Euphytica (2016) 207:571–592
DOI 10.1007/s10681-015-1550-9

Novel QTL associated with the Fusarium head blight resistance in Truman soft red winter wheat

Md. Sariful Islam · Gina Brown-Guedira · David Van Sanford · Herb Ohm · Yanhong Dong · Anne L. McKendry

Received: 19 August 2014 / Accepted: 30 August 2015 / Published online: 3 September 2015

Abstract Fusarium head blight (FHB) mainly caused by *Fusarium graminearum* Schwabe causes devastating losses in wheat globally. 'Truman' winter wheat, developed and released by the University of Missouri has excellent broad-based FHB resistance in a superior soft red winter wheat background. This research identified QTL associated with greenhouse type II resistance and field resistance for incidence, severity, Fusarium damaged kernels (FDK), and deoxynivalenol (DON) based on phenotypic data collected in Missouri, Kentucky and Indiana. Two years of replicated phenotypic data were collected on a set of 167 recombinant inbred lines. Genetic linkage maps were constructed using 160 SSR and 530 DArT polymorphic markers. Across years, QTL for type II resistance were identified on chromosomes 1BSc, 2BL, 2DS and 3BSc, for incidence on 2ASc, 2DS, and 3DS and for severity on 2DS and 3BSc. QTL were also detected for incidence on 1DLc and 2DS and for severity on 1BL, 3AL and 3BLC from data collected in Indiana and Kentucky, respectively. Common QTL for FDK on chromosomes 2ASc and 3BLc and for DON on chromosomes 2ASc and 2DS were identified from data from both Missouri and Kentucky, respectively with additional individual QTL for FDK and DON identified from tests at each independent location. All alleles were from Truman and associated with significant reductions in the respective traits. QTL on 2ASC, 2DS and 3DS may be novel and once further validated, should diversify the FHB gene pool globally and be useful for enhancing FHB resistance through marker assisted selection.

Keywords QTL · Wheat · FHB · Truman

Electronic supplementary material The online version of this article (doi:10.1007/s10681-015-1550-9) contains supplementary material, which is available to authorized users.

Md. S. Islam (✉)
Cotton Fiber Bioscience Research Unit, USDA-ARS-SRRC, 1100 Robert E Lee Blvd, New Orleans, LA 70124, USA
e-mail: md.islam@ars.usda.gov

Md. S. Islam · A. L. McKendry
Division of Plant Science, University of Missouri, 106 Curtis Hall, Columbia, MO 65211, USA

G. Brown-Guedira
Eastern Regional Small Grains Genotyping Lab, USDA-ARS, NCSU, Raleigh, NC 27695, USA

D. Van Sanford
Department of Plant and Soil Science, University of Kentucky, Lexington, KY 40546, USA

H. Ohm
Department of Agronomy, Purdue University, West Lafayette, IN 47907, USA

Y. Dong
Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA

Introduction

Fusarium graminearum Schwabe is the principal causal agent of Fusarium head blight (FHB) in wheat, causing severe losses in grain yield globally. In addition, FHB reduces grain quality through the accumulation of trichothecene mycotoxins such as deoxynivalenol (DON) (McMullen et al. 1997) which pose health risks to humans and monogastric (non-ruminant) animals (Okubara et al. 2002). Resistant cultivars are the most economical way to manage the disease, however, disease resistance is complex and incomplete, and most of the widely available sources of resistance are associated with negative traits including late maturity, excessive height and susceptibility to other diseases. Among the types of FHB resistance described by Mesterházy (1995) FHB reductions in both initial infection and disease spread in the spike are critical if a variety is to have a functional level of resistance under commercial production systems. In addition, retention of kernel quality (reduced Fusarium damaged kernels; FDK) and low DON accumulation are critical to the successful marketing and end-use of the grain. Therefore breeding for FHB resistance typically involves field and/or greenhouse selection for incidence and severity followed by evaluation of the grain for the percentage of FDK and DON.

Until 2000, most FHB resistance sources used by most breeders originated from Asia, Europe, or S. America (Rudd et al. 2001). Among sources that were most widely used was the Chinese spring wheat source, 'Sumai 3' which has excellent resistance to fungal spread (type II resistance) and good penetrance across genotypes (Bai and Shaner, 2004). Waldron et al. (1999) were the first to report QTL associated with FHB resistance in Sumai 3 identifying QTL on chromosomes 2AL, 3BS, 4BL, 5AS, and 6BS that were associated with type II resistance. The major QTL on 3BS was later designated *Fhb1* (Liu et al. 2006). This source of resistance has since been widely-studied and validated either in Sumai 3 directly or in germplasm carrying the Sumai 3 source of resistance (Bai et al. 1999; Zhou et al. 2002; Anderson et al. 2001; del Blanco et al. 2003; Buerstmayr et al. 2002, 2003; Yang et al. 2005). Several other Asian sources of resistance that differ by descent from Sumai 3 appear to carry QTL on 3BS, 5AS, and/or 6BS coincident with those in Sumai 3 including: 'Wangshuibai' (Lin et al. 2006; Zhang et al. 2004; Jia et al. 2005; Mardi et al. 2005; Ma et al. 2006; Yu et al. 2008), 'Wuhan-1' (Somers et al. 2003), 'Nyubai' (Somers et al. 2003), and 'W14' (Chen et al. 2006). Wuhan-1, however, also appeared to carry a QTL on chromosome 2DL that differs from those in other Asian sources (Somers et al. 2003).

Although both DON and FDK are recognized as critical components of resistance, there are fewer reports of QTL linked with these components of resistance in Asian germplasm (Buerstmayr et al. 2009; Liu et al. 2009). Somers et al. (2003) were among the first to report QTL on 2DS, 3BS, and 5AS associated with low DON accumulation in the Asian genotype 'Wuhan 1'. Resistance QTL on 3BS and 5AS have been confirmed in Sumai 3, its derivatives or from other Asian sources of resistance including: CM82036 (Lemmens et al. 2005), Wangshuibai (Ma et al. 2006), W14 (Chen et al. 2006) and CJ 9306 (Jiang et al. 2007b) while additional QTL on 3DL and 1AL appeared to be unique in the latter population. QTL associated with reduced FDK have been mapped in the same genomic regions. Yang et al. (2005) identified six QTL on 1DL, 2DS, 3BS, 3BSc, 4DL, and 6BS associated with reduced FDK in 'DH181' which carries Sumai 3 resistance. Of these regions the 1DL QTL appears to be unique. QTL on 3BS and 5AS were again confirmed for FDK in the Chinese landrace W14, and CJ 9306 (Chen et al. 2006; Jiang et al. 2007b) while QTL on 2A and 7D identified in Wangshuibai (Li et al. 2008) may be unique.

Among the QTL mapped in European wheat, most are in a winter wheat background. QTL for incidence, disease spread, DON and/or FDK have been mapped in 'Arina' (Paillard et al. 2004; Semagn et al. 2007; Draeger et al. 2007), 'Fundulea 201R', (Shen et al. 2003a), 'Renan' (Gervais et al. 2003), 'Remus' (Steiner et al. 2004), 'Dream', (Schmolke et al. 2005), and 'NK93604' (Semagn et al. 2007). In general, there was a lack of common QTL across these backgrounds. This lack of coincidence with the common Asian sources of resistance and those from the South American variety 'Frontana' (Steiner et al. 2004, Mardi et al. 2005) suggests these sources of resistance may be genetically different from those found in Asia and therefore, may be valuable resources for breeders wanting to pyramid genetically diverse resistance genes for FHB into wheat varieties.

Several potentially novel sources of FHB resistance have been identified in U.S. wheat varieties including 'Freedom' (Gooding et al. 1997), 'Goldfield' (Ohm et al. 2000), 'Roane', 'McCormick', 'Tribute' and 'Jamestown' (Griffey et al. 2001, 2005a, 2005b, 2010) and 'Ernie', 'Truman', and 'Bess' (McKendry et al. 1995, 2005, 2007), however, the genetics of these sources of resistance have not been widely studied or validated. Among the best studied is the FHB resistance in the cultivar Ernie developed and released from the University of Missouri (McKendry et al. 1995). Liu et al. (2007) identified two major QTL on 3BS and 5AS and two minor QTL on 2B and 4BL associated with type II resistance in Ernie. The QTL on 3BS, which is proximal to the centromere, differs from the distal 3BS QTL in Sumai 3 as does the 5AS QTL. More recently, Liu et al. (2013) reported three additional QTL on 4BS, 4DS, and 5AL associated with field incidence and severity in Ernie. A forth QTL on 2DS was associated with field severity but not with incidence. QTL on 4BS and 4DS were at or near height alleles *Rht-B1* and *Rht-D1* while the QTL on 2DS was near the photoperiod insensitivity allele *Ppd-D1a*. Abate et al. (2008) reported three QTL on chromosomes 3BSc, 4BL, and 5AS that were associated with lower DON and four QTL on 2B, 3BSc, 4BL and 5AS associated with reduced FDK in Ernie. All were coincidental with those identified by Liu et al. (2007). Bonin and Kolb (2009) also reported a 4B QTL associated with low DON in the Illinois experimental line 'IL 94-1653' and three QTL on 2B, 4B, and 6B that were associated with low FDK. Finally, Liu et al. 2012 reported major QTL on 2DL, 6A, and 5B that reduced the FHB index, FDK, and DON, respectively in the Virginia Polytechnic Institute and State University (Virginia Tech) variety 'VA00W-38'. Combining US native resistance alleles with those that have been well studied and validated from Asia may lead to enhanced levels of resistance in U.S. wheat and should diversify the FHB resistance gene pool globally. The identification of additional novel sources of resistance and QTL with major effects will facilitate that objective.

Truman soft red winter wheat, developed and released by the University of Missouri Agricultural Experiment Station in 2003 (McKendry et al. 2005), couples excellent broad-based FHB resistance with agronomic performance, broad adaptation, and grain quality necessary for production in soft red winter wheat regions. It serves as a resistant check variety in both the preliminary and advanced U.S. Northern Uniform Winter Wheat Scab Nurseries while its earlier maturing full sib Bess (McKendry et al. 2007) serves as the resistant check in the U.S. Southern Uniform FHB Nursery. The source of FHB resistance in Truman is highly penetrant (Abate and McKendry, 2010) and presumed to be novel because it carries none of the known FHB QTL alleles based on haplotype marker analysis (Brown-Guedira 2009—personal communication). This research was designed to identify QTL associated with FHB incidence, severity, FHBI (incidence × severity), FDK, and DON in Truman determined through both field and greenhouse analyses.

Materials and methods

Plant materials

A set of recombinant inbred lines (RILs) developed at the University of Missouri from the cross, Truman × MO 94-317, was used for this study. Truman originated from the cross, MO 11769 × 'Madison', made in 1990 where MO 11769 was from the cross, 'Kawvale'/'Vigo'//'Directeur Journee'/3/W7510/4/'NS 314'/'Stoddard'. W7510 is a full sib of 'Hart'. MO 94-317 is a highly inbred, FHB susceptible Missouri breeding line developed from the cross 'AgriPro Traveler'/'Pioneer Variety 2555'. A single seed was taken from each of 1200 random F_2 plants and advanced by single seed descent to the F_6. Phenotypic evaluations were conducted for type II resistance on a random set of 167 RILs in the F_8 and F_9 while phenotypic data for components of field resistance were conducted on RILs in the F_9 and F_{10}.

Disease evaluations

Components of FHB resistance were assessed over 2 years in replicated experiments conducted in the greenhouse (greenhouse severity) and field environments (field severity, incidence, FDK and DON content). For clarity, greenhouse severity will be referred to as type II resistance while severity estimated from field phenotyping will be referred to as field severity throughout the manuscript.

Type II resistance was phenotyped at Missouri in 2008 (3 replications) and 2009 (2 replications). In each year, lines were arranged as a randomized complete block design with eight plants per RIL per replication. At first anthesis of the main tiller, a single, central, basal, floret of each plant was point-inoculated with 10 μL of a macroconidial suspension of *F. graminearum* concentrated to 50,000 macroconidia/mL using an Oxford 8100 repeat dispensing syringe. A single isolate of *F. graminearum* from scabby kernels collected at Mt Vernon, Missouri was used for both greenhouse and field inoculation at Missouri. The isolate was previously tested for aggressivity and was used in this study because it caused higher levels of disease on our most resistant variety (Truman) than other isolates tested. Following inoculation, plants were incubated in a mist chamber at 100 % relative humidity for 72 h post-inoculation and then returned to the greenhouse bench at 25 °C under a 16-h photoperiod to promote disease development. Ratings were taken 21 days after inoculation. Data were recorded for flowering date, number of spikelets per spike, and number of diseased spikelets on the inoculated head. Type II resistance was determined as the percentage of diseased spikelets/total spikelets on the inoculated head.

Field phenotyping was conducted in 2009 and 2010 at the Bradford Research and Extension Center near Columbia, Missouri, and in collaboration with the University of Kentucky and Purdue University in Indiana. RILs were planted as single 3-month rows arranged as a randomized complete block design with two replications in each location year. At all locations, RILs were planted into corn residue and maintained under overhead mist irrigation. At Missouri, RILs were sprayed at 75 % heading of each individual RIL with a macroconidial suspension of *F. graminearum* concentrated to 70,000 macroconidia/mL. At Kentucky, disease levels were promoted through the use of infected corn grain-spawn produced from a mixture of Kentucky isolates applied to the nursery 3 weeks prior to first heading while at Purdue, natural infection from prevalent isolates was promoted by overhead mist irrigation of head rows planted into corn residue. Incidence was assessed at Missouri and Purdue on a random sample of 10 heads as the percentage of those heads showing FHB symptoms. At Missouri, it was assessed 10 days after inoculation while at Purdue it was assessed 21 days post-heading. Severity was assessed at Missouri and Kentucky 18–21 days after inoculation as the mean percentage of diseased spikelets on a 10-head sample. The FHB index (FHBI) was calculated at Missouri for each RIL as incidence × severity expressed as percentage. At Missouri, rows were harvested with a rice knife and threshed by hand to prevent loss of FDK, including kernels that were either shriveled or were tombstones. The percentage of FDK was visually estimated based on a standard set of samples. At Kentucky, hand-harvested and cleaned seed from each row were separated into healthy seed and FDK using air separation as described by Agostinelli et al. (2012). FDK were determined using the formula FDK $(\%) = [W_{SS}/(W_{SS} + W_{AS})] \times 100$; where W_{SS}, was the weight of scabby seed (g); and W_{AS}, was the weight of asymptomatic seed (g). Fifteen g of seed of each inoculated RIL from both locations were then sent to the Diagnostic Services Laboratory at the University of Minnesota where they were assessed for DON content using gas chromatography as described by Jones and Mirocha (1999).

DNA extraction and marker analysis

Leaf tissue from tillers of each RIL and the two parents was collected when plants reached the erect stem stage at Zadoks 30 (Zadoks et al. 1974). DNA was extracted using the CTAB method (Keim et al. 1988) and diluted to 25 ng/μL after quantification using a nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE 19810). Genome screening was conducted using SSR and DArT markers.

SSR assay

A whole genome polymorphism survey of the parents was done using a total of 885 SSR markers comprised of 191 *Xbarc*, 36 *Xcfa*, 75 *Xcfd*, 30 *Xgdm*, 238 *Xgwm* and 315 *Xwmc* markers (Somers et al. 2004; Röder et al. 1998; Song et al. 2005). Polymorphic SSR primers were then used to genotype the 167 RILs and two parents at the University of Missouri. The PCR reaction mix contained 50 ng of each SSR primer, 50 ng of genomic DNA, 10 μL of Jumpstart Ready Mix RedTaq™ PCR reaction mix (Sigma-Aldrich, St. Louis, MO) plus sterile distilled water to a volume of 20 μL per reaction. PCR conditions were as follows: 95 °C for 5 min; 95 °C for 45 s, 65 °C for 45 s and

72 °C for 1 min for first 15 cycles followed by a 1 °C decrease in the annealing temperature per cycle. Conditions thereafter were 95 °C for 45 s, 50 °C for 45 s, and 72 °C for 1 min, repeated for 25 cycles. Amplified products were separated by electrophoresis on 4.5 % super fine resolution agarose gel (Amresco, Solon, OH) comprised of 1× TBE buffer and 10 µL ethidium bromide (10 mg/mL), then run at 120 V.

DArT assay

DNA samples of each RIL and parents were sent to Diversity Arrays Technology Pty Ltd (University of Canberra, Kirinari Street, Bruce, ACT2617, Australia) for DArT analysis as described by Semagn et al. (2006). The locus designations used by Diversity Arrays Technology Pty. Ltd. were adopted in this paper and consisted of markers with the prefix '*wPt*' '*tPt*' and '*rPt*', followed by numbers corresponding to a particular clone in the genomic representation, where the prefixes w, t, and r were for wheat, triticale (×*Triticosecale Wittm.*) and rye (*Secale cereale*), respectively; P for *Pst*I (primary restriction enzyme used) and t for *Taq*I (secondary restriction enzyme).

Map construction

Both SSR and DArT marker data were used to construct linkage groups using JoinMap 2.0 (Van Ooijen and Voorrips 2001). Default parameters were LOD grouping thresholds ≥3.0 and a maximum distance of 50 cM. The Kosambi mapping function was used to determine the distance between markers (Kosambi 1944). Assignment of linkage groups was based on the wheat consensus map (Somers et al. 2004). All markers mapped to linkage groups were evaluated individually by the χ^2 test for goodness-of-fit against a 1:1 segregation ratio at a 0.01 probability level. SSR markers were used to anchor DArT markers. A linkage group was assigned to a chromosome when it contained at least two SSR loci that had been allocated to a particular chromosome according to the wheat consensus map (Somers et al. 2004). The order of markers on each chromosome was determined using the fixed order of SSR loci from Somers et al. (2004) using the following parameters: recombination threshold = 0.45; minimum LOD score for calculating map distance = 0.10; ripple value = 1; jump threshold = 5; and the Kosambi mapping function.

QTL analysis

Composite interval mapping (CIM) was performed to localize and detect resistance QTL using Windows QTL cartographer (WinQTLCart) version 2.5 (Wang et al. 2006). For CIM, forward and backward stepwise regressions were performed to select markers as cofactors with 10 cM window size and 1 cM walking speed. At each interval, the significance of the QTL-trait association was tested by likelihood ratio statistics. For each trait, a critical LOD threshold level was estimated by 1000 permutations at $p < 0.05$ using WinQTLCart 2.5. Significant LOD scores ranged from 2.9 to 3.5. The average LOD score for all traits (3.2) was used as a threshold to declare the significance of a putative QTL. QTL identified from CIM were further tested and additional QTL identified using Multiple Interval Mapping (MIM). The Bayesian Information Criteria default Model [BIC-M0 → c (n) = ln(n)] was used to identify significant QTL. MIM was also used to estimate the main additive effects of QTL and epistatic effects among QTL. R^2 values, representing the percentage of the phenotypic variance explained by the QTL, were determined from the MIM genetic models. Putative QTL and chromosome figures were created using Mapchart 2.2 (Voorrips 2002). Phenotypic effects of combinations of QTL alleles were estimated using closely linked markers. RILs having the same QTL were grouped. Phenotypic data were averaged across RILs within each group. Reductions in components of FHB resistance due to QTL allele combinations were estimated based on comparisons with disease levels in RILs carrying no known Truman alleles. QTL name and designation within the chromosome was provided on the basis consensus map of Somers et al. (2004) where 'A, B, and D' are genome names, 'S' is on short arm, 'L' is on long arm and 'c' is adjacent to centromere.

Statistical analysis

Tests of normality were conducted prior to analyses of variance (ANOVA) using PROC UNIVARIATE NORMAL PLOT (SAS Institute 2011). The log transformation was used to normalize type II resistance and DON data. Homogeneity of error variances were tested using Bartlett's test to determine whether the data could be combined across years. ANOVA were conducted on data using PROC GLM (SAS

Institute 2011). RILs were considered fixed while years, replications, and the RIL × year interactions were considered random effects. The RIL × year interaction was used to test significance of the variation due to year and RILs while the RIL × year interaction was tested for significance at 5 % level of probability against experimental error. Broad sense heritability estimates (H_{BS}) were determined from the ANOVA, on an entry mean basis as $H_{BS} = \sigma_g^2 / \left(\sigma_g^2 + \sigma_{gy/Y}^2 + \sigma_{e/RY}^2\right)$, where σ_g^2, σ_{gy}^2, and σ_e^2 reflect variance associated with genotype, genotype × year interaction, and error, respectively, and R and Y indicate the number of replications and years, respectively. Ninety-five percent confidence limits for the estimated heritabilities were determined following the procedure of Knapp et al. (1985). Minimum gene number was estimated following Cockerham's (1983) modification of Wright's (1968) formula.

Results

Analysis of phenotypic data

Parents Truman, and MO 94-317 differed significantly for all components of resistance assessed in all individual and combined greenhouse and field environments in each year of this study. Missouri data for all components are given in Table 1. For each of the components of resistance evaluated, phenotypic data for RILs were continuously distributed (Figs. S1). Tests for normality indicated that field severity and FHBI were normally distributed. Deviations from normality for incidence and FDK were minor and not expected to impact the QTL analysis because of the use of cofactor markers in CIM (Jansen and Stam 1994) and permutation testing for the determination of threshold LOD values for significance (Churchill and Doerge 1994; Yang et al. 2007). However, data for type II resistance and DON content were not normally distributed and therefore, were transformed using a log(x) transformation where x represented either the percentage of greenhouse severity or DON concentration (μg/g), respectively. These transformations were necessary in order to avoid over-estimation of the error variance for both ANOVA and QTL analyses (Miedaner et al. 2006; Semagn et al. 2007).

Analyses of variance (ANOVA) for data collected in all environments indicated significant genotypic effects for all components of resistance in both individual years and for data combined over years (Tables 2, 3). Year effects were significant for combined data for each trait under field conditions due to variation in temperature and humidity over years, both of which can impact disease development. RIL × year interactions under field conditions were also significant suggesting that there were differences in the response of some genotypes to environment over the 2 years of these experiments.

Broad sense heritabilities estimated from ANOVA from combined Missouri data ranged from 75 % for type II resistance to 62, 77, and 75 % for field incidence, severity and FHBI, respectively (Table 1). Estimates of gene numbers conditioning these traits ranged from 4.2 for type II resistance to 3.1, 3.9, and 4.1, for incidence, severity, and FHBI, respectively (Table 1). For seed traits heritability was also high averaging 80 % for FDK and 74 % for DON while gene numbers ranged from 3.5 to 4.4 for combined data for these traits, respectively. Correlation coefficients among traits for data combined over years varied (Table S1). Type II resistance was significantly correlated with field severity at $p < 0.001$ (0.43), but was not correlated with field incidence (0.22). Among field traits, incidence and severity were also highly correlated at $p < 0.001$ (0.71). FHBI, determined as the product of incidence and severity, integrates across these two parameters, and as expected was highly correlated at $p < 0.001$ with both incidence (0.85) and severity (0.96). Significant correlations at $p < 0.001$ were also detected for FDK and DON content at both the Missouri (0.47) and Kentucky (0.39) locations. Both seed traits were also correlated with incidence at $p < 0.01$ (FDK, 0.27; DON 0.49) and severity at $p < 0.001$ (FDK, 0.49; DON 0.71) in this RIL set at Missouri.

Genetic linkage map construction

SSR polymorphism in this population was low (20.8 %) due to the background genetic similarity that results when the parents used to develop mapping populations are derived from the same breeding program. Therefore 530 out of 647 (81.9 %) good DArT markers were used to more fully saturate the genome, although there were more polymorphic DArT

Table 1 Means, broad sense heritabilities (H_{BS}), and gene numbers for components of Fusarium head blight resistance of parents and 167 recombinant inbred lines (RILs) of the soft red winter wheat cross Truman × MO 94-317. Data are from replicated inoculated experiments conducted at Missouri in 2008, 2009 and 2010 from greenhouse (type II) or field (severity, incidence, FHBI, FDK and DON) environments

Trait[a]	Year	Truman (%)	MO 94-317 (%)	RIL mean ± SE (%)	H_{BS}^{b}	95 % CI[c]	No. of genes[d]
Type II	2008	5.4 ± 0.8	33.7 ± 9.1	17.5 ± 0.64	0.70	0.76–0.63	3.9
	2009	5.2 ± 0.5	33.1 ± 9.8	13.1 ± 0.52	0.60	0.69–0.48	3.4
	Combined	5.3 ± 0.5	33.9 ± 5.9	15.3 ± 0.52	0.75	0.81–0.68	4.2
Incidence	2009	65.0 ± 5.0	85.0 ± 5.0	71.6 ± 1.4	0.78	0.83–0.72	3.9
	2010	43.0 ± 12.5	88.0 ± 12.5	59.8 ± 1.3	0.67	0.74–0.57	3.4
	Combined	57.5 ± 9.5	82.5 ± 8.5	65.7 ± 1.2	0.62	0.71–0.51	3.1
Severity	2009	27.0 ± 2.9	80.0 ± 7.4	49.3 ± 1.5	0.85	0.88–0.80	3.5
	2010	18.0 ± 8.0	70.0 ± 2.2	39.1 ± 1.3	0.74	0.80–0.66	3.6
	Combined	23.0 ± 4.3	75.0 ± 4.3	44.2 ± 1.3	0.77	0.83–0.71	3.9
FHBI	2009	17.5 ± 0.5	68.3 ± 10.3	38.0 ± 1.6	0.86	0.89–0.82	3.6
	2010	10.7 ± 7.6	56.5 ± 15.8	25.1 ± 1.2	0.77	0.83–0.71	3.8
	Combined	14.1 ± 3.7	62.4 ± 8.4	31.6 ± 1.3	0.75	0.80–0.67	4.1
FDK	2009	43.3 ± 6.7	85.0 ± 1.7	64.0 ± 1.7	0.91	0.93–0.88	3.4
	2010	24.3 ± 14.7	94.2 ± 0.2	61.9 ± 1.6	0.84	0.87–0.79	4.0
	Combined	33.8 ± 8.6	89.6 ± 2.7	63.0 ± 1.5	0.80	0.85–0.75	3.5
DON	2009	10.8 ± 5.1	48.8 ± 18.4	19.8 ± 0.7	0.78	0.83–0.71	4.8
	2010	3.1 ± 0.5	12.4 ± 0.2	7.9 ± 0.4	0.66	0.74–0.56	4.6
	Combined	6.9 ± 3.0	30.6 ± 12.9	13.9 ± 0.5	0.74	0.80–0.66	4.4

[a] Type II resistance was determined following point-inoculation with 10 µL of *Fusarium graminearum* macroconidia concentrated to 50,000 spore/mL and rated 21 days post-inoculation for the percentage of diseased spikelets in the inoculated head. Incidence determined as the percentage of heads in a 10-head sample showing disease symptoms following spray inoculation with *Fusarium graminearum* macroconidia concentrated to 70,000 spore/mL and rated, 10-days post-inoculation; severity determined as mean percentage of diseased spikelets on the 10-head sample used to determine incidence and rated 18–21 days post-inoculation; FHBI determined as incidence × severity expressed as a percentage; FDK determined from a hand-threshed grain sample from inoculated parents or RILs assessed visually based on standard set of samples. DON content in µg/g from a 15 g sample of each inoculated parent or RIL determined at the Univ. of Minnesota using gas chromatography as described by Jones and Mirocha (1999)

[b] H_{BS} was calculated on an entry mean basis from the appropriate ANOVA

[c] 95 % confidence intervals (CI) for the heritabilities were estimated following Knapp et al. (1985)

[d] Gene numbers were calculated based on Wright (1968) as modified by Cockerham (1983)

markers. Ultimately, 160 SSRs, two known genes (*Ppd-B1* and *Ppd-D1*) and 530 DArTs were positioned on the 22 linkage groups, which spanned 2678.42 cM across 20 of the 21 chromosomes (Fig. S2). No markers were detected for chromosome 4D. Marker density varied from 2.4 cM/locus on chromosome 1A to 8.3 cM/locus on 5D, with an average distance of 3.7 cM between loci. Chromosomes 2D and 6A had the largest number of markers (63 each) while 1D and 4B (14 loci each) were the least saturated. Loci were more or less uniformly distributed along the chromosomes except for chromosomes 1A, 6D, 3A, and 4A which had distances between marker loci as large as 45.6, 41.1, 40.8, and 38.4 cM, respectively.. The final linkage map comprised 104 % of the wheat consensus map (Somers et al. 2004); 95 % of the wheat genetic map reported by Song et al. (2005); and 103 % of the wheat genetic map constructed using SSR, DArT and AFLP markers (Semagn et al. 2006).

QTL analyses for type II resistance

Four QTL associated with type II resistance were identified from combined data at Missouri (Table 4; Fig S3). QTL peaks were located near the centromere of the short arm of chromosomes 1B (1BSc) and 3B

Table 2 Analyses of variance for type II resistance, incidence, severity and the Fusarium head blight index (FHBI) of 167 recombinant inbred lines (RILs) of the soft red winter wheat cross Truman × MO 94-317 following inoculation with *Fusarium graminearum*. Experiments were conducted in greenhouse and field environments at Missouri in 2008, 2009 and 2010

Year	Source of variation	Mean square							
		df	Type II[b]	df	Incidence[c]	df	Severity[c]	df	FHBI[c]
2008 or 2009[a]	Rep	2	6.152***	1	1042.2**	1	21.5	1	303.1
	RILs	166	0.598***	166	686.8***	166	745.8***	166	878.9***
	Error	332	0.177	166	149.4	166	114.6	166	123.7
2009 or 2010[a]	Rep	1	1.376*	1	385.2	1	1627.7***	1	109.3
	RILs	166	0.437***	166	579.2***	166	552.4***	163	493.3***
	Error	165	0.174	158	193.5	159	143.4	153	111.6
Combined	Rep (year)	3	4.560***	2	694.9*	2	803.8**	2	206.2
	Years	1	15.550	1	23156.8*	1	16890.3*	1	27386.2***
	RILs	166	0.802***	166	915.5***	166	1059.3***	166	1096.6***
	RILS × year	166	0.201	166	348.1***	166	238.9***	166	277.2***
	Error	497	0.176	324	170.9	325	128.7	320	117.8

***, **, * Indicates significance at $p = 0.001$, $p = 0.01$, $p = 0.05$, respectively

[a] Type II determined in the greenhouse in 2008 and 2009 and combined over years; incidence, severity, and FHBI determined in the field in 2009, 2010 and combined over years

[b] Type II resistance following point-inoculation with 10 μL of *Fusarium graminearum* macroconidia concentrated to 50,000 spore/mL and rated 21 days post-inoculation for the percentage of diseased spikelets in the inoculated head

[c] Incidence determined as the percentage of heads in a 10-head sample showing disease symptoms following spray inoculation with *Fusarium graminearum* macroconidia concentrated to 70,000 spore/mL in an overhead misted nursery and rated, 10-days post-inoculation; severity determined as mean percentage of diseased spikelets on the 10-head sample used to determine incidence and rated 18–21 days post-inoculation; FHBI determined as incidence × severity expressed as a percentage

(3BSc) and on chromosome arms 2BL and 2DS. These QTL accounted for 10.9, 7.3, 16.1, and 19.9 % of the phenotypic variation for type II resistance, respectively. All QTL originated from the resistant parent, Truman, with additive effects ranging from −0.11 to −0.27 %. The QTL on 1BSc which accounted for 10.9 % of the variation in type II resistance was linked at the QTL peak with the SSR *Xwmc269*. The 2BL QTL which accounted for 16.1 % of the variation was linked with *Xwmc592* which was also at the QTL peak; however, flanking DArTs *wPt8548–wPt8916* spanned 18.9 cM. The QTL on 2DS was linked with the DArT marker *wPt666223* while *Ppd-D1* was 4.0 cM away from the QTL peak and fell inside the QTL region. This QTL accounted for 19.9 % of the variation, however, the length of the QTL region was large (43.8 cM) probably due to the lower marker saturation on chromosome 2D (Fig S3). Finally, a minor QTL near the centromere on 3BSc linked to *Xwmc615* and flanked by *Xgwm285–Xwmc625* spanned 11.4 cM.

QTL analyses for field incidence, severity, and the Fusarium head blight index

Error variances for data collected over years among different locations were heterogeneous but were homogeneous across years within each location. Therefore data were combined within locations but not across locations. Combined phenotypic data from Missouri, where complete field data for all traits were collected, were used for initial QTL analyses for incidence, severity, and FHBI.

Three QTL were identified on the short arms of chromosome 2AS, 2DS and 3DS that were associated with incidence (Table 4; Fig S4). For all QTL alleles from the resistant parent Truman were associated with decreased incidence. The QTL on chromosome arm 2ASc linked with the DArT marker *wPt8826* contributed 6.7 % of total phenotypic variation. The nearest SSR marker, *Xgwm095*, fell inside the QTL region, only 0.2 cM from the QTL peak. The 2ASc QTL was also

Table 3 Analyses of variance for Fusarium damaged kernels (FDK), and deoxynivalenol (DON) data from 167 recombinant inbred lines (RILs) of the soft red winter wheat cross Truman × MO 94-317 following spray inoculation with *Fusarium graminearum* macroconidia concentrated to 70,000 spore/mL in an overhead misted nursery. Experiments were conducted in the field at Missouri (MO) and Kentucky (KY) in 2009 and 2010

Year	Source of variation	FDK[a] (MO)		FDK[b] (KY)		Log DON[c] (MO)		Log DON[c] (KY)	
		df	Mean sq.	df	Mean sq.	df	Mean sq.	df	Mean sq.
2009	Rep	1	1121.5***	1	386.4*	1	2.35***	1	0.11**
	RILs	166	905.4***	165	192.1***	166	0.42***	164	0.32***
	Error	163	84.3	147	62.9	162	0.09	156	0.01
2010	Rep	1	4995.9***	1	105.5	1	2.20***	1	0.43**
	RILs	166	774.0***	163	250.1***	166	0.47***	163	0.11***
	Error	160	126.9	153	68.4	150	0.16	153	0.04
Combined	Rep (year)	2	3433.4***	2	246.0*	2	2.28***	2	0.27***
	Years	1	350.1	1	8737.1*	1	146.56*	1	46.50***
	RILs	166	1403.6***	166	345.5***	166	0.71***	166	0.29***
	RILs × year	166	274.4***	162	84.4*	166	0.19**	161	0.13***
	Error	323	105.4	300	65.7	312	0.13	309	0.03

***, **, * Significant at $p = 0.001$, $p = 0.01$, $p = 0.05$, respectively

[a] FDK determined from a hand-threshed grain sample from inoculated RILs assessed visually as the percentage of shriveled seed and tombstones based on standard set of samples

[b] Hand-harvested and cleaned seed from each row was separated into healthy seed and FDK using air separation as described by Agostinelli et al. (2012). FDK was determined from the formula FDK (%) = $(W_{SS}/(W_{SS} + W_{AS})) \times 100$; where W_{SS}, was the weight scabby seed (g); and W_{AS}, was the weight asymptomatic seed (g)

[c] Log(x) transformation of DON content in µg/g from a 15 g sample of inoculated RILs determined at the Univ. of Minnesota using gas chromatography as described by Jones and Mirocha (1999)

associated with FHBI, accounting for 8.2 % of the variability in this trait. The 2DS QTL was linked with the DArT marker *wPt666223* and accounted for 22.9 % of the phenotypic variance for incidence. The nearest marker, *Ppd-D1*, was 4.0 cM away from the QTL peak and fell inside the QTL region. The 2DS QTL had an equally large effect on both disease severity and FHBI, accounting for 23.0 and 25.3 % of the variability in those two traits, respectively. As previously noted, it was also associated with 19.9 % of the phenotypic variation in type II resistance. The QTL on 3DS appeared to be uniquely associated with reduced incidence. It was linked with the DArT marker *wPt5390* and accounted for 10.0 % of the phenotypic variation in this trait. The closest SSR marker, *Xcfd055*, was located 1.0 cM from the QTL peak. A flanking SSR marker, *Xwmc674,* was 1.6 cM distal from *Xcfd055*. Finally, a fourth QTL adjacent to the centromere on 3BS linked with the SSR marker *Xgwm285* was associated with both disease severity and FHBI but not with incidence. It explained 10.2 and 8.2 % of the variation in severity and FHBI, respectively. FHBI, determined as incidence × severity, integrates across these two traits and not unexpectedly, three common QTL on 2ASc, 2DS and 3BSc were associated with incidence, and/or severity and FHBI.

Incidence data on this set of RILs were also collected at Purdue University in Indiana and QTL analysis of that dataset confirmed the magnitude of the 2DS QTL where it accounted for 24.3 % of the variation in incidence. A second QTL on 1DLc linked with *XBarc229* that explained 14.2 % of the variation was unique to incidence data collected at Purdue. Both QTL were from Truman and were associated with decreased incidence. Finally, analysis of disease severity data collected at the University of Kentucky resulted in the identification of three minor QTL on chromosomes 1BL, 3AL and 3BLc. None co-localized with those detected from Missouri data.

QTL analysis for Fusarium damaged kernels (FDK) and DON

As was the case for error variances for incidence, severity and FHBI, error variances for data collected over years among different locations for FDK and DON were again heterogeneous but were

Table 4 Quantitative trait loci (QTL) associated with components of FHB resistance of recombinant inbred lines derived from the cross Truman × MO 94-317. Data used for analyses were combined from replicated experiments conducted over years of greenhouse (2008, 2009) or field (2009, 2010) evaluation at Missouri

Trait	Chromosome location	Position (cM)[c]	LOD	A[d]	R[e]	Marker[f]	Linked SSR[g]	Distance (cM)[h]
Type II[a]	1BSc	18.5	5.6	−0.1	10.9	*Xwmc269*	*Xwmc269*	0.0
	2BL	103.7	7.2	−0.1	16.1	*Xwmc592*	*Xwmc592*	0.0
	2DS	33.4	10.4	−0.2	19.9	*wPt666223*	*Ppd-D1*	3.0
	3BSc	59.4	3.5	−0.1	7.3	*Xwmc615*	*Xwmc615*	0.0
Incidence[b]	2ASc	33.3	4.3	−4.1	6.7	*wPt8826*	*Xgwm095*	0.2
	2DS	32.4	9.7	−7.3	22.9	*wPt666223*	*Ppd-D1*	4.0
	3DS	16.4	3.2	−4.6	10.0	*wPt5390*	*Xcfd055*	1.0
Severity[b]	2DS	34.1	10.2	−7.8	23.0	*wPt666223*	*Ppd-D1*	2.3
	3BSc	53.9	5.7	−5.1	10.2	*Xgwm285*	*Xgwm285*	0.0
FHBI[b]	2ASc	33.3	4.3	−3.5	8.2	*wPt8826*	*Xgwm095*	0.2
	2DS	33.4	12.2	−8.1	25.3	*wPt666223*	*Ppd-D1*	3.0
	3BSc	53.9	4.3	−4.2	8.2	*Xgwm285*	*Xgwm285*	0.0

[a] Type II resistance following point-inoculation in the greenhouse with 10 μL of *Fusarium graminearum* macroconidia concentrated to 50,000 spore/mL and rated 21 days post-inoculation for the percentage of diseased spikelets in the inoculated head. Data were not normally distributed and therefore were transformed with the Log(x) transformation prior to QTL analysis

[b] Incidence determined as the percentage of heads in a 10-head sample showing disease symptoms following spray inoculation in the field with *Fusarium graminearum* macroconidia concentrated to 70,000 spore/mL in an overhead misted nursery and rated, 10-days post-inoculation; severity determined as mean percentage of diseased spikelets on the 10-head sample used to determine incidence and rated 18–21 days post-inoculation; FHBI determined as incidence × severity expressed as a percentage

[c] The location of the QTL peak

[d] Additive effect of the marker linked to the QTL peak

[e] Proportion of total phenotypic variation explained by significant QTL based on multiple interval mapping (MIM) estimation

[f] Marker linked to the QTL peak. Markers beginning with '*X*' are single sequence repeat markers (SSRs) while those beginning with '*wpt*' are Diversity Array Technology markers (DArTs)

[g] Nearest SSR marker from the QTL peak

[h] Distance of SSR marker or *Ppd-D1* from the respective QTL peak

homogeneous across years within each location. Therefore data were combined within locations but not across locations. Based on phenotypic data combined across years within the Missouri and Kentucky locations, two common QTL on 2ASc and 3BLc were identified that were associated with FDK (Table 5; Fig. S5). A third QTL on 2DS was detected from Missouri data while Kentucky data revealed a third QTL on 1BLc. All QTL were from the resistant parent Truman and were associated with reduced FDK.

The 2ASc QTL linked with the DArT marker *wPt8826* accounted for 8.5 and 10.0 % of the variation in FDK based on Missouri and Kentucky data, respectively. The closest SSR was *Xgwm095*, located 1.1 cM from the QTL peak at both locations. This QTL was also significant for incidence and FHBI. The 3BLc QTL linked with the DArT marker *wPt9433* accounted for 9.6 and 5.0 % of the variation in FDK in Missouri and Kentucky, respectively. The closest single SSR marker was *XBarc164*, located 1.5 and 2.5 cM from the QTL peak at Missouri and Kentucky, respectively.

The 2DS QTL detected from Missouri data accounted for 7.5 % of the variation in FDK, was linked at the QTL peak with *Xgwm102*, however, it was not detected from the analysis of Kentucky data. Data from Kentucky did however, reveal a QTL on 1BLc that was not evident from the analysis of Missouri data. This QTL, linked with *Xwmc694*, accounted for 7.4 % of the phenotypic variation in FDK.

Table 5 Quantitative trait loci (QTL) associated with Fusarium damaged kernels (FDK), and deoxynivalenol (DON) of recombinant inbred lines developed from the soft red winter wheat cross Truman × MO 94-317. Data used for analyses were combined from replicated field experiments conducted at Missouri (MO) and Kentucky (KY) in 2009 and 2010

Trait[a]/location	Chromosome location	Position (cM)[b]	LOD	A[c]	R^{2d}	Marker[e]	Linked SSR[f]	Distance (cM)[g]
FDK (MO)	2ASc	32.4	4.5	−5.7	8.5	*wPt8826*	*Xgwm095*	1.1
	2DS	77.7	3.3	−5.2	7.5	*Xgwm102*	*Xgwm102*	0.0
	3BLc	74.9	4.6	−5.8	9.6	*wPt9433*	*XBarc164*	1.5
FDK (KY)	1BLc	21.8	3.7	−3.0	7.4	*Xwmc694*	*Xwmc694*	0.0
	2ASc	32.4	6.9	−3.4	10.0	*wPt8826*	*Xgwm095*	1.1
	3BLc	73.9	3.4	−2.4	5.0	*wPt9433*	*XBarc164*	2.5
DON (MO)	2ASc	32.4	6.4	−0.1	10.0	*wPt8826*	*Xgwm095*	1.1
	2DS	34.4	15.4	−0.3	30.7	*wPt666223*	*Ppd-D1*	2.0
	3BSc	60.9	6.2	−0.1	10.3	*Xwmc615*	*Xwmc615*	0.0
DON (KY)	2ASc	33.3	7.4	−0.1	12.3	*wPt8826*	*Xgwm095*	1.1
	2DS	32.4	9.4	−0.1	20.1	*wPt666223*	*Ppd-D1*	4.0
	6ALc	86.5	3.5	−0.1	6.7	*XBarc146*	*XBarc146*	0.0

[a] FDK at MO was determined from a hand-threshed grain sample from inoculated RILs assessed visually based on standard set of samples. At KY, hand-harvested and cleaned seed from each row was separated into healthy seed and FDK using air separation as described by Agostinelli et al. (2012). FDK was determined from the formula FDK (%) = $(W_{SS}/(W_{SS} + W_{AS})) \times 100$; where W_{SS}, was the weight scabby seed (g); and W_{AS}, was the weight of asymptomatic seed (g). DON content in μg/g from a 15 g sample of each inoculated parent or RIL determined at the Univ. of Minnesota using gas chromatography as described by Jones and Mirocha (1999). DON data were not normally distributed and therefore were transformed with the Log(x) transformation prior to QTL analysis

[b] The location of the QTL peak

[c] Additive effect of the marker linked to the QTL peak

[d] Proportion of total phenotypic variation explained by significant QTL based on multiple interval mapping (MIM) estimation

[e] Marker linked to the QTL peak. Markers beginning with '*X*' are single sequence repeats (SSRs) while those beginning with '*wpt*' are the DArTs

[f] Nearest SSR marker from the QTL peak

[g] Distance of SSR marker from the respective QTL peak

Two common QTL on 2ASc and 2DS were also identified for low DON across both screening environments (Table 5; Fig. S6). For both alleles from Truman were associated with reduced DON content in the grain. The 2ASc QTL linked with the DArT marker *wPt8826* explained 10.0 and 12.3 % of total phenotypic variation in DON, based on Missouri and Kentucky phenotypic data, respectively. The nearest SSR marker, *Xwmc095*, fell inside the QTL region that was located 1.1 cM from the QTL peak. This QTL was also significant for three other components of resistance including: incidence, FDK and FHBI. The QTL on 2DS was also common across locations. Analyses indicated that it was linked with *wPt666223*, and accounted for 30.7 and 20.1 % of the variation in DON at Missouri and Kentucky, respectively. Over years, the nearest marker was *Ppd-D1* which was within the QTL region, ranging from 2.0 to 4.0 cM away from the QTL peak.

Two additional QTL, one on 3BSc based on Missouri data and one on 6ALc based on Kentucky data were also identified. Again, alleles from Truman were associated with low DON. The 3BSc QTL, linked with *Xwmc615*, accounted for 10.3 % of the variation in DON and appears to coincide with a 3BSc associated with greenhouse type II resistance and field severity while the 6ALc QTL, linked with *Xbarc146*, accounted for 6.7 % of the variation in DON but does not appear to be associated with other FHB resistance traits in Truman.

A QTL on 2DS associated with flowering date was also common across data collected at Missouri and Kentucky (Fig. S7). Linked with *Xwmc453*, it accounted for 69.8 and 79.1 % of the variation in

flowering date based on data from Missouri and Kentucky, respectively. The known photoperiod sensitive gene *Ppd-D1* was within the QTL region, 2.5 cM away from the QTL peak.

Phenotypic effects of QTL allele combinations for type II resistance

Combined phenotypic data across years at Missouri were used to determine the phenotypic effects of Truman QTL alleles on type II resistance (Table 6). RILs were classified into five groups based on the number of resistant (T) and/or susceptible (M) alleles derived from the resistant (Truman) or susceptible parent (MO 94-317), respectively. Mean disease severity of RILs carrying all alleles from MO 94-317 (M_1M_1; M_2M_2; M_3M_3; M_4M_4) was 23.9 % whereas that for RILs carrying only Truman alleles for the four QTL (T_1T_1; T_2T_2; T_3T_3; T_4T_4) was 9.8 %. Truman alleles reduced disease severity by 59.0 % compared with MO94-317 alleles. Mean type II resistance of RILs with one Truman allele (18.9 %) reflected a mean reduction in severity of 20.9 % compared with RILs carrying no Truman alleles while type II resistance of RILs (35) with two Truman alleles averaged 14.7 %. The 2DS, 3BSc QTL combination had the greatest impact on type II resistance while the 2BL, 3BSc combination was least effective. Type II resistance in RILs (47) with three Truman alleles averaged 12.5 % which reflected a further 9.2 % reduction in severity over those with two alleles. The most effective three-allele combination (1BSc, 2BL, 2DS) reduced disease severity by 51.5 %. Finally, type II resistance for those RILs (19) with all four QTL from Truman averaged 9.8 % which equaled a reduction of 59.0 % in disease severity compared to RILs carrying no Truman alleles.

Table 6 Phenotypic effects of quantitative trait loci (QTL) allele combinations on type II Fusarium head blight resistance in 167 recombinant inbred lines (RILs) from the cross Truman × Mo 94-317. Analyses were conducted on combined phenotypic data collected from replicated greenhouse experiments conducted at Missouri in 2008 and 2009

QTL combination[a]	Markers linked to QTL[b]				No of RILs with QTL alleles	Type II resistance (%)[c]	Reduction in disease severity (%)[d]
	Xwmc269	*Xwmc592*	*wPt666223*	*Xwmc615*			
–, –, –, –	M_1M_1	M_2M_2	M_3M_3	M_4M_4	8	23.9 ± 3.8	–
–, –, –, 3B	M_1M_1	M_2M_2	M_3M_3	M_4M_4	5	21.1 ± 3.0	11.7
–, –, 2D, –	M_1M_1	M_2M_2	M_3M_3	T_4T_4	12	17.8 ± 1.3	25.5
–, 2B, –, –	M_1M_1	M_2M_2	T_3T_3	M_4M_4	2	15.9 ± 0.3	33.5
1B, –, –, –	T_1T_1	M_2M_2	M_3M_3	M_4M_4	11	20.8 ± 2.2	13.0
1B, 2B, –, –	T_1T_1	T_2T_2	M_3M_3	M_4M_4	6	13.4 ± 2.1	43.9
1B, –, 2D, –	T_1T_1	M_2M_2	T_3T_3	M_4M_4	7	14.4 ± 1.8	39.7
1B, –, –, 3B	T_1T_1	M_2M_2	M_3M_3	T_4T_4	2	14.7 ± 1.3	38.5
–, 2B, 2D, –	M_1M_1	T_2T_2	T_3T_3	M_4M_4	5	15.3 ± 0.9	36.0
–, 2B, –, 3B	M_1M_1	T_2T_2	M_3M_3	T_4T_4	6	17.2 ± 1.0	28.0
–, –, 2D, 3B	M_1M_1	M_2M_2	T_3T_3	T_4T_4	9	13.2 ± 1.2	44.8
1B, 2B, 2D, –	T_1T_1	T_2T_2	T_3T_3	M_4M_4	15	11.6 ± 1.0	51.5
1B, 2B, –, 3B	T_1T_1	T_2T_2	M_3M_3	T_4T_4	11	13.0 ± 1.4	45.6
1B, –, 2D, 3B	T_1T_1	M_2M_2	T_3T_3	T_4T_4	7	12.1 ± 2.1	49.4
–, 2B, 2D, 3B	M_1M_1	T_2T_2	T_3T_3	T_4T_4	14	13.3 ± 0.9	44.4
1B, 2B, 2D, 3B	T_1T_1	T_2T_2	T_3T_3	T_4T_4	19	9.8 ± 1.0	59.0

[a] Chromosome location of QTL associated with resistance alleles from Truman

[b] 'T' allele is from Truman and the 'M' allele is from MO 94-317 for all markers. Subscripts represent QTL alleles from 1BSc, 2BL, 2DS and 3BSc linked to *Xwmc269, Xwmc592, wPt666223*, and *Xwmc615,* respectively

[c] The mean type II resistance over years of Truman and MO 94-317 were 5.3 and 33.9 %, respectively

[d] Reduction in disease severity compared with the mean severity of eight RILs carrying four susceptible alleles from MO 94-317

Phenotypic effects of QTL allele combinations on field incidence, severity and the Fusarium head blight index

Phenotypic data for incidence, severity, and FHBI were all collected from replicated experiments in Missouri in 2009 and 2010, therefore, combined Missouri phenotypic data were used to determine the effects of QTL alleles associated with field resistance for these traits (Table 7). Individual trait data obtained from Purdue (incidence) and Kentucky (severity) were not included in these analyses.

Mean incidence of RILs (27) carrying all alleles from MO 94-317 was 77.5 % whereas that for RILs (21) carrying only Truman alleles was 54.0 %. Where RILs carried only one Truman allele, the 2DS allele had the greatest individual effect, reducing incidence by 19.5 % while the 3DS allele had the smallest effect on incidence (10.5 %). The addition of a second Truman allele resulted in a further reduction in incidence of 7.3 % with all allele combinations having a similar effect. Incidence in RILs carrying all three QTL was further reduced by 30.3 % compared with RILs carrying no Truman alleles.

For disease severity, when one Truman allele was substituted for a MO 94-317 allele, the average reduction on severity was 12.9 % with the 2DS allele having the greatest effect. Where RILs carried both Truman alleles, the mean reduction in severity was 27.1 % which averaged an additional reduction of 14.2 % over that observed with only one QTL allele from Truman.

Analyses of FHBI showed that where RILs (25) carried all three Truman alleles, FHBI was reduced by 61.9 % compared to RILs carrying no Truman alleles. Where one Truman allele was present, RILs averaged 35.8 % FHBI with the 2DS allele having the largest effect, while RILs carrying two Truman alleles, averaged 27.2 %. The combination of QTL on 2ASc and 2DS had the greatest impact on FHBI, whereas the 2ASc, 3BSc combination was least effective. Finally, where RILs carried all three Truman alleles, the FHBI was 17.4 %.

Phenotypic effects of QTL allele combinations on Fusarium damaged kernels, and DON content

Phenotypic data combined within location over years at Missouri and Kentucky were used to determine the effects of QTL alleles on FDK and DON content (Tables 8, 9). Locations are reported separately because of the heterogeneity of error variances across locations.

At Missouri, mean FDK of RILs (25) carrying only MO 94-317 alleles was 77.2 % whereas that for RILs (15) carrying only Truman alleles was 49.9 % (Table 8). A total of 87 RILs carried at least one allele from Truman. They averaged 66.7 % FDK, a reduction of 13.7 % compared to RILs carrying no Truman alleles. RILs (50) carrying two alleles from Truman averaged 54.3 % FDK or a 24.0 % reduction compared with RILs carrying only MO 94-317 alleles. Although standard errors for this trait were higher, it appeared that the 2A, 3B combination was most effective. Percentage FDK at the Kentucky location was lower than that for Missouri. Despite the lower range, RILs with higher numbers of QTL resistance alleles generally had a lower percentage of FDK than those with fewer alleles. RILs (69) with one allele from Truman averaged 20.3 % FDK which reflected an overall reduction of 19.0 % compared with RILs (23) having only susceptible alleles. The 2ASc QTL individually had the greatest effect, reducing FDK by 38.1 % while the 3BSc QTL had a significantly smaller effect. Where RILs (51) carried two resistance alleles, FDK was 15.9 % which represented a further reduction of 15.3 % over that realized with one allele. Truman alleles for QTL on 1BLc and 2ASc had the greatest impact, reducing FDK by 51.7 % compared with RILs carrying only alleles from the susceptible parent. Finally, RILs (17) with all three QTL alleles from Truman reduced FDK by 53.8 % but again, this level was not significantly different from the best 2-allele combination and was significantly higher that FDK in Truman itself (9.0 %).

Mean DON content of RILs (24) evaluated at Missouri that carried all alleles from the susceptible parent was 18.2 μg/g (Table 9) whereas that for RILs (25) carrying only Truman alleles was 9.4 ± 0.6 μg/g. DON content in RILs (49) that carried at least one Truman allele averaged 15.8 μg/g which represented an average reduction in DON of 13.4 %. The phenotypic effects of individual QTL however, ranged widely with the 3BS QTL having only a minor effect on DON content, while the 2DS and 2ASc alleles reduced DON by 18.1 and 15.4 % respectively. RILs (62) having two alleles from Truman averaged

Table 7 Phenotypic effects of quantitative trait loci (QTL) allele combinations on incidence, severity and Fusarium head blight index (FHBI) in167 recombinant inbred lines (RILs) from the soft red winter wheat cross, Truman × MO 94-317. Analyses were conducted on combined phenotypic data collected from replicated field experiments conducted at Missouri in 2009 and 2010

QTL combination[a]	Markers linked to QTL[b]				No. of RILs with QTL	Trait[c]	Reduction in disease resistance trait (%)[d]
	wPt8826	*wPt666223*	*Xgwm285*	*wPt5390*			
Incidence[e]							
–, –, –	M_1M_1	M_2M_2	–	M_3M_3	27	77.5 ± 1.8	
–, –, 3D	M_1M_1	M_2M_2	–	T_3T_3	13	69.4 ± 4.3	10.5
–, 2D, –	M_1M_1	T_2T_2	–	M_3M_3	23	62.4 ± 2.7	19.5
2A, –, –	T_1T_1	M_2M_2	–	M_3M_3	8	66.9 ± 5.6	13.7
2A, 2D, –	T_1T_1	T_2T_2	–	M_3M_3	20	60.7 ± 2.8	21.7
2A, –, 3D	T_1T_1	M_2M_2	–	T_3T_3	14	61.6 ± 3.9	20.5
–, 2D, 3D	M_1M_1	T_2T_2	–	T_3T_3	24	59.4 ± 2.5	23.4
2A, 2D, 3D	T_1T_1	T_2T_2	–	T_3T_3	21	54.0 ± 2.9	30.3
Severity[e]							
–, –	–	M_1M_1	M_2M_2	–	37	51.2 ± 2.6	–
–, 3B	–	M_1M_1	T_2T_2	–	29	45.6 ± 2.6	10.9
2D, –	–	T_1T_1	M_2M_2	–	42	43.6 ± 2.7	14.8
2D, 3B	–	T_1T_1	T_2T_2	–	54	37.3 ± 1.9	27.1
FHBI[e]							
–, –, –	M_1M_1	M_2M_2	M_3M_3	–	27	45.7 ± 2.8	–
–, –, 3B	M_1M_1	M_2M_2	T_3T_3	–	12	35.4 ± 2.8	22.5
–, 2D, –	M_1M_1	T_2T_2	M_3M_3	–	21	33.4 ± 3.7	26.9
2A, –, –	T_1T_1	M_2M_2	M_3M_3	–	10	38.5 ± 4.7	15.8
2A, 2D, –	T_1T_1	T_2T_2	M_3M_3	–	20	25.0 ± 3.6	45.3
2A, –, 3B	T_1T_1	M_2M_2	T_3T_3	–	15	30.4 ± 4.4	33.5
–, 2D, 3B	M_1M_1	T_2T_2	T_3T_3	–	29	26.3 ± 2.3	42.5
2A, 2D, 3B	T_1T_1	T_2T_2	T_3T_3	–	25	17.4 ± 1.6	61.9

[a] Chromosome locations of QTL associated with resistance alleles from Truman

[b] 'T' allele from Truman and 'M' allele from MO 94-317 for all markers. Subscripts represent QTL alleles from 1DLc, 2ASc, 2DS, 2DS and 3BSc linked to *XBarc229, wPt5251, wPt666223, wPt665644 and Xgwm285*, respectively

[c] Mean trait values over years for Truman and MO 94-317 of incidence 57.5 and 82.5 %, severity 23 and 75 %, and FHBI 14.1 and 62.4 %, respectively

[d] Reduction in incidence, severity or FHBI compared with the respective mean value of RILs carrying susceptible alleles from MO 94-317

[e] Incidence determined as the percentage of heads in a 10-head sample showing any disease symptoms; severity determined as percentage of florets on heads showing disease symptoms; FHBI determined as incidence × severity expressed as a percentage

12.0 μg/g of DON reflecting a 32.3 % reduction over those RILs carrying no Truman alleles.

Analysis of DON data from Kentucky identified two QTL that were common with those identified from Missouri data (2ASc and 2DS) but also detected a QTL on 6ALc that was associated with low DON. RILs with no alleles from Truman averaged 24.2 μg/g of DON, while those carrying all three Truman alleles averaged 15.4 μg/g. RILs (60) with one Truman allele averaged 20.5 μg/g of DON, reflecting a 15.4 % reduction over RILs (28) with no Truman alleles with the 2DS allele resulting in the largest reduction in DON. Those with two Truman alleles (60) averaged 18.4 μg/g of DON, further reducing the DON level. All two-allele combinations, however, appeared to have about the same effect.

Table 8 Phenotypic effects of quantitative trait loci (QTL) allele combinations on percentage Fusarium damage kernels (FDK) of recombinant inbred lines (RILs) from the cross Truman × MO 94-317. The experiment was conducted at Missouri (MO) and Kentucky (KY) in 2009 and 2010. Phenotypic data were combined data over years within location

QTL combination[a]	Markers linked to QTL[b]				No of RILs with QTL	Trait (%)[c]	Reduction in FDK (%)[d]
	Xwmc694	*wPt8826*	*Xgwm102*	*wPt9433*			
MO							
–, –, –	–	M_1M_1	M_2M_2	M_3M_3	25	77.2 ± 2.6	–
–, –, 3B	–	M_1M_1	M_2M_2	T_3T_3	11	64.7 ± 4.9	16.2
–, 2D, –	–	M_1M_1	T_2T_2	M_3M_3	30	67.2 ± 3.3	13.0
2A, –, –	–	T_1T_1	M_2M_2	M_3M_3	16	68.1 ± 4.4	11.8
2A, 2D, –	–	T_1T_1	T_2T_2	M_3M_3	21	56.1 ± 3.8	27.7
2A, –, 3B	–	T_1T_1	M_2M_2	T_3T_3	13	50.2 ± 4.6	22.3
–, 2D, 3B	–	M_1M_1	T_2T_2	T_3T_3	16	56.6 ± 5.1	21.9
2A, 2D, 3B	–	T_1T_1	T_2T_2	T_3T_3	15	49.9 ± 4.7	36.4
KY							
–, –, –	M_1M_1	M_2M_2	–	M_3M_3	23	28.6 ± 3.7	–
–, –, 3B	M_1M_1	M_2M_2	–	T_3T_3	12	23.9 ± 3.7	16.4
–, 2A, –	M_1M_1	T_2T_2	–	M_3M_3	27	17.7 ± 1.6	38.1
1B, –, –	T_1T_1	M_2M_2	–	M_3M_3	30	19.3 ± 1.5	32.5
1B, 2A, -	T_1T_1	T_2T_2	–	M_3M_3	22	13.8 ± 0.9	51.7
1B, -, 3B	T_1T_1	M_2M_2	–	T_3T_3	17	17.0 ± 2.1	40.6
–, 2A, 3B	M_1M_1	T_2T_2	–	T_3T_3	12	17.0 ± 2.0	40.6
1B, 2A, 3B	T_1T_1	T_2T_2	–	T_3T_3	17	13.2 ± 1.1	53.8

[a] Chromosome locations of QTL associated with resistance alleles from Truman

[d] 'T' allele from Truman and 'M' allele from MO 94-317 for all markers. Subscripts represent QTL alleles from 1BLc, 2ALc, 2DS, and 3BSc linked to *Xwmc694, Xwmc644, Xgwm122,* and *wPt9493,* respectively

[c] Mean FDK over years of Truman and MO 94-317 were 33.8 and 89.6 % at MO; 9.0 and 25.3 % at KY, respectively

[d] Percentage reduction in percentage FDK compared with the respective mean value of RILs carrying susceptible alleles from MO 94-317

Discussion

Combining various sources and types of resistance is a goal of most wheat breeding programs in areas where FHB is a recurring problem. This approach is expected to generate varieties with either more effective resistance under high inoculum loads or varieties in which resistance is more stable over broad geographic areas. The majority of studies to date have investigated FHB resistance in Asian germplasm and have revealed that much of this germplasm carries resistance QTL on chromosomes 3BS and 5AS (Buerstmayr et al. 2009; Liu et al. 2009) with additional, less frequent QTL on 2DL and 6BS. European cultivars on the other hand do not carry the Asian QTL yet still have broad diversity for FHB resistance QTL (Buerstmayr et al. 2009). Reports of FHB resistance have been published for several soft red winter wheat cultivars and, as is the case for European cultivars, none carry the predominant QTL alleles from Asian sources of resistance. Sneller et al. (2010) examined soft winter wheat entries in the uniform scab nurseries conducted by the U.S. Wheat and Barley Scab Initiative and concluded that this group of cultivars and/or experimental lines may provide additional unique genes for resistance that should diversify the FHB resistance gene pool globally.

Truman soft red winter wheat has broadly-based FHB resistance consisting of low greenhouse type II resistance, field incidence, severity, FDK and DON. All components of resistance in RILs were continuously distributed (Fig. S1), and controlled by 4–5

Table 9 Phenotypic effects of quantitative trait loci (QTL) allele combinations on deoxynivalenol (DON) content of recombinant inbred lines (RILs) from the cross Truman × MO 94-317. The experiment was conducted at Missouri (MO) and Kentucky (KY) in 2009 and 2010. Phenotypic data were combined data over years within location

QTL combination[a]	Markers linked to QTL[b]				No of RILs with QTL	Trait (μg/g)[c]	Reduction in DON %[d]
	wPt8826	*wPt666223*	*Xwmc615*	*XBarc146*			
MO							
–, –, –	M_1M_1	M_2M_2	M_3M_3	–	24	18.2 ± 1.8	–
–, –, 3B	M_1M_1	M_2M_2	T_3T_3	–	23	17.0 ± 1.3	6.6
–, 2D, –	M_1M_1	T_2T_2	M_3M_3	–	16	14.9 ± 1.7	18.1
2A, –, –	T_1T_1	M_2M_2	M_3M_3	–	10	15.4 ± 1.8	15.4
2A, 2D, –	T_1T_1	T_2T_2	M_3M_3	–	20	11.1 ± 1.3	39.0
2A, –, 3B	T_1T_1	M_2M_2	T_3T_3	–	15	13.4 ± 1.2	26.4
–, 2D, 3B	M_1M_1	T_2T_2	T_3T_3	–	27	11.4 ± 0.7	37.4
2A, 2D, 3B	T_1T_1	T_2T_2	T_3T_3	–	25	9.4 ± 0.6	48.4
KY							
–, –, –	M_1M_1	M_2M_2	–	M_3M_3	28	24.2 ± 0.9	–
–, –, 6A	M_1M_1	M_2M_2	–	T_3T_3	12	21.5 ± 1.6	11.2
–, 2D, –	M_1M_1	T_2T_2	–	M_3M_3	35	19.6 ± 0.8	19.0
2A, –, –	T_1T_1	M_2M_2	–	M_3M_3	13	20.3 ± 1.6	16.1
2A, 2D, –	T_1T_1	T_2T_2	–	M_3M_3	34	17.5 ± 0.7	27.7
2A, –, 6A	T_1T_1	M_2M_2	–	T_3T_3	11	18.8 ± 1.2	22.3
–, 2D, 6A	M_1M_1	T_2T_2	–	T_3T_3	15	18.9 ± 1.1	21.9
2A, 2D, 6A	T_1T_1	T_2T_2	–	T_3T_3	10	15.4 ± 0.7	36.4

[a] Chromosome locations of QTL associated with resistance alleles from Truman

[b] 'T' allele from Truman and 'M' allele from MO 94-317 for all markers. Subscripts represent QTL alleles from 2ASc, 2DS, 3BSc, and 6ALc linked to *wPt8826, wPt666223, Xwmc615,* and *XBarc146,* respectively

[c] The mean DON amount over years of Truman and MO 94-317 were 6.9 and 30.6 μg/g at MO; 13.0 and 24.1 μg/g at KY, respectively

[d] Percentage reduction in DON content compared with the respective mean value of RILs carrying susceptible alleles from MO 94-317

genes, with high heritability (Table 1). Earlier work on the resistance in Truman suggested that it was conditioned primarily by additive gene action with a smaller, but significant, dominance component (Abate and McKendry 2010). Of note in this work was the lack the epistatic interactions among genes that were detected for other sources of resistance. Combined phenotypic RIL data for type II resistance, incidence, severity, and DON (Table 1) were slightly lower than the mid-parental value while that for FDK approximated the mid-parental value, confirming earlier work by Abate and McKendry (2010) on gene actions conditioning FHB resistance in Truman. For all traits transgressive segregates were observed in RIL distributions (Fig. S1) again, confirming the predominance of additive variance associated with the FHB resistance in Truman. The relationship between incidence, severity, FDK and DON suggests an interdependence of FHB resistance traits in Truman which is consistent with results of a meta-analysis of 163 studies conducted by Paul et al. (2005) who reported highly significant correlations among FDK, DON and disease severity.

QTL analyses

A review of the literature suggests that the major QTL on 2DS associated with type II resistance, as well as field data for low incidence, disease severity, and DON, may be novel. This QTL was also detected for

DON at the Kentucky location and for incidence at the Purdue location. Although, several 2D QTL associated with FHB resistance have been reported in Chinese or Chinese-derived germplasm (Shen et al. 2003b; Somers et al. 2003; Handa et al. 2008; Yang et al. 2005; Ma et al. 2006; Jiang et al. 2007a; Mardi et al. 2005; Lin et al. 2006; Jia et al. 2005), only one QTL region related to disease severity remotely coincides with this 2DS QTL in Truman. Jia et al. (2005) identified a minor QTL ($R^2 = 10.6$ %) on 2D, that was flanked by *Xgwm261–Xgwm484*. The QTL originated from Wangshuibai but had a low LOD score and was inconsistent over years of the study. Yang et al. (2005) also reported a 2DS QTL associated with reduced incidence, however, it was not consistently detected and not linked with the 2DS QTL in this study (Somers et al. 2004). However, *Ppd-D1* was 4.0 cM away from the QTL peak and fell inside the QTL region. In examining this further, we mapped a flowering date QTL which partially overlapped this 2DS QTL. Usually *Ppd-D1* is associated with early head emergence coupled with shorter plant height (Liu et al., 2013) and initial infection and subsequent disease development can be influenced by flowering date of the genotypes being screened. Further research is necessary to determine whether or not the 2DS QTL is associated with FHB or is an artifact of flowering date.

The 2ASc QTL associated with reduced incidence and FHBI as well as with FDK and DON across locations may also be novel. A 2AL QTL associated with type II resistance was first identified by Waldron et al. (1999) and later verified by Anderson et al. (2001) in a population derived from the cross of Sumai 3 and 'Stoa'. In both cases, the 2AL QTL was derived from the susceptible parent Stoa. QTL on 2AS have been reported for type II resistance in the Chinese varieties Wangshuibai (Ma et al. 2006), and 'Ning 7840' (Zhou et al. 2002), however, in Wangshuibai, the right border remotely coincided with right border of the QTL in Truman while in Ning 7840 it was located distally on 2AS. Finally, Gervais et al. (2003) working with the European wheat 'Renan', identified a 2AL QTL associated with type II resistance that co-localized with the 2AL QTL reported in Stoa by Waldron et al. (1999) and was not linked with the 2ASc in Truman, based on the map of Somers et al. (2004). No known 2ASc QTL have been identified for reduced incidence and those that have been identified for type II resistance, map to regions outside the 2ASc region identified in this study. Thus, we believe this QTL in Truman may be novel.

A third potentially novel QTL in Truman is that on 3DS associated with incidence. Shen et al. (2003a) and Klahr et al. (2007) identified a QTL on 3DL associated with FHB spread in 'Patterson' and 'Cansas', respectively, but based on their map locations these 3DL QTL were 26 and 31 cM distal, respectively, from that identified from Truman. Yu et al. (2008) also reported a 3D QTL associated with FHB severity but it was distal on 3DL. No QTL have been identified on the short arm of 3DS and therefore, this QTL may be unique.

Two additional QTL were identified from analysis of phenotypic data collected at either Purdue or Kentucky. A 1DLc QTL associated with incidence at Purdue was not detected at Missouri, but should it be validated, it may also be unique. There are three known reports of a QTL on 1D linked with disease severity and kernel quality (Yang et al. 2005; Ittu et al. 2000; Klahr et al. 2007) but none co-localized with the 1DLc QTL from Truman that was detected from Purdue data. Analysis of Kentucky data also detected a centromeric QTL on 6AL that was solely associated with DON content. Although, QTL in the same region of 6AL associated with disease severity have been reported in both European and Chinese germplasm (Schmolke et al. 2005; Haberle et al. 2009 and Anderson et al. 2001) none have been associated with DON (Buerstmayr et al. 2009).

Finally, it is of interest to note that the 3BSc QTL identified for type II resistance, field severity, FHBI, and DON, although not unique, appears to be present in soft red winter wheat germplasm. It is co-localized with that identified in Ernie (Liu et al. 2007) and anecdotally, has also been detected in other U.S. soft red winter wheat germplasm (Brown-Guedira 2009—personal communication).

In summary, although there were some inconsistencies across locations, common QTL including those on 2ASc, 2DS, and 3BSc, were in fact identified across several FHB resistance traits and validated across Midwestern locations. This suggests that incidence, severity, FDK, and DON are interrelated in Truman. From a breeding perspective, this should accelerate the effort to enhance FHB resistance in winter wheat because screening for one trait, should in fact result in lower levels in correlated traits.

Similarly, once these QTL are further validated, fewer QTL will need to be pyramided into individual varieties for enhanced levels of most components of resistance.

Effect of QTL alleles on components of Fusarium head blight resistance

Although, four resistance alleles had a significant impact on type II resistance, disease severity in RILs carrying all four resistance alleles (9.8 %) was significantly higher than that of the resistant parent Truman (5.3 %). This may have been due to chance variation or minor QTL that failed to reach the LOD significance level of the experiment. Despite this, type II resistance of 9.8 % is a highly functional level of resistance in the field environment, therefore, once validated, these four QTL should contribute significantly to reducing losses associated with FHB when introduced into susceptible cultivars through MAS.

RILs carrying all three resistance QTL for incidence resulted in a reduction in disease to approximately the same level as was observed in the parental cultivar Truman (57.5 %). For Truman, this is consistent with research that has shown a smaller background epistatic effect for type II resistance than that found in other sources of resistance (Abate and McKendry 2010) and is also consistent with anecdotal evidence of a high level of recovery of offspring in the Missouri breeding program carrying resistance levels equal to Truman. For disease severity, results were not as clear. RILs with one QTL averaged 44.6 % infection while those with both QTL averaged 37.3 % infection. Although the R^2 values for the 3BSc and 2DS QTL were significant, in practice their combined impact did not reflect the proportion of the variation they explained individually. It is also of note that the mean disease severity of RILs with both Truman alleles did not reach the level of Truman (23.0 %) suggesting that a third QTL or QTL interaction may have gone undetected. In contrast, although FHBI of RILS carrying three Truman alleles (17.4 % ± 1.6) did not equal the FHBI of Truman itself (14.1 ± 3.7) it was within the standard error of the measurement. This reduction of 61.9 % in FHBI was a greater reduction than was observed for either incidence or severity independently and suggests the value of integrating over both incidence and severity in selecting for field resistance to FHB.

Phenotypic data for FDK in Truman and MO 94-317 ranged from 33.8 to 89.6 %, respectively when assessed at Missouri and from 9.0 to 25.3 %, respectively when assessed at Kentucky. These differences may have been due to the different techniques used to assess FDK at the two locations. At Missouri, FDK were determined as the percentage of tombstones and shriveled kernels following hand harvest and threshing assessed on a visual scale while at Kentucky, an aspiration technique was used to separate FDK from sound kernels. Regardless of the technique used, at both locations RILs with more QTL alleles generally had lower FDK than those with fewer QTL. At Missouri, there was no statistical difference between the phenotypic effects of two QTL, where it appeared that the 2ALc, 3BSc combination was most effective and those with three QTL. This was probably due to the fact that FDK typically has a higher standard error than other traits assessed because of a loss of tombstones or shriveled seed during the threshing process. At KY, RILs carrying all three alleles from Truman reduced FDK by 53.8 % which again, was not statistically different from the most effective two-allele combination that included QTL on 1BLc and 2ALc. Although QTL identified from Missouri data and those identified from Kentucky data reduced FDK, neither combination reduced this trait to the levels found in the resistant parent Truman. Again, this may have been due to a failure to identify all QTL associated with FDK because of the high error rate that is commonly associated with the measurement of this trait.

Phenotypic data for DON content also varied across locations ranging from 6.9 to 30.6 μg/g in Truman and MO 94-317, respectively based on Missouri tests and from 13.0 to 24.1 μg/g for Truman and MO 93-317, respectively based on Kentucky tests. This difference in range could have been due to harvest and seed preparation techniques at the two locations or to the respective isolates used for inoculation. It could also have been due to the fact that often, the DON levels in apparently sound kernels can be high despite a lack of FDK present in the sample (Sinha and Savard 1997). The latter would explain why the range of FDK was higher in Missouri while the DON levels were higher at Kentucky. Despite these differences, QTL on chromosomes 2ASc and 2DS that were identified from phenotypic data at both locations were the most effective two-allele combination resulting in 39.0 and

27.7 % reductions in DON at Missouri and Kentucky, respectively. With the addition of the 3BSc allele at Missouri, DON content was further reduced to 9.4 ± 0.6 μg/g which was within the standard error for the combined DON data for Truman and reflected a 48.4 % reduction in DON compared with RILs that had no Truman alleles. With the addition of the 6ALc QTL identified from data from Kentucky, again, DON was reduced to within the standard error for the combined DON data for Truman, a reduction of 36.4 % over RILs with no Truman alleles.

Conclusions

These studies have resulted in the identification of three potentially unique QTL on 2ASc, 2DS and, 3DS. The QTL on 2DS is associated with type II resistance, incidence, severity, FHBI and DON based on Missouri phenotypic data and is among the first major QTL identified from U.S. germplasm. It was confirmed for incidence from Purdue data and for DON from Kentucky data and clearly illustrates the interdependence of components of resistance in Truman. The QTL peak, however, is close to *Ppd-D1* and a flowering date QTL overlaps this QTL region on 2DS. Further work is currently underway to determine if this QTL is independent of flowering date. Should this be the case, it will be a valuable addition to those QTL currently in use for marker-assisted selection. QTL on 2ASc again is associated with many of the components of resistance in Truman including incidence, FHBI, FDK and DON. The significant contributions of this QTL to both FDK and DON have been confirmed from phenotypic data collected at Kentucky. The 3DS QTL has only been associated with incidence but accounts for 10 % of the variance in this trait and because of the lack of QTL for this trait, should again be useful for marker-assisted selection once it is validated. QTL on 1ASc, and 3BSc, although not unique, were also common across traits and screening environments which suggests an interrelationship among these traits that will be valuable for breeders attempting to enhance the FHB resistance in wheat. Truman is an adapted soft red winter wheat and experience has shown that the FHB resistance in Truman is highly penetrant. Both of these factors should be useful in accelerating the development of FHB resistant varieties. This population is also being genotyped using single nucleotide polymorphism (SNP) markers to further saturate the genome with co-dominant markers in an effort to further refine these QTL. Once completed and validated, these QTL should be valuable for both improving and diversifying FHB resistance in winter wheat.

Acknowledgments The authors thank Dr. Georgia Davis at the University of Missouri and Dr. John Perry Gustafson of the Plant Genetics Unit of the USDA-ARS for use of their laboratory facilities during genotyping and analysis. This material is based upon work supported in part by the U.S. Department of Agriculture under Agreement No. 59-0206-9-077. This is a cooperative project with the U.S. Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

References

Abate ZA, McKendry AL (2010) Diallel analysis of Fusarium head blight resistance in genetically diverse winter wheat germplasm. Euphytica 175:409–421

Abate ZA, Liu S, McKendry AL (2008) Quantitative trait loci associated with deoxynivalenol content and kernel quality in the soft red winter wheat 'Ernie'. Crop Sci 48:1408–1418

Agostinelli AM, Clark AJ, Brown-Guedira G, Van Sanford DA (2012) Optimizing phenotypic and genotypic selection for Fusarium head blight resistance in wheat. Euphytica 186:115–126

Anderson JA, Stack RW, Liu S, Waldron BL, Field AD, Coyne C, Moreno-Sevilla B, Fetch JM, Song QJ, Cregan PB, Frohberg RC (2001) DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theor Appl Genet 102:1164–1168

Bai GH, Shaner GE (2004) Management and resistance in wheat and barley to *Fusarium* head scab. Annu Rev Phytopathol 42:135–161

Bai GH, Kolb FL, Shaner GE, Domier LL (1999) Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology 89:343–348

Bonin CM, Kolb FL (2009) Resistance to Fusarium head blight and kernel damage in a winter wheat recombinant inbred line population. Crop Sci 49:1304–1312

Buerstmayr H, Lemmens M, Hartl L, Doldi L, Steiner B, Stierschneider M, Ruckenbauer P (2002) Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat I: resistance to fungal spread (Type II resistance). Theor Appl Genet 104:84–91

Buerstmayr H, Steiner B, Hartl L, Griesser M, Angerer N, Lengauer D, Miedaner T, Schneider B, Lemmens M (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

Buerstmayr H, Ban T, Anderson JA (2009) QTL mapping and marker-assisted selection for *Fusarium* head blight resistance in wheat: a review. Plant Breed 128:1–26

Chen J, Griffey CA, Saghai-Maroof MA, Stromberg EL, Biyashev RM, Zhao W, Chappell MR, Pridgen TH, Dong Y, Zeng Z (2006) Validation of two major quantitative trait loci for Fusarium head blight resistance in Chinese wheat line W14. Plant Breed 125:99–101

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Cockerham CC (1983) Covariance of relatives from self-fertilization. Crop Sci 23:1177–1180

Del Blanco IA, Frohberg RC, Stack RW, Berzonsky WA, Kianian SF (2003) Detection of QTL linked to *Fusarium* head blight resistance in Sumai 3-derived North Dakota bread wheat lines. Theor Appl Genet 106:1027–1031

Draeger R, Gosman N, Steed A, Chandler E, Srinivasachary MT, Schondelmaier J, Buerstmayr H, Lemmens M, Schmolke M, Mesterházy A, Nicholson P (2007) Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. Theor Appl Genet 115:617–625

Gervais L, Dedryver F, Morlais JY, Bodusseau V, Negre S, Bilous M, Groos C, Trottet M (2003) Mapping of quantitative trait loci for field resistance to Fusarium head blight in an European winter wheat. Theor Appl Genet 106:961–970

Gooding RW, Lafever HN, Campbell KG, Herald LD (1997) Registration of 'Freedom' wheat. Crop Sci 37:1007

Griffey CA, Starling TM, Price AM, Sisson WL, Das MK, Pridgen TH, Vaughn ME, Rohrer WL, Brann DE (2001) Registration of 'Roane' wheat. Crop Sci 41:1359–1360

Griffey CA, Rohrer WL, Pridgen TH, Brooks WS, Chen J, Wilson JA, Nabati D, Brann DE, Rucker EG, Behl HD, Vaughn ME, Sisson WL, Randall TR, Corbin RA, Kenner JC, Dunaway DW, Pitman RM, Bockelman HE, Gaines C, Long DL, McVey DV, Cambron SE, Whitcher L (2005a) Registration of 'McCormick' wheat. Crop Sci 45:417–419

Griffey CA, Rohrer WL, Pridgen TH, Brooks WS, Chen J, Wilson JA, Nabati D, Brann DE, Rucker EG, Behl HD, Vaughn ME, Sisson WL, Randall TR, Corbin RA, Kenner JC, Dunaway DW, Pitman RM, Smid AE, Bockelman HE, Gaines C, Long DL, McVey DV, Cambron SE, Whitcher L (2005b) Registration of 'Tribute' wheat. Crop Sci 45:419–420

Griffey CA, Thomason WE, Pitman RM, Beahm BR, Paling JJ, Chen J, Fanelli JK, Kenner JC, Dunaway DW, Brooks WS, Vaughn ME, Hokanson EG, Behl HD, Corbin RA, Hall MD, Liu S, Custis JT, Waldenmaier CM, Starner DE, Gulick SA, Ashburn SR, Whitt DL, Bockelman HE, Souza EJ, Brown-Guedira GL, Kolmer JA, Long DL, Yin Y, Chen X, Cambron SE (2010) Registration of 'Jamestown'' wheat. J Plant Reg 4:28–33

Haberle J, Schweizer G, Schondelmaier J, Zimmermann G, Hartl L (2009) Mapping of QTL for resistance against *Fusarium* head blight in the winter wheat population Pelikan//Bussard/Ning8026. Plant Breed 128:27–35

Handa H, Namiki N, Xu D, Ban T (2008) Dissecting of the FHB resistance QTL on the short arm of wheat chromosome 2D using a comparative genomic approach: from QTL to candidate gene. Mol Breed 27:71–84

Ittu M, Saulescu NN, Hagima I, Ittu G, Mustatea P (2000) Association of Fusarium head blight resistance with gliadin loci in a winter wheat cross. Crop Sci 40:62–67

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136:1447–1455

Jia G, Chen PD, Qin GJ, Bai GH, Wang X, Wang SL, Zhou B, Zhang SH, Liu DJ (2005) QTLs for Fusarium head blight response in a wheat DH population of Wangshuibai/Alondra's. Euphytica 146:183–191

Jiang GL, Shi J, Ward R (2007a) QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ9306: I. Resistance to fungal spread. Theor Appl Genet 116:3–13

Jiang GL, Dong Y, Shi J, Ward RW (2007b) QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ9306: II. Resistance to deoxynivalenol accumulation and grain yield loss. Theor Appl Genet 115:1043–1052

Jones RK, Mirocha CJ (1999) Qu ality parameters in small grains from Minnesota affected by Fusarium head blight. Plant Dis 83:506–511

Keim P, Olsen TC, Shoemaker RC (1988) A rapid protocol for isolating soybean DNA. Soybean Genet Newslett 15:147–148

Klahr A, Zimmermann J, Wenzel G, Mohler V (2007) Effects of environment, disease progress, plant height and heading date on the detection of QTLs for resistance to Fusarium head blight in an European winter wheat cross. Euphytica 154:17–28

Knapp SJ, Stroup WW, Ross WW (1985) Exact confidence intervals for heritability on a progeny mean basis. Crop Sci 25:192–194

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lemmens M, Scholz U, Berthiller F, Dall'Asta C, Koutnik A, Schuhmacher R, Adam G, Buerstmayr H, Mesterházy A, Krska R (2005) The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for Fusarium head blight resistance in wheat. Mol Plant Microbe Interact 18:1318–1324

Li C, Zhu H, Zhang C, Lin F, Xue S, Cao Y, Zhang Z, Zhang L, Ma Z (2008) Mapping QTLs associated with Fusarium-damaged kernels in the Nanda 2419× Wangshuibai population. Euphytica 163:185–191

Lin F, Xue SL, Zhang ZZ, Zhang CQ, Kong ZX, Yao GQ, Tian DG, Zhu HL, Li CJ, Cao Y, Wei JB, Luo QY, Ma ZQ (2006) Mapping QTL associated with resistance to Fusarium head blight in the Nanda2419× Wangshuibai population II: type I resistance. Theor Appl Genet 112:528–553

Liu S, Zhang X, Pumphrey MO, Stack W, Gill BS, Anderson JA (2006) Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Funct Integr Genom 6:83–89

Liu S, Abate Z, Lu H, Musket T, Davis GL, McKendry AL (2007) QTL associated with Fusarium head blight resistance in the soft red winter wheat Ernie. Theor Appl Genet 115:417–427

Liu S, Hall MD, Griffey CA, McKendry AL (2009) Meta-analysis of QTL associated with *Fusarium* head blight resistance in wheat. Crop Sci 49:1955–1968

Liu S, Christopher MD, Griffey CA, Hall MD, Gundrum PG, Brooks WS (2012) Molecular characterization of resistance to Fusarium head blight in U.S. soft red winter wheat breeding line VA00W-38. Crop Sci 52:2283–2292

Liu S, Griffey CA, Hall MD, McKendry AL, Chen J, Brooks WS, Guedira GB, Sanford DV, Schmale DG (2013) Molecular characterization of field resistance to Fusarium head blight in two US soft red winter wheat cultivars. Theor Appl Genet 126:2485–2498

Ma HX, Zhang KM, Gao L, Bai GH, Chen HG, Cai ZX, Lu WZ (2006) Quantitative trait loci for resistance to Fusarium head blight and deoxynivalenol accumulation in Wangshuibai wheat under field conditions. Plant Pathol 55:739–745

Mardi M, Buerstmayr H, Ghareyazie B, Lemmens M, Mohammadi SA, Nolz R, Ruckenbauer P (2005) QTL analysis of resistance to Fusarium head blight in wheat using a Wangshuibai derived population. Plant Breed 124:329–333

McKendry AL, Berg JE, Tague DN, Kephart KD (1995) Registration of Ernie wheat. Crop Sci 35:1513

McKendry AL, Tague DN, Wright RL, Tremain JA, Conley SP (2005) Registration of 'Truman' wheat. Crop Sci 45:421–423

McKendry AL, Tague DN, Wright RL, Tremain JA (2007) Registration of 'Bess' wheat. J Plant Reg 1:21–23

McMullen M, Jones R, Gallenberg D (1997) Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis 81(12):1340–1348

Mesterházy Á (1995) Types and composition of resistance of Fusarium head blight of wheat. Plant Breed 114:377–386

Miedaner T, Wilde F, Steiner B, Buerstmayr H, Korzun V, Ebmeyer E (2006) Stacking quantitative trait loci for Fusarium head blight resistance from non-adapted sources in an European elite spring wheat background and assessing their effect on deoxynivalenol content and disease severity. Theor Appl Genet 112:562–569

Ohm HW, Shaner G, Ratcliffe R, Huber D, Sharma H, Perry K, Buechley G, Cambron S (2000) Registration of 'Goldfield' wheat. Crop Sci 40:581–582

Okubara PA, Blechl AE, McCormick SP, Alexander NJ, Dill-Macky R (2002) Engineering deoxynivalenol metabolism in wheat through the expression of a fungal trichothecene acetyltransferase gene. Theor Appl Genet 106:74–83

Paillard S, Schnurbusch T, Tiwari R, Messmer M, Winzeler M, Keller B, Schachermayr G (2004) QTL analysis of resistance to Fusarium head blight in Swiss winter wheat. Theor Appl Genet 109:323–332

Paul PA, El-Allaf SM, Lipps PE, Madden LV (2005) Relationships between incidence and severity of Fusarium head blight on winter wheat in Ohio. Phytopathology 95(9):1049–1060

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MM (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Rudd JC, Horsley RD, McKendry AL, Elias EM (2001) Host plant resistance genes for Fusarium head blight: sources, mechanisms and utility in conventional breeding systems. Crop Sci 41:620–627

SAS Institute (2011) SAS/STAT user's guide. version 9.1, SAS Institute Inc., Cary, NC

Schmolke M, Zimmermann G, Buerstmayr H, Schweizer G, Miedaner T, Korzun V, Ebmeyer E, Hartl L (2005) Molecular mapping of Fusarium head blight resistance in the winter wheat population Dream/Lynx. Theor Appl Genet 111:747–756

Semagn K, Bjornstad A, Skinnes H, Guri Maroy A, Tarkegne Y, William M (2006) Distribution of DArT, AFLP, and SSR markers in a genetic linkage map of a doubled-haploid hexaploid wheat population. Genome 49:445–555

Semagn K, Skinner H, Bjornstad A, Guri Maroy A, Tarkegne Y (2007) Quantitative trait loci controlling Fusarium head blight resistance and low deoxynivalenol content in hexaploid wheat population from 'Arina' and NK93604. Crop Sci 47:294–303

Shen XR, Ittu M, Ohm HW (2003a) Quantitative trait loci conditioning resistance to Fusarium head blight in wheat line F201R. Crop Sci 43:850–857

Shen X, Zhou M, Lu W, Ohm H (2003b) Detection of Fusarium head blight resistance QTL in a wheat population using bulked segregant analysis. Theor Appl Genet 106:1041–1047

Sinha RC, Savard ME (1997) Concentration of deoxynivalenol in single kernels and various tissues of wheat heads. Can J Plant Pathol 19:8–12

Sneller CH, Paul P, Guttieri M (2010) Characterization of resistance to Fusarium head blight in an eastern U.S. soft red winter wheat population. Crop Sci 50:123–133

Somers DJ, George F, Savard M (2003) Molecular mapping of novel genes controlling Fusarium head blight resistance and deoxynivalenol accumulation in spring wheat. Genome 46(4):555–564

Somers DJ, Issac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R, Cregan PB (2005) Development and mapping of microsatellite (SSR) markers in wheat. Theor Appl Genet 110(3):550–560

Steiner B, Lemmens M, Griesser M, Scholz U, Schondelmaier J, Buerstmayr H (2004) Molecular mapping of resistance to Fusarium head blight in the spring wheat cultivar Frontana. Theor Appl Genet 109:215–224

Van Ooijen JW, Voorrips RE (2001) JoinMap 2.0 software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Waldron BL, Moreno-Sevilla B, Anderson JA, Stack RW, Frohberg RC (1999) RFLP mapping of QTL for Fusarium head blight resistance in wheat. Crop Sci 39:805–811

Wang S, Basten CJ, Zeng ZB (2006) Windows QTL cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh

Wright S (1968) Evolution and genetics of populations, vol 1. Genetics and biometric foundation, University of Chicago Press, Chicago

Yang Z, Gilbert J, Fedak G, Somers DJ (2005) Genetic characterization of QTL associated with resistance to Fusarium head blight in a double-haploid spring wheat population. Genome 48:187–196

Yang J, Zhu J, Williams RW (2007) Mapping the genetic architecture of complex traits in experimental populations. Bioinformatics 23:1527–1536

Yu JB, Bai GH, Zhou WC, Dong YH, Kolb FL (2008) Quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population of Wangshuibai/Wheaton. Phytopathology 98:87–94

Zadoks JC, Chang TT, Conzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421

Zhang X, Zhou M, Ren L, Bai GH, Ma H, Scholten OE, Guo P, Lu W (2004) Molecular characterization of Fusarium head blight resistance from wheat variety Wangshuibai. Euphytica 139:59–64

Zhou WC, Kolb FL, Bai GH, Shaner G, Domier LL (2002) Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. Genome 45:719–727