

QTL Analysis of a Putative Novel Source of Resistance to *Fusarium* Head Blight in Hard Winter Wheat

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ABSTRACT

Host plant resistance is a highly effective approach for managing *Fusarium* head blight (FHB), an important disease of wheat (*Triticum aestivum* L.) in many regions of the U.S. and worldwide. This study was conducted to identify QTL linked to an indigenous source of FHB resistance in a South Dakota – adapted winter wheat genotype, SD97060. A population was produced by hybridizing SD97060 with ‘Jagalene’ winter wheat and advancing generations using single seed descent. The F_{2:4}, F_{2:5} and F_{2:6} generations were evaluated after artificially inoculating plants in mist-irrigated field nurseries in 2006 and 2007 and in a greenhouse in 2008, respectively. Disease incidence, severity, *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) content were evaluated in the 114 recombinant inbred lines (RILs). Four QTL were detected using phenotypic data obtained from field and greenhouse trials. Two QTL, located at chromosomes 2BL and 4BL, exhibited stable expression across the two environments. The 2BL QTL conferred resistance to FHB; whereas, the 4BL QTL was associated with susceptibility to FHB. Two QTLs, which did not exhibit stable expression across environments were detected at the 3BS and 3BSc using field and greenhouse data. The 2BL QTL of SD97060 could serve as a valuable source to enhance FHB resistance in wheat without limiting yield potential.

Keywords: *Fusarium* head blight (FHB), quantitative trait loci (QTL), diversity array technology (DArT) marker, simple sequence repeat (SSR) marker

INTRODUCTION

Fusarium head blight (FHB) has negatively impacted U.S. wheat production over periods of time since the latter part of the 19th century (Arthur 1891; McMullen *et al.* 1997). The disease decreases yield, affects grain quality, and results in the production of mycotoxins in the grain. Approaches to managing the disease have included the use of host plant resistance, field cultivation, fungicide applications, and crop rotation. Field cultivation and the use of fungicides have only partially succeeded in helping manage the disease (da Luz *et al.* 2003; Mesterhazy 2003b). Hence, identifying and employing host plant resistance has been the focus of a more effective means of managing the disease. Snijders (1990) suggested that various FHB resistance genes originate from different geographical regions and could be categorized as being derived from: Eastern Europe, Italy, China, Japan and Brazil. ‘Frontana’, a Brazilian cultivar, is an important source, providing resistance to penetration and disease spread in the spike (Singh *et al.* 1995). The Chinese line ‘Sumai3’ and its derivatives, which exhibit resistance to disease spread in the spike have been widely used in breeding programs throughout the world (Bai and Shaner 1994; Rudd *et al.* 2001; Mesterhazy 2003a). Buerstmayr *et al.* (2002) reported that crossing with Sumai3 resulted in poor yield and low grain quality. Primarily because of the poor yield and quality associated with Sumai3, there has been an effort to identify indigenous sources of resistance to FHB within adapted U.S. germplasm.

The application of molecular markers and identification of quantitative trait loci (QTL) have helped to accelerate gain from selection in U.S. wheat breeding programs (Anderson *et al.* 2007), and FHB resistance is a complex trait that is influenced by various environmental factors. Complicating evaluations for FHB resistance is the fact that dis-

ease symptoms become evident at the adult plant stage, and therefore, phenotypic evaluations require an expenditure of field and greenhouse resources. Use of molecular markers can help optimize resources and to lessen the effects that the environment has on phenotypic expression. A number of studies with different sources of resistance have identified QTL linked to all wheat chromosomes, except 7D (Buerstmayr *et al.* 2009).

FHB infection results in the production of mycotoxins that are harmful to both humans and animals. Deoxynivalenol (DON), one of the major mycotoxins, causes feed refusal, emesis, and decreased weight gain in non-ruminants (Yoshizawa and Morooka 1973). Due to concerns of DON in human food products, countries have instituted standard guidelines that limited DON in wheat grain. For instance, the U.S. Food and Drug Administration (FDA) issued a guideline limiting mycotoxins to 1.0 ppm in finished wheat food products (FDA 1993). FHB epidemics have resulted in DON content levels of 10 ppm and higher (McMullen *et al.* 1997).

An advanced winter wheat breeding line developed at South Dakota, SD97060, has exhibited moderate resistance to the FHB. The source of resistance for SD97060 is unknown, and its pedigree does not predict that it would express a Sumai3 or Frontana resistance. Furthermore, SD97060 exhibits high grain yield and is otherwise competitive with adapted varieties for agronomic and grain quality performance. The objectives of the study were to (i) identify QTL associated with a reduced disease index, FDK and DON content in SD97060 and to (ii) confirm the novelty of these QTL compared with known FHB resistance sources.

Table 1 Pedigree and seed source of the parental genotypes used in this study.

Parents	Pedigree	Source
SD97060	'Maverick'/KS79397//''Norstar'/3/'Cody'/4/NE90574(='Rodeo'/'Brule' seln.//''Arapahoe')	South Dakota
Jagalene	'Abilene'/'Jagger'	Agripro Inc.

MATERIALS AND METHODS

Field evaluation

1. Plant material and transplanting

In 2006 and 2007, at least eight seeds from 114 F_{4,5} and F_{5,6} recombinant inbred lines (RILs), developed using single seed decent (SSD), from the cross SD97060/'Jagalene' were evaluated in mist-irrigated field nurseries at the Agronomy Farm, South Dakota State University, Brookings, SD (Latitude = 44° 16' N, Longitude = 96° 46' W). Nursery soils are characterized by a Barnes series (fine-loamy, mixed, superactive, frigid Calcic Hapludolls) soil type (Malo 2003; Schaefer 2004). Line SD97060 is a hard red winter wheat (HRW) that expresses a moderate level of resistance to FHB in South Dakota, and the source of resistance is unknown. Jagalene is a popular HRW that is highly susceptible to FHB in South Dakota (Table 1). The RILs were transplanted into the field in May 2006 and 2007. The plot size was a 12.7 cm row in the field. Seedlings were vernalized for 8 weeks at 4°C prior to transplanting. The field nurseries were planted to a randomized complete block design with two replications.

2. Inoculum production

An aggressive *Gibberella zeae* (anamorph *Fusarium graminearum*) isolate (Fg4 isolate) was multiplied and conidia were harvested as described by Zhang *et al.* (2001). The isolate was evenly spread on half strength potato dextrose agar media with lactic acid (1.6 ml L⁻¹). The plates were incubated at 25°C using a 12 h dark and light cycle for 7 days. Macroconidia were harvested in sterile water. The conidial suspension was adjusted to 70,000 spore ml⁻¹ with distilled water. TWEEN 80 (Fisher Scientific International Inc. Pittsburgh PA) wetting agent was added to the suspension at a rate of 400 µl L⁻¹.

Ten *Fusarium* isolates were used to produce infested maize (*Zea mays* L.) kernels for field nurseries. Maize kernels that filled one-fourth of a steel tray (49.5 × 29.2 × 6.4 [cm³]) were soaked in water overnight for 12 h. The water was drained and kernels were autoclaved for 45 min. For each isolate, two Petri plates of the previously colonized PDA were cut into approximately 1 cm² pieces and spread on each steel tray containing kernels. The inoculated maize kernels were incubated at room temperature for 14 days. The kernels were sun-dried inside a greenhouse for 5 days and stored at 4°C.

3. Inoculum application

Infested maize kernels were spread in the field at a rate of 5 g plot⁻¹ (1 m by 0.4 m) when wheat was at jointing (Growth Stage (GS) 31, Zadoks *et al.* 1974) and then at 7 days intervals until heading (GS 59). Field nurseries were mist-irrigated (0.4 L h⁻¹) on the same day the first infested kernels were spread. The mist irrigation system was run at a frequency of 2 min every 30 min from 7:00 pm to 7:00 am the following morning. Misting occurred each day until disease ratings were recorded for the latest maturing genotype.

At heading, a conidial suspension of 70,000 spore ml⁻¹ was applied to spikes using a backpack sprayer (0.5 L min⁻¹ at 207 kPa). To inoculate later flowering spikes, spraying was repeated 7 days after the initial spray.

4. Disease rating

Disease incidence and severity were recorded at 21 days after the first field and greenhouse inoculations, respectively. Disease ratings for each entry were averaged over the plants in the plot. Disease incidence was the percentage of number of spikes infected to total spikes. Disease severity was the percentage of infected spikelet(s)

in the spike. Disease severity was recorded based on a 0-9 scale (Stack and McMullen 1995). Disease index, calculated as the product of disease incidence and disease severity, was highly correlated with severity ($r \geq 0.99$, $P < 0.01$) in both years. Therefore, disease index was not used in the analysis.

The percentage of shriveled and bleached kernels was recorded in each RIL population after hand harvesting and threshing inoculated spikes from the field nurseries.

Mycotoxin concentrations were measured in the RILs grown in the field environment. The inoculated spikes from each population were hand harvested and threshed. Kernels from replicated plots were pooled for each population. A 20 g sample was ground in a coffee-grinder and sent from each RIL to North Dakota State University, Fargo, ND for mycotoxin measurements. Mycotoxins were analyzed using the GC/ECD method (Tacke and Casper 1996).

Greenhouse evaluation

1. Plant material and transplanting

In 2008, at least eight seed from F_{5,6} RILs were also evaluated in a greenhouse located at the main campus in Brookings, SD. The plot size and vernalization was same as described in the Plant material and transplanting under the field evaluation. The experiment was arranged in a completely randomized design with two replications.

2. Inoculum production and application

Conidial suspension was produced as described above. The F_{5,6} lines were evaluated by injecting a conidial suspension into a 10th or 5th (spike with less than 10 spikelets) spikelet. A suspension of 10 µl was injected with a micro-pipette into the spikelet at heading stage (GS 59). The spike was immediately covered by a plastic Ziploc bag for 72 h. The greenhouse temperature was maintained at 25 ± 3°C for the duration of the experiment.

3. Disease rating

In the greenhouse, disease severity was the percentage of the number of bleached and symptomatic spikelet(s) on each spike.

Genotyping

1. Diversity array technology (DArT)

Leaf samples were collected from plants at the seven-leaf stage (GS 17). DNA was extracted from the leaf samples following the CTAB method as described by Triticarte Pty. Ltd. (2009). Triticarte Pty. Ltd. (Canberra, Australia; <http://www.triticarte.com.au>) screened parents and RILs according to the method described by Wenzl *et al.* (2004) and Akbari *et al.* (2006). Each genotype was scored as either present "1" or absent "0" for DArT markers. Triticarte Pty. Ltd. assigned the prefix "wPt" (w stands for wheat, P stands for *PsI* and t stands for *TaqI*) followed by a unique four digit identification number for each of the DArT markers, and the same nomenclature of DArT markers is used in this publication.

2. Simple sequence repeat (SSR)

DNA was extracted from leaf samples following Saghai-Marooof *et al.* (1984) with minor modifications. The polymerase chain reaction mixture (13 µl) contained 0.05 µM of forward-tailed primer (5'-ACG ACG TTG TAA AAC GAC-3'), 0.05 µM 6-FAM/VIC/NED/PET-labelled M13 primer (5'-ACG ACG TTG TAA AAC GAC-3', Applied Biosystems), 0.10 µM reverse primer, 200 µM of deoxynucleotide, 2.5 mM MgCl₂, 1.5 U *Taq* polymerase, 200 ng of template DNA and 1X ammonium sulfate buffer. After heating the

mixture to 95°C for 5 min, the PCR reaction occurred over 35 cycles. The first five cycles consisted of denaturing at 96°C for 1 min, 68°C (-2°C/cycle) for 5 min, 72°C for 1 min, followed by five cycles of 96°C for 1 min, 58°C (-2°C/cycle) for 2 min, 72°C for 1 min, and the remaining 30 cycles consisted of 96°C for 1 min, 50°C for 1 min, 72°C for 1 min with a final extension step of 72°C for 5 min. PCR products were scanned with GeneScan-500 LIZ as an internal size standard (Applied Biosystems) in an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems), and the results were analyzed with GeneMarker software (Softgenetics LLC.).

Statistical analysis

An analysis of variance for single and combined years was conducted for disease, severity and FDK. Proc GLM of SAS (SAS Institute 2008) was used to analyze the data. Broad-sense heritability (h^2) was estimated by:

$$h^2 = \sigma_g / (\sigma_g + \sigma_{gy}/y + \sigma_e/ry)$$

where, σ_g = genotypic variance component, σ_{gy} = genotype × year interaction variance component, σ_e = residual error variance component, r = replications, and y = years.

The 90% confidence interval of heritability was calculated as described by Knapp *et al.* (1985). Pearson's correlation coefficient was computed to test the association between disease incidence, severity, index, FDK and DON content using Proc Corr of SAS (SAS Institute 2008).

QTL analysis

Marker map distance was constructed with Map Manager QTX (Manly *et al.* 2001) setting search linkage criterion at $P < 0.05$ and using Kosambi map functions. RECORD (Os *et al.* 2005) was used to reorder the marker position within the linkage group. Simple interval mapping with $P < 0.001$ was used to detect QTL in the Map Manager QTX. The linkage groups containing significant QTL were again reanalyzed in Windows QTL Cartographer 2.0 (Wang *et al.* 2007) software. Composite interval mapping (Zeng 1994) with a background control of 5 markers and a 10-cM window size was used for QTL analysis in the Windows QTL Cartographer. MapChart (Voorrips 2002) was used to draw the linkage map for the markers.

RESULTS

Statistical result

The RIL means for severity and FDK were correlated across years and the test for homogeneity of variance was not significant; hence, ANOVAs and heritability estimates were calculated for averaged values of severity and FDK across the two years (Table 2). While significant differences were detected among genotypes for severity and FDK, no significant genotype-by-year interaction was detected for either trait. As might be predicted based on the complexity of the trait, broad-sense heritability was intermediate for severity (0.51), and low for FDK (0.25).

Table 3 represents the correlation coefficients among disease assessment traits in different years and averaged across years. There was no significant correlation between DON content and any other measured evaluation trait both in the individual and combined across years. In 2006, 2007

Table 2 Analysis of variance and heritability estimates for severity and FDK (*Fusarium* damaged kernel) averaged across two years (2006 and 2007) in the field at Brookings, SD

Source	DF	Mean Squares	
		Severity	FDK
Rep (Year)	2	449.12*	80.13NS
Year (Y)	1	1195.68**	798.26**
Genotype (G)	113	264.73**	186.51**
G × Y	113	129.60NS	140.34NS
Error	120	107.32	114.68
h^2 (90% CI)		0.51 (0.35,0.64)	0.25 (0.00,0.45)

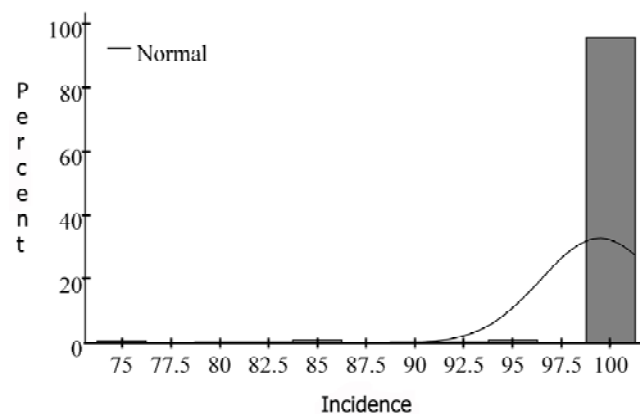


Fig. 1 Histogram of 115 RIL lines for incidence averaged across two years (2006-2007) in a field environment at Brookings, SD.

and combine across the years, there was a significant correlation between FDK and severity, but not between FDK and incidence. However, a significant correlation existed between severity and incidence in 2007 and combined across the years.

For the field nurseries in 2006 and 2007, histograms showing the distribution of RILs for incidence, severity, FDK and DON content are represented by Figs. 1, 2, 3, and 4, respectively. The incidence histogram was skewed toward susceptibility since there was very little resistance to initial FHB infection among the tested RILs. As an indication of this, the average incidence was 99.3%, with a range of 75 to 100%. A more continuous distribution was observed for FHB severity, FDK and DON content. There was a single histogram peak for severity and DON content; whereas, there were two distinct peaks for FDK. The average FHB severity for RILs was 61.0%, with minimum severity of 33.0% and maximum severity of 87.3%. The mean FDK and DON content for the RIL populations were 57.9% (range 35.0 to 90.0%) and 9.8 ppm (range 2.6 to 17.6 ppm) respectively. In the greenhouse, the average severity was 19.8% (range 6.0 to 53.3%) (Fig. 5). In both field and greenhouse environments, transgressive segregation was observed among RILs for severity, FDK, and DON content.

Markers and linkage map

Triticarte Pty. Ltd. detected 156 polymorphic DArT markers on the RILs based on the microarray evaluation of hexaploid wheat clones. Of the 156 polymorphic DArT markers, the locations of 31 markers were not assigned to any chro-

Table 3 Correlation coefficients among incidence, severity, FDK (*Fusarium* damaged kernel) and DON (deoxynivalenol) content in 2006, 2007 and averaged across two years (2006 and 2007) in the field at Brookings, SD.

	Incidence			Severity			FDK		
	2006	2007	Averaged	2006	2007	Averaged	2006	2007	Averaged
Severity	0.07NS	0.31**	0.32**	-	-	-	-	-	-
FDK	0.13NS	0.13NS	0.12NS	0.57**	0.49**	0.52**	-	-	-
DON	0.22NS	-0.18NS	-0.15NS	0.01NS	0.00NS	-0.05NS	0.13NS	-0.03NS	-0.01NS

NS non-significant at 0.05 probability level

** significant at 0.01 probability levels

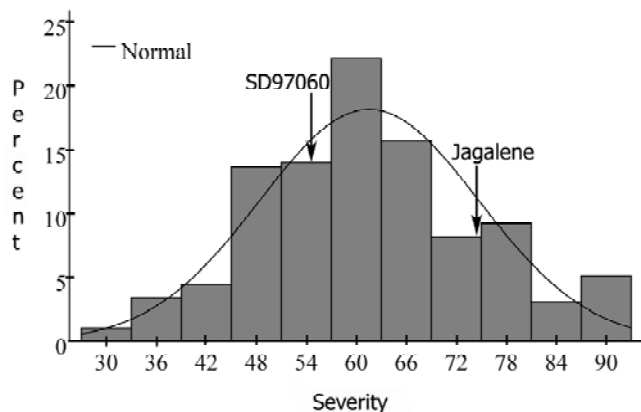


Fig. 2 Histogram of 115 RIL lines for disease severity averaged across two years (2006-2007) in a field environment at Brookings, SD.

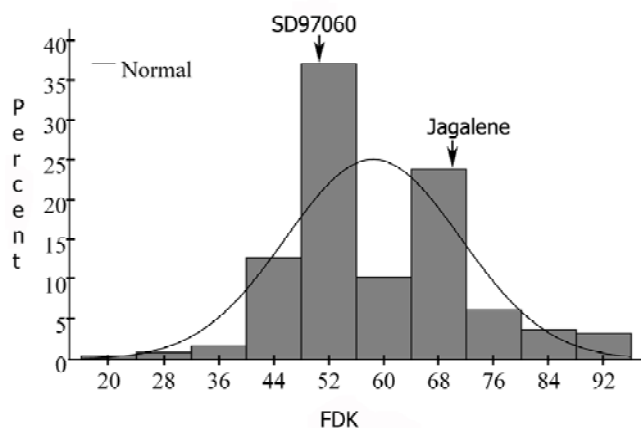


Fig. 3 Histogram of 115 RIL lines for *Fusarium* damaged kernels (FDK) averaged across two years (2006-2007) in a field environment in Brookings, SD.

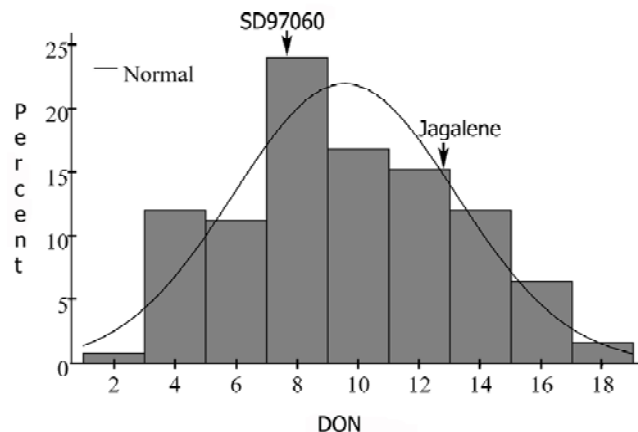


Fig. 4 Histogram of 115 RIL lines for DON content averaged across two years (2006-2007) in a field environment in Brookings, SD.

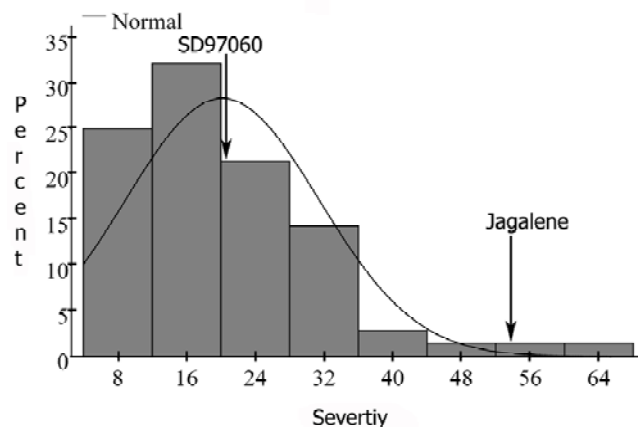


Fig. 5 Histogram of 115 RIL lines for disease severity in a greenhouse environment in Brookings, SD in 2008.

mosome. A 'Distribute' option in the Map Manager QTX was used to assign the markers into appropriate linkage groups. Three polymorphic SSR markers reported to be linked to the FHB QTL were also included to prepare the linkage maps. Twenty-five linkage groups were constructed from a total of 159 molecular markers (Figs. 6-9). Some of the linkage groups assigned to the same chromosome were vertically stacked over each other if the order of the DArT markers in the linkage groups matched the DArT order in the published hexaploid and tetraploid wheat maps (Akbari *et al.* 2006; Semagn 2006; Crossa *et al.* 2007; Mantovani *et al.* 2008). The total genetic map distance was the largest for the linkage group assigned to chromosome 6B (127.2 cM); whereas, the shortest linkage length was for chromosomes 3A and 7A (0.5 cM). The average length of a linkage group was 43.0 cM.

QTL mapping and analysis

Simple and composite interval mapping demonstrated that there were four QTL at three chromosomes for disease severity. These QTL were detected at 2BL, 3BS, 3BSc and 4BL, with LOD > 2.5 (Table 4). The QTL at 2BL was detected in both years and when using field and greenhouse data. It explained 11.3 and 32.8% of the variability for FHB severity in 2007 and 2006, respectively. The additive effects of the QTL ranged from -3.4 to -9.9. Two QTLs were detected at 3B. In the 2006 field environment, the 3BS QTL was detected in the marker interval of wPt-7984 to Xgwm533 (Table 4A). A different QTL at 3BSc and spanning the marker region from wPt-4209 to wPt-7142 was detected using the greenhouse data (Table 4B). The additive effects were negative (-14.2) for the 3BS QTL; whereas, they were positive (4.6) for the 3BSc QTL. A QTL at 4BL was detected

using data from the 2007 field nursery, from the combined data of the two field nurseries, as well as from the greenhouse data (Table 4). This 4BL QTL explained up to 52.2% of the phenotypic variation for disease severity in the greenhouse environment. The additive effects of this QTL ranged from 4.6 to 5.9.

DISCUSSION

Due to lack of disease incidence resistance in the parents, almost all RILs had high initial levels of disease and the distribution was skewed towards susceptibility. Thus, the data from the field represented observations for disease severity resistance. A significant correlation was observed between severity and FDK. It inferred that selecting for lower severity may also indirectly lower FDK in subsequent generations. However, there was no significant correlation between DON content and severity or FDK measured in the field nurseries. This result is in contrast to earlier findings, which demonstrated DON content was significantly correlated with severity and FDK (Malla 2005; Paul *et al.* 2006).

The QTL related to FHB response was detected with DArT markers for severity. No QTL were detected for either FDK or DON content in the population used in this study. Of the four QTL for severity, the one at 2BL was the most stable since it was observed across different inoculation methods, years, and evaluation environments. Based on the reported map of Akbari *et al.* (2006), this QTL should be in a 2BL region, similar to where a QTL from 'Ning7840' (Zhou *et al.* 2002), 'Strongfield' (Somers *et al.* 2006), 'Ernie' (Liu *et al.* 2007), and 'G19-92' (Schmolke *et al.* 2008) was located. The QTL at 2BL is apparently associated with a resistant reaction as the additive effects were

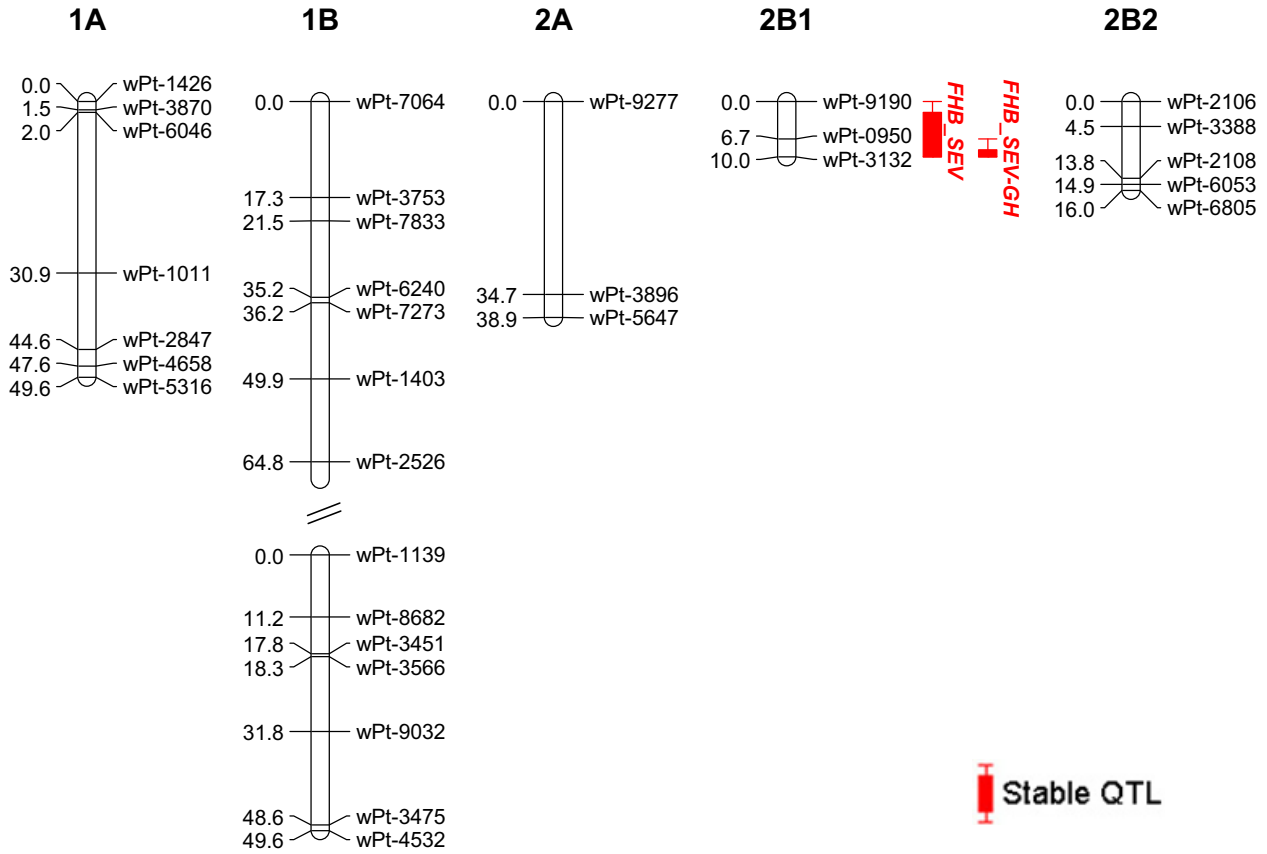


Fig. 6 Linkage map for specific chromosome (designated on top) and QTL position shown by red color on the right of the linkage map.

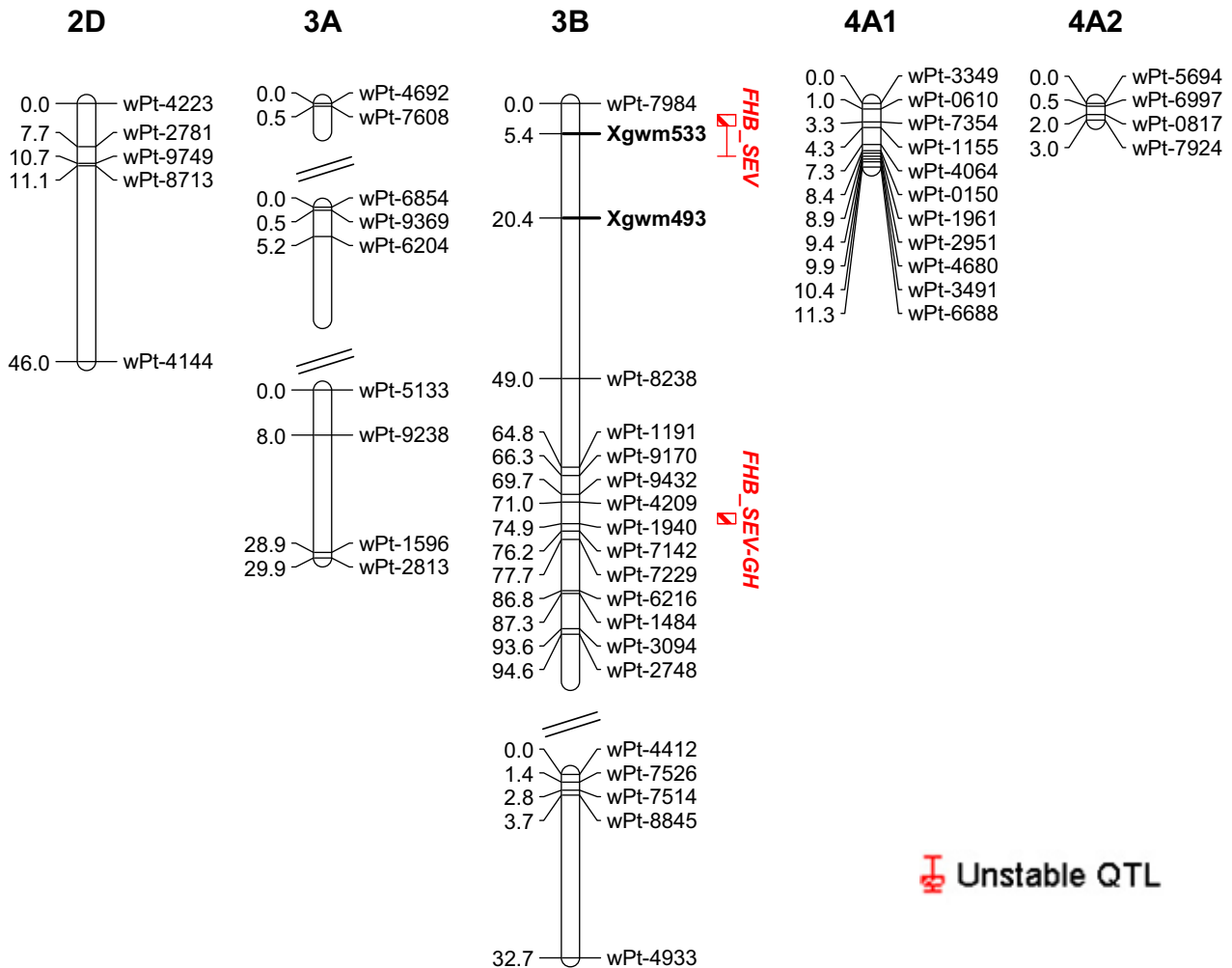


Fig. 7 Linkage map for specific chromosome (designated on top) and QTL position shown by red color on the right of the linkage map.

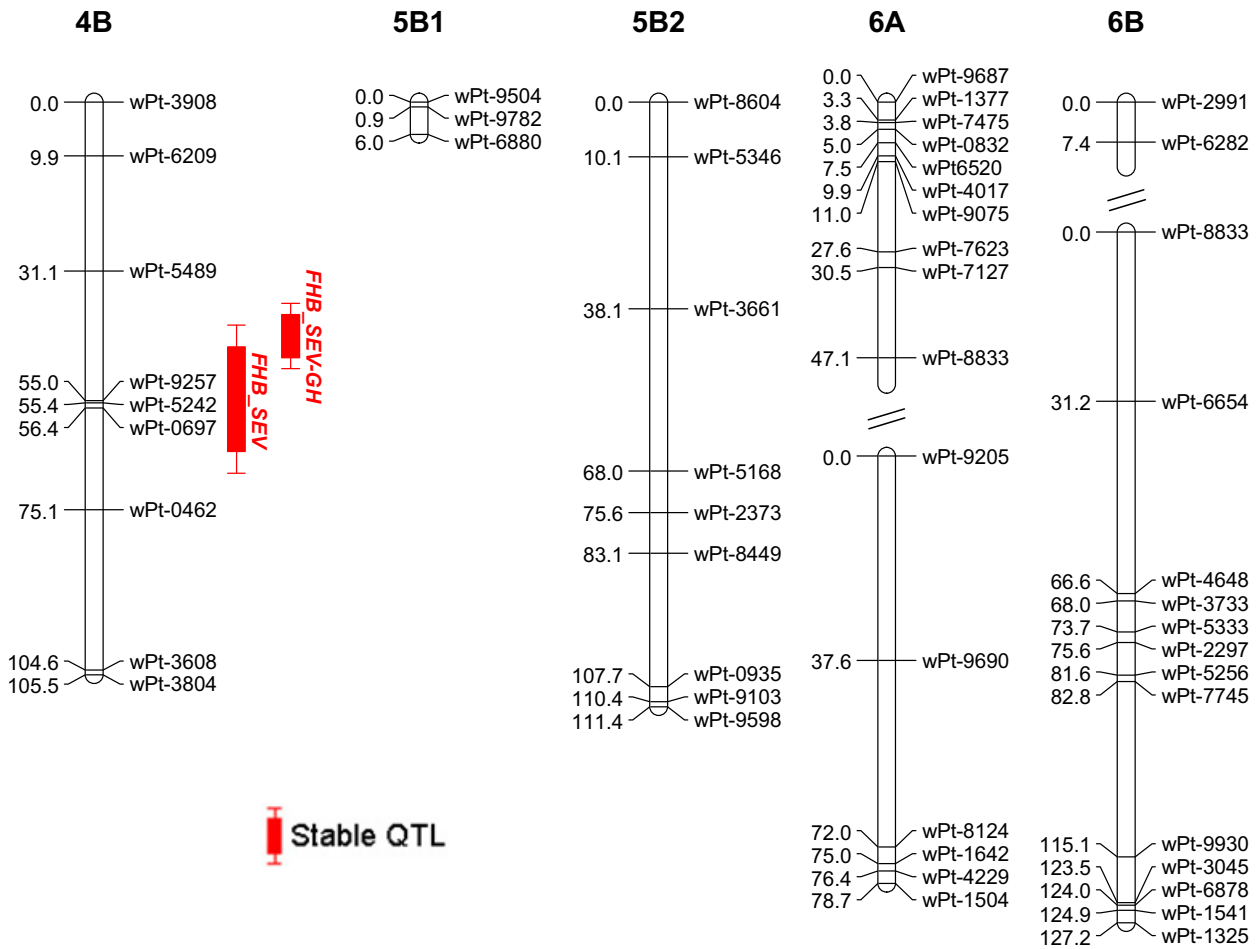


Fig. 8 Linkage map for specific chromosome (designated on top).

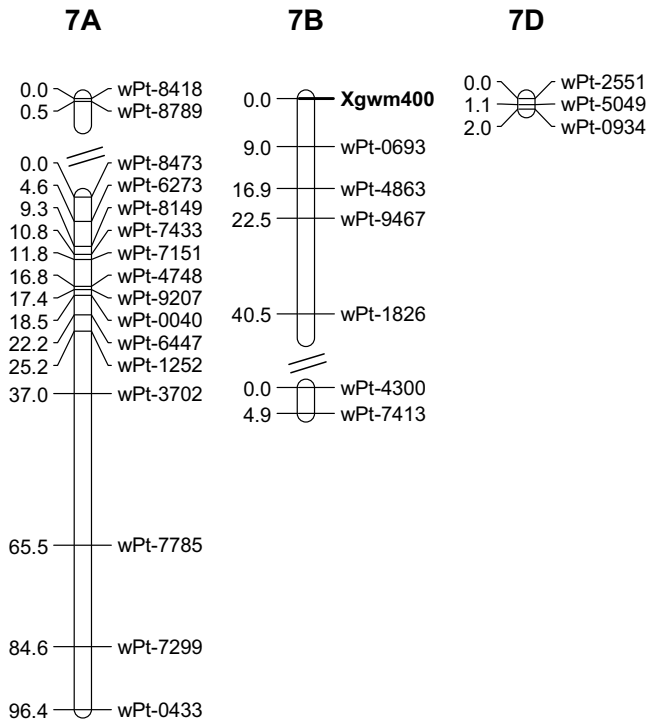


Fig. 9 Linkage map for specific chromosome (designated on top).

negative, and the results were in agreement with those reported by Liu *et al.* (2007) and Schmolke *et al.* (2008). A study using a reciprocal backcross monosomic analysis also found that chromosome 2B in Frontana expressed resistance to FHB (Berzonsky *et al.* 2007). In spite of non-significant

QTL effects using data from the 2006 field environment, the QTL at 4BL was also stable, as it was detected using the 2007 field as well as greenhouse data. This QTL was located at a similar region where other QTL were reported in previous studies (Somers *et al.* 2003; Jia *et al.* 2005; Yang *et al.* 2005; Lin *et al.* 2006; Liu *et al.* 2007). This QTL, which was likely contributed to the RILs by SD97060 was associated with susceptibility and a higher level of FHB severity. The result was in contrast to Liu *et al.* (2007), where 4BL QTL from Ernie was associated with resistance to disease severity. The two QTL detected at 3B are considered unstable in expression, as they were only expressed in the field or greenhouse environments, but not both. It is possible that the resistant QTL detected at 3BS might be *Fhb1*, a resistance QTL from Sumai3 and its derivatives (Waldron *et al.* 1999; Anderson *et al.* 2001; Zhou *et al.* 2002). The *Fhb1* gene has not previously been detected in any U.S. HRW variety. It is also possible, the QTL at 3BS for SD97060 might be the QTL common to 'Wangshuibai' (Zhou *et al.* 2004) and Ernie (Liu *et al.* 2007) that was associated with susceptibility in these genotypes, in contrast to the findings of Zhou *et al.* (2004) and Liu *et al.* (2007), who reported resistant QTLs at that chromosomal region. Environment likely influenced the expression of the 3BS and 3BSc QTL such that they were not consistently expressed over years. Three out of four QTL identified for SD97060 were also reported in other winter wheat genotypes. The QTL at 2BL, 3BSc and 4BL in this study were also reported in Ernie (Liu *et al.* 2007); whereas the QTL at 2BL was also reported in G16-92 (Schmolke *et al.* 2008). The results indicate that DArT markers were useful in detecting QTL with stable expression each at 2BL and 4BL and QTL at 3B which did not exhibit stable expression.

As in previous studies, QTL at 2BL, 3B and 4BL were involved with either the expression of resistance or susceptibility to FHB. Because of its stable expression, the resis-

Table 4A, B QTLs for resistance to FHB severity (type II) as detected by composite interval mapping in a SD97060/Jagalene population in field (A) and greenhouse (B) environments in Brookings, SD

A			2006			2007			2006 and 2007		
Chromosome	Marker interval	Region length (cM)	LOD	Additive effects	R ² (%)	LOD	Additive effects	R ² (%)	LOD	Additive effects	R ² (%)
2BL	wPt-9190 - wPt-3132	10.0	4.6	-9.9	32.8	2.6	-3.4	11.3	3.6	-4.3	15.4
3BS	wPt-7984 - Xgwm533	5.4	4.4	-14.2	32.1	-	-	-	-	-	-
4BL	wPt-5489 - wPt-0462	44.0	-	-	-	3.2	4.9	20.6	4.1	5.9	23.6

B			2008		
Chromosome	Marker interval	Region length (cM)	LOD	Additive effects	R ² (%)
2BL	wPt-9190 - wPt-3132	10.0	2.5	-4.2	11.7
3BSc	wPt-4209 - wPt-7142	5.2	3.6	4.6	16.2
4BL	wPt-5489 - wPt-9257	23.9	3.8	8.4	52.2

tant QTL at 2BL will likely be useful when utilized in marker-assisted selection for resistance to FHB. This will be valuable to breeding programs, particularly if it is confirmed that the QTL is from an indigenous source of resistance and breeders can successfully incorporate it into adapted genotypes with acceptable agronomic and quality characteristics.

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