference genetic map (Supplementary Figs. S1, S2, and S3) (Somers et al. 2004).

Mapping of major QTLs. From the 1000-run permutation test, the threshold of the LOD value was determined at 2.5, when the QTL possibility is greater than 95%. We detected two QTLs by composite interval mapping (CIM) using WinQTLCart 2.5 for PDS on chromosomes 2B and 3B: one on 2B and two closely linked QTLs on 3B. In the F_2 population, the QTL on 2B was flanked by markers *Xbarc55-2B* and *Xcn16-2B*, explaining 11.6% of the phenotypic variation, and the QTL on chromosome 3B was flanked by *Xwmc54-3B* and *Xwmc615-3B*. In the $F_{2:3}$ population, the QTL on 2B was flanked by *Xbarc55-2B* and *Xbarc1155-2B*, explaining 7.1% of the phenotypic variation, and the QTL on 3B was flanked by *Xwmc54-3B* and *Xgwm566-3B*, explaining 10% of the phenotypic variation. In addition, we also detected the QTLs for PDK and KGW in both populations at similar chromosomal locations (Table 3).

The data in Table 3 and Figure 3 show that the FHB resistance in this study was mainly controlled by two QTLs on 2B and 3B, designated *Qfhs-2B* and *Qfhs-3B*. *Qfhs-2B* was flanked by markers *Xbarc55-2B* and *Xbarc1155-2B* (Fig. 3), and *Qfhs-3B* was flanked by *Xwmc231-3B* and *Xwmc54-3B* (Fig. 3). Based on the major effect, we designated *Qfhs-2B* as *FhbL693a* and *Qfhs-3B* as *FhbL693b*.

Chromosome assignment of the major QTLs. Based on the published chromosomal locations of the linked markers (Röder et al. 1998 a, b; Somers et al. 2004; Song et al. 2002), *Qfhs-thin-2B* and *Qfhs-thin-3B* were located on chromosome 2B and 3B, respectively. We further verified the chromosomal location of the linked markers with Chinese Spring nulli-tetrasomic lines (Table 4). Seventeen out of the 22 markers linked with *Qfhs-2B* were specific

to chromosome 2B, and 14 out of 19 markers linked with *Qfhs-3B* were specific to chromosome 3B. Therefore, *Qfhs-2B (FhbL693a)* and *Qfhs-3B (FhbL693b)* were mapped on 2B and 3B, respectively.

DISCUSSION

The mode of inheritance of FHB resistance. Growing resistant cultivars is one of the most effective, economical, and environmentally sound measures for controlling wheat FHB; therefore, identifying new sources of FHB resistance is very important in wheat breeding programs (Zhang et al. 2008). PI 672538 showed resistance to wheat FHB in different locations and years (Fig. 1) (Yang et al. 2016). Although there was no difference in the PDS between PI 672538 and the known resistant controls Sumai 3 and Wangshuibai, the PDS of PI 672538 was slightly lower than that of Sumai 3 and Wangshuibai (Liu et al. 2015). In addition to FHB resistance, PI 672538 was resistant to stripe rust (Huang et al. 2014), powdery mildew (Liu et al. 2015; Ma et al. 2015). Therefore, PI 672538 is valuable wheat germplasm for the improvement of wheat disease resistance.

Elucidating the inheritance of FHB resistance in PI 672538 is critical to the utilization of PI 672538 in wheat breeding. FHB resistance has usually been treated as a quantitative trait controlled

by multiple genes (reviewed by Buerstmayr et al. 2009). Genetic analysis demonstrated that the genes for FHB resistance in Sumai 3 were assigned to chromosomes 2A, 5A, 7A, 1B, 2B, 3B, 6B, 6D, and 7D (Yao et al. 1996; Yu 1982). Consequently, further study revealed that FHB resistance was mainly conferred by the major QTL *Fhb1* (Cuthbert et al. 2006), which implied a few QTLs with a large effect on FHB resistance.

In this study, the PDS data distribution exhibited two peaks in the F_2 population in 2011 but only one peak in the $F_{2:3}$ population in 2012 (Fig. 2), which implies that the FHB resistance of PI 672538 is controlled by more than one gene, even though the number of genes responsible for this resistance is small. In addition, our previous studies showed that the resistance response of PI 672538 was different from those of other published germplasms (Liu et al. 2015), which further indicated that the genetic component of FHB resistance is possibly different from other FHB-resistant germplasms.

Chromosomal location and genetic mapping of FHB resistance QTLs. We detected two QTLs using WinQTLCart 2.5 software (Wang et al. 2007) in the F_2 and $F_{2:3}$ populations: *Qfhs-thin-2B* and *Qfhs-thin-3B* (Fig. 3). Almost all of the resistance parameters, including PDS, PDK, and KGW, exhibited good chromosomal agreement (Supplementary Fig. S4), which suggests that FHB QTLs exist in the chromosomal region. The locations of 17 out

TABLE 1. Means of Fusarium head blight resistance traits including the percentage of diseased spikelets (PDS), percentage of damaged kernels (PDK), and 1,000-grain weight (KGW) of the parent and population lines^z

Year	Line	PDS			PDK			KGW (g)		
		Mean	SE	Sig	Mean	SE	Sig	Mean	SE	Sig
2011	F_2 mean	0.457	0.166	b	0.206	0.110	b	19.837	0.504	b
	L661	0.739	0.034	c	0.484	0.097	a	12.679	1.671	a
	PI 672538	0.161	0.019	a	0.073	0.064	c	24.666	1.074	c
2012	$F_{2:3}$ mean	0.219	0.059	b	0.482	0.096	b	24.855	0.326	b
	L661	0.534	0.062	c	0.748	0.089	a	18.982	2.334	a
	PI 672538	0.104	0.010	a	0.266	0.039	c	33.670	1.509	c

^z SE stands for the standard error; Sig stands for the significance level; a, b, and c stand for significant differences at the 0.05 level for one trait within 1 year. Data for PDS and KGW were published previously (Liu et al. 2015).

Fig. 2. Distribution of Fusarium head blight-related traits in the L661 × PI 672538 F_2 population evaluated in 2011 and 2012. M, the mean of the population; P1, the parent line L661; P2, the parent line PI 672538. **A**, Distribution of the percentage of diseased spikelets (PDS). **B**, Distribution of the percentage of damaged kernels (PDK). **C**, Distribution of the 1,000-grain weight (KGW).

of the 22 markers linked with *Qfhs-thin-2B* were further verified on chromosome 2B using the Chinese Spring nulli-tetrasomic line (Table 4, Shen et al. 2015). At the same time, the location 14 out of the 19 markers linked with *Qfhs-thin-3B* were also verified on chromosome 3B using the same method (Table 4). The total genetic distance of the linkage mapping of *Qfhs-2B* was 130 cM, including 22 markers, and that of *Qfhs-3B* was 100 cM, including 19 markers.

To date, various researchers have detected the FHB resistance QTL on chromosome 2B from different plant materials, including Renan (Gervais et al. 2003) and Goldfield (Gilsinger et al. 2005) for type I resistance; Dream (Schmolke et al. 2005), Ernie (Liu et al. 2007), G16-92 (Schmolke et al. 2005), *Triticum durum*

‘Strongfield’ (Somers et al. 2006) and Ning 7840 (Zhou et al. 2002) for type II resistance; and Frontana (Steiner et al. 2004) for both type I and type II resistance. However, only two major QTLs have been reported on chromosome 2B to the best of our knowledge. *QFhs.inra-2b*, flanked by *Xgwm388-Xgwm257*, was located on the centromere regions of chromosome 2B mapped in the RILs derived from two European winter wheat cultivars, Renan and Récital (Gervais et al. 2003). The other QTL, named *Qfhs.unc-2B*, was linked with *Xgwm278-2B* on the chromosome arm 2BS and identified in the soft red winter wheat Ernie (Liu et al. 2007). We located a major QTL named *Qfhs-2B* on wheat chromosome 2B that explained 9.2 to 11.6% of the phenotypic variation of the PDS (Fig. 3; Table 3). An FHB resistance QTL was reported in the chromosomal region in which *Qfhs-2B* was mapped in *Triticum durum* (Somers et al. 2006), but there was no report of the genetic background of this wheat (reviewed by Buerstmayr et al. 2009). *Qfhs-2B* mapped in this study explained 9.2 to 11.6% of the phenotypic variation for the PDS (Fig. 3; Table 3). *Qfhs-2B* was located on the distal end of the chromosome arm 2BL, which is different from the previously reported QTLs; therefore, we designated *Qfhs-thin-2B* as *FhbL693a*.

FHB resistance QTLs on 3B have been widely reported and mainly derived from Sumai 3 and its derivatives (Anderson et al. 2001; Ban and Suenaga 2000; Buerstmayr et al. 2002; Makandar et al. 2006), Wangshuibai (Lin et al. 2006; Mardi et al. 2005), and Frontana and its derivatives (Han et al. 2005; Mardi et al. 2006). The well-known FHB resistance QTL on 3BS is *Fhb1*, which is derived

TABLE 2. Correlation analysis between the field test data: the percentage of diseased spikelets (PDS), percentage of damaged kernels (PDK), and 1,000-grain weight (KGW)^z

	PDS 2011	KGW 2011	PDK 2011	PDS 2012	KGW 2012	PDK 2012
PDS 2011						
KGW 2011	-0.649**					
PDK 2011	0.776**	-0.575**				
PDS 2012	0.846**	-0.522**	0.574**			
KGW 2012	-0.112	0.052	-0.01	-0.178**		
PDK 2012	0.522**	-0.253**	0.406**	0.557**	-0.222**	

^z ** Indicates significant correlation at the 0.01 level (bilateral); * indicates significant correlation at the 0.05 level (bilateral).

TABLE 3. Quantitative trait loci (QTLs) in the L661 × PI 672538 inbred F₂ and F_{2,3} line populations associated with the percentage of diseased spikelets (PDS), percentage of damaged kernels (PDK), and 1,000-grain weight (KGW)

Year	QTLs	Flanking markers	Range	Traits	Chr	Closest marker	Position (cM)	LOD ^z	Additive	Dominant	R ²
2011	<i>Qfhs-thin-1B</i>	<i>Xwmc419-1B-Xbarc187-1B</i>	9.01–16.88	KGW	1B	<i>Xwmc85.2-3B</i>	10.56	2.82	-2.498	2.248	6.9%
	<i>Qfhs-thin-2B</i>	<i>Xbarc55-2B-Xcnl6-2B</i>	115.4–128.25	PDS	2B	<i>Xbarc1064-2B</i>	120.40	5.02	0.122	-0.056	11.6%
	<i>Qfhs-thin-3B</i>	<i>Xwmc54-3B-Xwmc615-3B</i>	26.55–34.18	KGW	3B	<i>Xwmc54-3B</i>	27.55	5.50	3.292	4.461	8.7%
		<i>Xwmc54-3B-Xwmc615-3B</i>	26.55–37.18	PDK	3B	<i>Xcfd4-3B</i>	30.18	4.45	-0.073	-0.054	8.6%
		<i>Xwmc54-3B-Xwmc615-3B</i>	27.55–34.18	PDS	3B	<i>Xcfd4-3B</i>	29.18	5.92	-0.029	-0.141	0.4%
2012	<i>Qfhs-thin-1B</i>	<i>Xwmc419-1B-Xbarc187-1B</i>	0.01–25.88	KGW	1B	<i>Xgwm11-1B</i>	16.88	10.37	-3.117	1.758	27%
	<i>Qfhs-thin-2B</i>	<i>Xbarc1064-2B-Xbarc1155-2B</i>	117.4–134.78	KGW	2B	<i>Xcnl6-2B</i>	130.78	3.63	-2.100	0.748	9.2%
		<i>Xbarc55-2B-Xbarc1155-2B</i>	115.4–132.78	PDS	2B	<i>Xbarc1064-2B</i>	119.40	3.11	0.035	-0.008	7.1%
	<i>Qfhs-thin-3B</i>	<i>Xwmc54-3B-Xgwm566-3B</i>	26.55–44.56	PDS	3B	<i>Xcfd4-3B</i>	32.18	8.17	-0.043	-0.049	10%

^z The critical logarithm of the odds (LOD) threshold of 2.5 for the 0.05 level of probability was obtained through a 1,000-run permutation test.

Fig. 3. Quantitative trait loci (QTLs) for the four Fusarium head blight-related traits on the four linkage groups in 2011 (left) and 2012 (right). The x-axis is the Morgan distance (cM); the y-axis is the LOD value. A, Two QTLs on the linkage group of wheat chromosome 2B in 2011, and three QTLs in 2012. B, Three QTLs on the linkage group of wheat chromosome 3B in 2011, and one QTL in 2012.

from Sumai 3 and has been mapped between *Xgwm493* and *Xgwm533* (Cuthbert et al. 2006). In our study, *Qfhs-3B* was also mapped on the chromosome arm 3BS, which was flanked by *Xwmc231-3B* and *Xwmc54-3B* and was closer to the centromere region compared with *Fhb1* (Fig. 3), implying that *Qfhs-3B* is not *Fhb1* (Supplementary Fig. S5). In addition, the resistance response of PI 672538 also obviously differs from that of Sumai 3 (Zhang et al. 2011; Liu et al. 2015), and the pedigree of PI 672538 and L661 did not contain Sumai 3 or its derivatives (Liu et al. 2015; Zhang et al. 2011). Most importantly, we did not detect *Fhb1* in the parent lines PI 672538 and L661 using the diagnostic markers *cfb6058* and *cg8-Agro* developed by Professor Guihua Bai's group (G. Bai and Z. Su at Kansas State University, *personal communication*; Supplementary Figs. S6 and S7). Considering all of the evidence, we can conclude that *Qfhs-3B* is different from *Fhb1* and is therefore designated as *FhbL693b*.

In addition, we also observed differences in the contribution size and existence of QTLs detected on 2B and 3B between 2011 and 2012 (Table 3; Fig. 3) that possibly resulted from three main phenomena. First, the FHB resistance was mainly afforded by the QTLs, which can be easily influenced by environmental variations (Anderson et al. 2001). Second, the sample size in 2011 was small compared with that in 2012 because of the randomly segregated F_2

individuals. Finally and more importantly, the difference in the genetic components of F_2 and $F_{2,3}$ could have influenced the phenotypic data distribution.

Origin of FHB resistance and the putative mechanism of FHB resistance transfer. Alien chromosomal translocation is usually employed for transferring valuable genes from wild relative to wheat. Transferring large chromosome segments, often carrying genes for both desirable and undesirable traits and resulting in 'linkage drag', has not been widely used in wheat breeding (Young and Tanksley 1989). However, cryptic translocation with only the traits of interest and without detectable cytological or genetic changes has been reported (Kuraparthi et al. 2007). *Thinopyrum intermedium* is in the pedigree of both PI 672538 and L661. A recent study demonstrated that the YU25, PI 672538, and L661 wheat genotypes do not have a cytologically detectable alien chromosome segment and therefore may contain a cryptic translocation (Li et al. 2015). In addition, wheat-specific products were amplified by almost all of the wheat SSR primers, evenly distributed over all of the chromosomal arms with small spaces, which also indicates that a large foreign chromosomal segment is not present in PI 672538 or L661 (Huang et al. 2014; Li et al. 2015). This observation is similar to that observed in YU25 (Luo et al. 2009a, b). Therefore, we cannot exclude the possibility that the FHB resistance in PI 672538 was

TABLE 4. Linkage markers in groups 2B and 3B

Markers	Forward primer 5'-3'	Reverse primer 5'-3'	CM	Chromosome location	Type
<i>Xwmc764-1-2B^w</i>	AACAAAGCGTGCCTAATGCT	CCTGAAGCTCTGATGCCAAT	0	2B	Codominant
<i>Xwmc661-2-2B^w</i>	GAGAGGAGGCCTCACACTTTT	CATGTGGCATCGTGATGAAT	5.884	2B	Dominant
<i>Xgwm210-1-2B^w</i>	TGGAAGTCATGTGGCATTGT	CTCCAGTGTGTGGCACTATGG	6.546	2A, 2B, 2D	Dominant
<i>Xmag3930-2-2B^w</i>	TTGCATTGTCTTGATGATGG	CTGATCCCATGAACCAAGG	7.682	2B	Codominant
<i>Xwmc314-2B^x</i>	ACACGGGGTCTGATTGCTTTAC	ATCGCTTTTGTACAAGTGAGGC	8.603	2B	Dominant
<i>Xgpm2039-2B^x</i>	ACCGGGAGTCCTTGTCTTTT	AGCTTACCAGGTCGGACAAA	11.346	2B	Dominant
<i>Xgwm210-2B^x</i>	TGCATCAAGAATAGTGTGGAAG	TGAGAGGAAGGCTCACACCT	40.732	2A, 2D	Dominant
<i>Xwmc243-2B^x</i>	CGTCATTTCCTCAAACACACCT	ACCGGCATGTTGACAATAGT	52.781	2B	Codominant
<i>Xwmc154-2B^x</i>	ATGCTCGTCAGTGTGATGTTTG	AAACGGGAACCTACCTCACTCTT	64.174	2B	Codominant
<i>Xbarc200-2B^x</i>	AGCTTACCAGGTCGGACAAA	GCGATATGATTGTGAGCTGATTG	69.314	2B	Dominant
<i>BF478477^y</i>	AGCTACTCCACGCCCTCAGTA	AGCCCAGAGTAAGCAGCATC	71.972	2B	Dominant
<i>Xbarc318-2B^x</i>	CGACTAGACAATTTCATT	TGATTTTCGCTAACAAGG	73.266	2B	Codominant
<i>Xgpm4085-2-2B^w</i>	TTTTGAGTTGGGCTGGTTTC	AGGTCATGGCCTGTTGTTTC	85.083	2B	Dominant
<i>Xgpm1148-6-2B^w</i>	GCATGCATAACACCAACGTC	GCTCGGGTACATTTTGCTGCT	87.021	2B	Codominant
<i>Xgpm4307-4-2B^w</i>	TGCATGATGTAATAAATCCCTCA	CAAAGAGAAAGGGGGACACA	89.81	1D, 2B, 2D	Codominant
<i>Xgwm410-2B^x</i>	GCTTAGACCGGCACAGT	CGAGACCTTGAGGGTCTAGA	90.838	2B	Codominant
<i>Xgwm148-2B^x</i>	GTGAGGCAGCAAGAGAGAAA	CAAAGCTTGACTCAGACCAAA	92.777	2B	Codominant
<i>Xbarc55-2B^x</i>	GCATCTGGTACACTAGCTGCC	TCATGGATGCATCACATCCT	99.885	Ambiguous ^z	Codominant
<i>Xbarc1064-2B^x</i>	CGGTATTATCTCAAGATCAGAGGCACTA	GCGTTCTGGGGATTTCCTCTACCAATTAT	115.387	2B	Codominant
<i>Xwmc441-2B^x</i>	TCCAGTAGACACCTTTCATT	ATCACGAAGATAAACAACCG	124.238	Ambiguous ^z	Dominant
<i>Xcml6-2B^x</i>	GGACGAATGAAAAATCCCAAGG	GAGCGGTCTTCGGACTTTC	128.771	2B	Dominant
<i>Xbarc1155-2B^x</i>	TGCAAACATGAAACAATCCTCCAC	TCCACCTCGTCTCCTCTACACC	135.151	2B	Dominant
<i>Xbarc102.1-3B^x</i>	GGAGAGGACCTGCTAAATCGAAGACA	GCGTTTACGGATCAGTGTGGAGA	0	3B	Dominant
<i>Xbarc101a-3B^x</i>	GCTCCTCTCAGATCACGCAAG	GCGAGTCGATCACATATGAGCCAATG	22.97	3B	Dominant
<i>Xwmc623-3B^x</i>	ACGATTGGCCACAGAGGAG	CAGTGACCAATAGTGGAGGTCA	24.168	Ambiguous ^z	Codominant
<i>Xwmc54-3B^x</i>	TATTGTGCAATCGCAGCATCTC	TGCGACATTGGCAACCCTCT	25.54	3B	Codominant
<i>Xcfd4-3B^x</i>	TGCTCCGTCTCCGAGTAGAT	GGGAAGGAGAGATGGGAAAC	28.172	3B, 3D	Codominant
<i>Xwmc615-3B^x</i>	TGCCCACAACCTTATCTCAG	GGTAAGTGGCCAGGTAGT	37.552	3B	Dominant
<i>Xwmc231-3B^x</i>	CATGGCGAGGAGCTCGGTGGTC	GTGGAGCACAGGCGGAGCAAGG	44.822	3B	Codominant
<i>Xgwm566-3B^x</i>	TCTGTCTACCATGGGATTG	CTGGCTTCGAGGTAAGCAAC	47.46	3B	Dominant
<i>Xgpm1120-3B^x</i>	ATTGCGCAAGATTGGTAGGT	AAGCGATGCTACCCCATAAA	49.234	3B	Codominant
<i>Xpsp3030.1-3B^x</i>	ATTCGGCTGAGAAAAGAGGC	TTGTTGACTTGGTCAGTCGG	50.882	3B	Codominant
<i>Xgwm285-3B^x</i>	ATGACCCTTCTGCCAAACAC	ATCGACCGGGATCTAGCC	51.72	3B	Codominant
<i>Xwmc544-3B^x</i>	CCATTGTAGGTTTGGTCGCTAC	TATATGTGATTGTGCTGCCCC	52.22	3B	Dominant
<i>Xwmc505-3B^x</i>	AGGGGAGGAAAACCTTGTAATC	ACGACCTACGTGGTAGTTCTTG	53.247	3A, 3B	Dominant
<i>Xwmc540-3B^x</i>	CGGGGTCTGAACTACGGTGA	CCTGTAATGGGCAATATAAGT	54.181	3B	Dominant
<i>Xgpm1146a-3B^x</i>	CCACCTCCGTCTTCGAGTAG	GGGAAGGAGAGATGGGAAAC	57.749	3B, 7B	Codominant
<i>Xwmc777-3B^x</i>	GCCATCAAGCGGATCAACT	GTAGCGCCCTGTTTCACCTC	59.784	3B	Dominant
<i>Xbarc68-3B^x</i>	CGATGCCAACACACTGAGGT	AGCCGCATGAAGAGATAGGTAGAGAT	63.537	3B, 5A, 6B	Dominant
<i>Xwmc471-3B^x</i>	GGCAATAATAGTGCAAGGAATG	GCCGATAATGGGCAATATAAGT	75.792	3B	Codominant
<i>Xbarc344-3B^x</i>	GCGCGTCGACATGTATTCTTGAT	GCGTTTCATCTGGTATCTGGTGTAT	96.764	3B	Codominant

^w Newly developed simple sequence repeat (SSR) markers.

^x Published SSR markers.

^y Newly developed EST markers.

^z Marker location cannot be determined by nulli-tetrasomic lines.

transferred from *Thinopyrum intermedium* into wheat by cryptic translocation.

In addition, we also cannot exclude the possibility that the FHB resistance in PI 672538 resulted from DNA sequence changes in the wheat genome during wide crosses. In rice, DNA sequence deletion resulted in the production of a new blast resistance gene *Pi21* (Fukuoka et al. 2009). In PI 672538, a 36-bp deletion of a wheat DNA sequence on 1B resulted in a putative wheat copper-binding protein (*WCBP1*) that conferred resistance to strip rust (Li et al. 2015). Based on the available evidence, we cannot be certain whether *FhbL693a* and *FhbL693b* were directly transferred from *Thinopyrum intermedium* into wheat or were indirectly produced by DNA sequence changes, especially deletions. This problem will be clarified by our future work.

Potential utilization of the FHB resistance QTLs in wheat breeding programs. Concerning wheat FHB resistance, Sumai 3, Wangshuibai, and their derivatives are well-known sources of FHB resistance, and the major resistance QTL *Fhb1* is located on the chromosomal arm 3BS (Cuthbert et al. 2006). However, it is difficult to use these sources in wheat breeding because they have many undesired agronomic traits. In our study, PI 672538 was highly resistant to wheat stripe rust and powdery mildew, in addition to its FHB resistance. Therefore, this germplasm will play an important role in improving wheat disease resistance because more attention is usually paid to the disease resistance level and comprehensive agronomic traits than to the genetic mechanism. In addition, PI 672538 and its derivatives have been widely used in wheat breeding programs in Henan, Shandong, Beijing, and Sichuan provinces. Therefore, PI 672538 will be an important disease-resistant source in the future.

ACKNOWLEDGMENTS

We thank the National Natural Science Foundation of China (31271721 and 31571661), the Key Projects of the Education Ministry of China (2012146), the Specific Foundation of Agronomy (nyhyzx3-15, 201303016), the bureau of Science and Technology of Fuling of Chongqing (FLKJ-2015ABB1048), and the Ministry of Science and Technology, China (2013CB127701) for financial support. We thank G. H. Bai (Kansas State University) for critically reviewing a draft of this manuscript.

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