Near-Infrared Spectroscopic Method for Identification of *Fusarium* Head Blight Damage and Prediction of Deoxynivalenol in Single Wheat Kernels

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ABSTRACT

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Fusarium Head Blight (FHB), or scab, can result in significant crop yield losses and contaminated grain in wheat (*Triticum aestivum* L.). Growing less susceptible cultivars is one of the most effective methods for managing FHB and for reducing deoxynivalenol (DON) levels in grain, but breeding programs lack a rapid and objective method for identifying the fungi and toxins. It is important to estimate proportions of sound kernels and *Fusarium*-damaged kernels (FDK) in grain and to estimate DON levels of FDK to objectively assess the resistance of a cultivar. An automated single kernel near-infrared (SKNIR) spectroscopic method for identification of FDK and for estimating DON levels was evaluated. The SKNIR system classified visually sound and FDK with an accuracy of 98.8 and 99.9%, respectively. The sound fraction had no or very little accumulation of DON. The FDK fraction was sorted into frac-

tions with high or low DON content. The kernels identified as FDK by the SKNIR system had better correlation with other FHB assessment indices such as FHB severity, FHB incidence and kernels/g than visual FDK%. This technique can be successfully employed to nondestructively sort kernels with *Fusarium* damage and to estimate DON levels of those kernels. Single kernels could be predicted as having low (<60 ppm) or high (>60 ppm) DON with ≈96% accuracy. Single kernel DON levels of the high DON kernels could be estimated with $R^2 = 0.87$ and standard error of prediction (SEP) of 60.8 ppm. Because the method is nondestructive, seeds may be saved for generation advancement. The automated method is rapid (1 kernel/sec) and sorting grains into several fractions depending on DON levels will provide breeders with more information than techniques that deliver average DON levels from bulk seed samples.

Fusarium Head Blight (FHB), also referred to as scab or *Fusarium* ear blight, is a fungal disease that affects all classes of wheat, as well as other small grains such as rye, barley, and triticale (Parry et al 1995; McMullen et al 1997). In any growing season, FHB can reach epidemic proportions in the United States and in many other wheat growing regions of the world. The disease has threatened world food supplies due to outbreaks in Asia, Canada, Europe, and South America (Dubin et al 1997). Nganje et al (2004) estimated that from 1993 to 2001, the cumulative direct economic loss from FHB to wheat and barley across nine U.S. states was estimated to be $2.5 billion. Additionally, they estimated the combined direct and secondary economic loss for all cereals to be $7.7 billion.

FHB of wheat is caused by several fungal species of the filamentous ascomycetes genus, *Fusarium*; however, *F. graminearum* (sexual state: *Gibberella zeae*) is considered the predominate species causing FHB epidemics in North America (Parry et al 1995; Miedaner 1997; O'Donnell et al 2000). Disease development is favored when high humidity and rainfall occur during wheat flowering and grain-filling stages. Kernels of infected spikes are smaller than normal and often show changes in color and form, which results in a chalky or "tombstone" appearance, especially in cases of more severe infection. Thus, FHB reduces grain yield, but an even more serious threat to food safety is the potential accumulation of secondary fungal metabolites in the grain. Such trichothecene mycotoxins, which are mainly deoxynivalenol (DON) and its derivatives, make the grain unsafe for food or feed (Chelkowski 1991; Parry et al 1995; McMullen et al 1997).

Agronomic and chemical control measures are only partly effective in controlling *Fusarium* in wheat (Stack 1999). Epidemics are often sporadic, with spore production or infection cycles highly influenced by wheat genotypes, crop growth stage, temperature, and moisture; this complexity further confounds the feasibility of chemical control measures. The use of FHB-resistant cultivars, together with appropriate crop management practices, is regarded as perhaps the best method for controlling FHB (Parry et al 1995). Because of this, breeding FHB-resistant cultivars is a primary goal of wheat breeders in affected regions.

Resistance to FHB in wheat is quantitatively inherited (Ruckenbauer et al 2001; Miedaner et al 2003). Buerstmayr et al (1999) demonstrated that genes on chromosomes 6D, 6B, 5A, 4D, and 7A are frequently associated with scab resistance in wheat. Furthermore, the most prominent effect out of three identified genomics regions was detected on chromosome 3B, which accounted for ≤60% of phenotypic variance for Type II FHB resistance (Buerstmayr et al 2002). Mesterhazy et al (1999) proposed at least five different resistance reactions to *Fusarium*: 1) resistance to fungal invasion, 2) resistance to fungal spreading within the spike, 3) resistance to toxin accumulation, 4) resistance to kernel infection, and 5) tolerance. Mesterhazy (2001) extended the list of the components of physiological FHB resistance to include resistance to late blighting and resistance to head death above the infection site. Due to the ease of characterization, the majority of genetic studies and germplasm screening experiments have focused on resistance to spread within the spike. Although resistance to fungal invasion and toxin accumulation may be of equal or arguably greater importance, the difficulty and cost to assess these traits has limited routine applications.

Regulation of DON accumulation is a rather complicated process that depends on the host and fungal genotypes as well as ecological conditions (Mesterhazy et al 1999; Bai et al 2001; Miedaner et al 2003). Because of an increasing emphasis on limiting mycotoxins in wheat-based food and feed products, plant breeders and pathologists need precise information to adequately assess *Fusarium*-damaged kernels (FDK) and the DON content of FDK. *Fusarium* infection generally affects kernel appearance and can be visually assessed, but insect damage and other fungi can result in similar symptoms and obscure the level of damage due to FHB. Some kernels, possibly those infected at later stages of grain-filling, may have high DON levels but still appear asymptomatic.

Additionally, some kernels may have significant damage but have low levels of DON. Thus, visual inspection alone is not adequate for assessing FDK and the DON content of wheat cultivars. Moreover, there are many kernels that are difficult to visually categorize as either sound or *Fusarium*-damaged. An objective and more precise method to identify FDK and to relate FDK to DON content is needed for screening cultivars for FHB resistance.

Near-infrared spectroscopic (NIRS) methods have been developed for the evaluation of quality parameters of single wheat kernels (Dowell et al 2006, 2009). Delwiche (2003) and Dowell et al (1999) showed that NIR spectra from manually scanned kernels could be used to select kernels based on DON levels. Delwiche and Hareland (2004) and Delwiche and Gaines (2005) showed that NIRS could be used to distinguish sound kernels from FDK, either from manually scanning kernels or with high-speed bichromatic sorting systems. The work reported here describes further improvement of this technique by refining the calibration for the detection of FHB and by developing DON calibration for the automated single kernel near-infrared (SKNIR) spectroscopic system. The aim of this study was to determine whether the SKNIR system could automatically sort FDK from sound kernels and predict DON concentration in FDK.

MATERIALS AND METHODS

NIR Scanning of Wheat Kernels

The SKNIR system (Fig. 1A), an instrument developed by the USDA, ARS, CGAHR, Engineering and Wind Erosion Research Unit, Manhattan, KS, and commercialized by Perten Instruments (Stockholm, Sweden) was used for collecting NIR spectra of single kernels. This system automatically feeds single wheat kernels (Fig. 1C) to a spectrometer viewing area (Fig. 1E and F) at a rate of ≈1 kernel/sec. The kernel is illuminated with visible-NIR light through a fiber optic bundle. Reflected energy is gathered by the collecting optics of the same illumination fiber bundle and transmitted to a spectrometer with an indium-gallium-arsenide detector that measures absorbance at 950–1650 nm.

NIR Spectral Differences of *Fusarium*-Damaged and Sound Kernels

Wheat NIR spectra (950–1640 nm) were examined to see whether there were differences in NIR absorption between sound kernels and FDK. Five each of 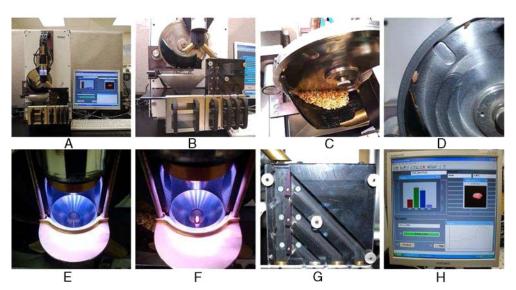visually sound kernels and FDK were scanned with three repacks (spectra recorded in three different random positions of each kernel). The absorbance (log [1/R]) spectra were averaged and second derivative NIR spectra (Savitsky-Golay second degree with 15-point convolution with data points spaced 1 nm) of sound kernels and FDK were computed using the derivative processing application of the GRAMS/AI 8.0 software package (Thermo Electron, Salem, NH). The second derivative of an absorbance spectrum can be used to resolve overlapping bands (Hruschka 1987). Inverted second derivative spectra were examined to see whether there were distinguishable differences between the two classes of kernels, and they were compared with regression coefficients of DON calibration.

FHB Calibration Model

Fusarium-damaged scabby kernels and sound kernels of 10 wheat cultivars received from the University of Arkansas were scanned under the data collection mode of the SKNIR system. Spectra used for FHB calibration included 431 spectra each from visibly sound kernels and FDK. The partial least squares (PLS) regression technique of the GRAMS/AI 8.0 spectroscopy software was used for calibration model development. Mean-centered spectra (Fig. 1H) with an assigned value of 1 for sound kernels and 2 for FDK as constituent values were used in model building (950–1640 nm) to discriminate the two classes of kernels.

The calibration was used on an independent sample for validation and thereafter it was used to sort FDK in submitted samples. When the calibration was loaded to the SKNIR for sorting, the predicted value was used to place the kernel into a specific bin (Fig. 1B and G). If the predicted value fell between –1.00 and 1.50, it was classified as a sound kernel and sorted into bin 1. Likewise, kernels with predicted values of 1.51–2.50, 2.51–3.50, and 3.51–5.00 were classified as FDK and were sorted into bins 2, 3, and 4, respectively. In this manner, the SKNIR instrument sorted kernels according to the severity of damage.

For three greenhouse seasons in 2008 and 2009, the calibration was used to sort sound kernels and FDK of 108 grain samples taken from control cultivars and reciprocal disomic lines of Frontana wheat, developed to detect chromosomes carrying resistance to FHB (Berzonsky et al 2007). These genotypes were artificially inoculated and separately evaluated for three seasons under greenhouse conditions for visual FDK%, FHB severity % (% infected spikelets/spike), FHB incidence % (% infected spikes/unit area), and number of kernels per gram (kernels/g). These assessments

Fig. 1. Single kernel near-infrared system and some external components. **A,** SKNIR system; **B,** close-up of singulator wheel, gates, and bins; **C,** singulator wheel and kernel feeder; **D,** singulator wheel with kernels attached; **E,** sample viewing trough; **F,** properly positioned grain on trough; **G,** diverters for directing kernels to buckets after prediction; **H,** computer screen showing progress of sorting (bottom left), histogram for frequency distribution into each bin (top left), spectrum (bottom right), and image of the kernel being scanned (top right).

were performed at the Department of Plant Science, North Dakota State University, by the same evaluator in all three seasons. In addition, samples were derived from cultivars that were either inoculated with water containing no fungal spores or were spray-inoculated with a spore suspension as described in Berzonsky et al (2007), or by injecting spikes with a spore suspension as described in Hartel et al (2004). Furthermore, the SKNIR-sorted fractions of 23 samples with heavy FHB incidence from the 108 samples in the first season and all 108 samples in the second season were analyzed for DON levels at the University of Minnesota using a gas chromatography-mass spectrometry (GC-MS) method (Mirocha et al 1998; Jiang et al 2006) to verify the success of SKNIR sorting in relation to DON levels of the sorted fractions. Visual FDK%, FHB severity %, FHB incidence %, and kernels/g recorded for the 108 samples were correlated with the SKNIR-sorted FDK% (SKNIR FDK%) for all three seasons to evaluate the potential of SKNIR sorting for the assessment of FHB resistance.

DON Calibration Model

A calibration for estimating DON content of single kernels was developed. Spectra were collected with the SKNIR instrument using 60 kernels each from two wheat cultivars (Wheaton and PI-69251). These kernels were extracted from artificially inoculated wheat spikes to get a broad range of DON content for calibration and validation of samples. Wheat spikes were artificially inoculated at anthesis by injecting a single central floret with 10 μL of macro conidia (1×10^5/mL) of *F. graminearum* isolate Z-3639 (NRRL accession 29169) and covering the spikes with plastic bags for 48 hr. Plants with inoculated spikes were grown under humid greenhouse conditions and harvested when mature. Kernels sampled from spikes with varying degrees of infection above and below the point of inoculation were collected after dissecting selected spikes with FHB. Sound kernels were obtained from uninoculated healthy wheat spikes grown in the same experiment. Each kernel was scanned in triplicate in three random positions. After scanning, DON levels of respective kernels were determined by the GC-MS method (Mirocha et al 1998; Jiang et al 2006). Mean centered spectra with corresponding single kernel DON concentrations (ppm) were used in developing a calibration using the PLS regression technique in GRAMS/AI 8.0. Calibration developed with Wheaton spectra was validated with PI-69251 spectra.

RESULTS AND DISCUSSION

NIR Spectra of *Fusarium*-Damaged and Sound Kernels

Use of NIR spectroscopy for classification of the sound and *Fusarium*-damaged wheat kernels depends on the existence of differences in NIR absorption by sound and damaged kernels. These differences in absorption may arise due to differences in chemical and physical properties of the kernels. Examination of second derivative spectra of sound kernels and FDK showed distinct NIR absorption patterns at ≈1160–1220 nm and 1395–1440 nm wavebands (Fig. 2). The difference spectrum shows an absorption band in the 1160–1220 nm region with a peak at 1195 nm that falls on the –CH second overtone region. Again, in the 1395–1440 nm region, the difference peak is at 1415 nm. This region corresponds with –OH first overtone and –CH first overtone combination vibrations, and absorption of many functional groups such as –CH, –CH$_2$, –CH$_3$, ArOH, and ROH fall within this region. The absorption peaks of the sound kernels and FDK are different under this waveband, possibly due to the interaction of moisture and other organic molecules of the two classes of kernels. Overall, the observed differences of NIR absorption patterns between sound and *Fusarium*-damaged wheat kernels may be due to changes in carbohydrate, lipid and protein reserves, and DON levels. Separate experiments with NIR absorption spectra of various concentrations of DON in acetonitrile solution also showed an NIR absorption peak of DON at ≈1410 nm (Peiris et al 2009).

These wavebands corresponded very well with the regression coefficients of the single kernel DON calibration (Fig. 2). This demonstrates that it may be possible to use NIRS to detect differences between FDK and sound kernels, irrespective of the visual appearance of the kernel and also to estimate DON levels of FDK.
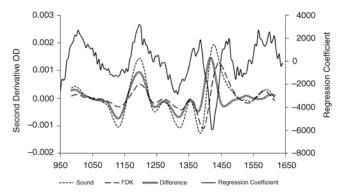
SKNIR *Fusarium*-Damage Sorting Performance

The FHB calibration ($R^2 = 0.64$ SECV = 0.28 with 10 PLS factors) developed for sorting of kernels was applied to sort grain samples of 108 reciprocal disomic lines developed at North Dakota State University to determine chromosomes carrying genes for resistance to FHB. The instrument was loaded with the FHB calibration and programmed to sort sound kernels into bin 1 and FDK into bins 2 or 3 depending on the severity of *Fusarium* damage (i.e., with bin 3 as higher severity). Kernels were sorted separately for each sample. Bin 4 was not used for these tests.

In the first season, after the machine sorted the kernels into sound (bin 1) and FDK (bins 2 and 3) fractions, each of these fractions were visually separated again into FDK (tombstones), "intermediate" kernels (which were neither unambiguously sound nor *Fusarium*-damaged), and sound kernels (Fig. 3). The number of kernels in each category was counted and recorded (Table I). After counting, the kernels were again separately packed into their respective bags. These counts were made on a subjective visual basis, and the counts may differ if another person were to repeat them. The 108 samples contained 25,655 kernels with 3,136 (12.2%) sorted as FDK and the remaining 22,519 (87.8%) sorted as sound (Table I). In relation to the subjective visual classification of kernels, the FDK fraction (bin 2 + bin 3) had 55.0% tombstone kernels and 2.0% sound kernels, while 43.0% of the kernels were intermediate.

The sound fraction had 98.8% sound kernels and 1.2% intermediate kernels. The percentage of tombstone kernels in the sound fraction was very low (0.01%). Hence, the instrument sorted visibly sound kernels with a level of accuracy of ≈99%. All except two tombstone kernels out of 1726 kernels were classified as FDK (Table I), indicating that tombstone kernels were classified with a level of accuracy of ≈100%. Because tombstone kernels are small and light in weight, they sometimes get picked up with a large kernel by the singulator wheel of the SKNIR instrument (Fig. 1D), which uses suction to pick up kernels. Proper position of the kernel in the bucket is also important (Fig. 1F). Mispositioned kernels in the spectrometer viewing area, particularly kernels placed crosswise in the kernel bucket, produce a poor spectrum that can lead to misclassification of kernels. It may be possible to further improve the classification accuracy by making modifications to the instrument on kernel singulating and positioning.

It is important to estimate the DON levels of each of the subfractions to conclude exactly how the instrument sorted kernels

Fig. 2. Inverted second derivative spectra of sound kernels and FDK with corresponding second derivative difference spectra and regression coefficients for Wheaton DON calibration.

based on DON levels in response to *Fusarium* infection. Hence, SKNIR-sorted fractions of 23 samples in the first season and all 108 samples in the second season were subjected to DON analysis by the GC-MS method. Results showed that in the first season, kernels classified as sound (those collected in bin 1) contained 0.8 ± 0.4 ppm DON, while FDK fractions collected in bins 2 and 3 had 98.2 ± 12.2 ppm and 680.1 ± 53.5 ppm of DON, respectively (Table II). In the second season, kernels sorted into bins 1, 2, and 3 had 2.1 ± 0.6, 70.6 ± 6.9, and 411.3 ± 109.9 ppm of DON, respectively. Hence, it was possible for the SKNIR instrument to sort kernels into sound and FDK fractions and to further sort FDK fractions into subgroups according to DON concentration.

Out of the 23 lines tested in the first season, lines 1, 2, and 3 had <5 ppm of DON in the composite samples (Table III). Relying on only a single composite sample to assess these lines for resistance to FHB or DON accumulation, line 1 would have appeared to be the most resistant line. However, if the capacity of a particular line to produce grains with nondetectable DON levels is

considered, lines 3, 7, 6, and 1 would be capable of producing 96.2, 96.0, 94.9, and 94.0%, respectively, by weight, of sound grain without DON. Line 3 had a DON level of 4.7 ppm, while line 7 had a comparatively higher DON level of 12.8 ppm. If tombstone kernels are separated and removed, as many producers often do by blowing away light kernels during combining or cleaning, lines 3 and 7 produce a total of 96.2 and 96.0% of uncontaminated grains, while line 1 produces only 94.0%. In this respect, lines 3 and 7 would be considered more resistant to FHB than line 1 because they produce a higher proportion of sound grains. However, the DON levels of the composite samples of these two lines were higher because a smaller fraction of FDK, by weight, exhibited higher DON levels.

The final DON level of a composite grain sample can be influenced by a small subsample of kernels with high DON content. The present data demonstrate that it is not reliable to depend on the DON value of a single composite sample to interpret the FHB resistance of a particular wheat cultivar, especially if geneticists

Fig. 3. Scabby seeds or "tombstones" in FDK fractions (top left); intermediate seeds in FDK fractions (top right); intermediate seeds in sound fraction (bottom left); and sound seeds in sound fraction (bottom right).

TABLE I
SKNIR Sorting of 108 Wheat Samples Containing Sound and *Fusarium*-Damaged Kernels (FDK)

Sorted Fraction	Sound		Intermediate		FDK		Actual Classification	
	No. of Kernels	% of All Kernels	No. of Kernels	% Sorted as Sound or FDK	No. of Kernels	% Sorted as Sound or FDK	No. of Kernels	% Sorted as Sound or FDK
Sound (bin 1)	22,519	87.78	22,237	98.75	280	1.23	2	0.01
FDK (bin 2+3)	3136	12.22	63	2.10	1349	43.02	1724[a]	54.96

[a] 337 of the FDK were in bin 3; all other kernels were in bin 2.

and breeders are interested in assessing the type of resistance to FHB. It is advisable to separate composite samples into sound and FDK fractions. Once an estimate is made of the DON levels of each fraction, cultivars can be evaluated for the proportion of FDK produced, the DON levels of the FDK, and the production of grain without DON.

This indepth assessment can be accomplished using SKNIR sorting technology in combination with a GC-MS technique to accurately estimate DON concentrations in small kernel fractions. This technique promises to provide geneticists and plant breeders with a more detailed characterization of host plant resistance mechanisms compared to characterizations based on DON analyses of composite samples.

When measuring bulk DON levels using current analytical techniques, the high cost and added time associated with analyzing many samples for DON content can prohibit the use of this information in assessing host plant resistance. Instead, criteria such as visual FDK% (V FDK %), FHB incidence (Incidence %), FHB severity (Severity %), and kernels/g are often used by plant breeders to assess FHB resistance. Therefore, after sampling 108 disomic lines over three seasons, we correlated SKNIR-sorted FDK% data (SKNIR FDK%) with respective V FDK% and other FHB assessment criteria. Coefficients of determination (R^2) values for various relationships are presented in Table IV. The R^2 values of the relationships varied considerably among the three seasons. Higher R^2 values were observed in the first season compared to the other two seasons. This variation among the seasons may be due to the influence of genotype-by-environmental (G×E) interactions on the expression of the FHB disease symptoms and

DON accumulation in infected kernels. Such G×E interactions may affect the relationship between FHB disease symptoms in spikes and kernels and subsequent DON levels in the infected kernels. Miedaner et al (2001) have also demonstrated that significant wheat genotype-by-environment interactions can exist in the expression of resistance to FHB.

Generally, FDK% determined by SKNIR showed a higher correlation with Severity %, Incidence %, and kernels/g compared with FDK% determined visually. This may be due to the ability of the SKNIR system to assess FDK levels of grain samples more consistently compared to visual assessments (Wegulo and Dowell 2008). Another factor could be the subjectivity and inconsistency associated with visual rating of FDK, particularly in seasons with severe FHB infections. For seasons 1, 2, and 3, the mean FHB severity of the disomic lines was 7.9, 27.4, and 29.6%, respectively, while the mean FHB incidence was 19.4, 49.1, and 49.6%, respectively. Wegulo and Dowell (2008) showed that, compared to using objective assessment of the SKNIR system, visual assessments underestimate FDK levels in samples with a low percentage of *Fusarium*-damaged grain, while assessments overestimate the FDK levels in samples with a high percentage of *Fusarium*-damaged grain. Because FHB incidence and severity are utilized as primary determinants of host plant resistance, this emphasizes the advantage of using SKNIR technology to objectively and comprehensively assess responses to FHB infection.
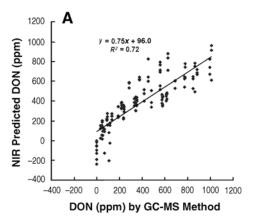
SKNIR Single Kernel DON Estimation

The single-kernel DON calibration ($R^2 = 0.72$; SECV = 154.2 ppm with 5 PLS factors) developed using Wheaton cultivar spectra was used to predict DON levels of PI-69251 kernels (Fig. 4A). Our results showed that this calibration could be used to separate kernels with low DON concentration (<60 ppm) from kernels with high DON concentration (>60 ppm) with 95.7 and 96.7% accuracy, respectively. Single-kernel DON levels of the high DON fraction (>60 ppm) were estimated fairly successfully, with $R^2 = 0.87$ and a standard error of prediction (SEP) = 60.8 ppm (Fig. 4B). Our single kernel DON analysis using the GC-MS method for the above DON calibration and validation datasets revealed that *Fusarium* infected kernels had high DON levels (min = 0.2 ppm, max = 1008.4 ppm, mean =298.8 ppm, SD = 282.1 ppm, and n = 71).

TABLE II
Deoxynivalenol Levels of SKNIR-Sorted Sound (Bin 1)
and FDK (Bins 2 and 3) Fractions for Two Seasons

Bin	DON (ppm ± SE)	
	Season 1	Season 2
1	0.8 ± 0.4	2.1 ± 0.6
2	98.2 ± 12.2	70.6 ± 6.9
3	680.1 ± 53.5	411.3 ± 109.9

TABLE III
Kernel Number, Weight, and DON Concentration of Kernels of 23 Breeder Lines as Sorted into Sound (Bin 1) and Two FDK Fractions (Bins 2 and 3), with Calculated Cumulative DON Concentration of Composite Sample

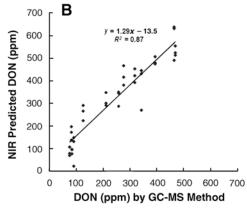| Line	Bin 1			Bin 2			Bin 3			Cumulative DON (ppm)
	# Kernels	Weight (g)	DON (ppm)	# Kernels	Weight (g)	DON (ppm)	# Kernels	Weight (g)	DON (ppm)	
1	244	9.003	0.0	36	0.550	31.9	5	0.021	641.0	3.2
2	257	9.644	1.3	31	0.630	29.4	5	0.015	661.7	4.0
3	215	6.758	0.0	24	0.270	123.6	0	na	na	4.7
4	211	7.981	0.4	32	0.320	151.8	14	0.052	712.3	10.6
5	111	3.818	2.5	39	0.342	84.7	6	0.018	623.5	11.9
6	163	5.827	0.0	38	0.293	179.8	7	0.021	986.6	12.0
7	193	7.203	0.0	25	0.269	233.2	7	0.028	1175.0	12.8
8	171	5.553	2.3	29	0.395	43.4	12	0.056	865.1	13.1
9	173	5.911	0.0	82	1.107	63.2	9	0.032	792.7	13.5
10	100	3.702	0.7	72	0.857	88.0	6	0.022	642.0	20.1
11	189	6.531	0.0	66	0.862	141.5	9	0.045	885.1	21.8
12	81	2.692	1.2	76	0.596	101.7	8	0.030	723.3	25.8
13	126	3.980	0.0	85	0.992	91.1	15	0.050	845.3	26.4
14	106	3.845	0.0	85	0.550	156.3	17	0.044	1034.0	29.6
15	80	3.392	0.5	61	0.775	130.7	15	0.077	530.6	33.9
16	97	3.257	0.2	120	1.027	72.3	41	0.124	602.8	34.0
17	43	1.433	4.4	92	1.021	52.9	15	0.062	587.2	38.5
18	78	2.882	4.0	117	1.048	100.7	23	0.085	449.8	38.7
19	40	1.105	5.0	91	0.716	25.5	18	0.052	1051.5	41.9
20	43	1.216	2.5	126	1.243	92.6	24	0.085	421.6	60.5
21	36	0.675	0.2	118	1.020	60.5	22	0.060	819.3	63.3
22	14	0.491	5.3	129	1.183	123.5	27	0.087	557.6	112.0
23	0	na	na	111	0.899	229.4	26	0.124	551.4	268.4

Fig. 4. A, Actual vs. predicted DON level of Wheaton calibration; **B,** DON predicted in PI-69251 kernels with > 60 ppm DON by Wheaton calibration.

TABLE IV
Coefficient of Determination (R^2) of Relationships Among
SKNIR FDK, Visual FDK, and FHB Severity, Incidence,
and Kernels/g for Three Greenhouse Seasons

	Season		
Relationship	**1**	**2**	**3**
SKNIR FDK % vs. Visual FDK %	0.726	0.274	0.387
SKNIR FDK % vs. Severity %	0.778	0.365	0.376
Visual FDK % vs. Severity %	0.755	0.284	0.661
SKNIR FDK % vs. Incidence %	0.642	0.278	0.559
Visual FDK % vs. Incidence %	0.581	0.133	0.554
SKNIRFDK % vs. Kernels/g	0.764	0.474	0.374
Visual FDK % vs. Kernels/g	0.457	0.274	0.061

These results indicate the potential of using SKNIR to estimate approximate DON levels of sorted FDK fractions that may have high DON levels.

CONCLUSIONS

A newly developed SKNIR technique is described that can be used to objectively assess wheat kernels for FHB damage. Kernels can be separated into sound and FDK fractions, and depending on the severity of kernel damage, FDK can be further subdivided by adjusting SKNIR sorting criteria. The SKNIR instrument is capable of analyzing 1 kernel/sec.

An estimation of DON level in high DON single kernels (>60 ppm) was also possible with $R^2 = 0.87$ and SEP = 60.8 ppm. This is a fairly good prediction for FDK with a mean DON level of 298 ppm with SD = 282 ppm. However, further studies are required to lower the DON detection limits and to reduce the SEP for DON estimates. The results of SKNIR sorting of sound kernels and FDK illustrate that this technique can be employed as a rapid, low-cost method for objectively obtaining detailed assessment of *Fusarium* damage in wheat kernels in response to FHB infection. Because the SKNIR method is nondestructive, plant breeders can use this technology to rapidly analyze many grain samples and obtain more information on responses to FHB infection while maintaining the seed for generation advancement. This technique may be particularly appropriate for conducting genetic studies designed to dissect different types of resistance, where large population sizes and required replication may hinder such detailed analyses by traditional methods.

Agronomists and pathologists evaluating fungicides and other FHB management practices could use this technique to evaluate the efficacy of treatments and practices in greater detail. Furthermore, the SKNIR technique may be modified such that it could be used to assess FHB damage and DON levels of small grains other than wheat.

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