Fusarium Head Blight Resistance in U.S. Winter Wheat Cultivars and Elite Breeding Lines

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ABSTRACT

Fusarium (*Fusarium graminearum*) head blight (FHB) is a destructive disease of wheat (*Triticum aestivum* L.) worldwide. To characterize FHB resistance in U.S. wheat germplasm, 363 U.S. winter wheat accessions were repeatedly evaluated for FHB resistance. A high correlation ($r = 0.73$, $P < 0.001$) for mean percentages of symptomatic spikelets (PSS) was observed between greenhouse and field experiments. The majority of tested accessions were either moderately or highly susceptible; only 7% of the accessions in the greenhouse and 6% of the accessions in the field showed a high level of resistance. Mean PSS for 19 accessions that carry markers for *Fhb1*, a major quantitative trait locus (QTL) from 'Sumai3', are 29.8% in the greenhouse and 25.1% in the field experiments. Fifty-four wheat accessions lacking *Fhb1* showed at least a moderately high level of FHB resistance in the greenhouse and/or field. These included three resistant accessions, 35 moderately resistant accessions, and 16 accessions that showed different levels of resistance in greenhouse and field experiments. Accessions without *Fhb1* that showed consistent resistance in both field and greenhouse experiments may be good sources for pyramiding native resistance QTLs from U.S. wheat with *Fhb1*.

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Abbreviations: FHB, Fusarium head blight; HWW, hard winter wheat; NIL, near-isogenic lines; PCR, polymerase chain reaction; PSS, percentage of symptomatic spikelets; QTL, quantitative trait locus; SWW, soft winter wheat.

Fusarium head blight (FHB, caused by *Fusarium* spp.) of wheat, also known as wheat scab, is one of the most destructive diseases in the humid and semihumid wheat–growing areas worldwide (Parry et al., 1995; Osborne and Stein, 2007). *Fusarium graminearum* Schwabe [teleomorph = *Gibberella zeae* (Schw.) Petch] is the prevailing wheat pathogen in the United States and many other countries (Bai and Shaner, 2004). Severe FHB epidemics occur when a susceptible host encounters abundant pathogen inocula in the presence of humid and warm weather during

wheat anthesis (Osborne and Stein, 2007). FHB epidemics can cause significant losses in both grain yield and quality. Harvested grain contaminated with mycotoxins, especially deoxynivalenol, produced by the pathogen is a serious safety concern to human and animal health (Bai and Shaner, 1994; Parry et al., 1995).

Use of resistant cultivars coupled with fungicide application is the most effective strategy to minimize disease losses. In China, a nationwide screening of germplasm and breeding lines identified 'Sumai 3' and its derivatives to have the best resistance (reviewed by Bai and Shaner, 1994, 2004), and they have become the major sources of FHB resistance in breeding programs worldwide. Quantitative trait loci (QTLs) for FHB resistance have been reported on all 21 chromosomes (Bai and Shaner 2004; Yu et al., 2008; Liu et al., 2009; Buerstmayr et al., 2009). However, only the *Fhb1* QTL on chromosome 3BS has a large effect mainly on type II resistance, resistance to fungal spread within a spike (Schroeder and Christensen, 1963), which has been stable across various genetic backgrounds (Bai and Shaner, 2004). In the United States, FHB epidemics originally occurred mainly in hard spring wheat in the northern Great Plains and in soft winter wheat (SWW) regions, and extensive screening of breeding materials from those regions has identified several U.S. cultivars with FHB resistance, such as 'Roane', 'Ernie', and 'Freedom' (Rudd et al., 2001, Griffey et al., 2001). QTL haplotype analysis indicates that these cultivars do not carry *Fhb1*, which means they may carry resistance QTLs that are different from those in Chinese sources (Liu et al., 2005). In the hard winter wheat (HWW) growing region of the Great Plains, FHB has not been a major issue until recent years; thus, systematic screening of HWW germplasm and breeding materials for FHB resistance has not been reported. Initial evaluation of some HWW identified several cultivars, including 'Heyne' and 'Hondo', with FHB resistance. QTLs in these cultivars may be different from those in Asian sources (Zhang et al., 2012). Combining U.S. native resistance genes with the resistance alleles at major QTLs from Asian sources may diversify the FHB resistance gene pool and significantly enhance FHB resistance levels in U.S. wheat. Therefore, characterizing U.S. winter wheat, especially HWW elite breeding lines, may provide important information to breeders for selecting good parents for breeding crosses. This study was designed to evaluate the effects of *Fhb1* on FHB resistance in U.S. winter wheat backgrounds, to identify native sources of FHB resistance, and to investigate wheat accessions with type I resistance (resistance to initial infection) and type II resistance by comparing reactions to FHB in greenhouse and field experiments.

MATERIALS AND METHODS
Plant Materials
A total of 363 winter wheat accessions, including 289 HWW and 74 SWW accessions, were evaluated for FHB resistance in both greenhouse and field experiments. HWW accessions were selected from five HWW nurseries: the 2008 and 2010 Southern and Northern HWW Regional Performance Nurseries, the 2010 HWW Regional Germplasm Observation Nursery, the 2010 Tri-state FHB Nursery, and the 2008 Yield Trial Nursery from the wheat breeding program at Oklahoma State University. SWW accessions were selected from Uniform Eastern Soft Red Winter Wheat Nurseries and Uniform Southern Soft Red Winter Wheat Nurseries. The project consisted of two sets of materials tested in different experiments: Set I had 207 accessions, including all the HWW and SWW entries from the 2008 nurseries and breeding lines from Oklahoma; and set II had 191 accessions, including 156 new accessions from the 2010 HWW nurseries and 35 selected accessions from experiment I. In both sets, Sumai3 (resistant), 'Wesley' (moderately susceptible), and 'Duster' (susceptible) were used as controls.

Evaluation of FHB Resistance
In the greenhouse experiments, six plants per line were transferred into a 13 by 13 cm Dura-pot (Hummert Int.) with a 12-h photoperiod after vernalization for 6 wk at 4°C in a cold chamber. Set I was tested in 2009 (spring and fall) and 2010 (spring) greenhouse experiments, and Set II was tested in 2011 (spring and fall) and 2012 (spring) greenhouse experiments. All experiments were arranged in a randomized complete block design with two replications (pots) of six plants in each experiment.

Conidial inocula of *F. graminearum* were prepared using field isolate GZ 3639 from Kansas. This isolate has showed consistent pathogenicity on a set of wheat cultivars for over a decade (G. Bai, unpublished data, 2013). Conidial suspension was adjusted to 100 spores μL^{-1} for inoculation. About six spikes with similar flowering time in each pot were inoculated by injecting 10 μL of the conidial suspension into a central spikelet of a spike at anthesis using a syringe. After inoculation, plants were moved into a moist chamber with 100% relative humidity for 48 h at 21 ± 5°C to initiate infection. Infected plants were then moved to a greenhouse bench for disease development at 21 ± 5°C during the day and 17 ± 2°C during the night. About 15 d post-inoculation, when the susceptible control was completely blighted, the numbers of infected and total spikelets in each inoculated spike were counted to calculate the percentage of symptomatic spikelets (PSS) in a spike.

Field experiments were conducted in the Rocky Ford FHB Nursery of the Department of Plant Pathology, Kansas State University (Manhattan, KS). Set I was evaluated for FHB in the springs of 2009, 2010, and 2011, and Set II was evaluated in the springs of 2011 and 2012. About 40 seeds per accession were planted in a 1-m-long single-row plot, and each experiment had two replications. The FHB nursery was inoculated using spawn inoculation, in which *F. graminearum*–infected corn (*Zea mays* L.) kernels were scattered on the soil surface at the booting stage and 2 wk afterward to facilitate initial infection. To ensure FHB infection in early flowering plants, needle inoculation was also conducted as described for greenhouse inoculation with six spikes per plot to assess type II resistance. From flowering through early dough stages, the nursery was misted by sprinklers 3 min h^{-1} from 2100 to 0600 h daily. PSS was estimated for all plots on the basis of overall performance of a plot at 21 d after needle inoculation. PSS data were rechecked after 3 d.

All accessions were classified into one of four categories based on their PSS: resistant, moderately resistant, moderately

susceptible, and susceptible. Classification decisions were made by comparing mean FHB rating of each accession with the 95% confidence intervals of resistant, moderately susceptible, and susceptible controls. Accessions falling between resistant and moderately susceptible were classified as moderately resistant.

DNA Extraction and Marker Analysis

Leaf tissue was collected at the two-leaf stage, and genomic DNA was isolated using a cetyltrimethyl ammonium bromide (CTAB) method (Zhang et al., 2012). A sequence tagged site marker, Xumn10, was used to identify whether the *Fhb1* resistance allele was present (Liu et al., 2008), and a single nucleotide polymorphism marker for *Fhb1*, Xsnp3BS-8, was analyzed to verify *Fhb1* resistance allele (Bernardo et al., 2012). DNA sequencing for Xsnp3BS-8 was done for these accessions that did not provide useful single nucleotide polymorphism results. Polymerase chain reaction (PCR) was performed following Sun et al. (2010), and DNA sequencing was done using a Big-Dye Terminator V1.1 sequencing kit (Applied Biosystems).

Data Analysis

Analysis of variance (ANOVA) and regression analysis were conducted using SAS ver. 9.2 (SAS Institute, Inc.). Because two sets of materials were selected from Regional Performance Nurseries in two different years (2008 and 2010), they were evaluated for FHB in different sets of greenhouse and field experiments. To investigate if any significant PSS differences existed between the two sets of experiments, ANOVA was conducted for both greenhouse and field PSS data for 35 common accessions that were tested in both sets of experiments.

RESULTS
Wheat Reactions to Fusarium Head Blight in Greenhouses

The difference in PSS for the 35 accessions that were common to both sets of materials was not significant between the two sets of greenhouse experiments, among three tests of each set, or between replications in each test (data not shown); thus, the two sets of materials were combined for further data analysis. Correlation coefficients of PSS for 363 accessions were highly significant among the three greenhouse experiments ($r = 0.53$–0.67, $P < 0.001$).

Wheat accessions showed significant variation in PSS after single floret inoculation (Fig. 1). Control cultivars Sumai3 (resistant), Wesley (moderately susceptible), and
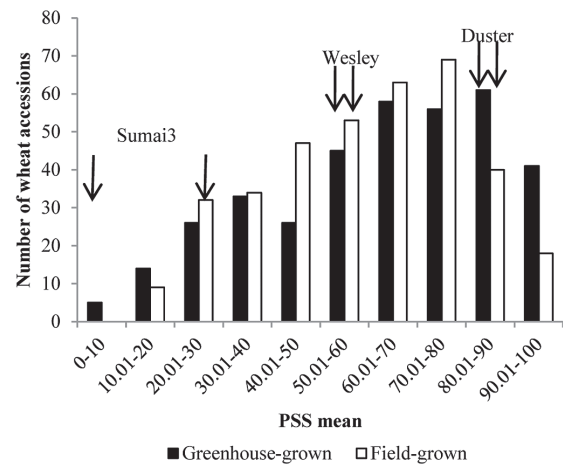

Figure 1. Frequency distribution of mean percentage of symptomatic spikelets (PSS) in a spike for 363 wheat accessions evaluated in greenhouse and field experiments at Manhattan, KS.

Duster (susceptible) had an average PSS of 8.6, 51.5, and 81.3%, respectively. Frequency distribution of PSS showed that most accessions (75.0%) were either as susceptible as Duster (43.0% with PSS $\geq$ 70.1%) or as moderately susceptible (32.0% with PSS between 45.1% and 70.0%) as Wesley (Fig. 1, Table 1). Among the 363 wheat accessions, only 25 (7.0%) were classified as resistant, with a PSS $\leq$ 23.0%, and 64 (18.0%) were moderately resistant, with a PSS between 23.1 and 45.0% (Table 1).

To test for the presence of the *Fhb1*-resistant allele in the resistant wheat accessions, marker Xumn10 was analyzed in all accessions. A total of 23 wheat accessions had the 258-bp marker allele associated with the *Fhb1* resistance allele. Among them, 16 were backcross-derived *Fhb1* near-isogenic lines (NILs) from the USDA marker-assisted backcross project (G. Bai, unpublished data, 2013). In these accessions, the *Fhb1* resistance allele had been transferred into three U.S. HWW cultivars (Wesley, 'Trego', and 'Harding') and one SWW cultivar ('Clark') (Table 2). They all showed a high level of resistance in greenhouse experiments, except for single *Fhb1* lines from Trego and Clark and two from Harding that had slightly higher PSS estimates. Among the seven other lines carrying the Xumn10 allele associated with *Fhb1* resistance, four lines (INW0411, P02444A1-23-9, NE08527,

Table 1. Reactions of two classes of U.S. winter wheat accessions, hard winter wheat (HWW) and soft winter wheat (SWW), to Fusarium head blight inoculation in the greenhouse and field experiments.

Wheat class	No. of accessions in greenhouse[†]					No. of accessions in field[†]				
	R	MR	MS	S	Total	R	MR	MS	S	Total
	(≤23.0%)	(23.1–45.0%)	(45.1–70.0%)	(≥70.1%)		(≤25.0%)	(25.1–50.0%)	(50.1–75.0%)	(≥75.1%)	
HWW	17	40	97	135	289	10	75	125	79	289
SWW	8	24	19	23	74	12	23	26	13	74
Total	25	64	116	158	363	22	98	151	92	363

[†] Phenotypic classification of accessions in greenhouse and field based on their reactions to *F. graminearum* by comparing their mean percentage of symptomatic spikelets (PSS) in a spike and 95% confidence intervals with resistant (R) control (Sumai3), moderately susceptible (MS) control (Wesley), and susceptible (S) control (Duster). Moderately resistant (MR) refers to accessions that had a PSS between Sumai3 and Wesley.

Table 2. A list of accessions that showed resistance and moderate resistance to Fusarium head blight as reflected by mean percentage of symptomatic spikelets (PSS) in a spike evaluated in greenhouse (GH) and field (F) experiments in Manhattan, KS, and that carry an *Fhb1* marker allele associated with FHB resistance.

Accession	Class†	PSS (GH)‡	PSS (F)‡	Xumn 10§	SNP¶
Wheat accessions with *Fhb1* allele					
INW0411	SWW	5.9 ± 2.6	27.8 ± 32.8	+	G
WesleyFhb1NIL09S-103#	HWW	8.1 ± 3.4	22.5 ± 8.9	+	G
WesleyFhb1NIL09S-104#	HWW	10.4 ± 2.8	23.8 ± 5.9	+	G
KS08FHB-78#	HWW	13.7 ± 8.4	23.8 ± 11.8	+	G
Wesley FHB1#	HWW	14.0 ± 6.1	18.1 ± 4.7	+	G
WesleyFhb1NIL09S-105#	HWW	15.9 ± 7.6	25.6 ± 9.2	+	G
TregoFhb1NIL09S-98#	HWW	21.0 ± 5.5	25.6 ± 10.3	+	G
TregoFhb1NIL09S-99#	HWW	21.6 ± 9.4	18.1 ± 3.4	+	G
ClarkFhb1NIL-75#	SWW	26.7 ± 7.1	13.8 ± 2.5	+	G
ClarkFhb1NIL09F-23#	SWW	27.1 ± 3.3	12.5 ± 2.7	+	G
ClarkFhb1NIL09F-45#	SWW	30.2 ± 21.0	18.1 ± 5.1	+	G
KS08FHB-31#	HWW	33.6 ± 21.1	20.6 ± 4.1	+	G
P02444A1-23-9	SWW	34.9 ± 30.4	21.2 ± 14.7	+	G
NE08527	HWW	35.3 ± 15.1	65.0 ± 11.2	+	C
P03112A1-7-14	SWW	35.6 ± 30.4	47.9 ± 24.8	+	G
HardingFhb1NIL09S-107#	HWW	44.7 ± 18.9	48.8 ± 9.5	+	G
TregoFhb1NIL09S-100#	HWW	45.6 ± 10.8	18.8 ± 5.9	+	G
BC01007-7	HWW	52.5 ± 19.2	58.8 ± 6.8	+	N
ClarkFhb1NIL09F-4#	SWW	53.9 ± 14.8	20.6 ± 9.2	+	G
HardingFhb1NIL09S-109#	HWW	59.1 ± 18.5	36.9 ± 15.6	+	G
HardingFhb1NIL09S-108#	HWW	64.7 ± 11.0	31.9 ± 12.6	+	G
VA05W-258	SWW	68.3 ± 10.2	50.7 ± 20.3	+	C
NX03Y2489	HWW	93.7 ± 10.7	92.9 ± 9.9	+	N
Mean PSS, %		35.5 ± 21.7	32.3 ± 19.3		
Resistant accessions without *Fhb1* allele in greenhouse experiments					
Freedom	SWW	7.6 ± 3.0	32.4 ± 25.2	–	–
MO040152	SWW	9.5 ± 1.7	25.2 ± 14.2	–	–
Roane	SWW	10.7 ± 4.8	19.3 ± 7.4	–	–
SD05085-1	HWW	14.6 ± 5.8	33.1 ± 11.6	–	–
T154	HWW	15.5 ± 7.8	18.1 ± 6.5	–	–
Bess	SWW	17.2 ± 14.4	36.8 ± 28.2	–	–
SD05210	HWW	18.4 ± 10.9	34.3 ± 20.1	–	–
Century	HWW	18.4 ± 9.6	51.4 ± 23.2	–	–
Heyne	HWW	18.6 ± 15.4	35.6 ± 7.4	–	–
P03207A1-7	SWW	18.8 ± 12.3	50.2 ± 26.7	–	–
KY96C-0769-7-3	SWW	19.9 ± 11.1	24.7 ± 7.9	–	–
Lyman	HWW	20.0 ± 8.8	26.9 ± 6.2	–	–
Everest	HWW	20.4 ± 12.5	27.3 ± 14.5	–	–
Harry	HWW	21.0 ± 13.0	38.1 ± 5.4	–	–
Atlas66	SWW	21.4 ± 13.4	43.9 ± 13.9	–	–
Husker	HWW	22.0 ± 14.9	76.9 ± 11.3	–	–
AP05T2413	HWW	22.1 ± 12.3	42.3 ± 25.3	–	–
Mean PSS, %		17.4 ± 4.3	36.3 ± 13.8		
Additional accessions without *Fhb1* but with FHB resistance in field experiments					
IL02-18228	SWW	54.7 ± 27.5	18.6 ± 10.2	–	–
M03-3616-C	SWW	31.5 ± 17.1	22.6 ± 9.0	–	–
G41732	SWW	42.2 ± 27.1	23.0 ± 11.7	–	–
USG 3555	SWW	25.2 ± 13.7	23.3 ± 12.4	–	–
Hitch	HWW	25.3 ± 15.3	23.8 ± 4.6	–	–
G61505	SWW	39.9 ± 33.6	24.1 ± 8.8	–	–
KS08IFAFS1	HWW	90.7 ± 4.0	25.0 ± 7.2	–	–
Moderately resistant accessions without *Fhb1* in greenhouse and field					
IL00-8530	SWW	23.6 ± 15.6	36.4 ± 19.5	–	–
SD08198	HWW	24.0 ± 9.8	37.5 ± 8.9	–	–
MD01W233-06-1	SWW	24.6 ± 17.9	27.2 ± 10.7	–	–
NI04420	HWW	26.0 ± 21.7	46.5 ± 10.9	–	–
SD05118	HWW	26.2 ± 22.4	40.9 ± 26.9	–	–
T153	HWW	26.5 ± 9.8	27.7 ± 7.9	–	–
M04*5109	SWW	26.6 ± 23.6	28.9 ± 8.2	–	–
MTS0531	HWW	27.2 ± 11.9	43.4 ± 19.9	–	–
G69202	SWW	27.6 ± 30.4	37.6 ± 26.4	–	–
Ernie	SWW	27.8 ± 14.0	26.7 ± 14.3	–	–
CO04W210	HWW	28.0 ± 12.0	38.3 ± 13.7	–	–
2008-193 Jagger (FHB3)	HWW	28.1 ± 6.4	42.5 ± 12.7	–	–
OK05128	HWW	28.3 ± 12.4	37.4 ± 11.0	–	–
OK05134	HWW	29.3 ± 17.6	42.7 ± 16.5	–	–
Aspen	HWW	30.2 ± 11.6	28.1 ± 6.8	–	–
OH02-12678	SWW	30.5 ± 14.8	35.0 ± 14.9	–	–
NE06545	HWW	32.8 ± 16.9	43.8 ± 13.8	–	–
Camelot	HWW	32.8 ± 18.2	45.0 ± 9.7	–	–
OH02-7217	SWW	32.8 ± 7.7	35.5 ± 10.7	–	–
U07-698-9	HWW	33.3 ± 15.9	31.6 ± 20.6	–	–
MD99W483-06-9	SWW	35.2 ± 18.8	43.5 ± 16.9	–	–
OK05723W	HWW	35.5 ± 25.7	49.8 ± 25.9	–	–
KY97C-0519-04-07	SWW	35.9 ± 27.2	32.9 ± 9.4	–	–
P04287A1-10	SWW	36.7 ± 15.8	35.2 ± 4.6	–	–
Endurance	HWW	36.8 ± 22.2	27.9 ± 9.4	–	–
Winterhawk	HWW	37.1 ± 11.2	46.3 ± 8.1	–	–
N02Y5117	HWW	39.1 ± 27.4	36.4 ± 20.1	–	–
OK06528	HWW	39.9 ± 34.1	48.5 ± 23.8	–	–
NW05M6011-6-1	HWW	40.0 ± 22.9	45.5 ± 5.5	–	–
Arapahoe	HWW	40.3 ± 14.5	43.1 ± 6.4	–	–

(cont'd)

and P03112A1–7–14) were resistant or moderately resistant to FHB, and three (BC01007-7, VA05W–258, and NX03Y2489) were moderately susceptible or susceptible.

To verify the presence of the *Fhb1* resistance allele in these accessions, the polymorphic nucleotide sequence at a recently developed single nucleotide polymorphism

Table 2. Continued.

Accession	Class[†]	PSS (GH)[‡]	PSS (F)[‡]	Xumn 10[§]	SNP[¶]
M04-4715	SWW	42.4 ± 29.2	32.3 ± 12.4	–	–
Overland	HWW	42.4 ± 19.9	40.0 ± 15.6	–	–
HV9W02-942R	HWW	42.6 ± 18.2	28.2 ± 11.2	–	–
MO011126	SWW	43.5 ± 15.5	28.0 ± 13.5	–	–
Jerry	HWW	44.4 ± 18.9	48.4 ± 17.7	–	–
Control cultivars					
Sumai3	SWW	8.6 ± 3.6	21.5 ± 18.0	–	G
Wesley	HWW	51.5 ± 22.2	55.9 ± 18.0	–	–
Duster	HWW	81.3 ± 18.7	85.9 ± 8.3	–	–

[†] HWW, hard winter wheat; SWW, soft winter wheat.

[‡] Mean of standard deviation.

[§] In Xumn10, '+' refers as *Fhb1* allele associated with FHB resistance, and '–' refers as non-*Fhb1* associated with FHB susceptibility.

[¶] In single nucleotide polymorphism (SNP) marker data derived from Xsnp3BS-8, G refers as *Fhb1* allele associated with FHB resistance, C refers as non-*Fhb1* associated with FHB susceptibility, and N refers as no polymerase chain reaction products in these lines carrying *Fhb1* resistant allele as predicted by Xumn10. '–' means that this marker was not analyzed for these lines without the resistance allele as predicted by Xumn10.

[#] Hard and soft winter wheat *Fhb1* near-isogenic lines.

marker, Xsnp3BS-8, was assayed. All 16 *Fhb1* NILs had the Sumai3 allele G (Table 2). Among the other seven *Fhb1* lines with the Xumn10 marker allele associated with resistance, only three (INW0411, P02444A1-23-9, and P03112A1-7-14) carry the Xsnp3BS-8 allele that is associated with resistance. Two (NE08527 and VA05W-258) carry the allele C associated with a susceptible reaction, and two (BC01007-7 and NX03Y2489) did not produce PCR products. Seventeen accessions did not carry the *Fhb1* resistance allele but still showed a high level of type II resistance, with a mean PSS of 17.4% (Table 2). These materials likely contain resistance QTLs other than *Fhb1* and include wheat accessions SD05085-1, T154, SD05210, 'Century', Heyne, 'Lyman', 'Everest', 'Harry', Freedom, and 'Atlas66' (Table 2). The mean PSS for the wheat accessions with the *Fhb1* resistance allele was 29.8% based on the two markers Xumn10 and Xsnp3BS-8 (Table 2). Therefore, *Fhb1* can significantly improve FHB resistance in many genetic backgrounds.

The percentage of resistant or moderately resistant accessions was higher in SWW (43.0%) than in HWW (20.0%) (Table 1). In HWW, the percentage was even lower (17.0%) after removal of *Fhb1* NILs; thus, HWW appears to have a much lower percentage of breeding lines or cultivars with FHB type II resistance than SWW (Table 2, Supplemental Table S1).

Wheat Reactions to FHB in the Field

In the two sets of field experiments, the difference in PSS for the 35 accessions common to both sets was not significant between the two sets of field experiments (data not shown), and the correlation coefficients of the 35 accessions

were significant (Supplemental Table S2). Therefore, they were combined for further statistical analysis. The field mean PSS for the three controls, Sumai3 (resistant), Wesley (moderately susceptible), and Duster (susceptible), increased slightly from the greenhouse data, so PSS ranges for the four phenotypic classes were adjusted accordingly for field data, with a PSS of 0 to 25.0% classified as resistant, 25.1 to 50.0% as moderately resistant, 50.1 to 75.0% as moderately susceptible, and above 75.0% as susceptible. Among the 363 accessions, only 22 were resistant (10 HWW and 12 SWW), and 98 were moderately resistant. A majority of accessions (67.0%) were either moderately susceptible (151) or susceptible (92). For the 289 HWW accessions, about 71.0% were moderately susceptible or susceptible to FHB in the field conditions (Table 1).

The 19 wheat accessions containing the *Fhb1*-associated alleles of both markers Xumn10 and Xsnp3BS-8 all had FHB resistance, with a mean PSS of 25.1% in the field experiments (Table 2). Among them, 16 *Fhb1* NILs had consistent resistance similar to that observed in the greenhouse experiments. Results confirmed that the *Fhb 1* resistance allele had a stable effect on reducing FHB severity both in greenhouse and field conditions. Among the 22 resistant accessions identified in the field experiments, three HWW and seven SWW accessions did not have the Xumn10 allele associated with *Fhb1* resistance (Table 1, Table 2). The HWW entries consisted of both breeding lines and released cultivars from different states, including T154, 'Hitch', and KS08IFAFS1. Resistant SWW cultivars or breeding lines from several states included IL02-18228, Roane, USG3555, and KY96C-0769-7-3 (Table 2).

Relationship of FHB Ratings between Greenhouse and Field Experiments

A significant correlation coefficient (r = 0.73, P < 0.001) of mean PSS for 363 wheat accessions was observed between greenhouse and field experiments (Fig. 2), suggesting that most wheat accessions with a low PSS in the greenhouse usually had a low PSS in the field (Fig. 2, Table 2). Correlation coefficients of PSS were significant (r = 0.45 to 0.64, P < 0.001) among the greenhouse experiments and among the field experiments. Significant correlations of PSS ratings were observed between three greenhouse and three field experiments, with r-values ranging from 0.40 to 0.96 (P < 0.001).

Comparing the resistant accessions identified from greenhouse and field experiments showed that 15 out of the 17 HWW accessions that demonstrated resistance in greenhouse experiments also had resistance or moderate resistance in field experiments, including the accessions developed from institutions or companies in South Dakota (SD05085-1, SD05210, Lyman), Nebraska (Harry), Kansas (T154, Heyne, Everest, and AP05T2413), and the USDA Genotyping Lab in Kansas (*Fhb1* NILs in Wesley or Trego backgrounds). Seven out of eight SWW

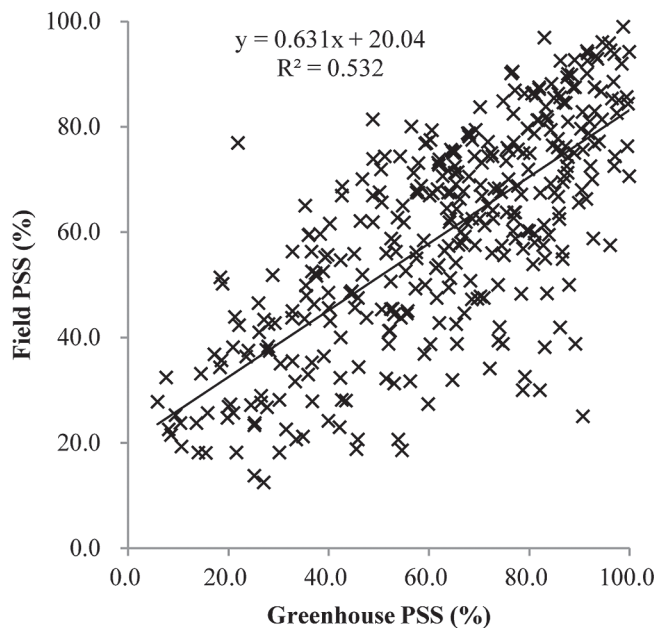$$y = 0.631x + 20.04$$
$$R^2 = 0.532$$

Fig. 2. Correlation of percentages of symptomatic spikelets in a spike (PSS) of 363 U.S. winter wheat accessions between greenhouse and field experiments conducted in Manhattan, KS.

accessions (INW0411, Freedom, MO040152, Roane, 'Bess', KY96C-0769-7-3, Atlas66) showed low PSS in both greenhouse and field experiments (Table 2). Accessions with a low PSS in the field usually also showed a low PSS in greenhouse, with a few exceptions.

Under both environments (greenhouse vs. field), most lines carrying *Fhb1* showed consistent resistance to FHB. For example, all the *Fhb1*-carrying NILs of Wesley, two of the three Trego *Fhb1* NILs, three of the four Clark *Fhb1* NILs, and one of three Harding *Fhb1* NILs showed consistent resistance in both environments (Table 2), suggesting that *Fhb1* is a reliable QTL for reduced PSS, and that it may contribute to both type I and type II resistance in the field. However, several accessions that did not carry *Fhb1* according to marker data also showed a high level of resistance. For example, one HWW, T154, and two soft red wheats, Roane and KY96C-0769-7-3, did not have *Fhb1* according to the allele at Xumn10, but showed a high level of resistance in all greenhouse and field experiments (Table 2). Another 35 accessions without the *Fhb1* resistance allele consistently showed moderate resistance in both greenhouse and field environments (Table 2). In addition, 16 accessions lacking *Fhb1*, such as SD05085-1, Heyne, Lyman, Everest, Harry, Hitch, Freedom, Bess, and Atlas66, had resistance in the greenhouse and moderate resistance in the field and moderate resistance in the greenhouse (Table 2, Supplemental Table S1). These accessions can be used either as parents in further breeding crosses or as FHB-resistant cultivars for commercial production to reduce FHB damage in epidemic years.

DISCUSSION
Repeatability of FHB Resistance in Field and Greenhouse Experiments

Systematic evaluation of wheat germplasm for FHB resistance has been reported in China and many other countries (Snijders, 1990; Miller et al., 1998; Buerstmayr et al., 2003; Bai and Shaner, 2004; Zhang et al., 2008; Oliver et al., 2008), but not for U.S. HWW, especially elite HWW breeding lines, so this study is the first attempt to systematically evaluate FHB resistance in U.S. winter wheat (mainly HWW) cultivars and breeding lines in both greenhouse and field experiments. The results provide valuable information that breeders can use to select resistant parents for crosses or to select elite breeding lines that could be released as FHB-resistant cultivars or germplasm.

To evaluate FHB resistance accurately, an effective evaluation protocol is crucial. Needle inoculation of a single spikelet in a spike is a common practice used for type II resistance, and FHB severity is usually scored using either PSS per spike (Bai and Shaner, 2004) or a 1 to 10 visual scale (Stack and McMullen, 1995). Spraying spores over spikes or scattering *Fusarium*-infected wheat or corn spawn in field is used to evaluate both type I and type II resistance, and incidence is scored by estimating proportion of diseased spikes per experimental unit (plot) to estimate type I resistance (Stack and McMullen, 1995). In field experiments, it is often impossible to distinguish between type I and type II resistance, so an FHB index is often used to reflect overall resistance (Seem, 1984; Bai and Shaner, 2004; Paul et al., 2005).

In this study, the experimental materials were repeatedly evaluated for FHB resistance in both greenhouse and field experiments. In the greenhouse, needle inoculation was performed and type II resistance was measured. Among the three greenhouse experiments, the correlation coefficients were highly significant. In the field studies, plants were inoculated by a combination of both needle and spawn inoculations, and were misted hourly from heading to dough stages to ensure that there would be enough moisture for infection. This procedure significantly reduced disease difference caused due to plant heights and flowering times of different wheat accessions. In Manhattan, KS, spawn inoculation with misting usually is effective in most years for inducing sufficient infection of most plants with high repeatability (Bockus et al., 2007), but spring weather conditions vary from year to year, especially with regard to ambient temperature. A warm early spring, for example, may lead to an early heading date, which may result in infection escape in early maturing accessions due to lack of inoculum. The needle inoculation technique can ensure that early flowering plants have an appropriate initial infection and can minimize flowering time effect on FHB level. Also, we scored FHB based on flowering time (21 d after needle inoculation),

needle–inoculated plants were scored when natural infection was low in these early flowering plants; thus, correlation coefficients among field experiments were similar to those among greenhouse experiments. The combination of needle and spawn inoculation methods can be recommended for field genetic studies, especially for genotypes with large differences in flowering times. Although we observed a slight difference in resistance ranking for some accessions between greenhouse and field experiments, the correlation coefficients between greenhouse and field experiments were still very high (Fig. 2). This result indicates that type II resistance is the major type of resistance for most accessions in field conditions, with a few exceptions, such as in Husker, Century, P03207A1-7, KS08I-FAFS1, and IL02-18228 (Table 2, Supplemental Table S1).

Husker, Century, and P03207A1-7 had a low PSS in the greenhouse experiments, indicating that they had type II resistance, but not type I resistance, as reflected by their high PSS in the field experiments, so they are not recommended for use in FHB resistance breeding. Only those accessions with low PSS in both field and greenhouse experiments should be used as resistant cultivars or breeding parents.

Impact of *Fhb1* on FHB Resistance

To date, although many different sources of FHB resistance have been reported worldwide (Bai and Shaner, 2004), the *Fhb1* gene has shown the largest effect on type II resistance in diverse genetic backgrounds and environments. Unfortunately, in this study, none of the released cultivars were shown to carry *Fhb1*, and only seven accessions (three HWW and four SWW) from regional nurseries carried the Xumn10 marker allele associated with *Fhb1*-mediated resistance (Liu et al., 2008). Among the seven accessions, NX03Y2489, VA05W-258, BC1007-7, and NE08527 are unlikely to carry *Fhb1* based on their pedigrees. One possible reason for the low frequency of *Fhb1* in U.S. winter wheat is that Sumai3 and its Chinese derivatives have many undesirable traits, so progenies with *Fhb1* usually inherit some of these. When breeders select for desirable agronomic traits and adaptation to North America, plants carrying the *Fhb1* gene might be discarded in field selection due to their poor agronomic traits. To solve this problem, the USDA Genotyping Laboratory in Manhattan, KS, successfully transferred *Fhb1* into four U.S. winter wheat backgrounds (Wesley, Trego, Harding, and Clark) using marker-assisted backcrossing. This successfully combined *Fhb1* with adapted agronomic traits and improved the resistance of U.S. winter wheat. Among the four recurrent parents, Clark is a soft red winter wheat, Trego is a hard white winter wheat, and Wesley and Harding are hard red winter wheats. In the greenhouse tests, four Wesley *Fhb1* resistant NILs had a mean PSS similar to Sumai3. Three Trego *Fhb1* resistant

NILs and four Clark *Fhb1* resistant NILs had a slightly higher PSS than Sumai3, but had a significant reduction in PSS compared with their recurrent parents. Significant PSS reduction in these NILs was also observed in the field experiments (Table 2, Supplemental Table S1). These NILs have an appearance similar to their recurrent parents, so transfer of *Fhb1* to U.S. winter wheat can quickly improve the level of FHB resistance. These selected *Fhb1* NILs should be good parents for future breeding crosses; however, *Fhb1* was not equally effective at enhancing FHB resistance in all genetic backgrounds. For example, the Harding *Fhb1* NILs had a PSS similar to Harding. Thus, selecting appropriate recurrent parents is important for successful use of *Fhb1*.

Among potential *Fhb1* carriers from the Regional Nurseries, INW0411, P02444A1-23-9, and P03112A1-7-14 displayed a high level of FHB resistance, whereas BC01007-7, VA05W-258, and NX03Y2489 were highly susceptible. NE08527 had only type II resistance, as shown in greenhouse experiments, but not in field experiments (Table 2). High susceptibility in some lines with the *Fhb1* resistance-associated allele of the Xumn10 marker was possibly due to Xumn10 not being a diagnostic marker for *Fhb1*. This assumption is supported by two factors: (i) the pedigrees of those lines do not have any connection with Sumai3 sources, and (ii) they all carry a susceptible allele that is associated with susceptibility or fail to amplify any PCR product at the Xsnp3BS-8 marker (Bernardo et al., 2012). All other lines with the Xumn10 allele linked to the *Fhb1* gene have the allele associated with resistance at Xsnp3BS-8 (Table 2). Thus, *Fhb1* as determined by both markers Xumn10 and Xsnp3BS-8 significantly improved type II resistance in these U.S. wheat backgrounds.

North American Sources of FHB Resistance in U.S. Winter Wheat

In this study, 17 accessions showed a similar or slightly lower level of type II resistance than Sumai3 in the greenhouse experiments, even though they do not carry the Xumn10 marker allele associated with *Fhb1* resistance allele and are not related to any Chinese sources of resistance in their pedigrees. This suggested that the resistance of these accessions to FHB might originate from North American sources. Among them, seven accessions are SWW types. Freedom (Gooding et al., 1997) and Roane (Griffey et al., 2001) have been major U.S. sources of FHB resistance of soft wheat in U.S. breeding programs (Liu et al., 2005). Other accessions, including MO040152, Bess, KY96C-0769-7-3, and Atlas66 had low PSS ratings in both greenhouse and field experiments. Those accessions are also good local sources of resistance for improvement of SWW FHB resistance. Ten such accessions were HWW. Among them, T154 showed the best resistance in both field and greenhouse experiments. SD05210, Heyne,

Lyman, Everest, and Harry also had relatively low PSS in both greenhouse and field experiments. These accessions are well-adapted to the Great Plains growing environments and are resistant to different diseases. Some of them have been released as commercial cultivars in the region, and thus are good native sources of resistance in HWW. To date, resistance QTLs from these sources have not been characterized, and identification of markers for the QTLs in those accessions will facilitate marker-assisted pyramiding of these QTLs in U.S. winter wheat.

In addition, HWW cultivars such as Hitch had a high level of field resistance as well as moderate resistance in greenhouse experiments. The released cultivars just mentioned not only have the desired adaptation to HWW regions, but also reasonable yield and quality, making them ideal parents for pyramiding *Fhb1* with resistance QTLs from North American sources to attain transgressive segregation. This list can be expanded to SD08198, T153, CO04W210, OK05128, 'Aspen', U07-698-9, 'Endurance', N02Y5117, and HV9W02-942R in HWW, and IL00-8530, MD01W233-06-1, M04★5109, Ernie, OH02-12678, and KY97C-0519-04-07 in SWW (Table 2). These accessions had slightly higher PSS than previously mentioned highly resistant cultivars in both field and greenhouse experiments, but they were all moderately resistant, which means they could be important breeding parents for improvement of FHB resistance in U.S. winter wheat.

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