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Fusarium head blight resistance loci in a stratified population of wheat landraces and varieties

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Abstract To determine if Chinese and Japanese wheat landraces and varieties have unique sources of Fusarium head blight (FHB) resistance, an association mapping panel of 195 wheat accessions including both commercial varieties and landraces was genotyped with 364 genome-wide simple sequence repeat and sequence-tagged site (STS) markers, and evaluated for type II FHB resistance in three greenhouse experiments using single floret inoculation. Population structure analysis stratified this population into five groups with Chinese landraces in four groups. Thirty-two of 51 Chinese landraces and 24 of 27 Japanese accessions were placed in one group. Association analysis using a mixed model identified 11 markers having significant associations with FHB resistance in at least two experiments. Most of these markers coincided with known quantitative trait loci (QTL) for FHB resistance with one potentially novel QTL associated with *Xgdm138-5DS* and *Xgwm358-5DS*. *Xbarc19-3AS* was significant in all three experiments, and the frequency of favorable alleles was more than 53 %. Chinese landraces and Japanese accessions had more favorable alleles at the majority of reproducible marker loci. Nine QTL combinations were identified according to the number of favorable alleles. Mean FHB severities increased with decreasing numbers of favorable alleles at reproducible loci. The resistance loci characterized here will further diversify the wheat FHB resistance gene pool, and provide breeders with additional sources of resistance for improvement of FHB resistance in wheat.

Dadong Zhang and Xiali Zhou have contributed equally to this work.

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Introduction

Fusarium head blight (FHB) caused mainly by *Fusarium graminearum Schwabe* [*teleomorph Gibberella zeae* (Schwein) Petch] is a destructive disease of wheat (*Triticum aestivum* L.) worldwide (Bai and Shaner 2004). Frequent FHB epidemics cause immense yield losses and quality deterioration problems that render infected grain unsuitable for human or animal consumption (Trail 2009). In China, FHB causes yield losses of up to 100 % in some locations (Zeng and Jiang 2013). The middle and lower reaches of the Yangtze River and northeastern spring wheat region are the most vulnerable regions for FHB epidemics. Since the 1980s, FHB has gradually spread to the wheat-growing areas between the Yellow and Huai River valleys due to expansion of irrigated areas and increased cultivation of maize with maize stubble being left in fields subsequently planted with wheat. Since 2000, FHB has been reported in more than 3.3 million ha of wheat in 9 of 11 years. In 2012, severe FHB epidemics affected more than 10 million ha of wheat (Zeng and Jiang 2013).

FHB resistance is quantitatively inherited and controlled by a few major genes and several modifying genes (Buerstmayr et al. 2009). Mapping studies using bi-parental populations have identified more than 200 quantitative trait loci (QTL) for FHB resistance on all 21 chromosomes, and most of them were for type II resistance, the most important type of resistance (Buerstmayr et al. 2009; Liu et al. 2009). Chromosomes 1B, 2D, 3B, 3A, 5A, 5B, 6B and 7A harbor more consistent and reliable QTL for FHB resistance across multiple mapping populations. Meta-analysis of QTL locations narrowed down the QTL regions of interest (Loffler et al. 2009; Liu et al. 2009; Haberle et al. 2009). *Fhb1*, a well-known QTL on chromosome 3BS in 'Sumai 3' and its derivatives, has been extensively utilized in breeding programs worldwide. Although progress in understanding the genetic components of FHB resistance has been made, the development of FHB resistant varieties with good agronomic performance is still a significant challenge because most published QTL have minor effects, chromosomal locations of these QTL are inconsistent among studies, and major sources of FHB resistance carry unfavorable agronomic traits.

Genome-wide association analysis (GWAS) is an alternative method that compensates for deficiencies of bi-parental linkage mapping and allows simultaneous identification multiple allelic variants among large numbers of varieties (Hall et al. 2010). GWAS was recently used in genome-wide mapping of QTL associated with FHB resistance in breeding populations of barley (Navara and Smith 2014; Massman et al. 2011) and wheat (Kollers et al. 2013; Ghavami et al. 2011; Miedaner et al. 2011).

Some Chinese and Japanese wheat accessions, particularly landraces, show high levels of FHB resistance and therefore might harbor unique QTL for FHB resistance (Yu et al. 2006b, 2008a; Li et al. 2011; Zhang et al. 2012). However, QTL have been confirmed in only a few Chinese or Japanese landraces and varieties (Ban and Suenaga 2000; del Blanco et al. 2003; Li et al. 2008, 2011, 2012; Lin et al. 2004, 2006; Jia et al. 2005; Ma et al. 2006; Mardi et al. 2005; Yu et al. 2008b; Zhang et al. 2004, 2012; Zhou et al. 2010; Zhuang et al. 2013). QTL for FHB resistance in most Asian wheat varieties, especially wheat landraces, remain to be investigated, and such studies may lead to identification of potentially novel QTL for FHB resistance to diversify the FHB resistance gene pool. The objectives of this study were to assess FHB responses in a set of wheat accessions to determine marker-trait associations for type II FHB resistance and to identify useful markers for use in marker-assisted selection to improve the level of wheat FHB resistance in wheat.

Materials and methods

Plant materials

The association mapping panel used in this study comprised 51 Chinese landraces, 72 Chinese commercial varieties, 27 Japanese accessions, 26 American accessions, and 19 accessions from other organizations and countries including France, Russia, Argentina, Brazil, Italy, Austria, Ukraine and the International Maize and Wheat Improvement Center (CIMMYT). 'Ning 7840' and 'Clark' were included as resistant and susceptible controls, respectively.

Evaluation of FHB responses

To evaluate FHB, the responses of these wheat materials, vernalized seedlings were grown in 12.5 × 12.5 cm plastic pots containing a soil mix on greenhouse benches at 17 ± 2 °C (night) and 22 ± 5 °C (day) with 12 h supplemental light at Manhattan, KS. *F. graminearum* conidial spore suspensions were prepared following Bai et al. (1999). Individual spikes were injected with 10 µl of spore suspensions (100 conidia/µl) into one floret of a central spikelet of a spike. Inoculated plants were placed in a mist chamber at 100 % relative humidity for 48 h, and then returned to greenhouse benches for disease development. Each experiment was arranged in a randomized complete block design with two replicates of five plants per pot. The experiment was repeated twice. The total number of spikelets and the number of symptomatic spikelets were counted for each inoculated spike at 21 days post-inoculation. The percentage of symptomatic spikelets (PSS) was calculated as FHB severity. Based on the PSS of controls, varieties were classified in four classes, highly resistant ($0 < \text{PSS} \leq 0.25$), moderately resistant ($0.25 < \text{PSS} \leq 0.50$), moderately susceptible ($0.50 < \text{PSS} \leq 0.75$) and highly susceptible ($0.75 < \text{PSS} \leq 1.00$).

Marker analysis

Genomic DNA was isolated from 2 weeks-old wheat leaves of each genotype using a modified CTAB method (Maguire et al. 1994). Harvested leaf tissue was dried in a freeze-dryer (ThermoSavant, Holbrook, NY) for 48 h and ground using a Mixer Mill (MM 300, Retsch, Germany) for DNA isolation. A total of 364 selected informative SSR and STS primer pairs were used to genotype the population. PCR amplification followed Li et al. (2012) and amplified PCR fragments were separated in an ABI3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Marker data were scored using GeneMarker 1.6 (Softgenetics Inc. LLC, State College, PA).

Data analysis

Broad sense heritability (H^2) was calculated for PSS based on analysis of variance (ANOVA) results using the formula $H^2 = \sigma_G^2 \Big/ \sigma_G^2 + \left(\sigma_{GE/e}^2\right) + \left(\sigma_{re}^2\right)$, where σ_G^2 is the genotypic variance, σ_e^2 is the residual error variance, σ_{GE}^2 is the genotype × environment variance, r is the number of replicates (pots) and e is the number of experiments (seasons) following Jayatilake et al. (2011). Multiple comparisons of PSS among groups of cultivars and landraces harboring different numbers of favorable alleles were conducted using least significant difference (LSD) at $\alpha = 0.05$. Statistical analyses were performed using Matlab software (MathWorks Inc., Natick, MA).

Number and frequency of alleles per locus, genetic diversity and polymorphism information content (PIC) were evaluated by Power Marker v3.25 (Liu and Muse 2005). Population structure was determined using STRUCTURE 2.1 (http://pritchardlab.stanford.edu/structure.html).

TASSEL v2.1 (Bradbury et al. 2007) was used to identify marker-trait associations (MTAs) for PSS using mixed linear models (MLM) to minimize spurious correlations (Yu et al. 2006a). The mixed model equation is $y = X\beta + S\alpha + Qv + Zu + e$, where Xβ represents fixed effects other than molecular markers under test and population structure; y is a vector of phenotypic observations; β is a vector of fixed effects other than molecular marker or population group effects; α is a vector of marker effects; v is a vector of population effects; u is a vector of polygenic background effects; e is a vector of residual effects; Q is a matrix from STRUCTURE relating y to v; and X, S and Z are incidence matrices of 1 and 0 s relating y to β, α and u, respectively. The variances of the random effects are assumed to be $\text{Var}(u) = 2KVg$, and $\text{Var}(e) = RV_R$, where K is an n × n matrix of relative kinship coefficients defining the degree of genetic covariance between a pair of individuals; R is an n × n matrix in which the off-diagonal elements are 0 and the diagonal elements are the reciprocal of the number of observations for which each phenotypic datapoint was obtained; Vg is the genetic variance; and V_R is the residual variance. Only markers that showed associations at a significance level of $p \leq 0.05$ were declared to be significant.

The descriptive statistical analysis, ANOVA, multiple comparisons, and analysis of genetic effects were performed using Matlab (MathWorks Inc., Natick, MA). The genetic effects were estimated as $pi = \sum xij/ni - \bar{x}$, where *pi* represents the phenotypic effect of the *i*th allele, *xij* indicates the phenotypic

value (PSS) of the *j*th accession carrying the *i*th allele, *ni* is the number of accessions carrying the *i*th allele, and $\bar{x}$ is the overall mean of PSS of all accessions carrying different alleles at a marker locus of interest. The allele with contradictory or non-significant effects across experiments was classified into an ineffective allele group. If the number of varieties carrying the *i*th allele was less than 3 %, it was regarded as a rare allele and the genetic effect of the *i*th allele was not analyzed. If *pi* was significantly lower than 0, the *i*th allele was defined as favorable (resistant) or otherwise defined as unfavorable (susceptible). Only those accessions carrying alleles with significant positive effects on FHB resistance were regarded as carriers of that allele.

Results

FHB severities and phenotypic variation

The mean PSS of 195 accessions ranged from 5 to 100 % over three experiments (Fig. 1) with a mean of 45 %. Phenotypic variation was significant among genotypes ($p = 4.92e^{-186}$), environments ($p = 7.14e^{-05}$), and genotype-by-environment interactions ($p = 1.4e^{-36}$). PSS were significantly correlated among the three experiments ($r_{1-2} = 0.751$, $r_{1-3} = 0.621$ and $r_{2-3} = 0.662$ at $p < 0.001$). The mean heritability (H^2) of PSS for 195 accessions was 0.76 over the three experiments. Based on the mean PSS values, 65 accessions were classified highly resistant (PSS = 13.5 %), 47 accessions were moderately resistant (PSS = 35.6 %), 45 were moderately susceptible (PSS = 63.1 %), and 38 accessions were highly susceptible (PSS = 87.6 %). The frequencies of accessions with high resistance, moderate resistance, moderate susceptibility, and high susceptibility were 0.33, 0.24, 0.23 and 0.20, respectively.

Genetic diversity and population structure

A total of 364 primer pairs amplified 3167 alleles across the 195 accessions, and the number of alleles amplified varied with primers with a mean of 8.7 alleles per primer pair. *Xgwm539-2D* amplified 33 alleles, but 37 primer pairs each amplified only two alleles. The genetic diversity and average PIC were 0.596 and 0.562, respectively. *Xwmc632-3B* showed the largest genetic diversity (0.930) and PIC (0.926).

Structure analysis indicated a maximum *LnP*(*D*)-value and stable α value at k = 5, thus the population was divided into five groups (G) using a mixed model (Supplementary Table S1). Nei's genetic distances between the groups ranged from 0.170 to 0.342. The longest genetic distance was observed between G2 and G5, and the shortest was between G1 and G4. FHB severities differed significantly among groups ($p = 8.87e^{-9}$). The mean PSS in order from the lowest to the highest was G2, G3, G4, G1 and G5 (Fig. 2). G2 included 12 Chinese accessions and three accessions from other countries. The mean PSS was 20.2 % with all accessions showing moderate to high FHB resistance except Clark that was highly susceptible (Supplementary Table S2). Most Chinese and Japanese accessions were in G3, including 68 % of

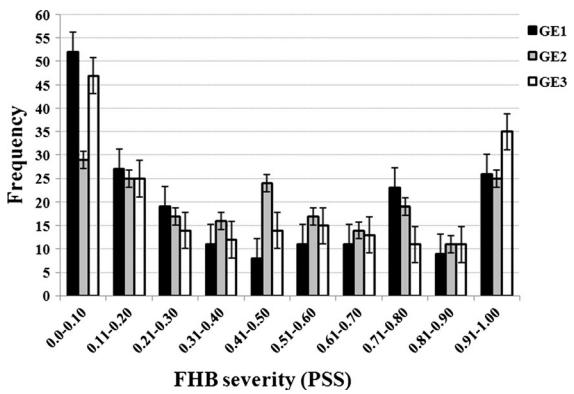

Fig. 1 Frequency distribution of proportion of symptomatic spikelets (PSS) for 195 wheat accessions evaluated in three greenhouse experiments (GE1, GE2, and GE3)

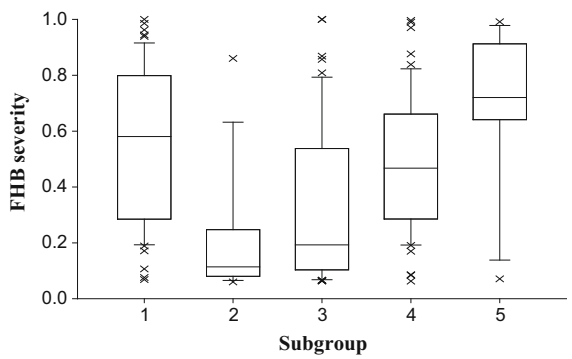

Fig. 2 Distribution of proportion of symptomatic spikelets per spike (PSS) for five subgroups of wheat accessions evaluated in three greenhouse experiments (×represents outliers)

Chinese landraces, 96 % of Japanese accessions, and one each of Chinese and Korean accessions. The mean PSS of G3 was 32.8 %, ranging from 6.3 to 100.0 % (Supplementary Table S2). The 51 accessions in G4 included 50 commercial varieties from China and one Brazilian variety (Supplementary Table S2). Among them, 39 have 'Funo' in their pedigrees. The mean PSS of G4 was 48.2 %, ranging from 6.3 to 99.6 %. Fifty-seven accessions in G1 included 24 accessions from the USA, 17 from China (including 7 landraces), 4 from Argentina, and 12 from Japan, Austria, France, Russia, Ukraine, South Korea, Brazil and CIMMYT (Supplementary Table S2). Accessions in G1 had the largest within-group PIC and genetic diversity (Supplementary Table S1), and mean PSS of 54.7 %, ranging from 6.9 to 100.0 %. G5 included 14 Chinese accessions, one accession each from Japan and the United Kingdom (Supplementary Table S2). All accessions were susceptible except for 'FK 17' and 'Xiangnong 3'. The mean PSS in this group was 69.0 %, ranging from 7.1 to 99.2 %.

Association between markers and PSS

Markers associated with PSS

In single experiments, 16, 22 and 19 markers were identified to be significantly associated with PSS explaining 0.70–4.71 %, 0.25–3.90 % and 0.41–7.31 % of phenotypic variations in three experiments (Supplementary Table S3). Eleven markers were significant in two or three experiments (Table 1). *Xbarc19* was consistently detected in all three experiments, explaining an average 2.16 % of the phenotypic variation. Seven markers with unique locations on chromosomes 2AS, 2DS, 3AL, 3BS, 3DL, 5DS and 7AL were detected in two of the three experiments. Four other markers, *Xwmc173.2*, *Xwmc273.1*, *Xgwm358* and *Xgdm138*, were significant in two experiments, but were assigned to multiple chromosome locations.

Table 1 The phenotypic variances explained by the 11 markers that were significant in two or three greenhouse experiments

Marker	Chromosome	Phenotypic variance (R^2)		
		GE1	GE2	GE3
Xwmc296	2AS	0.026	0.017	ns
Xgdm35	2DS	ns	0.039	0.054
Xbarc19	3AS	0.026	0.023	0.016
Xwmc428	3AL	0.017	ns	0.073
Xbarc133	3BS	0.031	ns	0.019
Xbarc71	3D	0.015	ns	0.024
Xcfa2019	7AL	0.014	0.009	ns
Xgdm138	5BL/5DS	0.018	0.011	ns
Xgwm358	2D/5D	ns	0.007	0.011
Xwmc273.1	7A/7D	0.031	ns	0.047
Xwmc173.2	1D/3A/4A/5A	ns	0.012	0.024

ns Not significant at $\alpha = 0.05$

Effects of significant marker alleles on PSS

A total of 90 alleles were identified at the 11 marker loci showing significant effects on PSS in at least two experiments. Among these alleles, 30 had significant effects on PSS ($p < 0.05$) with nine favorable (i.e. resistance) alleles, and 21 unfavorable (susceptibility) alleles (Table 2). Eight markers (*Xgdm138, Xgwm358*, *Xwmc173.2*, *Xbarc19*, *Xbarc133*, *Xwmc428*, *Xbarc71* and *Xwmc273.1*) carried contrasting alleles for resistance and susceptibility. Only alleles associated with FHB susceptibility were significant for markers *Xwmc296*, *Xcfa2019* and *Xgdm35*. *Xbarc19* carried one resistance, two susceptibility, and three neutral alleles.

Distribution of favorable alleles among wheat accessions

Frequencies of favorable alleles per marker ranged from 4.05 to 56.35 % (Table 3). Japanese accessions had the highest frequencies of favorable alleles for all of the reproducible markers. Favorable alleles $Xgdm138_{237}$ and $Xgwm358_{175}$ present only in Chinese and Japanese accessions at a frequency of less than 6 %, and were not detected in accessions from other sources. The favorable allele of $Xbarc133_{142}$ closely linked to *Fhb1* was identified in 56 accessions (28.4 %), including 10 Chinese landraces (such as 'Taiwan Wheat', 'Huangcandou', 'Shuilizhan' and 'Hongjianzi'), 18 Chinese commercial varieties (such as 'Sumai 3', 'Ning 7840', 'Wannian 2' and 'Siyang 936'), 21 Japanese accessions (such as 'Nyubai', 'Tokai 66' and 'Sanshukomugi') and six other accessions (such as 'Chukouku 81'). Favorable allele $Xbarc19_{156}$ presents in 111 accessions (56.3 %)

Table 2 Statistically significant alleles, their genetic effects and proportion of symptomatic spikelets (PSS) at significant marker loci identified in experiments GE1, GE2, and GE3

Marker	Allele size (bp)	Number of accessions	Effect			Mean PSS	Associated FHB response
			GE1	GE2	GE3		
Xbarc19	153	40	0.135	0.093	0.124	0.57	S
	156	111	−0.095	−0.092	−0.094	0.35	R
	159	24	0.166	0.19	0.153	0.62	S
Xbarc133	132	11	0.39	ns	0.251	0.73	S
	136	5	0.424	ns	0.302	0.77	S
	140	57	0.066	ns	0.11	0.50	S
	142	56	−0.168	ns	−0.185	0.25	R
Xbarc71	110	5	0.344	ns	0.121	0.66	S
	122	39	0.091	ns	0.191	0.58	S
	134	69	−0.103	ns	−0.129	0.32	R
Xwmc173.2	287	8	ns	0.405	0.324	0.83	S
	289	5	ns	0.296	0.284	0.75	S
	293	81	ns	−0.07	−0.083	0.37	R
Xwmc273.1	200	18	0.171	ns	0.102	0.55	S
	202	78	0.063	ns	0.121	0.53	S
	204	7	0.303	ns	0.034	0.67	S
	206	15	0.238	ns	0.302	0.71	S
	207	42	−0.188	ns	−0.262	0.22	R
	208	28	−0.252	ns	−0.179	0.22	R
Xwmc428	262	4	0.136	ns	0.486	0.75	S
	272	3	0.509	ns	0.491	0.94	S
	274	74	−0.09	ns	−0.101	0.35	R
Xgdm138	227	19	0.165	0.113	ns	0.59	S
	237	8	−0.32	−0.342	ns	0.12	R
Xgwm358	175	11	ns	−0.208	−0.121	0.30	R
	181	3	ns	0.468	0.538	0.97	S
Xwmc296	180	51	0.159	0.140	ns	0.59	S
Xgdm35	244	13	ns	0.187	0.252	0.68	S
	260	5	ns	0.434	0.389	0.93	S
Xcfa2019	214	37	0.103	ns	0.122	0.56	S

R Resistant; *S* susceptible; *ns* not significant

including 66 from China, 16 from Japan, and 29 from other countries. Favorable allele $Xbarc71_{134}$ was not detected in USA accessions, but was present at high frequencies in Chinese and Japanese accessions. Among different sources of accessions, Japanese accessions had the highest frequencies of favorable alleles across all markers of interest, ranging from 21.7 % at *Xgwm358* to 91.3 % at *Xbarc133* in 24 accessions tested (Table 3); Chinese landraces with favorable alleles ranked second with high frequencies at marker loci *Xbarc71*, *Xwmc428*, *Xwmc296*, *Xwmc273.1* and *Xgdm138*; and Chinese commercial varieties or breeding lines ranked third with high frequencies of favorable alleles at *Xbarc133*, *Xwmc173.2* and *Xgwm358*. *Xbarc19* had high frequencies of the favorable allele (53.2–69.6 %) across all sources.

Table 3 Numbers of varieties from different sources that carry favorable alleles of significant markers

Marker	Favorable allele (bp)	Source					Number of accessions	Freq. (%)
		China (landraces)	China (varieties)	Japan	USA	Others		
Xgdm138	237	1	1	6	0	0	8	4.06
Xgwm358	175	1	5	5	0	0	11	5.58
Xwmc273.1	208	7	9	8	2	2	28	14.21
Xwmc273.1	207	12	12	13	3	2	42	21.32
Xbarc133	142	10	19	21	2	4	56	28.43
Xbarc71	134	30	13	21	0	4	68	34.52
Xwmc428	274	26	9	21	11	7	74	37.56
Xwmc296	182	26	21	19	2	7	75	38.07
Xwmc173.2	293	17	34	14	9	7	81	41.12
Xbarc19	156	25	41	16	16	13	111	56.35

Table 4 Combinations of favorable and unfavorable alleles

Number of favorable alleles	Number of unfavorable alleles	Average number of unfavorable alleles	Number of accessions	Mean PSS*
8	0	0.00	4	0.098[a]
7	0	0.00	7	0.152[a]
6	0–2	0.50	12	0.158[a]
5	0–3	0.93	14	0.258[ab]
4	0–4	1.10	29	0.311[ab]
3	0–5	2.33	30	0.463[bc]
2	0–6	2.76	45	0.518[c]
1	0–7	3.47	36	0.553[c,d]
0	2–8	4.55	20	0.732[d]

*Combinations sharing the same letters are not significantly different at $P = 0.05$

Effects of different allelic combinations on PSS

The numbers of favorable and unfavorable alleles at the 11 reproducible marker loci varied among accessions (Supplementary Table S2). To investigate general trends in FHB response among the 195 accessions, the population was divided into nine combinations based on numbers of favorable alleles at the 11 marker loci (Table 4). The average PSS differed significantly among the nine combinations ($p = 9.71e^{-14}$). PSS increased as the number of favorable alleles decreased, and accessions that carried 8 favorable alleles and no unfavorable allele had the lowest PSS, whereas accessions carrying only unfavorable alleles had the highest PSS that was significantly higher than for groups carrying 2 or more favorable alleles (Table 4). Significant differences were observed between groups with 4 or more favorable alleles and groups with 2 or fewer favorable alleles. However, differences were not significant among groups with 4 to 8 favorable alleles or with 1 to 3 favorable alleles. Only Japanese accessions contained all 8 favorable alleles (Supplementary Table S2). Among accessions carrying 7 favorable alleles, 6 were from Japan and one from Korea (Supplementary Table S2). Among 12 accessions carrying 6 favorable alleles, 7 were Chinese landraces, 2 Japanese accessions, and 2 Chinese breeding lines or varieties ('Sumai 3' and 'DSumai 3'). Unfavorable alleles were not detected in 'Sumai 3' and 'DSumai 3'.

Discussion

Phenotypic variation and genetic diversity of the germplasm panel

Population diversity is of importance for association mapping in breeding populations to reduce spurious correlations (Yu et al. 2006a). The diversity of the current panel was manifested at levels of geographic origins of accessions, phenotypes, genotypes and breeding selection. The 195 accessions were collected from 12 countries, and included landraces, commercial varieties and elite breeding lines. FHB severity (PSS) ranging from 3 to 100.0 % indicated wide phenotypic variation. At the genotypic level, the population was divided into five distinct groups that also differed significantly in mean PSS ($p < 0.05$). About 92 % of USA accessions belonged to group G1, which had the largest within-group phenotypic and genotypic differences as well as geographic diversity, suggesting high diversity among USA accessions. Chinese landraces fell into G1, G2, G3 and G5, indicating that landraces *per se* have wide genetic diversity. Commercial varieties and elite breeding lines have undergone much higher artificial selection pressure during breeding than have landraces; however, landraces apparently harbor more resistance genes than selected varieties or elite breeding lines (Reif et al. 2005; Hao et al. 2011; Wingen et al. 2014).

Accessions harboring *Fhb1* (Buerstmayr et al. 2009; Liu et al. 2009; Li et al. 2012; Zhang et al. 2012) fell into two groups, G2 and G3. 'Taiwan Wheat', 'Sumai 3', 'Ning 7840' and 'F60096' were in G2, whereas several Chinese landraces (e.g. 'Wangshuibai', 'Huangfangzhu' and 'Baisanyuehuang') and Japanese accesions (e.g. 'Nyubai' and 'Tokai 66') were in G3. Pedigree analysis indicated 11 of 15 commercial varieties in G2 with 'Funo' in their pedigrees, suggesting close relationships among many accessions in this group. G3 consisted of 30 Chinese landraces, 25 Japanese accessions, one Korean accession and one Chinese commercial variety, implying the close genetic kinship among FHB resistance sources from Asian countries, particularly Chinese and Japanese accessions. *Fhb1* in these accessions might have the same origin.

QTL for FHB resistance

In the current study, we determined QTL repeatedly over experiments based on the markers that were significantly associated with FHB resistance in at least two experiments, such as *Xwmc296-2AS*, *Xgdm35-2DS*, *Xbarc19-3AS*, *Xwmc428-3AL*, *Xbarc133-3BS*, *Xbarc71-3D*, and *Xcfa2019-7AL* (Table 1). However, different markers were tightly linked and significant in two or three experiments based on established genetic maps (http://wheat.pw.usda.gov) were also used to identify repeatable QTL. For example, *Xumn10* (GE2), *Xbarc133* (GE1 and GE3), and *Xbarc147* (GE2) were closely linked markers on chromosome 3B and showed significant associations with *Fhb1* in different experiments (Supplemental Table S3), thus these three markers were considered together and *Xbarc133* was used to represent all three markers in determining repeatability of the linked QTL. Similarly, *Xwmc477-2BL* (GE1) and *Xgwm120-2BL* (GE3), *Xgdm35-2DS* (GE2 and GE3) and *Xgwm261-2DS* (GE3), *Xwmc291-3BL* (GE1) and *Xbarc84-3BL* (GE3), and *Xcfa2019-7AL* (GE1 and GE2) and *Xcfa2040.2-7AL* (GE2) are linked markers that were analyzed together in determining repeatability.

When significant markers were assigned to multiple chromosomal locations, the numbers, chromosomal locations and repeatability of QTL indicated by those markers were difficult to determine. For example, *Xwmc273.1* and *Xwmc173.2* were significant in two of the three experiments. The former was mapped to chromosomes 7A and 7D, and latter to chromosomes 1D, 3A, 4A and 5A. In these cases, their associations with other significant markers are unknown and remain to be investigated. In the current study, only reproducible markers with unique chromosome locations were compared with previously published data.

Two previously published QTL were located on chromosome 2AS, with one from 'Ning 7840', 'NK 93604' or 'Freedom' and the other from 'Ning 8026', 'Spark', 'Wangshuibai' and 'Rubens' (Buerstmayr et al. 2009). These two QTL were distinct from each other according to meta-analysis results from Liu et al. (2009). In the current study, *Xwmc296-2AS* was significant in two experiments and the associated QTL was probably allelic to, or the same as, the QTL reported in 'Ning 8026', 'Spark', 'Wangshuibai' and

'Rubens' based on the flanking markers on the wheat composite map (http://www.shigen.nig.ac.jp).

Xgdm35-2DS was significantly associated with FHB resistance in two experiments. This marker is linked to the distally located *Xgwm261-2DS*, which was also significant in GE3, with a genetic distance of 6.1 cM (http://www.shigen.nig.ac.jp). This marker was reported to be associated with a FHB resistance QTL in 'Sumai 3', 'Alondra', 'Nyubai', 'Romanus', 'Biscay', 'Gamenya' and 'Wangshuibai' (Buerstmayr et al. 2009). Therefore, the QTL linked to *Xgdm35* locus in this study might be the same locus as in 'Sumai 3'. However, the QTL might be distinct from the QTL in 'Wuhan 11', 'CJ 9306' and 'DH 181', because that QTL was mapped to the proximal region of 2DS (Buerstmayr et al. 2009).

Xbarc19-3AS was significant in all three experiments. This marker, together with *Xgwm32*, *Xgwm666*, *Xgwm674* and *Xbarc67* all within a <2.0 cM interval on chromosome 3AS, was significantly associated with an FHB resistance QTL in 'Frontana', 'Spark', 'Wangshuibai', 'Arina' and 'F201R', and are thus likely to be the same. Also, the favorable alleles of this marker locus had the highest frequencies across all sources of accessions analyzed in the study; thus the QTL associated with these markers may be the most frequent one among all accessions.

Fhb1 is one of the most important QTL conferring FHB resistance in multiple environments and genetic backgrounds. *Xbarc133* was significant in two experiments, whereas *Xumn10* and *Xbarc147* were each significant in only one experiment. These three markers have been extensively reported to be associated with *Fhb1* (Chen et al. 2006; Liu et al. 2008; Li et al. 2012; Bernardo et al. 2012; Hao et al. 2012).

Xbarc71-3DL was significant in two of the three experiments. The favorable allele of this marker was present in 30 Chinese landraces, 13 Chinese varieties, 21 Japanese accessions and 4 accessions in other sources. However, this marker was reported to be associated with FHB resistance only in 'Wangshuibai' (Yu et al. 2008b), suggesting relative novelty.

Xgdm138 and *Xgwm358* were both mapped to 5DS and both were linked to *Xgwm583-5DS*. The low frequencies (4.06 and 5.58 %) of favorable alleles for both markers suggest that this QTL is most likely on 5DS rather than 2DS or 5BS because the QTL on 2DS and 5BS were widely reported in germplasm and therefore high frequencies would be expected if either were present. Thus this QTL was postulated as an infrequent and novel QTL that might be specific to Chinese (such as 'Siyang 117') and Japanese (such as CAsozaira III', 'Itoukomugi', Minamikyushu 69', and 'Tokai 66') wheat germplasm. It was probably distinct from the QTL on 5DS in Chokwang (Yang et al. 2005) and Wangshuibai (Yu et al. 2008b) because *Xgwm358* was mapped more than 30 cM from *Xgwm292*, *Xgwm212* and *Xcfd57* that were tightly associated with the QTL on 5D in these two varieties. Moreover, none of the three markers was significant in any experiment.

Xcfa2019-7A was significant in two experiments. This marker is flanked by *Xgwm276* and *Xbarc121* that were previously reported to be associated with FHB resistance in 'Wangshuibai', 'Huangfangzhu', 'Ritmo', 'NK 93604', 'PI 478742', 'Spark' and 'Romanus' (Buerstmayr et al. 2009; Liu et al. 2009). However, neither *Xgwm276* or *Xbarc121* was significant in this study, thus *Xcfa2019* should be a better diagnostic marker for the QTL on 7A than the other two markers.

Allelic variation, genetic effects and allele carriers

In bi-parental mapping populations, individual SSR may have only two alleles, whereas more than two alleles per SSR marker is very common in an association mapping population consisting of diverse germplasm due to historical accumulation of recombination and mutation events. In the current study, each marker that was significantly associated with FHB resistance in at least two experiments had more than two alleles and the frequencies of significant alleles varied among markers. When all of the marker loci of interest are considered, Japanese accessions had the highest frequencies of favorable alleles among all sources tested, possibly because the samples from Japan were a selected set of resistant varieties. Chinese landraces also had favorable alleles at the majority of significant marker loci, suggesting that Chinese landraces and Japanese accessions are valuable sources of FHB resistance QTL for variety improvement. Resistance alleles linked to *Xbarc19*, *Xwmc173.2* and *Xwmc428* are more frequent in Chinese commercial varieties than in Chinese landraces, suggesting the QTL linked to these three markers may have undergone positive selection in modern breeding, and therefore might be more easily incorporated into modern high yielding cultivation systems.

Most of the loci identified in this study coincided with previously published QTL and there was a high negative correlation between PSS and number of favorable marker alleles at these loci (Table 4), indicating that the QTL identified were reliable and that most of the linked markers can be used for marker-assisted selection of the QTL. However, the number of QTL detected in the study was not exhaustive due to limited marker coverage and exclusion of significant markers with uncertain chromosome locations. Increased marker densities might permit more QTL and better markers to be identified.

Conclusion

The results of this study showed that the FHB severity of a particular accession was determined by a balance of favorable and unfavorable alleles at different loci. In breeding schemes, favorable alleles of interest are emphasized whereas unfavorable alleles are usually ignored, but might lower the efficiency of marker-assisted selection. Therefore, simultaneous selection of favorable alleles and removal of unfavorable alleles could significantly improve FHB resistance levels in breeding populations. Many Chinese and Japanese accessions, especially landraces, harbored novel FHB resistance alleles. In the panel of 195 accessions, the QTL for FHB resistance had been reported previously for only a small proportion of accessions (<10 %) Information on significant alleles and carriers identified here should provide breeders with more choice of FHB resistance sources for use in breeding programs.

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