Molecular Mapping of Fusarium Head Blight Resistance in the Spring Wheat Line ND2710

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ABSTRACT

ND2710 is a hard red spring wheat line with a very high level of resistance to Fusarium head blight (FHB). It was selected from the progeny of a cross between ND2603 (an advanced breeding line derived from the Sumai 3/Wheaton cross) and Grandin (a spring wheat cultivar). The FHB resistance of ND2710 is presumably derived from Sumai 3 because the other parents (Grandin and Wheaton) are very susceptible to FHB. To identify and map the quantitative trait loci (QTL) for FHB resistance in ND2710, we developed a mapping population consisting of 233 recombinant inbred lines (RILs) from the cross between ND2710 and the spring wheat cultivar Bobwhite. These RILs along with their parents and checks were evaluated for reactions to FHB in three greenhouse experiments and one field experiment during 2013 to 2014. The population was also genotyped with the wheat 90K iSelect single-nucleotide polymorphism (SNP) assay, and a genetic linkage map was developed with 1,373 non-cosegregating SNP markers, which were distributed on all 21 wheat

chromosomes spanning 914.98 centimorgans of genetic distance. Genetic analyses using both phenotypic and genotypic data identified one major QTL (*Qfhb.ndwp-3B*) on the short arm of chromosome 3B, and three minor QTL (*Qfhb.ndwp-6B*, *Qfhb.ndwp-2A*, and *Qfhb.ndwp-6A*) on 6B, 2A, and 6A, respectively. The major QTL on 3B was detected in all experiments and explained 5 to 20% of the phenotypic variation, while the three minor QTL on 6B, 2A, and 6A explained 5 to 12% phenotypic variation in at least two experiments, except for *Qfhb.ndwp-2A*, which was only detected in the field experiment. *Qfhb.ndwp-3B* and *Qfhb.ndwp-6B* were mapped to the genomic regions containing *Fhb1* and *Fhb2*, respectively, confirming that they originated from Sumai 3. The additive effect of the major and minor QTL may contribute to the high level of FHB resistance in ND2710. The SNP markers closely linked to the FHB resistance QTL will be useful for marker-assisted selection of FHB resistance in wheat breeding programs.

Fusarium head blight (FHB), also known as scab, is a devastating fungal disease affecting all classes of wheat in North America and many other regions of the world (Goswami and Kistler 2004). In North America, *Fusarium graminearum* Schwabe (= *Gibberella zeae* (Schwein.) Petch) is the primary causal agent of the disease (McMullen et al. 1997). Severe FHB epidemics can cause huge economic losses in wheat production by reducing yield and downgrading grain quality due to mycotoxin contamination (Bai and Shaner 2004; McMullen et al. 1997). Mycotoxin-contaminated food and feed pose high risk to both humans and animals (Korosteleva et al. 2007; Parry et al. 1995; Pestka 2010).

Conventional agronomical methods such as adjusting planting date, crop rotations, tillage, or the use of fungicides are only partly effective to minimize damages caused by FHB. Utilization of FHB-resistant wheat varieties together with fungicide application is generally considered to be the most practical and effective strategy to control this disease (McMullen et al. 2012). Therefore, developing wheat cultivars with a high level of FHB resistance has been of high priority in wheat breeding programs worldwide over the last decade (Buerstmayr et al. 2009). Although no wheat

varieties are immune to FHB, genotypes with relatively high levels of FHB resistance have been identified in different germplasm pools from Asia, Europe, and Latin America (Buerstmayr et al. 2009). Examples include Chinese cultivar Sumai 3 and its derivatives, Swiss cultivar Arina, and Brazilian cultivar Frontana (Buerstmayr et al. 2009; Paillard et al. 2004; Ruckebauer et al. 2001; Schroeder and Christensen 1963). Sumai 3 and its derivatives consistently showed a high level of FHB resistance across different genetic backgrounds and environments and have been widely used in wheat breeding programs worldwide (Anderson et al. 2001; Buerstmayr et al. 2002, 2003; Yang et al. 2005; Zhou et al. 2002, 2004). Indeed, most of the recently released FHB-resistant spring wheat varieties in the North Central Region contain the FHB resistance derived from Sumai 3 (Anderson et al. 2011).

FHB resistance in wheat is a complex and quantitative trait controlled by multiple genes with major and/or minor effects (Buerstmayr et al. 2009; Liu et al. 2009). The development of various molecular markers has greatly facilitated the genetic study and detection of quantitative trait loci (QTL) for FHB resistance. To date, more than 200 FHB resistance QTL have been identified on all 21 chromosomes of hexaploid wheat from a broad range of resistant sources; however, only 22 of them have been identified in multiple mapping populations and are considered stable QTL (Buerstmayr et al. 2009). The major QTL on 3BS (syn. *Qfhs.ndsu-3BS*, designated as *Fhb1*) from Sumai 3 is the strongest and most reliable and has been proved to be consistently expressed in different genetic backgrounds (Anderson et al. 2007; McCartney et al. 2007; Waldron et al. 1999). DNA markers tightly linked to *Fhb1* (syn. *Qfhs.ndsu-3BS*) such as *Xumn10* flanked by markers *Xgwm533* and *Xgwm493* have been routinely used for

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marker-assisted selection (MAS) of *Fhb1* (Liu et al. 2008). *Fhb1* was further fine mapped to a 1.1-Mb genomic region, with 28 candidate genes identified (Schweiger et al. 2016). The gene encoding chimeric lectin with agglutinin domains and a pore-forming toxin-like domain has been reported to be *Fhb1*, conferring resistance to FHB (Rawat et al. 2016). The *Fhb2* region on chromosome 6B was also dissected (Cuthbert et al. 2007) and six putative resistance genes were identified (Dhokane et al. 2016). However, effective markers have not been developed for other known and unknown FHB resistance QTL in wheat.

ND2710 was the first North Dakota hard red spring wheat experimental line generated in the process of introducing FHB resistance from Sumai 3 into adapted wheat varieties in the northern spring wheat region (del Blanco et al. 2003; Frohberg et al. 2004). It combines a high level of FHB resistance with relatively acceptable agronomic traits (Frohberg et al. 2004). ND2710 has been used as an FHB-resistant parent in the North Dakota State University (NDSU) spring wheat breeding program since 1994 (del Blanco et al. 2003). The objectives of this study were to verify the QTL for FHB resistance in ND2710, quantify the QTL effects using recombinant inbred lines (RILs) derived from the ND2710 × Bobwhite cross, and develop user-friendly markers for MAS of the FHB resistance in wheat breeding programs.

MATERIALS AND METHODS

Plant materials. To identify and map QTL for resistance to FHB in ND2710 (PI 633976), a mapping population (designated as BN) containing 233 RILs was developed from the cross between ND2710 and the FHB-susceptible spring wheat cultivar Bobwhite (PI 520368) using the single-seed descent method. ND2710 is a hard red spring wheat (*Triticum aestivum* L.) line combining a high level of FHB resistance derived from Sumai 3 with relatively acceptable agronomic traits (Frohberg et al. 2004). Grandin (PI 531005) and Alsen (PI 615543) were used as susceptible and moderately resistant checks, respectively.

Evaluation of reaction to FHB. The BN population and its parents (Bobwhite and ND2710) along with the checks were evaluated for type II resistance (resistance to fungal spread in the spikes) in three greenhouse experiments and one field environment using the procedures described by Chu et al. (2011). Greenhouse evaluations were conducted in the spring and winter seasons of 2013 (13GH1 and 13GH2) and the spring season of 2014 (14GH). Field evaluation was carried out in an FHB nursery located at Fargo, ND in 2014 (14FAR). In all experiments, the RILs were evaluated using a randomized complete block design with three replicates.

In greenhouse environments, the RILs and their parents were grown in clay pots with three plants per pot. The greenhouse was supplemented with artificial light for a 14-h photoperiod with the temperature maintained between 22 and 25°C. The inoculum was prepared at a concentration of 100,000 spores ml⁻¹ by mixing spores equally from four pathogenic *F. graminearum* strains collected from North Dakota (Puri and Zhong 2010). Inoculation was performed using the single-spikelet inoculation method described by Stack et al. (2002). Briefly, 10 µl of spore suspension was injected into a central spikelet of a spike using a syringe at anthesis. In each pot, 8 to 10 spikes at a similar developmental stage were inoculated. Inoculated plants were placed in a room with a misting system (1 min of misting in every half hour) to facilitate disease development. After 48 h of incubation, the plants were moved back to the greenhouse benches. In the field experiment, each line or genotype was planted in a hill plot with 8 to 10 seeds. At anthesis, at least eight spikes at a similar developmental stage in a hill were inoculated using the single-spikelet inoculation method, as described above. The nursery was misted for 5 min in 15-min intervals for 12 h daily (4:00 P.M. to 4:00 A.M.), until 14 days after anthesis of the latest maturing lines.

For both greenhouse and field experiments, disease ratings were conducted at 21 days postinoculation. The percentage of infected spikelets on each head was recorded as FHB severity using a 1-to-9 scale described by Stack and McMullen (1995) to reflect 0, 7, 14, 21, 33, 50, 67, 80, and 100% of symptomatic spikelets in an inoculated spike, respectively. The disease severity for each replicate (pot or hill) was calculated by averaging the severities of all heads.

The content of deoxynivalenol (DON) produced by *F. graminearum* in grains was determined in two greenhouse experiments (13GH2 and 14GH), designated as 13GH2-DON and 14GH-DON, respectively. To prepare the seed samples used for the DON test, all of the inoculated spikes of each line were harvested at maturity and threshed carefully to keep scabby seed. The threshed kernels of all three replicates from each line were combined, ground into powder, and sent to the Veterinary Diagnostic Laboratory of NDSU (Fargo) for DON analysis.

Genotyping and marker development. Genomic DNA was extracted from fresh leaf tissues of the parents and RIL population (F_{6,7}) using the method of Tai and Tanksley (1990) except that the leaf tissue was macerated with TissueLyser (Qiagen). DNA was diluted to approximately 50 ng/µl and used for genotyping with the wheat 90K iSelect assay (Wang et al. 2014). Genotypic clusters for every single-nucleotide polymorphism (SNP) were determined using the manual option of GenomeStudio (version 2011.1) with the polyploid clustering module v1.0 (Illumina), on the basis of the data from all of the described genotypes. DNA markers (*UMN10*, *Xgwm493*, *Xgwm533*, and *Xgwm644*) linked to *Fhb1* or *Fhb2* (Liu et al. 2008; Röder et al. 1998) were also used to genotype the BN population. Additional markers were developed for *Fhb2* using the six candidate genes (GENE1, GENE2, GENE3, GENE4, GENE5, and GENE6) for 4-coumarate CoA ligase, basic helix-loop-helix transcription factor, glutathione S-transferase (GST), ABC transporter-4, callose synthase, and cinnamyl alcohol dehydrogenase, respectively (Dhokane et al. 2016). The gene sequences were retrieved from wheat survey sequence annotation browser on the website of URGI (https://urgi.versailles.inra.fr/gb2/gbrowse/wheat_survey_sequence_annotation/) and used for designing primer pairs using Primer3 (<http://bioinfo.ut.ee/primer3-0.4.0/primer3/>). These primer pairs were used in polymerase chain reaction (PCR) to amplify DNA sequences from the two parental genotypes (ND2710 and Bobwhite). PCR products amplified from two parental genotypes were sequenced by Genescript (Piscataway, NJ). Sequences from the two parental wheat genotypes were aligned against each other to identify single nucleotide polymorphism (SNP), which were transformed into cleaved amplified polymorphic sequence (CAPS) markers. These markers, together with the SNP showing polymorphism between two parents identified from 90K iSelect assay, were used to genotype the mapping population.

Statistical analysis, linkage map construction, and QTL analysis. For testing distribution of disease severity and DON content in the BN population, the Shapiro-Wilk normality test was performed using PROC UNIVARIATE (SAS Institute 2011). Levene's test (Levene 1960) under the general linear model (GLM) procedure was used to test homogeneity of disease severity and DON content variances among all experiments using the SAS program, version 9.3 (SAS Institute 2011). Correlation coefficients between disease severity and DON content were calculated using the PROC CORR procedure (SAS Institute 2011). Broad-sense heritability for each trait was calculated using the restricted maximum-likelihood method in SAS (Holland et al. 2003). Heritability coefficients for single-season data were estimated using the equation $H = \delta_G^2 / (\delta_G^2 + \delta_E^2/r)$ and, for multiple seasons, the equation $H = \delta_G^2 / (\delta_G^2 + \delta_{GY}^2/y + \delta_E^2/\text{year})$ was used, where δ_G^2 = genotypic variation, δ_{GY}^2 = genotype-year interaction variance, δ_E^2 = residual variance, y = number of years, and r = number of replications.

A genetic linkage map was constructed using the computer program MapDisto (v1.7.7) (Lorieux 2012). The Kosambi mapping function was used and the threshold value of logarithm of odds

(LOD) score was set at 3.0 to claim linkage between markers, with a maximum fraction of recombination at 0.30.

The entire marker dataset was used to identify genomic regions associated with FHB resistance in the RIL population. According to the results of Levene's test, six phenotypic datasets were separately analyzed for QTL detection. Composite interval mapping was used to identify significant QTL using software QGene, v.4.3.10 (Joehanes and Nelson 2008). A QTL was claimed to be significant when a LOD value is equal or larger than 3.0. The percentage of phenotypic variance explained by a QTL and its additive effect were calculated. For the purpose of presentation, linkage groups possessing significant QTL were reevaluated using a set of nonredundant markers to generate the QTL figures. The results were

confirmed to be identical to the initial results with the entire marker dataset.

To determine whether resistance QTL, environment, and interactions between them affect FHB disease severity, genotypic data of markers at each QTL peak along with disease severity data of the BN population collected from each environment were used for variance analysis through the GLM procedure in SAS, with the environment set as the random factor. The RILs in the BN population were divided into five groups according to the genotypes of marker loci associated with QTL detected, and Fisher's least significant difference (LSD) test was used to determine significant differences in mean FHB severities among different groups.

Fig. 1. Frequency distribution of 233 recombinant inbred lines derived from the Bobwhite × ND2710 cross for Fusarium head blight (FHB) disease severity in the four environments. 13GH1, 13GH2, and 14GH represent the greenhouse experiments performed in spring and winter 2013 and spring 2014, respectively, while 14FAR indicates field experiment performed in the FHB nursery at Fargo, ND in 2014. Arrows indicate the disease severities of parents. Normality test was performed using the PROC UNIVARIATE procedure and Shapiro-Wilk test (SAS Institute 2011).

Fig. 2. Frequency distribution of 233 recombinant inbred lines derived from the cross of Bobwhite × ND2710 for deoxynivalenol (DON) content in two greenhouse experiments. 13GH2-DON and 14GH-DON represent DON tests from the experiments 13GH2 and 14GH, respectively. The DON content of parents were indicated by arrows. Normality test was performed using the PROC UNIVARIATE procedure and Shapiro-Wilk test (SAS Institute 2011).

RESULTS

FHB phenotyping across different environments. The 233 RILs in the BN population and their parents Bobwhite and ND2710 had variable reactions to FHB infection in different experiments (Fig. 1). The disease severities in 13GH2 were much higher than those in other experiments. In the greenhouse experiments, Bobwhite had disease severities of 91.4% in 13GH1, 74.2% in 13GH2, and 62.5% in 14GH, with an average of 76.0%, whereas ND2710 had disease severities of 7.8% in 13GH1, 12.5% in 13GH2, and 9.7% in 14GH, with an average of 10.0% (Fig. 1). In the field nursery (14FAR), the average disease severities for Bobwhite and ND2710 were 51.8 and 10.9%, respectively (Fig. 1). The distribution of disease severity of the BN population varied among different environments as well. The average disease severity for the population was 30.4, 49.9, 32.4, and 25.1% in the experiments 13GH1, 13GH2, 14GH, and 14FAR, respectively (Fig. 1). Over 80% of the RILs had a disease severity of 50% or less in all experiments, except for 13GH2, with 50% of lines having a disease severity of 50% or less (Fig. 1). DON tests (13GH2-DON and 14GH-DON) performed in the two greenhouse experiments (13GH2 and 14GH) indicated that ND2710 had a much lower DON content than Bobwhite and the RILs showed distribution patterns similar to those for the disease severity (Fig. 2).

The normality test revealed that the distribution of disease severity and DON concentration in the RIL population was significant in all experiments except for 13GH2 (Fig. 1), suggesting that the disease severity of the population in most of the FHB inoculation experiments deviated from a normal distribution. Thus, Levene's test (Levene 1960), which was less sensitive to nonnormal distribution, was used to test homogeneity of disease severity variances across all experiments. The results showed that the variances among the three greenhouse experiments and the field nursery were heterogeneous ($P < 0.01$, $df = 3$), and variances among three greenhouse experiments were also heterogeneous ($P < 0.01$, $df = 2$). Only the variance between 13GH1 and 14GH was homogeneous ($P = 0.36$, $df = 1$). The variance between the two DON tests for greenhouse experiments was heterogeneous. There was a

low to moderate correlation between measured FHB traits among environments, ranging from 0.38 to 0.76 (Table 1). The correlation coefficient was 0.67 between disease severity of 13GH2 and 13GH2-DON and 0.76 between disease severity of 14GH and 14GH-DON, each being highly significant ($P < 0.0001$), suggesting that RILs with a low disease severity usually had a low DON concentration in the greenhouse.

The heritability for disease severity and DON concentration were calculated. Heritability for disease severity was high, ranging from 0.70 to 0.99 for four experiments, with an overall mean of 0.74, whereas it was low for DON concentration, with a value of 0.30.

Linkage map construction. In total, 7,413 polymorphic SNP markers were identified in the BN population using the 90K iSelect assays. After removal of cosegregating markers, 1,370 unique SNP markers were used for further genetic analysis. In addition, three simple-sequence repeat (SSR) markers (*Xumn10*, *Xgwm493*, and *Xgwm533*) associated with *Fhb1*, one SSR marker (*Xgwm644*) linked to *Fhb2*, and two CAPS markers (*Fhb2-CAPS3* and *Fhb2-CAPS6*) developed from two candidate genes (GENE3 and GENE6) for *Fhb2* were used to genotype the mapping population. With a total of 1,376 markers, a genetic linkage map was developed, which consisted of 35 linkage groups. In all, 8 of the linkage groups were assigned to genome A, 11 to genome B, and 16 to genome D (Supplementary Table S1). The total genetic map length was 914.98 centimorgans (cM), partitioned into 385.43 cM for genome A, 384.74 cM for genome B, and 144.82 cM for genome D. Average distance between markers was 0.66 cM.

QTL analysis for FHB resistance and DON content. QTL analysis of six datasets (13GH1, 13GH2, 14GH, 14FAR, 13GH2-DON, and 14GH-DON) led to identification of four ND2710-derived QTL for FHB resistance on chromosome 3B, 6B, 2A, and 6A, respectively (Table 2). *Qfhb.ndwp-3B* peaked at marker *SNP4343* was detected from all datasets, explaining 5 to 20% of the phenotypic variation, with an LOD value between 2.8 and 11.5 (Fig. 3; Table 2). *Qfhb.ndwp-6B* peaked at marker *Fhb2-CAPS3* was detected from 13GH1, 14GH, and 14GH-DON, explaining 6, 12, and 7% of the phenotypic variation, respectively. *Qfhb.ndwp-2A* peaked at *SNP79083* was only detected from the field experiment (14FAR), explaining 6% of phenotypic variation. *Qfhb.ndwp-6A* peaked at marker *SNP74620* was detected from 13GH2 and 13GH2-DON, explaining 5 and 8% of phenotypic variation, respectively. The QTL for resistance to FHB and DON accumulation on 3BS, 6BS, and 6AL were coincident (Fig. 3; Table 2).

Effects of QTL on type II resistance and DON accumulation. Under greenhouse (13GH1, 13GH2, and 14GH) and field (14FAR) conditions, the average disease severity of the RILs carrying the ND2710 alleles at *SNP4343* on 3B and *Fhb2-CAPS3* on 6B were 17.4 and 19.6%, respectively, while the average disease severity of the RILs carrying the Bobwhite alleles were 45.4 and 30.2%, respectively. In the two DON tests, the average DON content for RILs carrying ND2710 alleles at *SNP4343* on 3B and *Fhb2-CAPS3* on 6B were 2.3 and 3.7 ppm, respectively, while the average DON

TABLE 1. Correlation coefficients among Fusarium head blight traits from field and greenhouse environments of the Bobwhite × ND2710 population^a

Experiment	13GH1	13GH2	14GH	14FAR	13GH2-DON	14GH-DON
13GH	...	...	...	...	...	...
13GH2	0.64***	...	...	...	...	...
14GH	0.68***	0.67***	...	...	...	...
14FAR	0.43***	0.54***	0.45***	...	...	...
13GH2-DON	0.47***	0.67***	0.49***	0.38***	...	...
14GH-DON	0.55***	0.49***	0.76***	0.43***	0.44***	...

^a Asterisks (***) indicate significance at $P < 0.001$. GH = greenhouse, FAR = field, and DON = deoxynivalenol.

TABLE 2. Quantitative trait loci associated with Fusarium head blight (FHB) resistance in the Bobwhite × ND2710 population^a

Experiment ^b	<i>Qfhb.ndwp-3B</i>			<i>Qfhb.ndwp-6B</i>			<i>Qfhb.ndwp-2A</i>			<i>Qfhb.ndwp-6A</i>		
	LOD	R ²	Add	LOD	R ²	Add	LOD	R ²	Add	LOD	R ²	Add
13GH1	6.3	0.12	-7.2	3.3	0.06	-5.7	...	...	...	...	...	...
13GH2	11.5	0.20	-7.3	...	...	...	...	...	...	2.7	0.05	-3.9
14GH	9.8	0.18	-8.2	6.3	0.12	-7.1	...	...	...	...	...	...
14FAR	4.9	0.09	-4.0	...	...	...	3.3	0.06	-3.5	...	...	...
13GH2-DON	3.9	0.07	-0.5	...	...	...	...	...	...	4.1	0.08	-0.5
14GH-DON	2.8	0.05	-3.3	3.9	0.07	-4.1	...	...	...	...	...	...

^a The critical logarithm of odds (LOD) threshold of 3.0 for the 0.05 level of probability was obtained through a permutation test with 1,000 iterations. Add = additive effect. A negative value indicates an increasing allele for disease severity derived from Bobwhite; then, the resistance effects were derived from ND2710.

^b 13GH1, 13GH2, and 14GH were experiments conducted in the greenhouse in spring and winter 2013 and spring 2014, respectively; 14FAR was the experiment conducted in the field FHB nursery at Fargo, ND in 2014; and 13GH2-DON and 14GH-DON represent the content of deoxynivalenol (DON) produced by *Fusarium graminearum* calculated from two greenhouse experiments 13GH2 and 14GH.

content of RILs carrying the Bobwhite alleles were 3.7 and 17.1 ppm, respectively. For the other two markers on 2A and 6A, the average disease severity of the RILs with ND2710 alleles in greenhouse and field experiments were 27.9 and 20.3%, compared with 34.9 and 30.1% for those carrying the corresponding Bobwhite alleles. The average DON contents of the RILs with ND2710 alleles were 2.4 and 6.8 ppm, compared with 3.9 and 13.1 ppm for those with the Bobwhite alleles (Fig. 4). The lower average disease severity and DON content in RILs with ND2710 alleles than in those with Bobwhite alleles confirmed that all favorable alleles for FHB resistance were contributed by ND2710. *Qfhb.ndwp-3B* contributed the largest effect on FHB resistance, followed by *Qfhb.ndwp-6B*.

To elucidate the effect of single and combined QTL on FHB response, the RILs were divided into five groups: group 1 (G1 = 16 RILs) carried the ND2710 alleles at the peak marker loci associated with all four QTL detected, group 2 (G2 = 7 RILs) contained the

ND2710 alleles associated with *Qfhb.ndwp-3B* and *Qfhb.ndwp-6B*, group 3 (G3 = 16 RILs) carried the ND2710 allele associated with *Qfhb.ndwp-3B* only, group 4 (G4 = 8 RILs) carried the ND2710 allele associated with *Qfhb.ndwp-6B* only, group 5 (G5 = 17 RILs) carried the ND2710 alleles associated with the two minor QTL *Qfhb.ndwp-2A* and *Qfhb.ndwp-6A*, and group 6 (G6 = 15 RILs) carried the Bobwhite alleles for all four QTL. For greenhouse experiments (GH_MEAN), the average disease severities of G1 and G2 were significantly lower (LSD, $\alpha = 0.05$) than those of G4, G5, and G6, while there were no significant differences among G1, G2, and G3 (Fig. 4). G6 showed significantly higher disease severity than the other groups. For the field experiment, G1 and G2 showed significantly lower disease severity than G4 and G6, but there were no significant differences either between G1 and G2, or between G4 and G6. G3 and G5 showed a similar level of disease severity. For the DON tests, G1 and G2 had a significantly lower DON content than G6 but the differences among G1, G2, and G3

Fig. 3. Regions of linkage maps for chromosome 3B, 6B, 2A, and 6A harboring quantitative trait loci (QTL) for Fusarium head blight resistance detected in the Bobwhite $\times$ ND2710 (BN) population. All QTL were derived from the resistant parent ND2710. The centimorgan distances between marker loci are indicated on the left sides, while the positions of marker loci are marked on the right sides of the linkage maps. The logarithm of the odds significance threshold of 3.0 is represented by a vertical dotted line. Different lines indicate greenhouse seasons (13GH2, 13GH1 and 14GH), the field experiment (14FAR), and greenhouse experiments that determined content of deoxynivalenol (DON) (13GH2-DON and 14GH-DON).

Fig. 4. Effect of quantitative trait loci (QTL) on Fusarium head blight (FHB) severity and deoxynivalenol (DON) content. To elucidate the effect of single and combined QTL on FHB response, the recombinant inbred lines (RILs) were divided into six groups: group 1 (G1) contained 16 RILs with the ND2710 alleles associated with all four QTL, group 2 (G2) contained 7 RILs carrying the ND2710 alleles associated with the 3B and 6B QTL only, group 3 (G3) contained 16 RILs carrying the ND2710 allele associated with the 3B QTL, group 4 (G4) contained 8 RILs carrying the ND2710 allele associated with 6B, group 5 (G5) contained 17 RILs harboring the two minor QTL on 2A and 6A, and group 6 (G6) contained 15 RILs carrying the Bobwhite alleles for all four QTL. GH-Mean = mean FHB severity of each group across the greenhouse experiments, 14FAR = mean FHB severity of each group in the field experiment in the Fargo location, 13GH2-DON = mean DON content of each group from the experiment 13GH2, and 14GH-DON = mean DON content of each group from the experiment 14GH. Fisher's least significant difference test was used to determine significant differences among means of different groups. The same letters above the histogram indicate no significant difference at the 0.05 level of probability.

were not significant whereas the difference between G5 and G6 was significant.

DISCUSSION

In this study, we genotyped and phenotyped the RIL population derived from the cross between ND2710 and Bobwhite, and identified four QTL for FHB resistance contributed by the hard-red spring wheat line ND2710. These QTL were mapped to chromosomes 3BS, 6BS, 2AS, and 6AL and saturated with SNP markers, which will be useful for MAS of the FHB resistance QTL from ND2710.

The QTL on 3BS and 6BS in ND2710 were mapped to the same genomic regions as *Fhb1* and *Fhb2*, respectively. Considering the fact that ND2710 was a progeny of crosses with Sumai 3 in the pedigree (Frohberg et al. 2004) and SSR markers for *Fhb1* and *Fhb2* mapped to the QTL regions in the BN population, the high level of FHB resistance in ND2710 was mainly due to these two QTL derived from Sumai 3, although the other two minor QTL may have some effect on the resistance as well. *Fhb1* and *Fhb2* were first reported in Sumai 3 (Waldron et al. 1999) and Ning 7840 (Bai et al. 1999), and also detected in many different wheat genotypes related to Sumai 3 (Buerstmayr et al. 2009; Liu et al. 2009). Due to the large effect on FHB resistance, *Fhb1* was fine mapped as a single Mendelian gene within a 1.2-cM interval (Cuthbert et al. 2006). Liu et al. (2008) developed an SSR marker (*Xumn10*), which has been widely used to predict the presence of *Fhb1* in various genetic studies and in breeding programs. Recently, genome sequencing, gene annotation, and function characterization of the *Fhb1* region have led to identification and cloning of the candidate gene responsible for the FHB resistance (Rawat et al. 2016; Schweiger et al. 2016). These studies provide valuable resources to further understand the mechanism of the FHB resistance gene and accelerate its application in wheat breeding programs.

Among the six candidate genes identified by Dhokane et al. (2016) for *Fhb2*, two markers (*Fhb2-CAPS3* and *Fhb2-CAPS6*) were developed and mapped to the *Qfhb.ndwp-6B* region in the population derived from the cross between Bobwhite and ND2710. Interestingly, the marker *Fhb2-CAPS3* developed from the gene for GST is localized at the peak of the *Fhb2*-QTL region. It should be a useful additional marker for MAS of the *Fhb2* QTL in wheat breeding programs. GST catalyze the conjugation of electrophilic molecules to glutathione and have been implicated for detoxification of DON in barley (Gardiner et al. 2010). Several other studies also indicated that GST genes play a role in resistance to fungal plant pathogens (Dean et al. 2003, 2005; Han et al. 2016). Dhokane et al. (2016) speculated that GST is involved in FHB resistance by detoxification of DON and reducing the pathogenicity of the pathogen. However, further studies are needed to characterize the function of GST in FHB resistance and to clone and characterize the candidate genes for *Fhb2*.

The QTL (*Qfhb.ndwp-2A*) on 2A was only detected in the field inoculation experiment, whereas *Qfhb.ndwp-6A* was detected from one greenhouse experiment and the corresponding DON test. They are considered minor QTL, which are inconsistent among different environmental conditions. However, these minor QTL are still important for further improvement of FHB resistance in wheat cultivars because commercially released spring wheat cultivars with *Fhb1* alone or even with *Fhb1* and *Fhb2* combined did not have the same high and stable level of FHB resistance as ND2710 (Anderson et al. 2011; Frohberg et al. 2006). QTL for FHB resistance on chromosome 2A have been reported in several sources; they were associated with either type II resistance or DON content reduction and explained 3 to 26.7% of the phenotypic variation in different experiments (Anderson et al. 2001; Gervais et al. 2003; Paillard et al. 2004; Steiner et al. 2004; Waldron et al. 1999; Zhou et al. 2002). *Qfhb.ndwp-2A* in ND2710 peaked at 41.7 cM on the genetic map of this study, which is approximately at 38.7 cM on the 9K consensus SNP

map (Cavanagh et al. 2013); thus, this QTL may be the same as the 2A QTL identified from winter wheat cultivar Freedom, with confidence intervals (CI) between 39.5 and 43.6 cM (Gupta et al. 2001), and the 2A QTL from Chinese spring wheat cultivar Ning7840, with CI between 29.1 and 53.6 cM (Zhou et al. 2002). All three QTL previously identified on 2A condition type II resistance.

FHB resistance QTL were previously identified on chromosome 6AS from the U.S. wheat line ND2603 (Anderson et al. 2001), French bread wheat cultivar Apache (Holzapfel et al. 2008), German winter wheat cultivar Dream (Häberle et al. 2007; Schmolke et al. 2005), and U.K. winter wheat cultivar Spark (Gosman et al. 2007). These reported 6AS QTL condition type II resistance and explained 7.8 to 19% of phenotypic variation. ND2710 has a pedigree with ND2603 but no QTL on 6AS was identified in this study; instead, a QTL on 6AL was detected from dataset 13GH2 and the corresponding DON test 13GH2-DON. The 6AL QTL may be novel.

Over 200 QTL for FHB resistance have been identified from different resistance sources worldwide and they confer one or more of type I, II, III, and IV resistance to FHB (Buerstmayr et al. 2009). However, most of the QTL are responsible for one type of resistance, whereas some of them confer more than one type of resistance (Buerstmayr et al. 2009; Liu et al. 2009). For example, QTL identified from Sumai3, CJ9306, Wangshuibai, Ernie, and Arina condition both type II and III resistance (Abate et al. 2008; Draeger et al. 2007; Handa et al. 2008; Jiang et al. 2007a,b; Yu et al. 2008). In the present study, *Qfhb.ndwp-2A* conditioned type II resistance, while the QTL on chromosome 3B, 6A, and 6B were associated with both type II and II resistances to FHB and DON accumulation. These results are in line with previous reports showing that QTL identified in various sources may contribute to one or more of the four currently recognized types of FHB resistance (Buerstmayr et al. 2009).

In summary, our data showed that FHB resistance in ND2710 was contributed by a combination of four QTL. Three of them were reported previously in different germplasms whereas one QTL was identified to be novel. The effects of these QTL were additive. The QTL on chromosome 3B showed a major effect on FHB resistance and DON reduction and was consistently detected in multiple experiments. The RILs combining ND2710 alleles at the QTL on 3B and 6B had significantly lower disease severity and DON content than RILs without these alleles in both greenhouse and field experiments. ND2710 had the same high level of FHB resistance as Sumai 3 but its agronomic traits were much closer to those of North Dakota varieties (Frohberg et al. 2004). It has been a very useful source for improving wheat FHB resistance, especially in the Northern Great Plains (del Blanco et al. 2003) (unpublished data). The identified DNA markers associated with the four QTL will further facilitate the selection and use of FHB resistance from ND2710 in breeding programs.

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