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ORIGINAL PAPER

Molecular mapping of kernel shattering and its association with Fusarium head blight resistance in a Sumai3 derived population

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Abstract Kernel shattering (KS) can cause severe grain yield loss in wheat (*Triticum aestivum* L.). The introduction of genotypes with Fusarium head blight (FHB) resistance has elevated the KS importance. 'Sumai3,' the most commonly used FHB-resistant germplasm worldwide, is reported to be KS susceptible. The objectives of this study were to detect quantitative trait loci (QTLs) for KS and to determine the relationship between KS and FHB. A recombinant inbred line population derived from a cross between Sumai3 and 'Stoa' was evaluated for KS in five environments and FHB in two field trials, separately. Four genomic regions on chromosomes 2B, 3B, and 7A were associated with KS. Of them, two major KS QTLs were detected consistently over three environments and each located proximal to the centromere on chromosomes 3B and 7A. The resistant alleles at these two QTLs together can reduce KS by 66.1% relative to the reciprocal alleles and by 41.1% compared to the population mean. The field FHB data revealed four QTLs on chromosomes 2B, 3B, and 7A. Three of these FHB QTLs coincided with and/or linked to the KS QTLs with opposite allele effects in the corresponding genomic regions, which may explain the negative correlation ($r = -0.29$ and $P < 0.01$) between the KS and FHB infection found in this study. The results in this study indicate that KS and FHB in Sumai3 are, in part, inherited dependently. However, the correlation between KS and FHB is not strong, and the major FHB resistance QTL on chromosome arm 3BS was not linked to any KS QTL. Our results showed that pyramiding of the two major KS-resistant alleles and the unlinked major FHB-resistant allele could produce lines with both low values of KS and FHB infection.

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Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most important food grain crop (FAO 2006). In 2004 for instance, wheat was grown on more land area worldwide than any other crops (FAO 2004). Shattering is essential for the survival of wild plants in nature, but it causes severe grain yield loss for cultivated crops. In a broad sense, shattering in wheat refers to both losses of entire spikelets or spikes and loss of kernels from their enveloping glumes before harvest. Loss of entire spikelets or spikes mainly occurs in the wild wheat species due to the brittle rachis. After domestication, cultivated wheat has evolved with tough rachises. The shattering loss in wheat cultivars is mainly due to the loss of kernels (Clarke 1981), which is referred to as kernel shattering (KS) in this study. Cultivars with KS susceptibility have the potential to lose all kernels. Therefore, KS resistance is an important trait to be considered when developing new wheat cultivars (Poehlman and Sleper 1995). However, information on the genetics of KS is limited and inconsistent. Lewicki (1929) reported KS resistance was controlled by a single recessive gene in the most F_2 crosses that he studied. Hughes (1940) concluded that two genes possibly controlled the shattering reaction. Later, Porter (1959) suggested that KS resistance might be controlled by two major dominant genes together with unknown number of minor genes or polygene depending on the genotypes. Recently, the development and use of molecular markers have greatly facilitated genetic studies by locating quantitative trait loci (QTLs) and esti-

mating their effects. However, studies on QTL mapping for KS in wheat are limited. In the only published study, Marza et al. (2006) reported six KS QTLs in a wheat population Ning7840 × Clark, which indicates the complex inheritance of KS.

Much progress has been achieved in breeding KS-resistant cultivars (Kadkol et al. 1989). However, KS has resurfaced with the introduction of Fusarium head blight (FHB, caused by *Fusarium graminearum* Schwabe) resistant genotypes. FHB is a fungal disease of cereals that causes severe losses in both grain yield and quality worldwide. The United States Department of Agriculture ranked FHB as the worst disease of wheat and barley (*Hordeum vulgare* L.) in 1999 (Woods et al. 1999). Host resistance is the most practical, effective, and economic means of FHB control. However, only limited FHB-resistant sources in wheat are available. 'Sumai3' (PI 481542) is the most commonly used FHB-resistant source, but it is susceptible to KS (Rudd et al. 2001). Preliminary field observations indicate that Sumai3-derived progenies had great variations for KS (unpublished data). Alsen (Frohberg et al. 2006), the first released FHB-resistant cultivar derived from Sumai3, showed significant KS in 2002 when harvest was delayed by the late season rain (unpublished data). These observations prompted us to investigate the relationship between FHB resistance and KS.

Inheritance studies indicated that FHB resistance is a complex trait conditioned by oligogenes or polygenes (van Ginkel et al. 1996; Ban and Suenage 2000; Gervais et al. 2003). Molecular markers have been used to identify QTLs conditioning FHB resistance, and a number of FHB-resistant QTLs have been found (reviewed by Bai and Shaner 2004). Schroeder and Christensen (1963) suggested that FHB resistance mainly consisted of two components: the resistance to initial infection (type I) and the resistance to spread within the infected spike (type II). Field resistance is the combination of types-I and II resistances, which is critical in the development of FHB-resistant cultivars. To our knowledge, QTL mapping in Sumai3 itself has only been conducted for the type-II resistance (Waldron et al. 1999; Anderson et al. 2001). Therefore, it is of great interest to further study the field FHB resistance in Sumai3 by QTL mapping. Hence, the objectives of this study were to (1) detect QTLs conferring the KS resistance, (2) identify QTLs for the field FHB resistance, which might coincide with or link to the KS QTLs, and (3) determine the relationship between KS and FHB.

Materials and methods

Plant materials

A population of 108 F_5-derived recombinant inbred lines (RILs) from the cross of Sumai3 and 'Stoa' was developed by single seed descent and used in this study. This population was previously studied by Waldron et al. (1999) and Anderson et al. (2001) for the type-II FHB resistance. During the seed multiplication, four RILs were lost (original population had 112 RILs). Sumai3, originated from China, is susceptible to KS and well known for the type-II FHB resistance (Rudd et al. 2001). Stoa, a hard red spring wheat cultivar released by North Dakota State University in 1984, is KS resistance and FHB susceptibility.

Field experiments for kernel shattering

The RILs and their parents were grown at Prosper and Casselton, ND, in 2004 and 2005; and Carrington, ND, in 2004. The experiments were arranged in a randomized complete-block design with two replicates. Each plot was comprised of two rows, 17 cm apart and 2.4 m long. It is well documented (McMullen and Stack 1999) that severe FHB infection results in shriveled kernels, whereas plump kernels tend to increase KS (Clarke and DePauw 1983). Therefore, all experiments for evaluating KS were sprayed with the fungicide Tebuconazole at a 0.31 l/ha rate at Feekes' stage 10.5.1 to minimize the possible confounding effect of FHB on KS. The fungicide was sprayed as described by Hofman et al. (2000). KS was evaluated in the field using the method of KS from spikes (SS), as described by Zhang and Mergoum (2007). Briefly, 20 spikes per plot in 2004 and ten spikes per plot in 2005 were collected 3 weeks after Feekes' stage 11.4, and the shattered kernels from the spikes were counted. The KS was determined as the percentage of shattered kernels per spike.

Field experiments for Fusarium head blight

The RILs and parents were grown in the FHB nursery at Prosper, ND, in 2004 and 2005. The experiments were arranged in a randomized complete-block design with two replicates. The experimental units consisted of hill plots, each having at least 15 plants. The lines were inoculated by the grain spawn method, as described by Stack et al. (1997). Briefly, corn (*Zea mays* L.) grain spawn was produced in the laboratory. The grain spawn was spread in the field at Feekes' stage 9 and then another two times at weekly intervals. The overhead mist irrigation was applied for 1 min every half an hour from 24:00 to 08:00 to enhance disease development once the grain spawn was spread. The FHB was evaluated 21 days after Feekes' stage 10.3 for each line based on their heading date. Ten to fifteen spikes per hill plot were evaluated individually for the visual symptom based on a scale of 0–100% (Stack and McMullen 1995). The field FHB infection was determined as a mean score of the scored spikes in each plot, including the spikes that did not show any FHB symptoms. There-

fore, the field FHB infection data combined the effects of the disease incidence (type I) and disease spread (type II).

DNA extraction and marker analysis

Five plants from each RIL and their parents were grown in the greenhouse in 2005 and bulk harvested at their three-leaf or four-leaf stages for DNA isolation. DNA was extracted as described by Zhou et al. (2002b). DNA concentration was quantified with a Hoefer DyNA Quant 200 fluorometer (Hoefer Inc., San Francisco, CA). Initially, 427 micro-satellite (or simple sequence repeat, SSR) markers were screened for parental polymorphism. To accelerate the identification of genomic regions containing KS QTLs, selective genotyping approach (Lebowitz et al. 1987) was used at the beginning. Twenty lines (the ten most resistant and ten most susceptible RILs based on KS average data across the five environments) were selected to further screen the markers that showed parental polymorphism. The marker allele frequencies from those two groups were compared using a two-sample *t*-test. Markers associated ($P = 0.05$) with KS were used to genotype the entire population. Then the population data were subjected to the marker regression analysis using Map Manager QTXb20 (Manly et al. 2001), and the putative markers associated with KS were identified. Based on the previously published genetic maps (Somers et al. 2004; Liu et al. 2005), the potential genomic regions containing KS QTLs were determined and saturated with additional molecular markers. Finally, composite interval mapping (CIM) was performed to detect QTLs for KS and FHB on these potential genomic regions for KS.

The PCR was performed as described by Röder et al. (1998) and run in a Techne TC-412 thermal cycler (Techne at Barloworld Scientific Ltd., Staffordshire, UK). The PCR products were separated on 6% non-denaturing polyacrylamide gels using the electrophoresis unit DASG-400-50 (C.B.S. Scientific Co., Del Mar, CA). Gels were stained with ethidium bromide, visualized under UV light, and photographed.

Statistical and QTL analysis

Analysis of variance (ANOVA) was performed for the KS and FHB in each environment using the GLM procedure of SAS (2003). Error homogeneity was determined using the Bartlett's chi-square ($P = 0.005$). Combined ANOVA was performed considering RIL as a fixed effect and environment as a random effect. The broad sense heritability based on the entry mean was calculated according to Fehr (1987). The difference between two parents was compared using a *t*-test ($P = 0.05$). Pearson correlation between traits was calculated based on line means across environments using CORR procedure of SAS (2003). Linkage maps were constructed using Map Manager QTXb20 (Manly et al. 2001) with a LOD score of 3 and the Kosambi function. Assignment of linkage groups to the specific chromosomes was based on common SSR markers in the previously published genetic maps (Somers et al. 2004; Liu et al. 2005). The CIM analysis was performed in each environment based on the line means using QTL Cartographer V2.5 (Wang et al. 2006). The CIM analysis use markers other than the interval being tested as cofactors to control the genetic background (Zeng 1994). Standard model Zmapqtl 6 was used to select markers as cofactors. The walking speed chosen for CIM was 2 cM. The empirical LOD threshold at 5% probability level was determined by a 1,000-permutation test (Churchill and Doerge 1994). The QTL consistently detected over 50% environments is considered as a major QTL in this study.

Results

Phenotypic analysis

Phenotypic values of parents and RILs in single environments and across environments for KS and FHB were summarized and listed in Table 1. Sumai3 had significantly ($P < 0.05$) higher KS and lower FHB infection than Stoa in all environments ($P < 0.05$). The ANOVA (data not shown) in each environment showed significant ($P < 0.01$) genetic variation among RILs for both traits. Combined ANOVA (data not shown) showed significant ($P < 0.01$) interactions between RIL and environment for both traits. Wide variations between environments for both traits were observed. In general, severe shattering occurred at Casselton in 2004 with a population mean of 37.2%, while much less shattering was observed at Carrington with a population mean of 7.7%. Less FHB infection occured in 2005 than in 2004 (population mean 19.5% vs. 11.0%). The heritability of FHB across the environments was intermediate (0.60). A high heritability (0.86) was observed for KS across the five environments. However, the heritability of KS varied from 0.41 to 0.94 among environments, which could be explained by the significant interaction between genotype and environment. The frequency distributions for both traits were continuous, but skewed toward the resistant parent (Fig. 1). Based on the least significant difference ($LSD_{0.05}$), there was no transgressive segregation for KS and FHB in all environments except FHB in 2004 (Table 1). Although not strong, a negative and significant correlation ($r = -0.29$ and $P < 0.01$) was found between KS and FHB infection, which suggests that KS-resistant lines tend to have higher FHB infection.

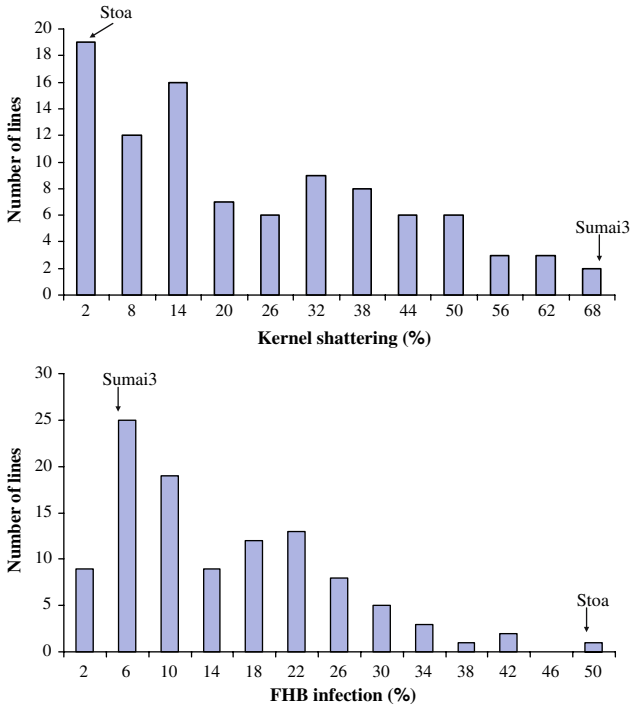

Fig. 1 Frequency distributions of 108 recombinant inbred lines derived from the Sumai3 × Stoa cross for kernel shattering (*KS*) and Fusarium head blight (*FHB*) infection based on the line means across environments in North Dakota

Marker analysis and QTL identification

Initially, 128 SSR markers showing parental polymorphism were genotyped on the 20 lines (ten resistant and ten susceptible). Of these markers, 11 markers showed possible associations with KS at $P = 0.05$ using the *t*-test. These markers were then genotyped on the entire RIL population. Further analysis with the whole population data suggested six markers were still significantly associated with KS at $P = 0.05$ (Table 2). Based on the common SSR markers in the previously published genetic maps (Somers et al. 2004; Liu et al. 2005), three chromosomes 2B, 3B, and 7A were determined to contain putative KS QTLs. Then, all available polymorphic markers in these genomic regions were genotyped on the entire population. In total, eight, fifteen, and seven markers were mapped on chromosomes 2B, 3B, and 7A, respectively. On chromosome 3B, 15 markers formed two linkage groups. One linkage group, consisting of four markers and designated as 3BS, is located near the distal end of the short arm. The other linkage group, consisting of 11 markers and designated as 3BC, includes the centromeric region.

Two major and two minor QTLs were detected for KS, and the Sumai3 allele contributed to the increase of KS in all four loci (Table 3; Fig. 2). One major KS QTL was located near the centromere on linkage group 7A. This QTL was consistently detected in four of the five environments, explaining 8.9–54.6% of the phenotypic variation. The other major QTL resided close to the centromere on linkage group 3BC and was consistently detected in three environments, accounting for 11.6–14.3% of the phenotypic variation. Both major QTLs were also detected in the combined analysis across environments. Two additional minor QTLs for KS were only revealed in a single or two environments and were not detected in the combined analysis across environments. One minor QTL was located at the distal end of the linkage group 7A. The other minor QTL was located on linkage group 2B. The molecular markers

Table 1 Phenotypic values of Sumai3, Stoa, and their 108 recombinant inbred lines (RILs) for kernel shattering (KS) and Fusarium head blight (FHB) infection investigated in 2004 and 2005

Trait	Environment[a]	Parents[b]		RILs population			
		Sumai3	Stoa	Mean	Range	SE[c]	H^{2d}
KS (%)	2004 CAS	93.8a	7.0b	37.2	0.0–97.3	10.7	94.2
	2004 PRO	63.3a	3.8b	16.3	0.0–73.4	17.7	41.0
	2004 CAR	22.7a	2.5b	7.7	0.1–48.1	5.5	81.4
	2005 CAS	95.3a	2.7b	23.0	0.0–91.3	12.9	85.5
	2005 PRO	80.9a	6.0b	29.3	0.4–96.6	13.9	85.6
	Mean	71.2a	4.4b	22.1	0.5–69.4	9.6	86.3
FHB infection (%)	2004	6.3a	57.4b	19.5	2.5–81.4	10.8	70.8
	2005	2.1a	36.7b	11.0	0.0–43.4	7.9	61.5
	Mean	4.2a	47.1b	15.3	1.2–48.3	9.0	60.2

[a] Environment: *CAS* Casselton, *PRO* Prosper, *CAR* Carrington; 2004 and 2005, years 2004 and 2005

[b] Means of parents followed the same letter within the same environment are not significantly different at $P = 0.05$

[c] Standard error

[d] Broad sense heritability, %

Table 2 List of markers linked to kernel shattering in a recombinant inbred line population derived from Suma3 × Stoa based on line means across five environments in North Dakota using the single marker regression analysis

Marker	*Xbarc160*	*Xbarc1064*	*Xbarc68*	*Xbarc139*	*Xbarc49*	*Xbarc121*
Chromosomes	2B	2B	3B	3B	7A	7A
R^2 %[a]	7.0	10.0	6.0	13.0	15.0	21.0
P-value	0.00494	0.00073	0.013	0.00013	<0.000001	0.00002

[a] Proportion of phenotypic variation explained by each marker

Table 3 Summary of QTLs for kernel shattering and Fusarium head blight (FHB) infection detected in a recombinant inbred line population derived from Sumai3 × Stoa in investigated environments using the composite interval mapping

LG[a]	QTL peak position[b]	Marker interval[c]	Increase effect[d]	2004 CAS[e]		2004 PRO		2004 CAR		2005 CAS		2005 PRO		Combine	
				LOD	R^{2f}	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2
Kernel shattering															
2B	46.1 (*Xbarc1064*)	*Xbarc1064–Xbarc101*	Sumai3									2.6	15.4		
3BC	27.2 (*Xbarc1111*)	*Xbarc139-Xwmc787*	Sumai3	2.8	11.6					3.4	14.3	3.6	12.6	3.1	12.5
7A	12.0 (*Xbarc195*)	*Xbarc23–Xbarc192*	Sumai3	6.9	54.6	2.9	19.4	2.1	8.9			2.0	14.5	3.8	19.2
7A	56.7 (*Xwmc633*)	*Xgwm282–Xwmc633*	Sumai3	2.6	10.6					2.3	8.8				
FHB infection															
2B	42.1 (*Xbarc1064*)	*Xgwm319–Xgwm388*	Stoa	-	-			-	-	-	-	4.3	14.9	4.0	12.4
3BS	13.0 (*Xbarc102*)	*Xwmc754–Xgwm493*	Stoa	-	-	2.5	7.3	-	-	-	-	3.5	13.8	5.7	19.9
3BC	30.9 (*Xgwm77*)	*Xbarc68–Xwmc787*	Stoa	-	-	4.1	12.4	-	-	-	-				
7A	48.6 (*Xgwm282*)	*Xbarc192–Xwmc633*	Stoa	-	-	2.0	6.4	-	-	-	-				

[a] Linkage group

[b] Peak position in the most significant environment is expressed in cM and the closest marker is indicated in bracket

[c] Marker interval for the most significant environment

[d] Increase effect is the source of the allele causing an increase in the trait

[e] *CAS* Casselton, *PRO* Prosper, *CAR* Carrington; 2004 and 2005, years 2004 and 2005; trait not measured in specific environment are marked with '-'

[f] R^2 , percentage of phenotypic variation explained by a QTL

closest to these four KS QTLs were analyzed in a multiple regression model based on the line means across the five environments. The minor QTL on linkage group 2B is not significant ($P > 0.05$) and is excluded from the multiple regression model. The remaining three loci in this model collectively explain 36.9% of the phenotypic variation. Given the broad sense heritability of 0.86 (across the five environments), they account for 42.8% of the genetic variation.

Four QTLs for FHB were found using the same genotypic data for KS (Table 3; Fig. 2). Sumai3 contributed the alleles conferring FHB resistance in all four loci. One major QTL on linkage group 3BS was consistently detected in both years explaining 7.3 and 13.8% of the phenotypic variation in 2004 and 2005, respectively. This QTL does not coincide with or link to any KS QTLs detected in this study. Three additional minor FHB QTLs were each detected on linkage groups 2B, 3BC, and 7A. These three FHB QTLs were each present only in a single year and accounted for 14.9, 12.4, and 6.4% of the phenotypic variation, respectively. The FHB QTLs on linkage groups 2B and 3BC coincided with the KS QTLs in the corresponding genomic regions. Similarly, the FHB QTL on linkage group 7A was located between two KS QTLs and linked to the minor KS QTL.

Alternate allele effects for the major KS and FHB QTLs are shown in Table 4. Molecular markers closest to the QTLs are used in the analysis. Either of two major KS-resistant alleles (from Stoa) can significantly decrease KS. Selecting of both KS-resistant alleles further decreases KS, which is 66.1% less relative to the reciprocal alleles (from Sumai3) and 41.1% less than the population mean (22.1%). However, these lines with both KS-resistant alleles are much more susceptible to FHB than the lines with the reciprocal alleles (20.8% vs. 11.9% of the FHB infection). This might be explained by the coincident and/or linked QTLs for KS and FHB on linkage groups 3BC, and 7A. However, if these two major KS-resistant alleles are

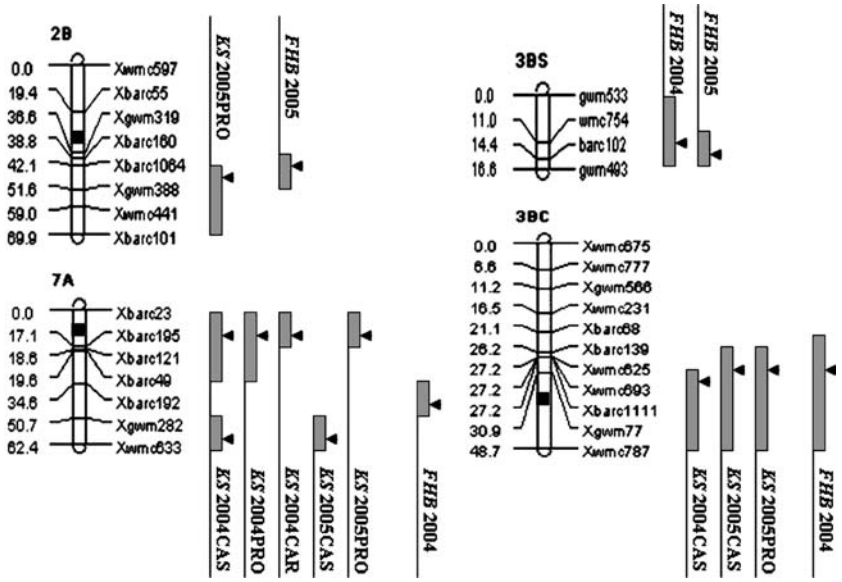

Fig. 2 The locations of QTLs identified by composite interval mapping for kernel shattering (*KS*) and Fusarium head blight (*FHB*) in a recombinant inbred lines population derived from the Sumai3 × Stoa cross. *Each bar* indicates the QTL for a specific trait in a specific environment. The *length* of the *bar* indicates the marker interval, and the *triangle* indicates the LOD peak position. Abbreviations for environments are following the trait names (KS or FHB): *CAS* Casselton, *PRO* Prosper, *CAR* Carrington; 2004 and 2005, years 2004 and 2005. The *black box* on the chromosome indicates the possible centromere based on the previously published genetic maps (Somers et al. 2004; Liu et al. 2005)

Table 4 Effects of alternative alleles at two major kernel shattering (KS) QTLs and one major Fusarium head blight (FHB) QTL using line mean values of KS and FHB infection across environments

QTL alleles			Number of lines	Mean[b]	
7A *Xbarc195*	3BC *Xbarc1111*	3BS *Xbarc102*		KS	FHB infection
Stoa	Stoa	–[a]	46	13.0d	20.8a
Stoa	Sumai3	–	17	23.5bc	12.0b
Sumai3	Stoa	–	12	25.1b	9.9b
Sumai3	Sumai3	–	25	38.4a	11.9b
Stoa	Stoa	Sumai3	14	14.1cd	13.7b

[a] Allele is not considered

[b] Means followed by the same letter within the same column are not significantly different at $P = 0.05$

stacked with the major FHB-resistant allele on linkage group 3BS (from Sumai3), which is not linked with the KS QTLs, the lines would have both low FHB infection (13.7%) and KS (14.1%) values.

Discussion

The KS in cultivated wheat is different from the shattering in wild wheat species caused by the brittle rachis. There are three types of brittle rachis, W, B, and spike, based on different breaking points on the rachis (Li and Gill 2006), which are due to the formation of abscission zones or layers on the rachis (Matsumoto et al. 1963). However, in cultivated wheat, abscission zones are not formed on the rachis. The occurrence of KS is triggered by the loose glumes, which could be easily separated wider and even be broken off with the outer forces such as wind, rain, or mechanical forces, causing kernels to fall out. Lewicki (1929) suggested that the degree of glume separation was associated with KS. However, very limited information on the microscopic structure of glumes in wheat is available. Vogel (1938) reported the breaking point at the glume base in cultivated wheat. Therefore, the possibility exists that the abscission zone in cultivated wheat is formed at the glume base, which might has an effect on KS. In rice (*Oryza sativa*

L.), various morphologies of abscission layers among cultivars have been found at the basal portions of the grains, causing different degrees of seed shattering (Ji et al. 2006).

Evaluation of KS under field conditions is difficult, time-consuming, and inconsistent because it is influenced by various factors (Harrington and Waywell 1950). The micro-environmental changes in the field might result in large error variances in evaluating KS (Clarke and DePauw 1983). Vogel (1941) suggested that lodging could protect the spikes from the wind force, and therefore affect the occurrence of KS. Head disease such as FHB could cause shriveled kernels resulting in little shattering (Zhang and Mergoum 2007). All these may cause difficulties in phenotyping and identifying QTLs for KS. In this study, severe lodging and FHB infection was observed at Prosper and Carrington in 2004, respectively, which significantly lowered the KS levels. The low heritability of KS at Prosper in 2004 further reflected the difficulties in evaluating KS in certain environments. Consequently, only the major QTL on linkage group 7A was detected with smaller effects in those two environments. At Casselton in 2004, however, little lodging and FHB infection were observed to be coupled with favorable environmental conditions for KS. This resulted in a high KS level and heritability as well, leading to the detection of a few KS QTLs. Among the four QTLs detected in this study, two were present in most test environments. However, Marza et al. (2006) recently observed a high inconsistency of QTL detection for KS, which they attributed to the environmental conditions. In their study, only one QTL had been detected in three of the seven test environments. Three were detected only in a single environment and the remaining two in two environments. This high inconsistency might also be explained by the use of a different evaluation method and scoring date (visual estimation right at the harvest maturity) in their study.

Porter (1959) suggested that KS was controlled by polygene in genotype 'Cimarron.' This study used a similar evaluation method as Porter (1959) and found a continuous frequency distribution for KS, which indicates that KS is quantitatively inherited. In our study, two major and two minor QTLs for KS were discovered, which agrees with Porter (1959), who reported that KS in genotype 'Blackhull' was controlled by two major genes together with some minor genes. However, early studies (Lewicki 1929; Hughes 1940) suggested that KS was controlled by one or two genes. This might be explained by the different evaluation method employed in their studies, where minor genes might be difficult to detect because genotypes were only visually classified as resistant and susceptible. The genetic variation explained by the QTLs detected in our study is 42.8%. The unexplained variation may be attributed to the undetected minor QTLs and/or epistatic effects, which is difficult to identify with the selective genotyping methodology. It is also possible that there are still more major QTLs undetected because of the low coverage of SSR markers in the genome in our study. Marza et al. (2006) have reported that chromosomes 4B, 5A, 6A, 6B, 7A, and 7D were all associated with KS. This indicates that more genomic regions may be involved in controlling KS except the ones found in our study.

One of the two major KS QTLs found in this study is located near the centromere on chromosome 7A, where Marzar et al. (2006) also detected one KS QTL tagged by SSR marker *Xbarc108* in the Ning7840 × Clark population, where Ning7840 is a Sumai3 derivative. Based on the wheat consensus map (Somers et al. 2004), *Xbarc108* is located near the centromere on chromosome 7A. Therefore, the QTL on 7A identified in our study is more likely to be identical to the one reported by Marza et al. (2006). Our results confirmed that the genomic region on chromosome 7A is associated with KS.

The other major KS QTL identified in this study is located near the centromere on chromosome 3BC. Interestingly, genes for the brittle rachis in wheat have been detected on group 3 chromosomes by various studies (Chen et al. 1998; Iqbal et al. 2000; Watanabe et al. 2002, 2006; Li and Gill 2006; Nalam et al. 2006). Iqbal et al. (2000) revealed a gene for spike-type brittle rachis on chromosome arm 3AS. Watanabe et al. (2006) and Nalam et al. (2006) located genes for W-type brittle rachis on homoeologous chromosome arms 3AS and 3BS. Li and Gill (2006) further suggested that the genes for the spike-type and W-type brittle rachis were located in the same genomic region on chromosome arm 3AS based their franked markers. A gene for B-type brittle rachis was also identified on chromosome arm 3DL (Li and Gill 2006). Comparative mapping analysis further suggested that the major loci for brittle rachis between wheat and barley were homoeologous (Nalam et al. 2006). All these facts indicate that there might be one gene family on group 3 chromosomes. This gene family has the same function: formation of abscission layers. But each gene in this gene family may control the development of abscission layers at a specific position. It might be possible that the KS QTL on linkage group 3BC in this study also belongs to this gene family, and it controls the formation of abscission layers at the glume base. The abscission layers at the glume base might make the glume be broken off easily, and therefore cause more KS. Nalam et al. (2006) located one gene for W-type brittle rachis 28 cM distal from *Xgwm77*, which is in the centromeric region on chromosome 3B. In our study, the KS QTL on linkage group 3BC is about 3 cM proximal from *Xgwm77*. The QTL in our study seems different from the brittle rachis gene reported by Nalam et al. (2006) since the loci are located more than 20 cM away. This is in agreement with the different characteristics of these two traits. A similar finding in barley was

reported by Kandemir et al. (2000): genes controlling two types of brittle rachis were both located on the short arm of chromosome 3H, but they were not closely linked.

Resistance to FHB is a complex trait, which can be confounded by environmental conditions, such as temperature and humidity, particularly at flowering. Therefore, artificial inoculation at flowering stage is a prerequisite to accurately evaluate FHB resistance. Three inoculation methods have been developed to evaluate FHB (Rudd et al. 2001). Point (single floret) inoculation is used to evaluate type-II resistance. Spraying and grain spawn inoculation could be used to measure the combination of types-I and II resistance and closer to the natural epidemics. Most studies detected the type-II FHB QTLs using the point inoculation (Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002b, 2004; del Blanco et al. 2003; Shen et al. 2003; Lin et al. 2004). Evaluating only for type-II resistance simplifies the analysis of this complex disease; however, it does not provide information on the field resistance. The type-II resistance in Sumai3 and its derivatives has been well characterized through QTL mapping (Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002b). These studies revealed a major QTL for type-II resistance on chromosome arm 3BS. Using the grain spawn method in our study, a QTL for field resistance in the same genomic region on 3BS were consistently detected. However, the QTL effect (19.9% of the phenotypic variation across environments) detected in our study is much lower than the one (41.6% of the phenotypc variation) detected by Anderson et al. (2001). This suggests that the FHB QTL on 3BS might mainly contribute to the type-II resistance. This finding concurred with the conclusion of Buerstmayr et al. (2003) when comparing between the point and spraying inoculation results.

The results from our study allowed us to detect another three FHB QTLs belonging to linkage groups 2B, 3BC, and 7A. These QTLs are new and have not been reported in this population by Waldron et al. (1999) and Anderson et al. (2001), who studied the same population using the point inoculation method. This might be explained by either these QTLs were not mainly for type-II resistance or these genomic regions have not been covered in their studies. However, these three QTLs were each detected only in a single year. These QTLs are reported because FHB QTLs in similar genomic regions have been detected in previous studies. Zhou et al. (2002a), using the substitution lines, suggested that chromosomes 2B and 7A in Sumai3 conferred type-II resistance. A type-II resistant QTL in similar genomic region on 2B was detected in Ning7840 in only one of four environments (Zhou et al. 2002b). Schmolke et al. (2005) detected a field resistant QTL with small effect on chromosome arm 2BL in 'Dream' (PI 539570) based on the mean across four environments. Similarly, the centromeric region on chromosome 3B has been reported to contain a field resistant QTL in 'DH181' (a Sumai3 derivative) (Yang et al. 2005) and 'Maringa' (PI 542436) (Somers et al. 2003), and a type-II resistant QTL in Sumai3 derived lines (del Blanco et al. 2003) and 'Wangshuibai' (Zhou et al. 2004). But these QTLs generally explained only <10% of the phenotypic variation and were significant only in a single environment. Both Zhou et al. (2004) and Jia et al. (2005) identified a type-II resistant QTL on chromosome arm 7AL in Wangshuibai, which are located close to the QTL position reported in our study. Both QTLs reported in their studies were not consistently detected and had only small effects. All these similar QTLs were detected in different studies indicate that the minor FHB QTLs detected in our study might be real, although they were inconsistently detected and had only small effects.

The FHB-resistant source Sumai3 introduced from China possesses unfavorable agronomic traits, including KS (Rudd et al. 2001). Most studies focused on the FHB resistance from Sumai3 or its derivatives. None of the studies has determined the relationship between FHB resistance and KS. Our study is the first one to report a significant and negative association between KS and FHB infection using a Sumai3 derived RIL population. The coincident and/or linked QTLs between KS and FHB on chromosomes 2B, 3B, and 7A with opposite allele effects provides further evidence that low FHB severity is associated with high KS. Either close linkages or pleiotropic effects between FHB and KS at the loci on linkage groups 2B and 3BC are possible. At the level of map resolution in this study, it is difficult to distinguish between the close linkage and pleiotropic effect. This undesirable association might cause difficulties in breeding adapted FHB resistant cultivars. However, only minor FHB QTLs are associated with the KS QTLs in this study, and the correlation between KS and FHB is not strong. The major FHB QTL on chromosome arm 3BS was not linked to any KS QTLs. Breeding cultivars with reasonable resistance for both FHB and KS is possible by pyramiding appropriate resistant QTLs for both KS and FHB. This has been demonstrated by the recent release of hard red spring wheat cultivars with moderate FHB resistance and high resistance to KS such as 'Glenn' (Mergoum et al. 2006) and 'Faller.' However, further investigations are warranted to fully understand these genomic regions containing both KS and FHB QTLs before attempting to pyramid these QTLs.

Evaluation of both KS and FHB is challenging, particularly under field conditions. Marker-assisted selection (MAS) can be a useful and powerful tool for breeders to select for these two traits given that robust markers linked to these traits are identified. This is the first study to report two major QTLs conditioning KS resistance and the linked SSR markers for these two KS QTLs. This makes MAS for

KS resistance feasible. Stacking different FHB resistant QTLs is the way to maximize the FHB resistance level. However, caution is needed when introgressing FHB-resistant QTLs from Sumai3 into adapted cultivars because of the undesirable linkage between FHB and KS at several loci.

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