Fusarium-Damaged Kernels and Deoxynivalenol in *Fusarium*-Infected U.S. Winter Wheat

Feng Jin, Guihua Bai, Dadong Zhang, Yanhong Dong, Lingjian Ma, William Bockus, and Floyd Dowell

First and fifth authors: College of Agronomy, Northwest A&F University, Yangling, 712100, Shaanxi, China; first, second, and third authors: Department of Agronomy, Kansas State University, Manhattan 66506; second author: United States Department of Agriculture–Agricultural Research Service (USDA-ARS), Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506; fourth author: Department of Plant Pathology, University of Minnesota, St. Paul 55108; sixth author: Department of Plant Pathology, Kansas State University, Manhattan; and seventh author: USDA-ARS Engineering and Wind Erosion Research Unit, Manhattan, KS 66502.

Accepted for publication 20 December 2013.

ABSTRACT

Jin, F., Bai, G.-H., Zhang, D.-D., Dong, Y.-H., Ma, L.-J., Bockus, W., and Dowell, F. 2014. *Fusarium*-damaged kernels and deoxynivalenol in *Fusarium*-infected U.S. winter wheat. Phytopathology 104:472-478.

Fusarium head blight (FHB) is a devastating disease that threatens wheat (*Triticum aestivum*) production in many areas worldwide. FHB infection results in *Fusarium*-damaged kernels (FDK) and deoxynivalenol (DON) that dramatically reduce grain yield and quality. More effective and accurate disease evaluation methods are imperative for successful identification of FHB-resistant sources and selection of resistant cultivars. To determine the relationships among different types of resistance, 363 (74 soft and 289 hard) U.S. winter wheat accessions were repeatedly evaluated for FDK and DON concentration in greenhouse and field experiments. Single-kernel near-infrared (SKNIR)-estimated FDK and DON were compared with visually estimated FDK and gas chromatography-mass spectroscopy-estimated DON. Significant correlations were detected between percentage of symptomatic spikelets and visual FDK in the greenhouse and field, although correlations were slightly lower in the field. High correlation coefficients also were observed between visually scored FDK and SKNIR-estimated FDK (0.72, $P < 0.001$) and SKNIR-estimated DON (0.68, $P < 0.001$); therefore, both visual scoring and SKNIR methods are useful for estimating FDK and DON in breeding programs.

Fusarium head blight (FHB) is a destructive disease of wheat worldwide. In the United States, *Fusarium graminearum* Schwabe (teleomorph = *Gibberella zeae* (Schwein.) Petch) is the prevailing species responsible for FHB (4). FHB causes significant reductions in both grain yield and quality through *Fusarium*-damaged kernels (FDK) and mycotoxins, especially deoxynivalenol (DON), produced in harvested grain infected by the pathogen (4). DON contamination is a serious health concern to humans and livestock. Maximum allowable DON concentration in wheat grain for human consumption ranges from 0.5 to 2.0 mg/kg depending on the country (4,23); therefore, in the regions where FHB epidemics are frequent and severe, FHB threatens wheat production.

Fusarium infection results in FDK: whitish, shrived kernels or tombstones that not only reduce kernel weight but also damage the protein quality. Wheat FDK are contaminated with DON in most FHB epidemic regions (4,14). Resistant cultivars usually have reduced DON concentrations (type III resistance) and fewer FDK (type IV resistance) than susceptible cultivars after infection (15). To date, these two types of resistance have not been systematically characterized in U.S. winter wheat. Some reports have suggested that FHB severity and FDK could predict DON concentration but the conclusions on the relationships among these three measurements of FHB remain equivocal (3,10,12, 19,25).

Although a worldwide effort has been made to identify FHB-resistant germplasm (4), only Sumai 3 and its derivatives have been identified as having a major quantitative trait locus (QTL) (*Fhb1*) for type II resistance (FHB spread within a spike); this QTL has the greatest and most stable effect on FHB resistance across different genetic backgrounds and environments among the FHB resistance sources identified to date (1,2,5,13,24) and has been used extensively as a major source of resistance in breeding programs (4,22). The level of the *Fhb1* contribution to lessen FDK and DON concentration in U.S. winter wheat remains unknown.

A number of technologies are available to quantify DON concentration in *Fusarium*-contaminated grain and each has its strength and weaknesses (3,17,21). All these measuring methods are complicated; however, a simpler visual scoring method may be sufficient for breeders to quickly estimate DON concentration in breeding materials. This study was designed to identify an effective method for determining FDK and DON by evaluating the relationships among different measurements of FHB resistance. Meanwhile, this study also identified U.S. wheat germplasm with low DON concentration and FDK and assessed the effect of *Fhb1* on FDK and DON concentration in these germplasm lines.

MATERIALS AND METHODS

Plant materials and FHB inoculation. Two sets of wheat materials consisting of 363 wheat accessions were evaluated for FHB resistance in both the greenhouse and field experiments from 2009 through 2012 as described by Jin et al. (9). Set I had

Corresponding authors: G.-H. Bai; E-mail address: guihua.bai@ars.usda.gov
L.-J. Ma; E-mail address: malingjian@nwsuaf.edu.cn

*The *e*-Xtra logo stands for "electronic extra" and indicates that the online version contains one supplemental table.

http://dx.doi.org/10.1094/PHYTO-07-13-0187-R

207 accessions, including all of the hard winter wheat (HWW) and soft winter wheat (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 set I. Greenhouse experiments were conducted at Kansas State University. After vernalization at 5°C in a cold chamber for 8 weeks, six wheat seedlings per accession were transferred into a 13-by-13-cm Dura-pot (Hummert Int., Earth City, MO) and grown at 19 ± 2°C at night and 22 ± 2°C during the day with a 12-h photoperiod. Each experiment used a randomized complete block design with two replications. Six spikes per replication (pot) were inoculated at anthesis by injecting 10 µl of conidia (≈100 spores/µl) with a syringe into a central spikelet of each spike. Inoculum was a field isolate (GZ 3639) of *F. graminearum* from Kansas (6), which was increased in a mung bean broth for inoculation (9). Inoculated plants were enclosed in a moist chamber for 48 h at 22 ± 2°C to initiate infection, then moved to a greenhouse bench for disease development under the same conditions.

Field experiments were conducted in the FHB Nursery of the Plant Pathology Department, Kansas State University at Rocky Ford in Manhattan. In all, ≈40 seeds per accession were planted in a 1-m-long single-row plot, and each experiment used a randomized complete block design with two replications. Spawn inoculation was implemented twice by scattering *F. graminearum*–infested corn kernels on the soil surface before boot stage (Feekes 8) and 2 weeks after (Feekes 10.1). In addition, single-spikelet inoculation was used to inoculate six spikes per plot using the same inoculum and the same method as described for the greenhouse experiments. The FHB nursery was sprinkled for 3 min every hour from 1900 to 0600 h daily from flowering to early dough stages to improve initial infection using an overhead impact sprinkler system.

Evaluation of FDK. In greenhouse experiments, inoculated plants from each pot were bulk harvested and manually threshed to insure that all infected shriveled kernels were collected. To score FDK, samples with 1, 5, 20, 50, 75, 95, and 100% FDK were prepared as checks. FDK in each sample (at least 100 kernels) were visually inspected by comparing the grain sample with the checks. Each sample was graded by two scientists and the mean from the two readings was used for statistical analysis. Only accessions in set II were evaluated for FDK and DON in the greenhouse experiments.

In the field experiments, all plants from each plot (replication) were harvested by hand and threshed using a single plant thresher (Almaco, Nevada, IA) by leaving the air blower slightly open to keep as many tombstones as possible, then hand-cleaned to remove leftover trash. FDK were visually scored based on all grain (at least 1,000 kernels) harvested in each plot using the same method as that used in the greenhouse experiments.

DON analysis. In the greenhouse experiments, DON concentration (milligrams per kilogram) was measured for set II of 191 accessions (9) using gas chromatography-mass spectrometry (GC-MS) (7,17) at the University of Minnesota, St. Paul. All grains harvested from inoculated plants in each line were weighed and extracted by soaking and shaking in acetonitrile/water (84/16, vol/vol) at a ratio of solvent (milliliters)/seed (grams) ≥12 for 24 h. The extract was passed through a column packed with C_{18} and aluminum oxide, and 1.5 ml of filtrate was evaporated to dryness under nitrogen at room temperature, and derivatized by 25 trimethylsilyl ether (TMS) reagent (TMSI/TMCS = 100/1, vol/vol). DON was analyzed by a GC-MS (Shimadzu GCMS-QP2010; Shimadzu Corporation, Kyoto, Japan) using selected ion monitoring mode. The fragment ion (*m/z* value) of 235.10 was used as target ion and 259.10 and 422.10 ions were used as reference ions. Concentration of DON in each sample was calculated using a 10-point standard calibration curve of 0.025 to 15 ng/ml generated with each set of samples.

In the field experiments, DON was measured by GC-MS using randomly sampled grain collected from each plot. All samples in set I collected in 2010 and 2011 and set II in 2011 and 2012 field experiments were evaluated for DON. In brief, a 4-g ground sample was extracted with 16 ml of acetonitrile/water (84/16, vol/vol) for 1 h. Extract (4 ml) was passed through a column packed with C_{18} and aluminum oxide, and 1 ml of filtrate was evaporated to dryness under nitrogen at room temperature and derivatized by 100 µl of TMS reagent (TMSI/TMCS = 100/1, vol/vol). DON was quantified using GC-MS with quantification limit at 50 ng/g.

Data analysis. Analysis of variance and correlation coefficient were calculated using the Statistical Analysis System 9.2 (SAS Institute, Inc., Cary, NC). Percentage of symptomatic spikelets (PSS) reported in a previous study (9) was used to analyze the relationship between disease severity and FDK or DON concentrations. The contribution of *Fhb1* in four different genetic backgrounds (Clark, Trego, Wesley, and Harding) was analyzed by comparing the mean FHB ratings of the *Fhb1*-containing near-isogenic lines (NILs) with their parents.

Single-kernel near-infrared for FDK and DON prediction. A subset of samples (100 accessions) from set I grown in the field in 2010 and all 191 samples from set II grown in the field in 2011 were evaluated for FDK, and 27 random samples from set I and 121 random samples from set II were evaluated for DON concentration using the single-kernel near-infrared (SKNIR) method. For the SKNIR method, 200 kernels/sample were separated into sound (bin 1) and FDK (bins 2 and 3) and the DON concentration was estimated in each kernel (21). The SKNIR system calibration for both FDK and DON followed Peiris et al. (21). Correlation coefficients were calculated among SKNIR, visually scored FDK, and SKNIR- and GC-MS-estimated DON concentrations.

RESULTS

FDK in the greenhouse and field experiments. In the greenhouse experiments, FDK varied significantly among 191 HWW accessions tested after *Fusarium* spp. infection, and ≈75% of accessions had mean FDK >50%, indicating that most U.S. HWW was susceptible to FHB infection. Only 10% of accessions tested had FDK <25%, and 15% had FDK of 25 to 50%. Among the 10% low-FDK accessions, 5%, including eight *Fhb1* NILs and KS08FHB-78 (Table 1), carry the *Fhb1* resistance allele, as determined by the closely linked markers (9).

Average FDK from the field experiments was much less than that from the greenhouse, and ≈73.6% of total tested accessions (n = 363) had FDK <50.0%, 33.1% showed FDK <25.0%, and only 15.4% had ≤15.0% FDK in the field experiments. In all, ≈12.5% of the 15.4% low-FDK accessions carry the *Fhb1* resistance allele from the backcrossing project and had FDK <25% in greenhouse experiments; meanwhile, 12.5% of other accessions without the *Fhb1* resistance allele (T154, SD05085-1, Everest, Lyman, SD05118, Husker, and Overland) also showed a similar low FDK in the greenhouse experiments (Table 1). Among the remaining 75% accessions, 23.2% had FDK of 25 to 50% in the greenhouses, including four *Fhb1* resistance allele carriers and nine non-*Fhb1* accessions such as Aspen, Heyne, Arapahoe, and so on; 51.8% other accessions had >50% FDK (51.7 to 98%) in the greenhouses (Table 1), indicating that the accessions with low FDK (≤15%) in the field experiments might have relatively high FDK in the greenhouse experiments.

DON concentration of FHB-inoculated wheat in both the greenhouse and field. In the greenhouse experiments, all the samples from set II were evaluated for DON concentration using GC-MS. DON concentration was 0.42 to 1,003.4 mg/kg, and only 1.0% of tested accessions had DON <2.0 mg/kg. DON concentration was arbitrarily classified into five groups (<2.0, 2.01 to 5.0, 5.01 to 50.0, 50.01 to 100.0, and >100.0 mg/kg); only 3.1% had DON concentration <5.0 mg/kg and the majority (96.9%) of

accessions had >5.0 mg/kg DON, including 22% the tested accessions that had DON concentrations of 5.01 to 50.0 mg/kg, 19.4% that had >50.0 mg/kg but <100.0 mg/kg, and the remaining 55.5% that had DON concentrations >100 mg/kg. In the 3.1% accessions with low DON concentration (<5.0 mg/kg), two-thirds carried *Fhb1,* including three WesleyFhb1 progenies and KS08FHB-78, and the remaining one-third (T154 and Everest) did not carry *Fhb1* (Table 1).

TABLE 1. List of accessions that displayed low levels of percentage of symptomatic spikelets in a spike, visually estimated *Fusarium*-damaged kernels (FDK), and gas chromatography-mass spectroscopy–measured deoxynivalenol (DON) content evaluated in greenhouse (GH) and field experiments[a]

Accession name	Type[b]	GH FDK (%)	GH DON (mg/kg)	Field FDK (%)	Field DON (mg/kg)
GH FDK ≤25%					
KS08FHB-78[c]	HWW	5.3 ± 3.7	4.08 ± 3.01	23.8 ± 15.6	3.60 ± 2.37
WesleyFhb1NIL09S-103[c]	HWW	5.3 ± 3.7	0.42 ± 0.27	9.0 ± 8.7	2.53 ± 1.15
WesleyFhb1NIL09S-104[c]	HWW	8.7 ± 5.8	2.19 ± 2.12	23.8 ± 21.6	4.23 ± 2.71
WesleyFhb1NIL09S-105[c]	HWW	10.0 ± 7.1	2.22 ± 1.40	4.1 ± 3.8	2.30 ± 0.92
TregoFhb1NIL09S-98	HWW	10.3 ± 7.8	5.15 ± 5.44	11.3 ± 10.8	6.46 ± 1.70
Wesley FHB1	HWW	15.0 ± 4.1	6.93 ± 3.95	6.5 ± 6.2	2.70 ± 1.60
TregoFhb1NIL09S-99	HWW	15.0 ± 4.1	9.57 ± 11.86	5.5 ± 2.9	4.07 ± 1.41
ClarkFhb1NIL09F-23[d]	SWW	23.3 ± 4.7	24.22 ± 24.63	6.5 ± 3.8	0.88 ± 0.35
ClarkFhb1NIL-75[d]	SWW	23.3 ± 4.7	14.55 ± 8.26	13.3 ± 21.2	0.49 ± 0.28
T154[c]	HWW	5.3 ± 3.7	1.68 ± 0.54	13.8 ± 6.5	3.36 ± 1.77
SD05085-1	HWW	12.3 ± 9.5	5.07 ± 3.0	14.3 ± 10.2	4.97 ± 1.08
Everest[c]	HWW	13.3 ± 6.2	3.26 ± 2.22	8.3 ± 4.1	2.30 ± 0.70
Lyman	HWW	17.0 ± 12.0	8.24 ± 10.04	4.3 ± 3.7	2.57 ± 0.37
Harry	HWW	20.0 ± 14.1	19.43 ± 24.37	52.5 ± 20.5	11.65 ± 2.28
SD05118	HWW	20.0 ± 8.2	20.13 ± 11.78	14.0 ± 9.3	6.43 ± 1.45
Husker	HWW	20.3 ± 15.9	15.03 ± 17.58	13.8 ± 7.4	7.14 ± 2.53
T153	HWW	23.3 ± 12.5	6.26 ± 3.39	17.5 ± 5.6	4.10 ± 1.92
Overland	HWW	25.0 ± 4.1	7.42 ± 0.42	15.0 ± 10.8	4.48 ± 1.86
SD08198	HWW	25.0 ± 17.8	9.93 ± 3.27	15.9 ± 15.8	6.82 ± 3.64
Field FDK ≤15% or DON <2 mg/kg					
ClarkFhb1NIL09F-45[d]	SWW	26.7 ± 20.1	26.20 ± 34.86	3.0 ± 2.0	0.59 ± 0.18
KS08FHB-31	HWW	28.3 ± 15.5	42.76 ± 39.41	3.4 ± 1.8	2.56 ± 1.23
TregoFhb1NIL09S-100[d]	HWW	33.3 ± 4.7	8.58 ± 7.38	4.1 ± 3.8	1.82 ± 0.63
ClarkFhb1NIL09F-4[d]	SWW	60.0 ± 14.1	33.69 ± 15.61	5.0 ± 0.0	1.16 ± 0.52
HardingFhb1NIL09S-108	HWW	70.0 ± 14.1	99.04 ± 38.46	6.9 ± 5.9	4.87 ± 3.26
HardingFhb1NIL09S-109	HWW	30.0 ± 16.3	11.28 ± 3.38	10.0 ± 6.1	5.48 ± 2.98
NE08527	HWW	43.3 ± 20.5	31.07 ± 32.04	4.3 ± 3.7	2.81 ± 1.52
Jerry	HWW	43.3 ± 20.9	32.92 ± 22.48	6.8 ± 6.0	3.26 ± 2.23
KS08IFAFS1	HWW	98.0 ± 2.2	343.95 ± 80.29	7.6 ± 5.4	2.51 ± 1.52
Kharkof	HWW	65.0 ± 21.2	38.76 ± 15.36	7.8 ± 8.1	2.48 ± 1.96
MTS0713	HWW	51.7 ± 31.7	172.46 ± 230.58	7.8 ± 8.0	3.34 ± 1.92
Heyne	HWW	31.7 ± 14.3	19.22 ± 20.65	9.0 ± 7.1	5.67 ± 1.48
CA9W08-856	HWW	56.7 ± 20.5	77.84 ± 67.21	9.0 ± 12.2	3.09 ± 1.42
CA9W07-817	HWW	86.7 ± 10.3	259.04 ± 44.77	9.0 ± 12.2	3.23 ± 2.57
Bess	SWW	–	–	9.2 ± 5.3	5.23 ± 1.67
Aspen	HWW	26.7 ± 12.5	11.20 ± 2.32	9.5 ± 3.6	2.91 ± 2.06
B030543	SWW	–	–	10.0 ± 4.1	3.72 ± 1.22
Karl 92	HWW	80.0 ± 16.3	236.20 ± 191.66	10.3 ± 9.1	4.03 ± 1.40
SD07126	HWW	67.5 ± 27.5	63.10 ± 44.40	10.4 ± 9.6	4.53 ± 3.62
SD05W030	HWW	44.3 ± 38.0	91.22 ± 126.62	10.8 ± 9.3	2.93 ± 1.58
SD07220	HWW	51.7 ± 34.2	24.01 ± 25.11	11.3 ± 5.4	4.48 ± 0.95
U07-698-9	HWW	43.3 ± 9.4	15.61 ± 9.49	11.3 ± 5.4	6.66 ± 2.37
Camelot	HWW	36.7 ± 20.9	53.53 ± 58.82	11.4 ± 8.8	5.59 ± 4.04
HV9W04-1594R	HWW	88.3 ± 9.4	161.93 ± 79.85	11.5 ± 9.3	5.27 ± 2.45
IL02-18228[d]	SWW	–	–	11.7 ± 8.5	1.88 ± 1.22
KS08P1-108	HWW	98.0 ± 2.2	265.43 ± 50.36	11.8 ± 8.3	3.99 ± 1.28
KY96C-0769-7-3	SWW	–	–	12.5 ± 6.3	4.88 ± 2.32
MD01W233-06-1[d]	SWW	–	–	12.5 ± 6.3	1.79 ± 0.53
HV9W05-1125R	HWW	91.3 ± 8.2	316.09 ± 153.39	12.5 ± 9.0	5.37 ± 2.83
NE05548	HWW	87.5 ± 7.5	155.65 ± 6.29	12.8 ± 11.2	3.76 ± 0.71
Hondo	HWW	53.3 ± 26.2	66.25 ± 47.24	13.0 ± 12.5	2.97 ± 0.83
Winterhawk	HWW	31.7 ± 10.3	10.11 ± 4.59	13.3 ± 6.3	6.63 ± 0.54
Arapahoe	HWW	38.3 ± 10.3	39.81 ± 16.63	13.3 ± 6.3	5.96 ± 2.40
SD07W053	HWW	69.0 ± 34.7	169.81 ± 113.89	13.3 ± 9.4	5.66 ± 0.69
Hawken	HWW	88.0 ± 8.0	62.56 ± 19.85	13.3 ± 7.2	3.72 ± 1.80
KS030024-K-4	HWW	97.7 ± 1.9	274.91 ± 31.89	13.3 ± 4.7	4.93 ± 1.72
Roane	SWW	–	–	13.3 ± 5.5	2.42 ± 1.80
Ike	HWW	83.3 ± 10.3	96.49 ± 23.82	14.5 ± 9.9	4.74 ± 1.91
ART	HWW	60.0 ± 21.6	67.26 ± 79.52	14.5 ± 14.9	4.74 ± 1.62
Santa Fe	HWW	93.3 ± 2.4	143.88 ± 53.13	14.5 ± 5.5	5.47 ± 2.19
P04287A1-10	SWW	–	–	15.0 ± 7.1	3.79 ± 1.47
NW05M6015-25-4	HWW	81.7 ± 22.5	122.02 ± 86.03	15.0 ± 14.6	5.22 ± 3.81
INW0411[d]	SWW	–	–	30.0 ± 18.9	1.93 ± 0.52
P02444A1-23-9[d]	SWW	–	–	30.0 ± 17.3	1.99 ± 0.48

[a] Values represent mean trait value and its standard error for each lines; – indicates missing data.
[b] HWW = hard winter wheat and SWW = soft winter wheat.
[c] Accessions with DON at <5 mg/kg in greenhouse experiments.
[d] Accessions with DON at <2 mg/kg in field experiments.

A much lower DON concentration (<30.0 mg/kg) was detected from field samples than from greenhouse samples (Supplemental Table 1). Among all tested accessions in the field experiments, only 2.5% had a DON concentration <2.0 mg/kg, of which seven carried *Fhb1* and two (MD01W233-06-1 and IL02-18228) did not (Table 1). A total of 26.2% of the screened accessions had DON concentrations of 2.01 to 5.0 mg/kg, including 17.7% HWW and 8.5% SWW; thus, most U.S. winter wheat breeding lines and cultivars (71.1% of tested accessions) had DON at >5.0 mg/kg in the field-irrigated FHB nursery.

Relationship between FDK and DON in the greenhouse and field experiments. In the greenhouse experiments, 19 accessions with FDK <25% also had low DON concentration (<25.0 mg/kg), in which six accessions had both reduced FDK (<15%) and reduced DON concentration (<5.0 mg/kg) (Table 1). In addition, 23 of the 29 accessions that had FDK of 25 to 50% also had relatively low DON concentrations (<50.0 mg/kg). High correlation coefficients were observed between FDK and DON concentration (r = 0.704, P <0.001) (Fig. 1A), between PSS and DON concentration (r = 0.762, P < 0.001) (Fig. 1B), and between PSS and FDK (r = 0.928, P < 0.001) (Fig. 1C), suggesting that wheat accessions with low PSS and low FDK usually have a low DON concentration in greenhouse experiments.

In the field experiments, correlation was also significant between mean FDK and DON concentration (r = 0.628, P < 0.001) (Fig. 2A). Although it was lower than that from the greenhouse experiments, it was greater than the correlation between PSS and DON concentration (r = 0.503, P < 0.001) (Fig. 2B) and between PSS and FDK (r = 0.58, P < 0.001) (Fig. 2C) from the same experiments. The results suggest that FDK is a more accurate estimate for DON concentration than PSS in field experiments.

Contribution of *Fhb1* to different FHB measurements. Four parental cultivars used in the *Fhb1* backcross project showed different levels of FHB infection, including Wesley (moderately susceptible in the both greenhouse and field), Trego and Clark

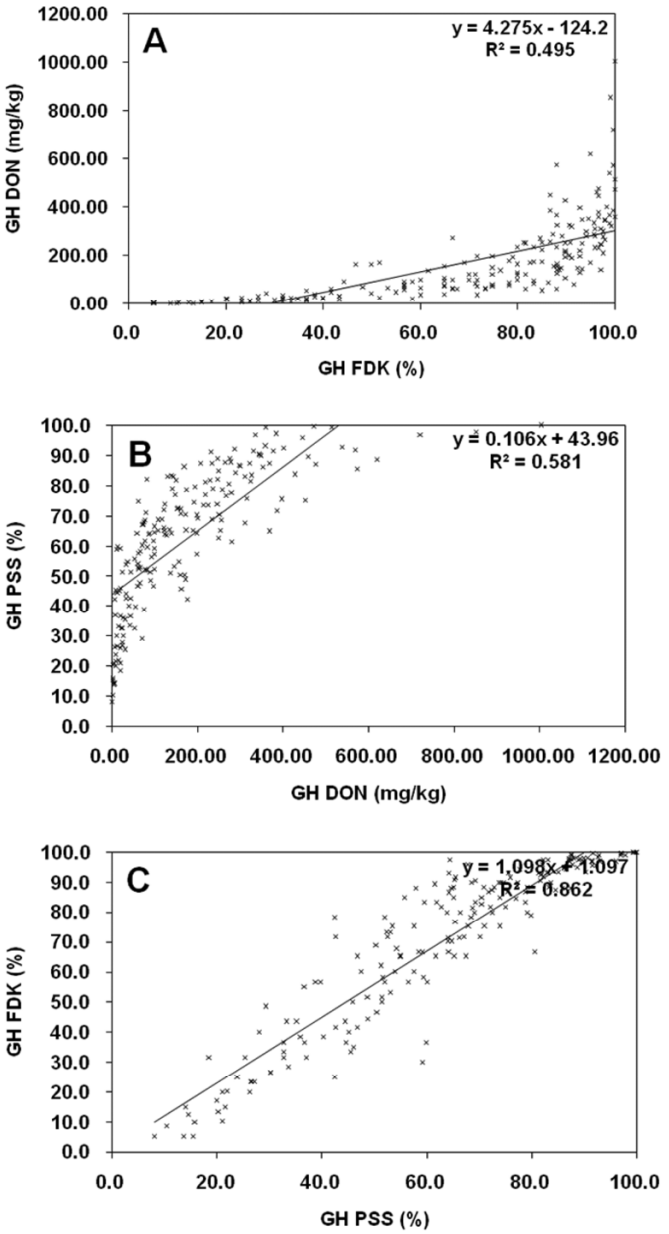

Fig. 1. Correlation coefficients of 191 accessions tested in the greenhouse (GH) experiments. **A,** Visually estimated *Fusarium*-damaged kernels (FDK) and deoxynivalenol (DON); **B,** DON and percentage of symptomatic spikelets (PSS) (9); and **C,** PSS (9) and visually estimated FDK.

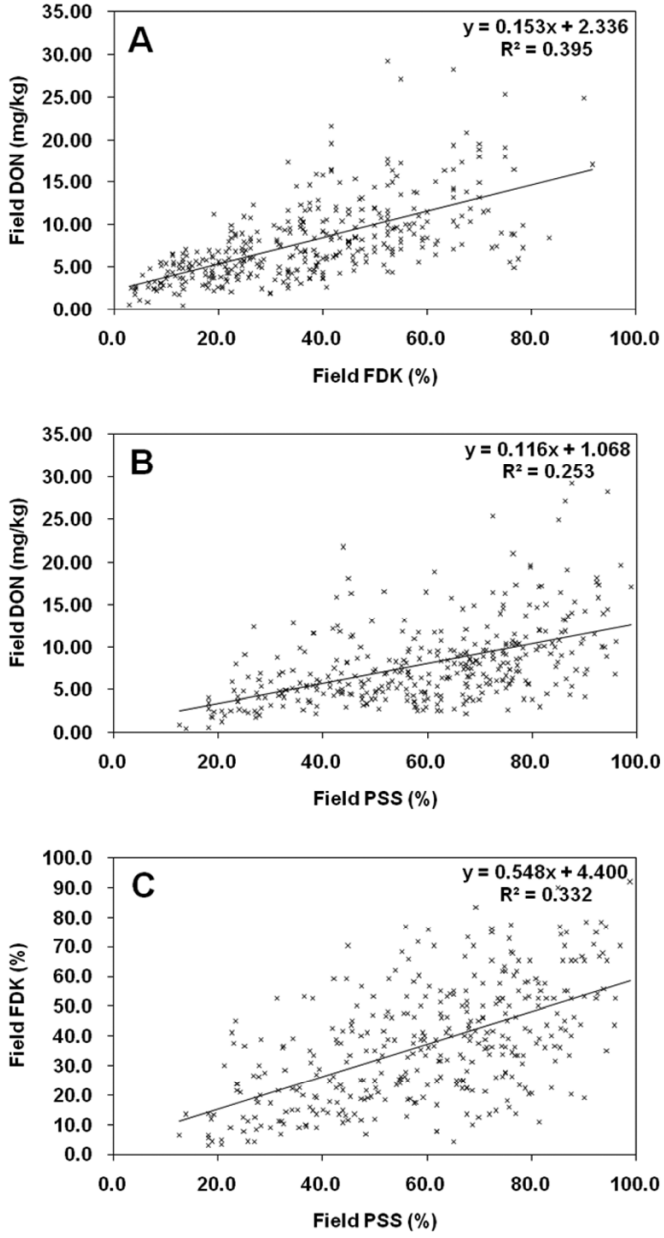

Fig. 2. Correlation coefficients of 363 wheat accessions tested in field experiments. **A,** Visually estimated *Fusarium*-damaged kernels (FDK) and deoxynivalenol (DON); **B,** percentage of symptomatic spikelets (PSS) (9) and DON; and **C,** PSS (9) and visually estimated FDK.

(highly susceptible in the greenhouse and field), and Harding (moderately susceptible in the greenhouses but moderately resistant in the field) (Table 2). All WesleyFhb1 resistant NILs had significantly lower DON concentrations and FDK than their recurrent parent. Similar results were obtained for ClarkFhb1 and TregoFhb1 resistant NILs (Table 2). HardingFhb1 NILs showed the lowest reduction in both DON and FDK but still showed significant reductions compared with Harding; therefore, *Fhb1* significantly reduces both FDK and DON concentration in U.S. winter wheat genetic backgrounds.

SKNIR for FDK and DON prediction. In total, 291 wheat accessions were evaluated for FDK via SKNIR in 2011, including 100 accessions selected from set I harvested in the 2010 field experiment and 191 from set II harvested in the 2011 field experiment. The mean FDK evaluated via SKNIR was 0.8 to 86.8%, similar to visual FDK estimates of 3.0 to 91.7%, and the correlation between the two methods was significant (r = 0.58, $P < 0.001$) (Fig. 3).

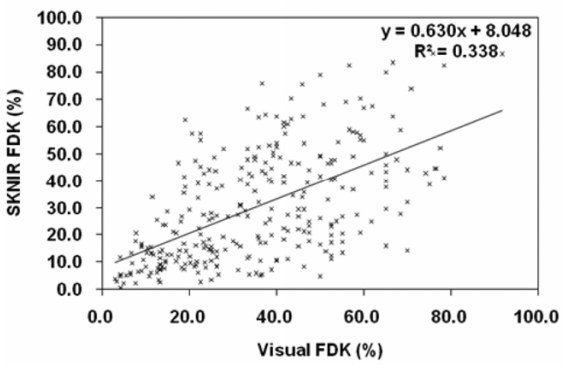

Fig. 3. Correlation between two *Fusarium*-damaged kernels (FDK) estimation methods: visually estimated FDK and single-kernel near-infrared (SKNIR)-measured FDK in field experiments.

A subset of 148 wheat accessions from the samples used for SKNIR FDK analysis was selected for DON estimation, including 27 accessions from set I and 121 accessions from set II. The mean DON concentration estimated by SKNIR was 0.36 to 70.18 mg/kg, with 6.8% of the accessions having >30.0 mg/kg, whereas the GC-MS method provided a narrower range of DON concentrations for the same set of samples (0.49 to 29.25 mg/kg). The correlation coefficient between the two methods was significant (r = 0.46, $P < 0.001$) (Table 3). Meanwhile, significant correlations were also observed among visual FDK, GC-MS-measured DON concentration, SKNIR-measured FDK, and SKNIR-measured DON concentration (Table 3).

DISCUSSION

A previous study evaluated PSS in both greenhouse and field experiments (9), whereas the same accessions were analyzed for FDK and DON concentration in the current study. In the green-

TABLE 3. Correlation coefficients among gas chromatography-mass spectroscopy (GC-MS)- and single-kernel near-infrared (SKNIR)-measured deoxynivalenol (DON) content and visually estimated *Fusarium*-damaged kernel (FDK) and SKNIR-measured FDK for 148 selected wheat accessions from both set I and set II field experiments[a]

Traits	PSS	FDK (visual)	DON (GC-MS)	FDK (SKNIR)
FDK (visual)	0.62***	…	…	…
DON (GC-MS)	0.57***	0.74***	…	…
FDK (SKNIR)	0.38***	0.72***	0.49***	…
DON (SKNIR)	0.36***	0.68***	0.46***	0.95***

[a] PSS = percentage of symptomatic spikelets and **** indicates significant at the 0.001 probability level.

TABLE 2. Contribution of *Fhb1* allele to low *Fusarium*-damaged kernel (FDK) and deoxynivalenol (DON) content in *Fhb1* near-isogenic lines (NILs)

		FDK (%)		DON (mg/kg)	
Accession[a]	Class	Greenhouse	Field	Greenhouse	Field
Clark					
ClarkFhb1NIL09F-4	SWW	60.0	5.0	33.69	1.16
ClarkFhb1NIL09F-23	SWW	23.3	6.5	24.22	0.88
ClarkFhb1NIL09F-45	SWW	26.7	3.0	26.20	0.59
ClarkFhb1NIL-75	SWW	23.3	13.3	14.55	0.49
NIL mean	…	33.3	7.0	24.67	0.78
Clark	SWW	86.1	33.3	193.00	2.54
Reduction (%)	…	61.3	79.0	87.2	69.3
Trego					
TregoFhb1NIL09S-100	HWW	33.3	4.1	8.58	1.82
TregoFhb1NIL09S-99	HWW	15.0	5.5	9.57	4.07
TregoFhb1NIL09S-98	HWW	10.3	11.3	5.15	6.46
Nil mean	…	19.5	7.0	7.77	4.12
Trego	HWW	88.0	37.5	234.20	14.70
Reduction (%)	…	77.8	81.3	96.7	72.0
Wesley					
Wesley FHB1	HWW	15.0	6.5	6.93	2.7
WesleyFhb1NIL09S-103	HWW	5.3	9.0	0.42	2.53
WesleyFhb1NIL09S-104	HWW	8.7	23.8	2.19	4.23
WesleyFhb1NIL09S-105	HWW	10.0	4.1	2.22	2.3
Nil mean	…	9.8	10.9	2.94	2.94
Wesley	HWW	50.0	27.8	44.67	8.14
Reduction (%)	…	80.4	60.8	93.4	63.9
Harding					
HardingFhb1NIL09S-109	HWW	30.0	10.0	11.28	5.48
HardingFhb1NIL09S-108	HWW	70.0	6.9	99.04	4.87
HardingFhb1NIL09S-107	HWW	36.7	19.0	14.67	6.18
Mean	…	45.6	12.0	41.66	5.51
Harding	HWW	78.3	17.8	82.41	5.87
Reduction (%)	…	41.8	32.6	49.4	6.1

[a] Clark and its derived NILs are soft winter wheat (SWW) and all others are hard winter wheat (HWW). Reduction (%) refers to percent reduction in FHB-related trait values due to *Fhb1* and is calculated by the following formula: 100 × [(recurrent parent value – mean value of all its NIL)/recurrent parent value].

house experiments, a very high correlation was obtained between PSS and FDK (Fig. 1C), indicating high consistency between both estimates of FHB damage, which agrees with a previous report (21). High correlations detected between DON concentration and PSS and between DON and FDK (Fig. 1) supported several previous reports (3,10,19,25). Visual PSS rating is done directly on growing plants; therefore, it is quicker and easier than FDK and DON estimation that needs to be done on harvested grain; thus, PSS may be a more useful estimate of FHB damage than FDK and DON measured by GC-MS in greenhouse experiments.

In contrast with the greenhouse experiments, a lower but significant correlation was detected between PSS and DON (Fig. 2B) in the field experiments. In some previous studies, significant correlation was not found between visual FHB rating and DON concentration in the field experiments (12,26). The poor correlation might be because some tombstones were blown away during threshing. Threshing procedures that easily remove heavily infected grain can reduce DON concentration by reducing the proportion of DON-contaminated FDK. This is supported by a higher correlation ($r = 0.628$) between visual FDK and DON concentration than that ($r = 0.503$) between PSS and DON concentration (Fig. 2) in this study, where the thresher might have blown away some shriveled and very light grain. In addition, visual FDK was evaluated by comparing samples with known standards; therefore, FDK evaluation was more robust than the PSS evaluation in the field. In addition, field PSS scoring was based on PSS estimation of a whole plot at a specific day, not on individual plants, and personal experience can significantly affect PSS scores (21). However, threshing carefully and using a skilled person to score the PSS can improve the level of consistency between PSS and DON concentration. Visual FDK appears to be a better estimate of DON concentration than PSS in the field (12,19,25).

Standard DON testing (GC-MS) is expensive and destructive, which renders it unsuitable for quick screening of a large number of materials in breeding programs; clearly, an inexpensive and effective method is needed for quick DON estimation by grain quality inspectors and wheat breeders. SKNIR spectroscopy recently has been proposed for such an application (21). A significant correlation was established between the DON concentrations that were estimated by GC-MS and SKNIR spectroscopy (0.46, $P < 0.001$) in the current study; however, the correlation was much lower than those ($r > 0.849$) from a previous report (21). It was also lower than those reported between GC-MS and other methods such as high-performance liquid chromatography (3) and enzyme immunoassay (16). GC-MS measured DON concentration in a sample of bulked kernels, whereas the SKNIR method estimated DON concentration in a single kernel; thus, estimating DON concentration taking sample weight in consideration may improve accuracy of the SKNIR method (20). Therefore, an improved SKNIR method can be a quick and nondestructive alternative for grain quality inspectors to roughly estimate DON concentration in wheat samples and for breeders to remove DON-contaminated FDK in segregating populations.

The highest correlation was observed between SKNIR-estimated FDK and DON (Table 3); thus, SKNIR FDK can be used directly to predict SKNIR DON concentration. High correlations between visual and SKNIR-estimated FDK and between visual FDK and SKNIR-estimated DON (Table 3) suggest that both visual and SKNIR FDK scores can be used to assess DON concentration in wheat samples. In addition, PSS had better correlation with visual FDK than with SKNIR FDK (Table 3); therefore, visual FDK or PSS can be used to estimate DON in most breeding programs that have no access to a SKNIR system.

In most U.S. wheat breeding programs, although Sumai 3 or its derivatives have been used as resistant parents for approximately two decades, this study shows that the majority of U.S. winter wheat, especially HWW, does not carry *Fhb1* and remains susceptible to FHB. Due to increased severity and frequency of FHB infection in HWW in the central Great Plains (18), transferring resistant genes from Sumai 3 and others is an effective method to quickly improve FHB resistance in U.S. HWW. In a case study, we used marker-assisted backcross to transfer *Fhb1* into four U.S. winter wheat cultivars and found that most selected *Fhb1* NILs showed significant improvement in FHB resistance. Results from the current study showed that *Fhb1* NILs had significantly lower FDK and DON than their recurrent parents in both the greenhouse and the field (Table 2), indicating that *Fhb1* can significantly reduce both FDK and DON in these genetic backgrounds. Lemmens et al. (11) reported that *Fhb1* detoxified DON by converting DON into nontoxic DON-3-O-glucoside and proposed detoxification as a major FHB resistance mechanism of *Fhb1* through either encoding a DON-glucosyltransferase or regulating enzyme expression. In another study, differential expression of UDP-glucosyltransferases between two NILs supported the hypothesis but DON concentration was not different between the NILs (8); thus, *Fhb1* detoxification as a major FHB resistance mechanism remains to be validated.

It is possible that the difference in DON concentration among genotypes contrasting in *Fhb1* alleles is due to a difference in DON production rather than detoxification. In this study, a high correlation coefficient was observed between FDK and DON concentration. A much higher proportion of FDK in harvested grain of parents than their *Fhb1* NILs suggests that a greater amount of fungal biomass in the parents may be responsible for a higher level of DON production than their NILs; however, this was not measured in this study.

Effect of *Fhb1* on reducing FDK and DON was slightly different in HardingFhb1 NILs. Although an obvious reduction was observed for both FDK and DON in both the greenhouse and field conditions, the degree of reduction was much lower (≤50.0%) than in the other three sets of NILs (Table 2). The same trend was noticed for visual FHB rating (9). Thus, selecting the right recurrent parents is important for successful use of *Fhb1* in breeding for FHB resistance.

In this study, 10 HWW accessions that do not carry *Fhb1* were also determined to have low FDK levels (≤25.0%) and DON concentrations (<20.13 mg/kg) in the greenhouse experiments. Among them, T154 showed the lowest FDK (5.3%) and DON concentration (1.68 mg/kg), followed by Everest. SD05085, T153, Overland, Lyman, and SD08198 also had DON concentration <10.0 mg/kg and low FDK (Table 1). These accessions with reduced FDK and DON concentration are important native sources of resistance and the QTL underlining the resistance should be investigated further. Also, they are either newly released cultivars such as Everest and Overland or elite breeding lines that have good adaptation to U.S. HWW growing environments; thus, they are good sources of resistance to be used in FHB resistance breeding. In addition, most non-*Fhb1* accessions from the United States that had low FDK (<50%) and DON concentration (<50.0 mg/kg) in the greenhouse or had low FDK (<15.0%) and DON concentration (<5.0 mg/kg) in the field experiments (Table 1) are well adapted for local wheat production; therefore, they may also contain some minor resistance QTLs and can be good parents for combining *Fhb1* and other Asian sources of resistance QTLs to enhance FHB resistance in U.S. HWW.

ACKNOWLEDGMENTS

This project was partly funded by the U.S. Wheat and Barley Scab Initiative and the National Research Initiative Competitive Grants CAP project 2011-68002-30029 from the USDA National Institute of Food and Agriculture. The United States Department of Agriculture (USDA) is an equal opportunity provider and employer. Mention of trade names or

commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. Contribution number 13-403-J from the Kansas Agricultural Experiment Station.

LITERATURE CITED

1. Anderson, J. A., Stack, R. W., Liu, S., Waldron, B. L., Fjeld, A. D., Coyne, C., Moreno-Sevilla, B., Mitchell Fetch, J., Song, Q. J., Cregan, P. B., and Frohberg, R. C. 2001. DNA markers for *Fusarium* head blight resistance QTLs in two wheat populations. Theor. Appl. Genet. 102:1164-1168.
2. Bai, G. H., Kolb, F. L., Shaner, G. E., and Domier, L. L. 1999. Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89:343-348.
3. Bai, G. H., Plattner, R., Desjardins, A., and Kolb, F. 2001. Resistance to Fusarium head blight and deoxynivalenol accumulation in wheat. Plant Breed. 120:1-6.
4. Bai, G. H., and Shaner, G. E. 2004. Management and resistance in wheat and barley to *Fusarium* head blight. Annu. Rev. Phytopathol. 42:135-161.
5. Buerstmayr, H., Lemmens, M., Hartl, L., Doldi, L., Steiner, B., Stierschneider, M., and Ruckenbauer, P. 2002. Molecular mapping of QTLs for *Fusarium* head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). Theor. Appl. Genet. 104:84-91.
6. Desjardins, A. E., Proctor, R. H., Bai, G. H., McCormick, S. P., Shaner, G., Buechley, G., and Hohn, T. M. 1996. Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. Mol. Plant-Microbe Interact. 9:775-781.
7. Fuentes, R. G, Mickelson, H. R., Busch, R. H., Dill-Macky, R., Evans, C. K., Thompson, W. G., Wiersma, J. V., Xie, W., Dong, Y., and Anderson, J. A. 2005. Resource allocation and cultivar stability in breeding for Fusarium head blight resistance in spring wheat. Crop Sci. 45:1965-1972.
8. Jia, H. Y., Cho, S., and Muehlbauer, G. J. 2009. Transcriptome analysis of a wheat near-isogenic line pair carrying *Fusarium* head blight-resistant and susceptible alleles. Mol. Plant-Microbe Interact. 22:1366-1378.
9. Jin, F. Zhang, D. D., Bochus, W., Baenziger, P. S., Carver, B., and Bai, G. H. 2013. *Fusarium* head blight resistance in U.S. winter wheat cultivars and elite breeding lines. Crop Sci. 53:2006-2013.
10. Lemmens, M., Buerstmayr, H., Krska, R., and Schuhmacher, R. 2004. The effect of inoculation treatment and long-term application of moisture on *Fusarium* head blight symptoms and deoxynivalenol contamination in wheat grains. Eur. J. Plant Pathol. 110:299-308.
11. Lemmens, M., Scholz, U., Berthiller, F., Dall'Asta, C., Koutnik, A., Schuhmacher, R., Adam, G., Buerstmayr, H., Mesterhazy, A., Krska, R., and Ruckenbauer, P. 2005. The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for *Fusarium* head blight resistance in wheat. Mol. Plant-Microbe Interact. 18:1318-1324.
12. Liu, W. Z., Langseth, W., Skinnes, H., Elen, O. N., and Sundheim, L. 1997. Comparison of visual head blight ratings, seed infection levels, and deoxynivalenol production for assessment of resistance in cereals inoculated with *Fusarium culmorum*. Eur. J. Plant Pathol. 103:589-595.
13. Ma, H. X., Zhang, K. M., Gao, L., Bai, G. H., Chen, H. G., Cai, Z. X., and Lu, W. Z. 2006. Quantitative trait loci for resistance to *Fusarium* head blight and deoxynivalenol accumulation in Wangshuibai wheat under field conditions. Plant Pathol. 55:739-745.
14. McCartney, C. A., Somers, D. J., Fedak, G., DePauw, R. M., Thomas, J., Fox, S. J., Humphreys, D. G., Lukow, O., Savard, M. E., McCallum, B. D., Gilbert, J., and Cao, W. 2007. The evaluation of FHB resistance QTLs introgressed into elite Canadian spring wheat germplasm. Mol. Breed. 20:209-221.
15. Mesterházy, A., Bartók, T., Mirocha, C. G., and Komorócay, R. 1999. Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding. Plant Breed. 118:97-110.
16. Miedaner, T., Schneider, B., and Geiger, H. H. 2003. Deoxynivalenol (DON) content and *Fusarium* head blight resistance in segregating population of winter rye and winter wheat. Crop Sci. 43:519-526.
17. Mirocha, C. J., Kolaczkowski, E., Xie, W. P., Yu, H., and Jelen, H. 1998. Analysis of deoxynivalenol and its derivatives (batch and single kernel) using gas chromatography/mass spectrometry. J. Agric. Food Chem. 46:1414-1418.
18. Nopsa, J. F. H., Baenziger, P. S., Eskridge, K. M., Peiris, K. M., Kamarange, H. S., Dowell, F. E., Harris, S. D., and Wegulo, S. N. 2012. Differential accumulation of deoxynivalenol in two winter wheat cultivars varying in FHB phenotype response under field conditions. Can. J. Plant Pathol. 34:380-389.
19. Paul, P. A., Lipps, P. E., and Madden, L. V. 2005. Relationship between visual estimates of *Fusarium* head blight intensity and deoxynivalenol accumulation in harvested wheat grain: a meta-analysis. Phytopathology 95:1225-1236.
20. Peiris, K. H. S., Dong, Y., Bockus, W. W., and Dowell, F. E. 2013. Single-kernel NIR analysis for evaluating wheat samples for Fusarium head blight resistance. Cereal Chem. Online publication. doi.org/10.1094/CCHEM-11-12-0157-R
21. Peiris, K. H. S., Pumphrey, M. O., Dong, Y., Maghirang, E. B., Berzonsky, W., and Dowell, F. E. 2010. Near-infrared spectroscopic method for identification of *Fusarium* head blight damage and prediction of deoxynivalenol in single wheat kernels. Cereal Chem. 87:511-517.
22. Rudd, J. C., Horsley, R. D., McKendry, A. L., and Elias, E. M. 2001. Host plant resistance genes for *Fusarium* head blight: sources, mechanisms, and utility in conventional breeding systems. Crop Sci. 41:620-627.
23. Shaner, G. E. 2003. Epidemiology of *Fusarium* head blight of small grain cereals in North America. Pages 84-119 in: Fusarium Head Blight of Wheat and Barley 2003. American Phytopathological Society, St. Paul, MN.
24. Waldron, B. L., Moreno-Sevilla, B., Anderson, J. A., Stack, R. W., and Frohberg, R. C. 1999. RFLP mapping of QTL for *Fusarium* head blight resistance in wheat. Crop Sci. 39:805-811.
25. Wegulo, S. N., Bockus, W., Nopsa, J. H., De Wolf, E. D., Eskridge, K. M., Peiris, K. H. S., and Dowell, F. E. 2011. Effects of integrating cultivar resistance and fungicide application on *Fusarium* head blight and deoxynivalenol in winter wheat. Plant Dis. 95:554-560.
26. Wisniewska, H., Perkowski, J., and Kaczmarek, Z. 2004. Scab response and deoxynivalenol accumulation in spring wheat kernels of different geographical origins following inoculation with *Fusarium culmorum*. J. Phytopathol. 152:613-621.