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RESEARCH

Validation of *Fhb1* and *QFhs.nau-2DL* in Several Soft Red Winter Wheat Populations

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ABSTRACT

Exotic resistance quantitative trait loci (QTL) such as *Fhb1* and *QFhs.nau-2DL* provide one strategy for breeding wheat (*Triticum aestivum* L.) cultivars resistant to Fusarium head blight (FHB). The first objective of this study was to evaluate the effectiveness of these QTL in reducing FHB in diverse genetic backgrounds and to measure their impact on agronomic and quality traits. Lines from five susceptible × resistant crosses were evaluated in the FHB nursery at Lexington, KY, in 2010 and 2011. The populations were also grown in yield trials at Lexington (2010 and 2011) and Princeton (2011), KY, to measure agronomic and quality traits. *Fhb1* reduced *Fusarium* damaged kernels (FDK) by 32% and deoxynivalenol (DON) concentration by 20%. *QFhs.nau-2DL* reduced FDK by 29% in two of five populations and DON by 24% in four of five populations. Significant QTL effects ($P < 0.05$) on agronomic and quality traits were observed although impact was small. One cycle of direct or indirect simulated phenotypic selection was effective at reducing DON levels. The frequency of *Fhb1*-homozygous resistant lines among the phenotypically selected lines was higher than the frequency of *QFhs.nau-2DL*-homozygous resistant lines. The second objective was to assess the effectiveness of near-infrared reflectance (NIR) to estimate damage from FHB. Near-infrared reflectance-based predictions of FDK and DON showed that FDK measured by NIR was at least as good as FDK measured by air separation in predicting DON in four of five populations.

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Abbreviations: DON, deoxynivalenol; FDK, *Fusarium* damaged kernels; FHB, Fusarium head blight; GC–MS, gas chromatography with mass spectrometry; GPI, gluten performance index; H, heterozygous; H^2, broad-sense heritability; LEX, Lexington, KY; NIR, near-infrared reflectance; PRN, Princeton, KY; QTL, quantitative trait loci; R, resistant; RCB, randomized complete block; RR, homozygous for resistance alleles at both loci; RS, resistant at *Fhb1* and susceptible at *QFhs.nau-2DL*; S, susceptible; SRC, solvent retention capacity; SRW, soft red winter; SS, susceptible at both *Fhb1* and *QFhs.nau-2DL*; TWT, test weight.

In the United States, Fusarium head blight (FHB), or head scab, is primarily caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.) Petch; syn. *Gibberella saubinetti*] both in wheat [*Triticum aestivum* L. and *Triticum turgidum* L. subsp. *durum* (Desf.) Husn. (syn. *Triticum durum* Desf.)] and barley (*Hordeum vulgare* L.). Yield and test weight (TWT) reduction, contamination with the mycotoxin deoxynivalenol (DON), and additional costs of cleaning seed to improve grain quality have resulted in severe economic losses in the billions of dollars since the early 1990s (McMullen et al., 2008, 1997).

Breeding for FHB resistance is widely regarded as one of the most important strategies for reducing the impact of this disease.

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However, the quantitative nature of FHB resistance, its frequent association with undesirable agronomic traits, and the large effect of the environment make resistance breeding very difficult (Bai and Shaner, 2004). In addition, screening for scab resistance is time consuming and expensive. Molecular markers can be used to complement phenotyping and classical breeding to select for major resistance quantitative trait loci (QTL) (Agostinelli et al., 2012; Kang et al., 2011; Buerstmayr et al., 2002, 2009). The most widely used resistance QTL are exotic, deriving from Asian sources such as the Chinese spring wheat cultivar Sumai-3 (Bai and Shaner, 1994; Rudd et al., 2001). Their use provides a key strategy for FHB resistance breeding although the success of this approach depends on (i) effectiveness of the QTL in diverse genetic backgrounds and (ii) the effects of the QTL on agronomic and quality traits (Van Sanford et al., 2001). Fusarium head blight resistance QTL have been reported on almost all wheat chromosomes (Buerstmayr et al., 2010). Marker-assisted selection combines both phenotypic and QTL information and it assumes that not all of the QTL for the trait of interest are known (Bernardo, 2002). Quantitative trait loci analysis of Sumai-3 and other Asian cultivars shows that almost all of them have a QTL for resistance on chromosome 3BS at the *Qfhs.ndsu-3BS* locus (Sneller et al., 2010) also known as *Fhb1*. VA01W-476, derived from resistant cultivars Roane and W14, combines two exotic QTL, *Fhb1* and *QFhs.nau-2DL*, with additional resistance (Agostinelli et al., 2012).

Fhb1 has been reported to explain 29% of the phenotypic variance in severity and to be associated with fungal spread within the spike (Type 2 resistance) in F_1 derived doubled-haploid spring wheat lines from a resistant (carrying Sumai-3 resistance) and a susceptible parent (Buerstmayr et al., 2003). Pumphrey et al. (2007) estimated 27% reductions in *Fusarium* damaged kernels (FDK) associated with *Fhb1* in near-isogenic spring wheat lines. In a soft red winter (SRW) wheat population derived from a single cross of a high yielding susceptible parent and a resistant parent, Agostinelli et al. (2012) found that *Fhb1* reduced FDK and DON by 32 and 25%, respectively. The authors also evaluated *QFhs.nau-2DL* and they found 40 and 55% reduction in FDK and DON, respectively. When combined, both QTL complemented one another reducing FDK and DON more than each individual QTL. Kang et al. (2011) studied the effects of *Fhb1*, and QTL on chromosomes 2DL and 5A on eight SRW wheat near-isogenic lines developed by marker assisted backcrossing in Maryland and Kentucky. The combination of *Fhb1* and the 2DL QTL resulted in lower levels of DON, similar to all three QTL together. The researchers concluded that *Fhb1* and the 2DL QTL together would be useful in breeding for FHB resistance in the mid-Atlantic region. The limited number of populations in which these genes were evaluated means that more robust information on the effects of *Fhb1* and *QFhs.nau-2DL* in multiple SRW wheat genetic backgrounds is required.

The greatest challenge in breeding for FHB resistance is to release adapted FHB resistant cultivars that combine competitive yield and acceptable end-use quality (Bai and Shaner, 2004; Buerstmayr et al., 2009). Soft red winter wheat is mainly used for cakes, cookies, crackers, donuts, and flat breads (Beuerlein, 2001). Traits used to characterize soft wheat quality include flour yield, flour protein, softness equivalent, and flour solvent retention capacity (SRC). One of the concerns of soft wheat breeders is that these exotic QTL derived from spring wheats might impart deleterious quality attributes to the progeny of these types of crosses.

The objectives of this study were (i) to evaluate the impact of *Fhb1* and *QFhs.nau-2DL* on FHB traits, agronomic traits, and milling and baking quality in five different SRW wheat populations, (ii) to simulate progress from direct and indirect phenotypic selection and genotypic selection using data from 2 yr, and (iii) to assess the utility of near-infrared reflectance (NIR) for estimating FDK and DON.

MATERIALS AND METHODS

Plant Material

Five sets of inbred lines derived from two- and three-way crosses were evaluated in this study: (i) 26R58/VA01W-476//KY97C-0574-01 (n = 21 lines), (ii) 25R54/VA01W-476//KY97C-0574-01 (n = 24 lines), (iii) 25R54/VA01W-476//KY97C-0554-02 (n = 36 lines), (iv) 25R78/VA01W-476 (n = 45 lines), and (v) KY93C-1238-17-1/VA01W-476 (n = 29 lines). Crosses were made between FHB-susceptible parents and FHB-resistant VA01W-476, a doubled haploid line derived from the cross Roane × W14. These crosses represent typical resistant × susceptible crosses used in SRW wheat breeding programs. Each population consisted of lines chosen from an initial group of 27 crosses genotyped at the Regional Small Grains Genotyping lab in Raleigh, NC (http://www.ars.usda.gov/saa/psru, verified 22 Feb. 2011), in 2007. The lines included in this study were a subset of the lines in each population and were developed as follows. We evaluated F_2 plants for the presence of resistance alleles at *Fhb1* and *QFhs.nau-2DL*. Polymorphic markers used were Xgwm533 (Röder et al., 1998) for *Fhb1* and Xcfd233 (Grain genes 2.0 [http://wheat.pw.usda.gov/GG2/index.shtml, verified 13 Feb. 2009]) for *QFhs.nau-2DL*. In the F_2 populations, resistant (R) and susceptible (S) *Fhb1* homozygotes were selected. We did not select only homozygous *QFhs.nau-2DL* plants but included *QFhs.nau-2DL* heterozygotes because there were not enough *QFhs.nau-2DL* homozgotes that were also homozygous for *Fhb1*. Genotyped F_2 plants were increased in the greenhouse and head rows seeded in the fall of 2008. Ten heads from each $F_{2:3}$ head row were threshed in bulk in 2008 and increased in rows in 2009 to provide seed for this study.

The five crosses were selected out of all those genotyped according to two criteria: (i) pedigree—we chose pedigrees that were expected to generate lines with agronomic fitness—and (ii) marker behavior, including segregation ratios to confirm that the data were complete and ratios conformed to expectation.

To evaluate FHB traits, $F_{2:5}$ and $F_{2:6}$ lines were planted in headrows in a scab nursery at Spindletop Research Farm (38°7′37.81″ N, 84°29′44.85″ W) (Maury silt loam [fine, mixed, semiactive, mesic Typic Paleudalfs]) near Lexington, KY (LEX). The populations were also grown in yield trials at LEX (2010 and 2011) and Princeton, KY (PRN), (2011) at the West Kentucky Research and Educational Center (37°6′7.37″ N, 87°52′13.62″ W) (Crider silt loam [fine-silty, mixed, active, mesic Typic Paleudalfs]) to measure agronomic traits.

Lexington Scab Nursery

Two replicates of each line were sown in a randomized complete block (RCB) experiment in the 2009/2010 Lexington scab nursery for FHB evaluation and bulked for planting in 2010/2011. Lines were planted in rows 1.2 m long spaced 30 cm apart. The scab nursery was planted on 12 Oct. 2009 and 20 Oct. 2010. To provide favorable conditions for the disease, rows were misted with an overhead mist irrigation system on an automatic timer. Mist irrigation was on from 11 May to 16 June for periods of 5 min every quarter hour from 2000 to 2045 h, 2100 to 2145 h, 0200 to 0245 h, 0500 to 0530 h, and 0830 h (e.g., the equipment operated from 2000 to 2005 h the first time and the last time in the misting cycle was from 0830 to 0835 h). Both years the nursery was inoculated with *Fusarium graminearum*-infected corn (*Zea mays* L.) (Verges et al., 2006). Inoculum comprised 27 isolates taken from scabby wheat seed collected from 2007 to 2010 in multiple locations across Kentucky. For inoculum preparation, dry corn was set to imbibe water for 16 h before autoclaving. After autoclaving, the corn was inoculated with potato dextrose agar plugs of *Fusarium graminearum* mixed with 0.2 g streptomycin in 50 mL sterile water, covered, and incubated at room temperature for 3 wk until fully colonized by the fungus. At this point, the corn was spread on a sterilized plastic sheet until dry, put in mesh bags, and stored in the freezer until used. On 14 April the corn was spread between rows at a rate of 11.86 g m^{-2}. Liquid N fertilizer (28% urea ammonium nitrate) was applied in the spring at a rate of 105 kg N ha^{-1} in split applications. Harmony Extra herbicide (thifensulfuron methyl [methyl 3-[[[[(4-methoxy-6-methyl-1,3,5- triazin-2-yl) amino]carbonyl]amino] sulfonyl]-2-thiophenecarboxylate]; E.I Dupont de Nemours and Company) was applied on 20 April and a second application of *Fusarium graminearum*-infected corn was applied on 21 April.

Heading date was recorded when 50% of the spikes in the row had emerged. Plant height was measured at the soft dough stage. Fusarium head blight traits measured included incidence, severity, FHB index (severity × incidence, expressed as percentage), visual rating (0–9, in which 0 equals absence of FHB symptoms and 9 equals an FHB index ≥90%), FDK, and DON concentration (mg kg^{-1}). Incidence was estimated by counting the number of blighted spikes in a random sample of 20 spikes in each row 24 d after heading. Severity was measured as the proportion of infected spikelets per total spikelets per spike in 10 heads per row 24 d after heading. Samples were mechanically threshed and cleaned with low air flow to ensure minimal loss of scabby kernels. Approximately 45-g grain samples from each row were cleaned by hand and subsequently evaluated for FDK using an air separation machine specifically developed from a Precision Machine head thresher and a Shop-Vac vacuum to separate scabby kernels from healthy ones as described in Agostinelli et al. (2012). *Fusarium* damaged kernels was expressed as the weight of scabby kernels divided by total weight. A 20-g sample in which scabby kernels had been recombined with sound kernels was analyzed for DON at the University of Minnesota DON testing laboratory using gas chromatography with mass spectrometry (GC–MS) (Mirocha et al., 1998). To predict FDK and DON, samples were also run on a near-infrared analyzer (DA7200; 950–1650 nm wavelength range) manufactured by Perten Instruments. A calibration built in 2007 by the University of Kentucky Wheat Breeding Program and the manufacturers showed strong positive correlations between FDK and DON measured with traditional methods and NIR. Coefficients of determination (R^2) of FDK measured using NIR and FDK and DON measured by traditional methods were 0.67 and 0.59, respectively (A. Agostinelli, personal communication, 2010). This calibration was used to run 2010 samples and it was updated on the basis of 2010 FDK and DON data measured by air separation and GC–MS, respectively, to run 2011 samples. The amount of seed required was approximately 20 g.

Yield Plots

The five populations were grown in six row plots 3 m long. The experimental design was a RCB with three replications, grown in LEX in 2010 and 2011 and PRN in 2011, planted on 12 Oct. 2009, 20 Oct. 2010, and 14 Oct. 2010, respectively. All experimental plots received 105 kg N ha^{-1} applied in the spring as described previously. Recommended agricultural practices for wheat production in Kentucky were followed (Lee et al., 2009). Heading date, foliar disease ratings, FHB ratings, and plant height measurements were taken in yield plots. Each plot was harvested with a mechanical combine for yield and TWT determination.

Quality Analyses

The SRC tests predict baking performance by measuring the weight of solvent (water, lactic acid, sucrose, or sodium carbonate) retained as a percentage of the flour weight (Smith et al., 2011). In a recent review, Kweon et al. (2011) suggest using the gluten performance index (GPI), that is, the ratio between flour lactic acid SRC:(flour sodium SRC plus flour sucrose SRC), as an overall performance predictor. Taking minimum gold standard targets published in this review, the GPI would be 57%. A higher percentage given by lower flour sucrose or sodium carbonate or higher flour lactic acid SRC is better for baking cookies. A 100-g sample from each replication was analyzed for milling and baking quality at the USDA-ARS Soft Wheat Quality Laboratory, Wooster, OH. All grain was tempered at 15% moisture before milling. Flour yield was calculated as the bran weight subtracted from the grain weight, divided by the grain weight times 100 as described in Souza et al. (2008). Softness equivalent was calculated from the fraction of mill product that is in the mids (ground meal smaller than bran and larger than flour) that is subtracted from the adjusted flour yield. Water SRC, sucrose SRC, sodium carbonate SRC, and lactic acid SRC were estimated using approved AACC International method 56-11.02 (AACC, 2010) and were used to calculate GPI as described by Kweon et al. (2011).

Data Analysis

Analysis of variance was performed using the general linear models procedure (Proc GLM; SAS Institute, 2002) to determine line and QTL effects. The model used was

$$Y_{ijk} = \mu + ENV_i + R(ENV)_{ij} + QTL + G_k(QTL) + ENV_i \times G_k + E_{ijk},$$

in which Y_{ijk} is the observation in the kth genotype in the jth replication in the ith environment, μ is the overall mean, ENV_i is the effect of the ith environment, $R(ENV)_{ij}$ is the effect of jth replication within ith environment, QTL is the effect of the QTL, $G_k(QTL)$ is the effect of the kth genotype within QTL, $ENV_i \times G_j$ is the effect of the interaction of the ith environment with the kth genotype, and E_{ijk} is the residual error. Fisher's LSD was used to assess significant differences among QTL combination classes.

Broad-sense heritability of FHB and agronomic traits was estimated on an entry mean basis using the following model:

$$Y_{ijk} = \mu + ENV_i + R(ENV)_{ij} + G_k + ENV_i \times G_j + E_{ijk},$$

in which Y_{ijk} is the observation in the kth genotype in the jth replication in the ith environment, μ is the overall mean, ENV_i is the effect of the ith environment, $R(ENV)_{ij}$ is the effect of jth replication within the ith environment, G_k is the effect of the kth genotype, $ENV_i \times G_k$ is the effect of the interaction of the ith environment with the kth genotype, and E_{ijk} is the residual error.

Data was analyzed using the general linear models procedure (Proc GLM; SAS Institute, 2002). Genotypic and phenotypic variances were estimated from the expected mean squares and heritability estimates were computed as

$$H^2 = V_g/V_p,$$

in which H^2 is broad-sense heritability, V_g is genotypic variance, and V_p is phenotypic variance.

Confidence intervals (90%) were calculated after Knapp et al. (1985) as

$$UL = 1 - [MS_3/MS_2 \times F_{UL\ (0.10,\ v1\ and\ v2\ df)}] - 1 \text{ and}$$

$$LL = 1 - [\ MS_3/MS_2 \times F_{LL\ (0.90,\ v1\ and\ v2\ df)}] - 1,$$

in which UL is the upper limit of the confidence interval, LL is the lower limit of the confidence interval, MS_3 is the entry mean square, MS_2 is the residual mean square, F_{UL} and F_{LL} are the F value for the upper and lower limits calculated using the FINV function of Microsoft Excel (Microsoft, 2007), and v1 df and v2 df refer to the genotype and genotype × environment mean square degrees of freedom, respectively.

Proc CORR (SAS Institute, 2002) was used to analyze the relationship among traits on an entry mean basis. Entry means were plotted using Microsoft Excel (Microsoft, 2007) to study the relationship among traits and calculate R^2.

Selection Simulation

Selection was simulated to estimate genetic gain for various selection criteria. Lexington, KY, 2010 was treated as the selection environment and LEX 2011 was treated as the validation environment for FHB traits. For agronomic traits, an average of LEX 2011 and PRN 2011 was used as the validation environment. Quality traits were validated in PRN 2011. The mean of the selected lines after one cycle of selection in 2011 was compared to the mean of the population in the absence of selection in 2011. Selection for low DON was performed directly by retaining lines with the lowest DON levels in 2010 and indirectly by retaining lines with low FDK, NIR FDK, FHB index, or visual rating. Selection pressure was 20 and 10%. A genotypic selection scenario was performed by retaining lines homozygous resistant at both *Fhb1* and *QFhs.nau-2DL*. An additional selection scenario was performed by discarding undesirable lines based on heading date, plant height, and test weight and by selecting for high yield and low DON.

RESULTS AND DISCUSSION

Weather Conditions and Disease Levels

Wet and warm conditions were favorable for *Fusarium graminearum* infection in the three environments with precipitation levels much higher than historical averages during flowering (data not shown). Fusarium head blight traits varied among and within populations (Table 1). As expected, susceptible parents showed higher disease levels than the resistant parent VA01W-476 in both years. Transgressive segregants with DON levels lower than the resistant parent were observed in three populations in 2010 and in two populations in 2011. Disease levels in 2011 were higher than in 2010 both in the five populations and the parents that were used as checks (Table 1). Moderate to high levels of leaf rust [causal agent: *Puccinia triticina* Eriks; syn. *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* (Eriks. & E. Henn.) D.M. Henderson] and leaf blotch [disease complex caused by *Septoria tritici* Roberge in Desmaz (teleomorph *Mycosphaerella graminicola* (Fuckel) J. Schröt and *Phaeosphaeria nodorum* (E. Müller) Hedjaroude (anamorph *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano; syn. *Septoria nodorum* (Berk.) Berk. in Berk. & Broome] were observed in yield plots in LEX 2010. In 2011, leaf rust symptoms were negligible with low to moderate ratings of leaf blotch and FHB in both locations. Moderate to high and high levels of glume blotch [*Stagonospora nodorum* (teleomorph: *Phaeosphaeria nodorum*)] were observed in LEX and PRN 2011, respectively (data not shown). Even though no significant differences for non-FHB diseases were found between homozygous susceptible and resistant lines at each QTL, it is reasonable to assume a certain level of yield reduction associated with foliar diseases and of TWT reduction associated with both foliar diseases and glume blotch.

Fhb1 Effects on Fusarium Head Blight Traits

Fhb1 effects were studied on a class mean basis (S or R) for each population averaged across 2010 and 2011 (Table 2). In all populations, there was a significant reduction in FHB traits when comparing R with S classes. The

Table 1. Mean, maximum, and minimum *Fusarium* damaged kernels (FDK), deoxynivalenol (DON) level, and Fusarium head blight (FHB) index for five wheat populations and their parents in the Lexington, KY, scab nursery in 2010 and 2011.

Population	2010			2011		
	FDK	DON	FHB index	FDK	DON	FHB index
	%	mg kg^{-1}	%	%	mg kg^{-1}	%
Population 1 (*n* = 21)						
Mean	15.5	15.3	51.5	23.0	25.8	33.2
Max.	57.1	28.3	95.9	46.9	56.4	72.3
Min.	7.0	9.9	12.7	9.5	8.3	11.9
Population 2 (*n* = 24)						
Mean	12.8	13.1	53.9	17.8	24.6	29.0
Max.	26.8	21.3	86.3	32.8	37.0	65.1
Min.	5.9	5.0	21.8	6.1	14.0	8.5
Population 3 (*n* = 36)						
Mean	11.3	9.1	31.7	21.7	21.4	38.0
Max.	60.8	20.5	89.2	44.8	42.5	87.0
Min.	3.7	3.5	4.7	9.1	10.7	7.2
Population 4 (*n* = 45)						
Mean	11.8	9.3	37.2	25.2	19.1	30.3
Max.	30.6	16.6	93.5	70.3	34.6	65.8
Min.	2.7	3.5	2.8	4.4	7.7	0.2
Population 5 (*n* = 29)						
Mean	8.7	8.4	22.7	16.3	17.3	32.8
Max.	17.7	15.4	85.3	37.1	33.4	84.1
Min.	2.1	3.5	5.5	4.4	7.9	7.2
25R54	15.7 (1.7)†	13.2 (1.4)	55.3 (10.4)	25.8 (3.2)	31.9 (2.8)	29.4 (5.9)
25R78	18.7 (2.9)	14.1 (2.4)	48.3 (18.0)	31.1 (4.5)	31.5 (4.0)	35.3 (8.3)
26R58	21.2 (2.9)	23.1 (2.4)	73.1 (18.0)	28.2 (4.5)	30.6 (4.0)	42.0 (8.3)
KY93C-1238-17-1	13.3 (2.9)	15.6 (2.4)	57.5 (18.0)	30.2 (4.5)	30.0 (4.0)	41.2 (8.3)
KY97C-0554-02	8.6 (2.9)	13.4 (3.4)	39.3 (18.0)	29.6 (4.5)	30.2 (4.0)	67.2 (8.3)
KY97C-0574-01	13.3 (2.0)	18.2 (1.7)	61.8 (12.7)	25.0 (3.2)	42.3 (2.8)	54.2 (5.9)
VA01W-476	6.5 (1.3)	3.6 (1.1)	28.4 (9.6)	13.7 (2.2)	8.0 (2.0)	27.2 (4.2)

†Standard errors in parentheses.

presence of *Fhb1* resistance alleles significantly reduced FDK by 32% on average. These results are comparable to the 31, 32, and 27% FDK reductions reported by Cardwell (2011), Agostinelli et al. (2012), and Pumphrey et al. (2007), respectively. This QTL reduced DON by an average of 20%, similar to the 25% observed by Agostinelli et al. (2012) and lower than the 40% reported by Cardwell (2011). Index was significantly reduced by 28% on average in populations 2 through 5. These *Fhb1*-associated FDK and DON reduction levels indicate that exotic resistance is effective in lowering FHB impact in diverse genetic backgrounds. In this study, *Fhb1* tended to be more effective in lowering DON in populations derived from single crosses (populations 4 and 5) than in the three-way crosses (populations 1, 2, and 3). *Fhb1*-associated FDK and DON reduction levels were higher in 2010 than in 2011, when disease pressure was higher (Balut, 2012). For this reason, it might be expected that under lower natural field infection levels, *Fhb1* would show better levels of FHB control than the results we observed in the inoculated scab nursery with extremely high disease pressure.

QFhs.nau-2DL Effects on Fusarium Head Blight Traits

A similar analysis was conducted to study the effects of *QFhs.nau-2DL* classes (Table 2). For this QTL, high frequencies of lines derived from heterozygous (H) F_2 plants were present in the five populations as noted previously. Some H lines were as resistant as R lines while others were as susceptible as S lines; no clear pattern was evident. For example, DON levels in H lines in populations 1 and 4 were statistically the same as in the R lines and in population 3 were statistically the same as R and S lines. In populations 2 and 5, DON levels in H lines in were the same as in the S lines. When comparing the S against the R classes, *QFhs.nau-2DL* significantly reduced FDK by 29% on average in populations 1 and 4. Other studies showed both higher *QFhs.nau-2DL*-associated FDK reduction (Agostinelli et al., 2012) and nonsignificant effects (Cardwell, 2011). In the present study a significant ($P < 0.05$) 24% DON average reduction was observed in all populations with the exception of population 3. These DON reductions were lower than the 50% reported in other studies under both similar and lower levels of FHB pressure (Cardwell,

Table 2. Mean *Fusarium* damaged kernels (FDK), deoxynivalenol (DON) levels, and Fusarium head blight (FHB) index for homozygous susceptible (S), resistant (R), and heterozygous (H) wheat lines at *QFhs.nau-2DL* and *Fhb1* in the Lexington, KY, scab nursery in 2010 and 2011.

QTL† class	*n*	FDK	DON	FHB index
		%	mg kg^{-1}	%
Population 1				
Fhb1				
S	12	21.3 a‡	21.7 a	40.7 a
R	9	16.5 b	19.0 b	44.6 a
QFhs.nau-2DL				
S	9	21.9 b	21.9 b	47.0 b
H	8	16.3 a	18.3 a	37.3 a
R	2	16.7 a	16.7 a	40.3 ab
Population 2				
Fhb1				
S	13	17.9 a	20.0 a	46.6 a
R	11	12.5 b	17.7 b	35.3 b
QFhs.nau-2DL				
S	4	14.5 a	20.6 b	37.1 a
H	15	15.6 a	19.3 b	41.7 a
R	4	14.1 a	15.0 a	42.1 a
Population 3				
Fhb1				
S	15	20.2 a	17.7 a	41.4 a
R	21	13.8 b	13.6 b	30.2 b
QFhs.nau-2DL				
S	17	15.0 a	14.7 a	33.2 ab
H	9	18.6 b	15.8 a	38.1 b
R	7	16.2 ab	15.4 a	29.3 a
Population 4				
Fhb1				
S	24	21.9 a	16.1 a	39.3 a
R	21	14.4 b	11.9 b	27.5 b
QFhs.nau-2DL				
S	12	21.9 b	16.3 b	35.3 a
H	20	16.6 a	13.2 a	31.5 a
R	7	14.3 a	12.7 a	34.5 a
Population 5				
Fhb1				
S	15	15.8 a	14.8 a	32.7 a
R	14	9.0 b	10.8 b	22.8 b
QFhs.nau-2DL				
S	6	11.4 a	13.3 b	23.5 a
H	15	14.0 b	14.0 b	31.0 b
R	8	10.5 a	10.5 a	25.1 ab
QTL effect,§ %				
Fhb1		–32	–20	–28
QFhs.nau-2DL		–29	–24	0

†QTL, quantitative trait loci.

‡Means (by population and by QTL) within the same column followed by different letters are significantly different at $P < 0.05$.

§Mean QTL effect based on significant differences between homozygous R and S classes.

2011; Agostinelli et al., 2012). Although average *QFhs.nau-2DL* associated DON reduction in this study was similar to that associated with *Fhb1*, when looking at each population individually, *QFhs.nau-2DL* was more stable across populations and reduced DON by 21 to 27% whereas *Fhb1* ranged from 12 to 27% (Table 2). In 2011, under higher disease pressure, *QFhs.nau-2DL* was associated with a more generalized DON reduction (4 of 5 populations) than in 2010 (2 of 5 populations) (Balut, 2012). However, the largest DON reduction level (43%) was observed in 2010 whereas in 2011 DON reduction associated with this QTL ranged from 17 to 27% (Balut, 2012). In the present study, significant differences between homozygous S and R lines at *QFhs.nau-2DL* were detected in a larger number of genetic backgrounds at higher FHB pressure conditions (Lexington 2012). There were significant differences for FHB index between H lines and both S and R lines (Table 2).

Agronomic Traits

In the analysis of populations per se, significant ($P < 0.05$) genotype × environment interaction was observed for most yield and quality traits (Balut, 2012); therefore, data from each environment are presented separately in Table 3. Higher mean yields were observed in PRN 2011 than in LEX 2010 and LEX 2011 (Table 3). The resistant parent, VA01W-476, was always outyielded by the other parents. Heading date was the least variable of agronomic traits; population means ranged from 125.3 to 127.5 Julian days (data not shown). Transgressive segregants were found for all agronomic traits in the five populations (Table 3)

Fhb1 Effects on Agronomic Traits

Yield was significantly higher for the R-*Fhb1* class in populations 2 (5%), 3 (8%), and 5 (2%) (Table 4). Most of the effects on TWT were in the desired direction but very small in absolute value. In contrast with previous reports of *Fhb1* associated with increased plant height, in this study mean height was reduced for the R class in two populations and increased in only one population (data not shown) although these differences were less than 2%. Although heading date was significantly ($P < 0.05$) affected in three of four populations, differences were less than one Julian day (data not shown).

QFhs.nau-2DL Effects on Agronomic Traits

The effects of *QFhs.nau-2DL* on yield varied among populations (Table 4). For example, in population 3 where yield was 5% lower for the R class than for the S class, the H class was not significantly different from the R one. In population 4, a similar scenario was observed but with a 3% yield increment for both R and H classes. Population 1 presented a different situation in which the H class outyielded the other classes by 2%. Although statistically significant, these differences were small in absolute value (100–200 kg ha^{-1}) with little agronomic significance. *QFhs.nau-2DL* increased plant height in two populations but it was associated with height reduction in other populations (data not

Table 3. Mean, maximum, and minimum yield and test weight (TWT) for five wheat populations and their parents in Lexington, KY (LEX), in 2010 and 2011 and Princeton, KY (PRN), in 2011. Mean flour yield (FY), softness equivalent (SEQ), and gluten performance index (GPI) in LEX 2010 and PRN 2011.

Population	LEX 2010		LEX 2011		PRN 2011		LEX 2010 and PRN 2011		
	Yield	TWT	Yield	TWT	Yield	TWT	FY	SEQ	GPI
	kg ha^{-1}	kg hL^{-1}	kg ha^{-1}	kg hL^{-1}	kg ha^{-1}	kg hL^{-1}	%		
Population 1 (n = 21)									
Mean	4041	72.4	3899	68.9	4380	71.1	68.8	55.8	57.9
Max.	4743	75.8	5114	74.1	5249	74.3	71.8	61.9	65.6
Min.	3084	67.9	2114	63.0	2263	66.6	66.3	45.5	46.1
Population 2 (n = 24)									
Mean	4117	72.2	3839	71.4	4339	72.2	66.9	53.6	58.4
Max.	4886	76.1	5085	74.8	5419	76.0	69.2	62.0	66.5
Min.	2237	66.4	2440	62.1	2966	63.3	63.6	46.5	49.4
Population 3 (n = 36)									
Mean	4069	71.8	4059	69.7	4486	71.7	69.8	52.4	61.2
Max.	5307	75.8	5367	74.6	5471	75.0	73.2	63.9	74.6
Min.	2634	65.3	1758	58.8	3044	67.0	65.9	42.2	49.7
Population 4 (n = 45)									
Mean	4055	72.4	3492	68.7	3927	70.9	66.9	58.1	62.9
Max.	5219	76.8	4971	74.6	5003	75.5	69.0	64.3	75.9
Min.	2661	67.6	1846	57.1	1598	60.8	63.5	50.7	50.5
Population 5 (n = 29)									
Mean	3626	83.6	4075	70.3	4383	72.8	67.9	57.7	53.8
Max.	4536	99.1	5328	74.9	5157	76.4	71.7	63.6	64.2
Min.	2584	68.6	3031	65.3	3326	65.6	65.2	51.0	46.5
25R54	3775 (141)†	69.0 (0.6)	4055 (162)	67.2 (0.6)	4393 (190)	68.8 (0.5)	70.1 (0.2)	62.1 (0.5)	60.7 (0.0)
25R78	5098 (215)	71.8 (0.8)	3372 (229)	66.6 (0.8)	4579 (268)	70.8 (0.6)	69.2 (0.3)	59.1 (0.7)	58.6 (0.0)
26R58	4193 (215)	66.7 (0.8)	4284 (229)	63.9 (0.8)	5020 (268)	68.2 (0.6)	68.4 (0.3)	58.2 (0.7)	51.0 (0.0)
KY93C-1238-17-1	4091 (215)	72.7 (0.8)	4504 (229)	67.0 (0.8)	4991 (268)	70.4 (0.6)	70.5 (0.3)	61.9 (0.7)	47.3 (0.0)
KY97C-0554-02	4998 (215)	72.7 (0.8)	4789 (229)	70.1 (0.8)	4856 (268)	70.2 (0.6)	72.8 (0.3)	48.0 (0.7)	59.7 (0.0)
KY97C-0574-01	4231 (152)	71.7 (0.6)	4317 (162)	69.6 (0.6)	4801 (190)	72.1 (0.5)	69.0 (0.2)	56.5 (0.5)	60.2 (0.0)
VA01W-476	2946 (107)	74.5 (0.4)	2881 (115)	71.6 (0.4)	3482 (134)	73.1 (0.3)	64.8 (0.2)	54.8 (0.4)	65.3 (0.0)

†Standard errors in parentheses.

shown). Although heading date was significantly delayed in two populations ($P < 0.05$), the impact was less than one Julian day. In population 2, the R *QFhs.nau-2DL* class was 2 d earlier than the S class (data not shown).

Fhb1 Effects on Milling and Baking Quality

Mean flour yield, softness equivalent, and GPI are presented in Table 3 with their corresponding ranges and parental means. In these genetic backgrounds, *Fhb1* had small and variable impacts on quality traits that varied among populations (Table 4). Although significant reductions were found between S and R *Fhb1* classes in all five populations for flour yield, differences were 1% or less. *Fhb1* resistance alleles were associated with higher softness equivalent in population 1 (3%) and 1 to 2% lower softness equivalent levels in the other four populations. More importantly, mean softness equivalent in each QTL class was within the acceptable 50 to 60% minimum range for this trait (Everts et al., 2001) and the QTL effects were very small in absolute values. Gluten performance index was 4% higher for the R-*Fhb1* class in population 2, which reflects a better balance between lactic acid, sucrose, and sodium carbonate SRC, but had the opposite impact by 2% in populations 1 and 3. In a different genetic background, Cardwell (2011) found no significant differences between S and R lines for *Fhb1* for quality traits such as softness equivalent, flour yield, flour protein, and the different SRC tests. In hard spring wheat, FHB-resistant QTL effects on quality traits varied according to the QTL, the source of the QTL, and the population into which the QTL was introgressed (McCartney et al., 2007). In that context, *Fhb1*-R alleles were associated with a slight reduction in flour yield and a reduction in falling number in one of the three genetic backgrounds evaluated. No significant impact was found on other traits such as TWT, weight of 1000 kernels, grain protein concentration, sodium dodecyl sulfate sedimentation, and mixograph parameters.

The present study shows a consistent association of *Fhb1* with effective FHB levels reduction and negligible impact on agronomic and quality traits that highlights its usefulness in breeding programs.

Table 4. Mean yield, test weight (TWT), flour yield (FY), softness equivalent (SEQ), and gluten performance index (GPI) for homozygous susceptible (S), resistant (R), and heterozygous (H) wheat lines at *Fhb1* and *QFhs.nau-2DL* in Lexington in 2010 and 2011 and Princeton in 2011.

QTL† class	n	Yield‡	TWT	FY	SEQ	GPI
		kg ha^{-1}	kg hL^{-1}	——— % ———		
Population 1						
Fhb1						
S	12	4139 a§	71.0 a	68.9 a	55.0 b	58.5 a
R	9	4064 a	70.5 b	68.6 b	56.7 a	57.2 b
QFhs.nau-2DL						
S	9	4059 a	70.0 a	68.5 a	56.6 b	57.1 a
H	8	4193 b	71.5 b	68.9 b	56.1 a	59.9 b
R	2	3971 a	71.4 b	68.3 a	55.7 a	57.8 a
Population 2						
Fhb1						
S	13	4014 b	71.5 b	67.1 a	53.9 a	57.2 b
R	11	4191 a	72.5 a	66.8 b	53.3 b	59.7 a
QFhs.nau-2DL						
S	4	4060 a	70.8 a	67.3 c	56.5 c	56.5 a
H	15	4071 a	72.0 b	66.9 b	53.9 b	59.6 b
R	4	4154 a	72.5 b	66.5 a	50.4 a	56.7 a
Population 3						
Fhb1						
S	15	4025 b	70.1 b	70.1 a	52.9 a	62.1 a
R	21	4334 a	71.8 a	69.6 b	52.1 b	60.6 b
QFhs.nau-2DL						
S	17	4314 b	71.7 b	69.7 a	51.8 a	60.5 a
H	9	4085 a	70.3 a	70.2 c	52.2 b	63.6 b
R	7	4071 a	70.7 a	70.0 b	53.7 c	60.0 a
Population 4						
Fhb1						
S	24	3816 a	70.2 b	67.0 a	58.4 a	62.7 a
R	21	3834 a	71.2 a	66.7 b	57.6 b	63.3 a
QFhs.nau-2DL						
S	12	3756 a	70.1 a	66.9 b	58.1 b	62.4 a
H	20	3868 b	71.2 b	66.8 b	57.7 a	62.7 ab
R	7	3903 b	71.0 b	66.6 a	57.7 ab	63.6 b
Population 5						
Fhb1						
S	15	3975 b	72.3 b	68.2 a	58.2 a	54.1 a
R	14	4085 a	72.9 a	67.7 b	57.1 b	53.7 a
QFhs.nau-2DL						
S	6	4025 a	73.0 b	67.8 b	57.3 a	54.4 a
H	15	4053 a	72.6 b	68.3 c	57.6 a	54.0 a
R	8	3984 a	72.1 a	67.4 a	58.1 b	53.5 a
QTL effect,¶ %						
Fhb1		5	1	–1	–1	0
QFhs.nau-2DL		–1	1	0	–2	2

†QTL, quantitative trait loci.

‡Yield and TWT were measured in Lexington in 2010 and 2011 and Princeton in 2011. FY, SEQ, and GPI were measured in Lexington in 2010 and Princeton in 2011.

§Means (by population and by QTL) within the same column followed by different letters are significantly different at $P < 0.05$.

¶Mean QTL effect based on significant differences between homozygous R and S classes

QFhs.nau-2DL Effects on Milling and Baking Quality

As observed in the *Fhb1* analysis, in general, *QFhs.nau-2DL* had small effects on quality traits (Table 4). Although *QFhs.nau-2DL* effects on flour yield were significant in most populations, this effect was by 1% or less. The R-*QFhs.nau-2DL* class showed both reduction and increase in softness equivalent. Once again, all softness equivalent means were within the acceptable 50 to 60% minimum range for this trait (Everts et al., 2001). Gluten performance index was only affected in population 4 and in a positive manner. Cardwell (2011) found that the presence of 2DL R alleles were associated with a 3% decline in lactic acid SRC that translated into a 1% reduction in GPI. This QTL was also associated with an 11% lower milling quality score and softness equivalent, an 8% lower baking quality score, and a 2% reduction in flour yield. With the exception of population 3, *QFhs.nau-2DL* was slightly more effective than *Fhb1* in DON reduction (24 vs. 20%) with negligible impact on agronomic and quality traits.

Heritability Estimates

Broad-sense heritabilities and their corresponding confidence intervals were estimated on an entry mean basis for each population separately (Table 5). Deoxynivalenol H^2 ranged from 0.59 to 0.75, which are lower than the estimates reported by Agostinelli et al. (2012). *Fusarium* damaged kernels H^2 estimates were moderate to high (0.58 to 0.82), lower than those reported by Agostinelli et al. (2012) and higher than reported by Verges et al. (2006). Fusarium head blight index H^2 estimates were more variable among populations and less precise, which suggests that selection based on DON or FDK should be more effective than that based on FHB index. Heritability of quality traits was high both overall and in each individual population in agreement with estimates reported in the literature (Smith et al., 2011). Flour yield and softness equivalent had H^2 greater than or equal to 0.85. Gluten performance index was also highly heritable and ranged from 0.79 to 0.95 among the different populations.

Combined Effects of *Fhb1* and *QFhs.nau-2DL*

To investigate the joint effects of both QTL, the data from all five populations were combined to ensure a large enough sample size for each *Fhb1* and *QFhs.nau-2DL* class combination. Six classes were compared in total where the first letter of the class combination corresponds to *Fhb1* (S or R) and the second to *QFhs.nau-2DL* (S, H, or R). The RR class, for example, comprised genotypes homozygous for resistance alleles at both loci. The SS (i.e., susceptible at both *Fhb1* and *QFhs.nau-2DL*) combination was significantly higher in FDK and DON than all other possible combinations (data not shown). The effect of resistance alleles at both QTL (RR class) resulted in a significant reduction by 50, 40, and 30% for FDK, DON, and FHB index when compared against the double susceptible class (SS). In lines with the RR genotypes we measured an additional 10% reduction in DON when compared against RH (resistant at *Fhb1*

Table 5. Heritabilities and their 90% confidence intervals (in parentheses) based on 2 yr ANOVA of five wheat populations. Traits evaluated were *Fusarium* damaged kernels (FDK), deoxynivalenol (DON), and Fusarium head blight (FHB) index (Lexington in 2010 and 2011) and flour yield (FY), softness equivalent (SEQ), and gluten performance index (GPI) (Lexington in 2010 and Princeton in 2011).

	DON	FDK	FHB index	FY	SEQ	GPI
Overall	0.75 (0.80–0.69)	0.65 (0.72–0.57)	0.33 (0.46–0.18)	0.94 (0.95–0.92)	0.95 (0.96–0.94)	0.93 (0.94–0.91)
Population 1	0.71 (0.84–0.48)	0.82 (0.90–0.68)	0.51 (0.73–0.12)	0.85 (0.92–0.74)	0.93 (0.96–0.88)	0.95 (0.97–0.91)
Population 2	0.63 (0.79–0.37)	0.60 (0.77–0.31)	0.26 (0.57–0.28)	0.90 (0.94–0.84)	0.98 (0.99–0.96)	0.95 (0.97–0.92)
Population 3	0.59 (0.74–0.37)	0.63 (0.76–0.43)	0.41 (0.62–0.08)	0.95 (0.97–0.93)	0.98 (0.99–0.96)	0.93 (0.95–0.89)
Population 4	0.75 (0.83–0.62)	0.58 (0.72–0.38)	0.59 (0.72–0.39)	0.88 (0.92–0.82)	0.91 (0.94–0.86)	0.79 (0.86–0.69)
Population 5	0.59 (0.75–0.34)	0.67 (0.80–0.47)	0.66 (0.79–0.44)	0.93 (0.96–0.88)	0.89 (0.93–0.82)	0.80 (0.88–0.67)

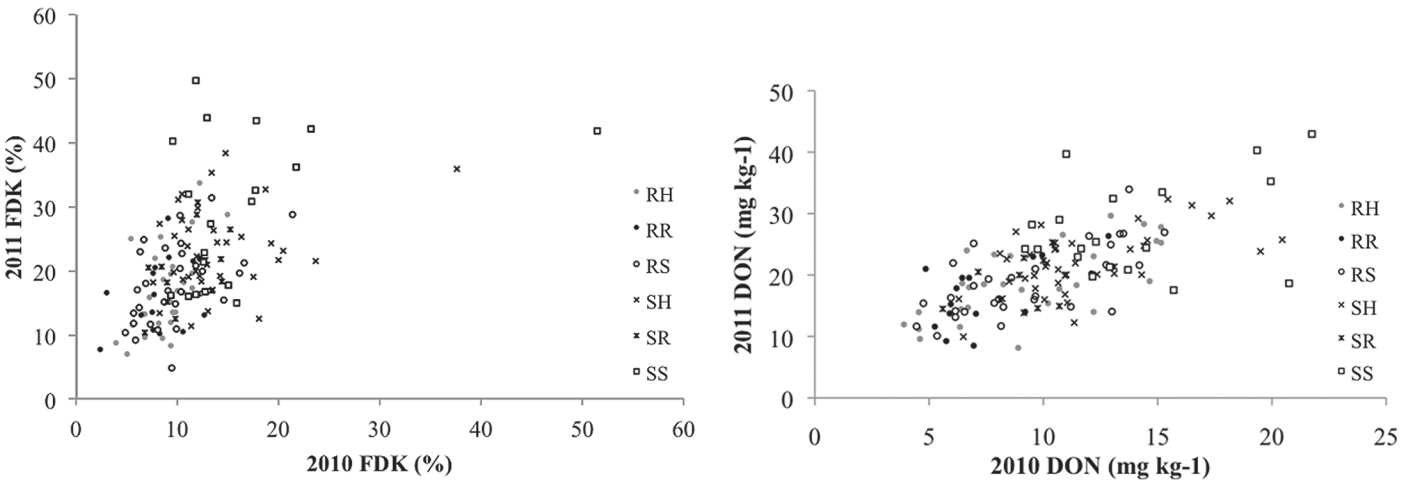

Figure 1. Relationship between 2010 and 2011 measurements of *Fusarium* damaged kernels (FDK) (a) and deoxynivalenol (DON) (b) by quantitative trait loci class combination: homozygous for resistance alleles at *Fhb1* and *QFhs.nau-2DL* (RR), resistant at *Fhb1* and heterozygous at *QFhs.nau-2DL* (RH), resistant at *Fhb1* and susceptible at *QFhs.nau-2DL* (RS), susceptible at *Fhb1* and resistant at *QFhs.nau-2DL* (SR), and susceptible at both *Fhb1* and *QFhs.nau-2DL* (SS). SH, susceptible at *Fhb1* and heterozygous at *QFhs.nau-2DL*.

and heterozygous at *QFhs.nau-2DL*) and an additional 10% reduction in both FDK and DON when compared against RS (resistant at *Fhb1* and susceptible at *QFhs.nau-2DL*) lines (data not shown). Significant QTL × year interaction was found in populations 3, 4, and 5 for *Fhb1* and in populations 1 and 5 for *QFhs.nau-2DL* (data not shown). However, when 2010 means were plotted against 2011 means, double resistant genotypes tended to cluster within the lower FDK and DON levels (Fig. 1).

One of the most vexing questions facing breeders is whether QTL will impart sufficient resistance to progeny in the absence of background resistance that is widely available in the soft winter wheat gene pool, frequently referred to as "native resistance." In this study, lines KY97C-0574-01 and 25R58 used in population 1 are good examples of highly susceptible parents with no apparent native resistance. In this population, *Fhb1* reduced FDK and DON by 23 and 12%, respectively, and *QFhs.nau-2DL* reduced both FHB traits by 24% (Table 2). Population 4 is another example that provides evidence that resistance derived from these exotic QTL could be sufficient. The parent 25R78 used in this population is also highly susceptible and in this context, *Fhb1* reduced FDK and DON by 34 and 26%, respectively (Table 2), and *QFhs.nau-2DL* reduced both FHB traits by 35 and 22%, respectively (Table 2). Trangressive segregates for DON or segregates that are close in DON and FDK to VA01W-476 support this idea of exotic resistance being enough to reduce FHB levels in well-adapted material. For example, segregates in populations 2 through 5 showed lower FDK levels than VA01W-476 in 2010 and segregates in all populations met this criterion in 2011 (Table 1). Minimum DON levels found in populations 3, 4, and 5 in 2010 were essentially the same as those of VA01W-476 DON levels. In 2011, minimum DON levels in populations 4 and 5 were consistent with 2010, showing slightly lower levels than VA01W-476, and minimum DON level in population 1 was close to the resistant parent as well (Table 1).

Deoxynivalenol and *Fusarium* Damaged Kernels Assessment

Both DON and FDK are expensive and time consuming to quantify; therefore, rapid and nondestructive methods for predicting these traits are of great interest. Near-infrared reflectance detects the absorption response by overtone and combination frequencies of O-H, C-H, and N-H molecular vibrations (approved methods 39-00.01 and 39-25.01; AACC, 2011) as well as physical properties

Table 6. Mean deoxynivalenol (DON) reduction after one cycle of simulated selection, proportion of the population selected (Pop.), number of lines selected (n) for different selection criteria, and lines selected from the following genotypic categories: homozygous for resistance alleles at *Fhb1* and *QFhs.nau-2DL* (RR), resistant at *Fhb1* and heterozygous at *QFhs.nau-2DL* (RH), resistant at *Fhb1* and susceptible at *QFhs.nau-2DL* (RS), susceptible at *Fhb1* and resistant at *QFhs.nau-2DL* (SR), susceptible at *Fhb1* and heterozygous at *QFhs.nau-2DL* (SH), susceptible at both *Fhb1* and *QFhs.nau-2DL* (SS), resistant for *Fhb1* and unknown for *QFhs.nau-2DL* (R?), and susceptible for *Fhb1* and unknown for *QFhs.nau-2DL* (S?).

Selection criterion†	*p*	*n*	DON reduction	Lines selected from each category							
				RR	RH	RS	SR	SH	SS	R?	S?
			%	15‡	29	29	13	38	19	3	9
Direct phenotypic selection	0.20	31									
DON			28	8	10	9	1	2	–	1	–
Indirect phenotypic selection											
FDK			25	7	10	10	2	1	–	–	1
NIR FDK			22	6	11	9	1	3	1	–	–
Visual rating			18	5	10	8	1	5	1	1	–
FHB index			18	7	4	9	3	6	2	–	–
Direct phenotypic selection	0.10	15									
DON			36	5	4	4	1	–	–	1	–
Indirect phenotypic selection											
FDK			34	3	5	7	–	–	–	–	–
NIR FDK			29	3	6	5	–	–	1	–	–
Visual rating			11	2	6	3	–	2	1	1	–
FHB index			26	4	3	7	–	1	–	–	–
Genotypic selection											
Homozygous resistant lines at *Fhb1* and *QFhs.nau-2DL* (RR)			19	15	–	–	–	–	–	–	–

†FDK, *Fusarium* damaged kernels; NIR, near-infrared reflectance; FHB, Fusarium head blight.

‡Number of lines selected from each category.

such as grain shape, size, and color (Peiris et al., 2010). With the appropriate calibration, an NIR instrument can measure FDK and DON on a whole kernel basis at the same time and 100 samples can be run in 3 h. If NIR can be used to identify probable high DON samples, then actual DON analysis (approximately $8–10 per sample; Yanhong Dong, personal communication, 2009) need not be run, which is a significant cost savings. In our study the regression of DON on FDK varied among populations (R^2 = 0.24–0.62; data not shown) as well as NIR FDK-DON (data not shown). Near-infrared reflectance FDK was more effective than FDK in predicting DON in two of five populations, indicating that the NIR instrument may be somewhat more accurate in distinguishing scabby from healthy kernels. The fact that the ability of FDK or NIR FDK to predict DON varied among populations illustrates a relative weakness of their predictive value. However, NIR predictive ability can be easily improved by updating the calibration equations with samples of diverse genetic backgrounds and DON wet chemistry.

Selection Simulation

When only FHB resistance was taken into account in the phenotypic selection simulation, either directly on DON or indirectly on NIR FDK or FDK, the majority of the lines kept were homozygous resistant for *Fhb1* with a lower frequency of R lines for *QFhs.nau-2DL* (Table 6). Moreover, when direct selection on DON or indirect selection based on NIR FDK or FDK was implemented at P = 0.20 (top 20% selected), very few *Fhb1*-S lines were retained (only 3 and 5). Visual rating and FHB index based selections retained a larger number of *Fhb1*-S lines (7 and 11, respectively) than the other indirect selection criteria. The number of *QFhs.nau-2DL*-R lines retained was in most cases lower than the number of *QFhs.nau-2DL*-H or -S lines. This indicates that in these diverse genetic backgrounds, DON reduction was more closely associated with *Fhb1* than with *QFhs.nau-2DL*. This contrasts with a previous study (Agostinelli et al., 2012) in which the effect of *QFhs.nau-2DL* was more pronounced than that of *Fhb1* and phenotypic selection enriched the population with a similar proportion of R lines for both QTL.

Direct phenotypic selection on DON, regardless the selection pressure, was the most effective strategy for lowering the toxin levels in the next generation. However, indirect selection using FDK and NIR FDK, which are less expensive traits to measure, showed acceptable levels of DON reduction. Among the phenotypic selection criteria, visual rating and FHB index showed the lowest DON reduction (Table 6) indicating that these traits are less effective for selecting for resistance to DON contamination. Although less effective, visual ratings and FHB index are less expensive than DON assessments; the data are available before harvest, and there is no need to

exhaustively clean samples to separate grain from chaff as is required for DON, FDK, or NIR FDK determinations.

A genotypic selection scenario was conducted by retaining only the R lines at both *Fhb1* and *QFhs.nau-2DL* (i.e., RR genotypes). Under this scenario, DON was reduced less than under phenotypic selection using DON, FDK, NIR FDK, or FHB index (Table 6). Genotypic selection was less effective than direct phenotypic selection in lowering DON, in agreement with Agostinelli et al. (2012) who found that retaining the 30% lower DON lines equalized retaining all R lines for both *Fhb1* and *QFhs.nau-2DL* (12% of the population).

A more realistic selection scenario was simulated by first retaining lines with desirable agronomic characteristics. As short early lines (<93 cm tall and ≤130 Julian days heading date) with ≥70.8 kg hL^{-1} TWT were considered acceptable, the population size was reduced from 155 to 125. From this subpopulation, the top 25% high yielding lines were selected (n = 31). Under this scenario, mean DON levels of the selected population remained the same as the nonselected population (21 mg kg^{-1}), suggesting that yield and susceptibility to FHB are not necessarily associated in this genetic background (Fig. 2). In agreement with these results, correlation coefficients between yield and DON was weak (r = 0.20) in both years. Four of these 31 lines were in common with the top 20% subpopulation selected for low DON (Table 6). These four lines identified with low DON constituted the 13% of the 31 lines that had been selected for high yield. By selecting these four lines, grain yield increased by 8% and DON was reduced by 30% (Fig. 2). It is important to highlight that the four lines are high yielding and have low DON levels. Moreover, two lines carry homozygous R alleles at both *Fhb1* and *QFhs.nau-2DL* (RR), and the other two lines carry homozygous R alleles at *Fhb1* (RS) or *QFhs.nau-2DL* (susceptible at *Fhb1* and resistant at *QFhs.nau-2DL* [SR]). Quality traits for these four lines were within the acceptable ranges for soft wheat for the most part. One of the four lines presented acceptable flour yield, softness equivalent, and GPI. The other three lines presented acceptable quality with the exception of a 5% lower flour yield in one of the lines, a 4% lower softness equivalent in a different line, and a 5% lower GPI in the third one (data not shown). These results suggest that by building good quality from the selection of the parental material and including some sort of rapid assessment of quality in earlier generations to enrich the population with the desired quality parameters, plant breeders can succeed in selecting for resistance to FHB using the exotic QTL evaluated in this study while preserving quality standards and improving yield.

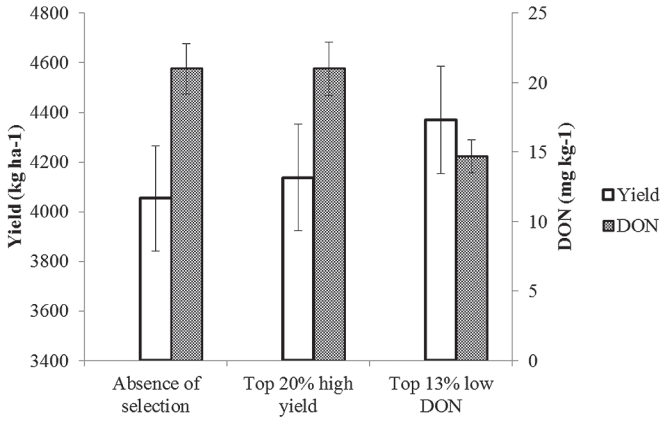

Figure 2. Mean yield (white bars) and deoxynivalenol (DON) levels (black bars) for wheat lines before selection for these traits (absence of selection), after the top high yielding 25% lines were selected, and after a subsequent top 13% of the lines was selected for low DON.

CONCLUSIONS

Fhb1-derived resistance reduced FDK and DON, the two most direct measurements of FHB impact, in all five populations by 32 and 20%, on average. Index was also reduced by 28% in four of five populations. *QFhs.nau-2DL* reduced FDK by 29% on average in two of five populations and DON by 24% on average in four of five populations. *Fhb1* effects on yield were significant and positive but small in absolute value (5% increase on average in 3 populations). *Fhb1* effects on TWT were also small and ranged from a 1% reduction to a 2% increase. In four of five populations *Fhb1* was associated with increases in TWT. In four of five populations *QFhs.nau-2DL* effects on TWT were positive. In this study, the exotic resistance conferred by *Fhb1* and *QFhs.nau-2DL* was effective in reducing DON in all populations with the exception of population 3 where no significant differences were found between homozygous S and R *QFhs.nau-2DL* classes. The effects of these QTL on agronomic and quality traits appear to be small and depend on genetic background; negative effects can be balanced with adequate preservation of genetic variation and selection in the desired direction. High heritability of quality traits reported here and in other studies indicates that it is feasible to select for the desirable end-use quality while selecting for FHB resistance. One cycle of either direct or indirect simulated phenotypic selection was effective in reducing DON levels and enriching the population with *Fhb1* homozygous resistant lines.

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References

AACC International (AACC). 2010. Approved methods of the AACC, 10th ed. AACC Int., St. Paul, MN.

AACC International (AACC). 2011. Approved methods of analysis, 11th ed. AACC Int., St. Paul, MN. http://methods.aaccnet.org/toc.aspx (accessed 24 Mar. 2012).

Agostinelli, A.M., A.J. Clark, G. Brown-Guedira, and D.A. Van Sanford. 2012. Optimizing phenotypic and genotypic selection for Fusarium head blight resistance in wheat. Euphytica 186:115–126. doi:10.1007/s10681-011-0499-6

Bai, G., and G. Shaner. 1994. Scab of wheat: Prospects for control. Plant Dis. 78:760–766. doi:10.1094/PD-78-0760

Bai, G., and G. Shaner. 2004. Management and resistance in wheat and barley to Fusarium head blight. Annu. Rev. Phytopathol. 42:135–161. doi:10.1146/annurev.phyto.42.040803.140340

Balut, A.L. 2012. Validation of Fhb1 and QFhs.nau-2DL in several soft red winter wheat populations. M.S. thesis. University of Kentucky, Lexington, KY.

Bernardo, R. 2002. Breeding for quantitative traits in plants. Stemma Press, Woodbury, MN.

Beuerlein, J. 2001. Classes and uses of wheat. Ohio State University Extension Fact Sheet. Horticulture and Crop Science AGF 146-0. Ohio State University, Columbus, OH. http://ohioline.osu.edu/agf-fact/0146.html (accessed 24 Mar. 2012).

Buerstmayr, H., T. Ban, and J.A. Anderson. 2009. QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: A review. Plant Breed. 128:1–26. doi:10.1111/j.1439-0523.2008.01550.x

Buerstmayr, H., M. Lemmens, L. Hartl, L. Doldi, B. Steiner, M. Stierschneider, and P. Ruckenbauer. 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. doi:10.1007/s001220200009

Buerstmayr, H., B. Steiner, L. Hartl, M. Griesser, N. Angerer, D. Lengauer, T. Miedaner, B. Schneider, and M. Lemmens. 2003. Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theor. Appl. Genet. 107:503–508. doi:10.1007/s00122-003-1272-6

Buerstmayr, H., B. Steiner, and M. Lemmens. 2010. Current knowledge on the genetics of Fusarium head blight resistance in wheat – Implications for resistance breeding. In: S. Canty, A. Clark, A. Anderson-Scully, E. Ellis, and D. Van Sanford, editors, Proceedings of the 2010 National Fusarium Head Blight Forum, Milwaukee, WI. 7–9 December 2010. University of Kentucky, Lexington, KY. p. 132–133.

Cardwell, L.A. 2011. Scab resistance QTLs are associated with quality and agronomic traits of soft red winter wheat. Ph.D. diss. University of Maryland, College Park, MD. http://drum.lib.umd.edu//handle/1903/11481 (accessed 24 Mar. 2012).

Everts, K.L., S. Leath, and P.L. Finney. 2001. Impact of powdery mildew and leaf rust on milling and baking quality of soft red winter wheat. Plant Dis. 85:423–429. doi:10.1094/PDIS.2001.85.4.423

Kang, J., A. Clark, D. Van Sanford, C. Griffey, G. Brown-Guedira, Y. Dong, J.P. Murphy, and J. Costa. 2011. Exotic scab resistance quantitative trait loci effects on soft red winter wheat. Crop Sci. 51:924–933. doi:10.2135/cropsci2010.06.0313

Knapp, S.J., W.W. Stroup, and W.M. Ross. 1985. Exact confidence intervals for heritability on a progeny mean basis. Crop Sci. 25:192–195. doi:10.2135/cropsci1985.0011183X002500010046x

Kweon, M., L. Slade, and H. Levine. 2011. Solvent retention capacity (SRC) testing of wheat flour: Principles and value in predicting flour functionality in different wheat-based food processes in wheat breeding – A review. Cereal Chem. 88:537–552. doi:10.1094/CCHEM-07-11-0092

Lee, C., J. Herbek, D. Van Sanford, and W. Bruening. 2009. Cultural practices. In: ID 125 A comprehensive guide to wheat management in Kentucky. Cooperative Extension Service, University of Kentucky, College of Agriculture, Lexington, KY. p. 13–19.

McCartney, C.A., D.J. Somers, G. Fedak, R.M. DePauw, J. Thomas, S.L. Fox, D.G. Humphreys, O. Lukow, M.E. Savard, B.D. McCallum, J. Gilbert, and W. Cao. 2007. The evaluation of FHB resistance QTLs introgressed into elite Canadian spring wheat germplasm. Mol. Breed. 20:209–221. doi:10.1007/s11032-007-9084-z

McMullen, M., R. Jones, and D. Gallenberg. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340–1348. doi:10.1094/PDIS.1997.81.12.1340

McMullen, M., Z. Shaobin, and S. Neate. 2008. Fusarium head blight (scab) of small grains. Plant Disease Management NSDU Extension Service. PP-804 (revised). North Dakota State Univ. Extension Service, Fargo, ND. http://www.ag.ndsu.edu/pubs/plantsci/smgrains/pp804.pdf (accessed 24 Mar. 2012).

Microsoft. 2007. Microsoft Office Excel 2007. Microsoft Corp., Redmond, WA.

Mirocha, C.J., E. Kolaczkowski, W. Xie, H. Yu, and H. Jelen. 1998. Analysis of deoxynivalenol and its derivatives (batch and single kernel) using gas chromatography/mass spectrometry. J. Agric. Food Chem. 46:1414–1418. doi:10.1021/jf970857o

Peiris, K.H.S., M.O. Pumphrey, Y. Dong, E.B. Maghirang, W. Berzonsky, and F.E. Dowell. 2010. Near-infrared spectroscopy method for identification of Fusarium head blight damage and prediction of deoxynivalenol in single wheat kernels. Cereal Chem. 87:511–517. doi:10.1094/CCHEM-01-10-0006

Pumphrey, M.O., R. Bernardo, and J. Anderson. 2007. Validating the Fhb1 QTL for Fusarium head blight resistance in near-isogenic Wheat lines developed from breeding populations. Crop Sci. 47:200–206. doi:10.2135/cropsci2006.03.0206

Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy, and M. Ganal. 1998. A microsatellite map of wheat. Genetics 149:2007–2023.

Rudd, J.C., R.D. Horsley, A.L. McKendry, and E.M. Elias. 2001. Host plant resistance genes for Fusarium head blight: Sources, mechanisms and utility in conventional breeding systems. Crop Sci. 41:620–627. doi:10.2135/cropsci2001.413620x

SAS Institute. 2002. The SAS System for Windows, release 9.1. SAS Inst., Cary, NC.

Smith, N., M. Guttieri, E. Souza, J. Shoots, M. Sorrells, and C. Sneller. 2011. Identification and validation of QTL for grain quality traits in a cross of soft wheat cultivars Pioneer Brand 25R26 and Foster. Crop Sci. 51:1424–1436. doi:10.2135/cropsci2010.04.0193

Sneller, C.H., P. Paul, and M. Guttieri. 2010. Characterization of resistance to Fusarium head blight in an eastern U.S. soft red winter wheat population. Crop Sci. 50:123–133. doi:10.2135/cropsci2008.09.0569

Souza, E.J., C. Griffey, M. Kweon, and M.J. Guttieri. 2008. Sources of variation for long-flow milling. Crop Sci. 48:1432–1440. doi:10.2135/cropsci2007.10.0562

Van Sanford, D., J. Anderson, K. Campbell, J. Costa, P. Cregan, C. Griffey, P. Hayes, and R. Ward. 2001. Discovery and deployment of molecular markers linked to Fusarium head blight resistance: An integrated system for wheat and barley. Crop Sci. 41:638–644. doi:10.2135/cropsci2001.413638x

Verges, V.L., D. Van Sanford, and G. Brown-Guedira. 2006. Heritability estimates and response to selection for Fusarium head blight resistance in soft red winter wheat. Crop Sci. 46:1587–1594. doi:10.2135/cropsci2005.11-0412