Validation of Molecular Markers for Use With Adapted Sources of Fusarium Head Blight Resistance in Wheat

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Abstract

Genetic control of resistance to Fusarium head blight (FHB) is quantitative, making phenotypic selection difficult. Genetic markers to resistance are helpful to select favorable genotypes. This study was conducted to determine if *Fhb1* and *Fhb5* present in the Sumai 3 source of FHB resistance occur in Sumai 3-derived North American spring wheat cultivars and to understand the appropriateness of using markers to select for the favorable alleles at these loci in breeding. Sumai 3-derived parents Alsen, ND3085, ND744, Carberry, and Glenn were used in crosses to generate 14 doubled haploid breeding populations. The parents and progeny were genotyped with five *Fhb1* and three *Fhb5* microsatellite markers. Progeny were selected based on performance relative to parents and other control cultivars in FHB nurseries near Portage la Prairie and Carman, MB. χ^2 and *t* test analyses were performed on marker and FHB data. The χ^2

test frequently determined the proportion of lines carrying molecular variants associated with FHB resistance increased following nursery selection for FHB. Similarly, the *t* test regularly demonstrated that selection for FHB resistance lowered the mean level of disease associated with resistant marker haplotypes. The study affirmed FHB resistance sources Alsen, Carberry, ND3085, and ND744 have *Fhb1* and *Fhb5* loci like Sumai 3, but no evidence was found that Glenn carries *Fhb1* and *Fhb5* resistance alleles. The results justified use of *Fhb1* and *Fhb5* markers for marker assisted selection in populations derived from Alsen, Carberry, ND3085, and ND744, but not Glenn. Combined or individual application of *Xgwm493* and *Xgwm533* in selection of genotypes carrying *Fhb1*, and *Xgwm150*, *Xgwm304*, and *Xgwm595* for *Fhb5* will enhance FHB resistance in wheat.

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe (telomorph: *Gibberella zeae* Schw. [Petch]), is a destructive disease resulting in serious losses of grain yield and quality in wheat (McMullen et al. 1997). Yield reduction results from the pathogen interfering with seed development, causing Fusarium damaged kernels (FDK). The pathogen contaminates the grain with mycotoxins (deoxynivalenol [DON]), making grain unsafe for use as food and feed (Snijders 1990; Somers et al. 2003). The deployment of resistant varieties is the most effective, economical, and environmentally safe way to control FHB in wheat (Mergoum et al. 2007).

The inheritance of the expression of resistance in wheat to FHB is polygenic, with quantitative trait loci (QTL) reported on all wheat chromosomes except 7D (Buerstmayr et al. 2009). Several sources of FHB resistance are known, but few are as resistant as the Chinese cultivar Sumai 3 (Rudd et al. 2001). In Sumai 3, major FHB resistance QTL are *Fhb1* (*Qfhs.ndsu-3BS*) on chromosome 3BS (Anderson et al. 2001; Waldron et al. 1999) and *Fhb5* (*Qfhs.ifa-5A*) on 5A (Buerstmayr et al. 2003). Other QTL occur on chromosomes 6BS (*Fhb2*: Cuthbert et al. 2007), 3AL, 6AS (Anderson et al. 2001), 2B, and 7A (Zhou et al. 2002).

The expression of a single locus itself can be complex such as with the Sumai 3 QTL on 3BS, which demonstrates resistance to initial infection, restriction of hyphal spread, and reduction of FDK and DON (McCartney et al. 2007). When exotic parents are used in breeding, desirable alleles for traits such as FHB resistance may be linked to undesirable alleles of other traits, in which case some of the resistance may be lost due to selection against the undesirable traits. Conversely, selection with markers for a QTL done to enrich the allele

frequency of FHB resistance may inadvertently enrich detrimental linked alleles.

Selecting for favorable linkages between agronomic traits and resistance to FHB is a challenging task. Yet, several cultivars have been developed with improved resistance to FHB that are adapted to the Northern American Great Plain region. Alsen (Frohberg et al. 2006), Carberry (DePauw et al. 2011), ND3085 (International Wheat Information System [IWIS]), ND744 (Mergoum et al. 2005), and Glenn (Mergoum et al. 2006) are a few adapted cultivars with improved FHB resistance relative to contemporaries. Alsen and ND3085 share an FHB resistant parent ND2710, which was developed from a cross involving Sumai 3 (Frohberg et al. 2004). FHB resistance in Carberry derives from Sumai 3 through Alsen (DePauw et al. 2011). However, it has been difficult to reconstitute the full resistance of cultivars such as Sumai 3. This presents a dilemma for marker assisted breeding. The example of Sumai 3 and Carberry can help us to understand the problem. Derived cultivars such as Carberry, which have improved resistance to FHB but are not as resistant as Sumai 3, presumably have not acquired all of the resistance alleles that Sumai 3 possesses. As derived cultivars are used in breeding programs, from the perspective of marker assisted breeding, the question becomes which loci with resistance alleles were acquired by cultivars such as Carberry. Many markers have been developed for Sumai 3 resistance, but without knowledge of which FHB resistance loci were transferred to Carberry, the breeder cannot know which markers to apply. Because FHB resistance is a quantitative trait, various combinations of favorable alleles may provide similar or the same phenotype. Given QTL markers are only associated with the gene, crossing over between markers and the gene can occur, therefore the haplotype of Sumai 3 is not an absolute in knowing whether or not a resistant allele is present in a Sumai 3-derived cultivar.

The complexity of FHB resistance not only presents challenges in reconstituting the resistance during cultivar improvement, it presents challenges in DNA marker development and application of markers to breeding. In the implementation of markers for marker assisted selection, validation of the markers in the targeted gene pool takes into account such factors as: linkage drag, pleiotropy, background effects,

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proximity of the markers to the gene, transferability of the markers across laboratories, and polymorphism among potential parents (Knox and Clarke 2007). Validation of markers is important for determining the potential to select for FHB resistance while not diminishing selection of desirable traits.

This study was conducted to determine if *Fhb1* and *Fhb5* present in Sumai 3 occur in Sumai 3-derived North American spring wheat cultivars that are being used as sources of FHB resistance and to understand the appropriateness of using markers to select for the favorable alleles at these loci in breeding. *Fhb1* and *Fhb5* marker-cultivar combinations were validated through the actual process of breeding and selection in populations derived from Alsen, Carberry, ND3085, ND744, and Glenn.

Materials and Methods

Plant materials. Fourteen wheat (*Triticum aestivum* L.) doubled haploid (DH) populations, targeting the Canada Western Red Spring (CWRS) market class, used for this study are listed in Table 1 by the pedigree of the parents. For example, Alsen/Superb represents the population derived from a cross of Alsen as female and Superb as male parents. The populations were developed using the maize pollen method (Knox et al. 2000) on the F₁ plants of the wheat crosses shown in Table 1. The population parents Alsen (Frohberg et al. 2006), Carberry (DePauw et al. 2011), Glenn (Mergoum et al. 2006), ND3085, and ND744 (Mergoum et al. 2005) have Sumai 3 in their genealogy (IWIS), while the other parents were Canadian CWRS cultivars or breeding lines. The populations were originally produced for evaluation within the breeding program of the Swift Current Research and Development Centre (SCRDC). First generation double haploid (D₁) lines of the 14 populations except Alsen/Waskada and Alsen/Helios were first grown near Swift Current, SK, and the D₂ lines underwent seed multiplication in New Zealand the following winter. The D₁ lines of Alsen/Waskada and Alsen/Helios were multiplied in New Zealand only. The seeds of the lines multiplied were grown in FHB nurseries near Portage la Prairie (PLP) and Carman, MB.

Field evaluation. Table 1 shows the progress of the DH populations through PLP and Carman nurseries for field evaluation. The lines of populations AC Barrie/Alsen, BW796/ND3085, BW796/ND744, Infinity/ND3085, Infinity/ND744, Alsen/Helios, Infinity/Glenn, Somerset/Glenn, BW796/Alsen, and Carberry/AC Cadillac were grown in an FHB inoculated nursery near PLP, MB. The primary purpose was to select lines with improved response to FHB relative to control cultivars while also enriching the populations for other disease and agronomic traits. Within the nurseries, the population lines were selected that appeared within the range of FHB

response of intermediate resistant check cultivars AC Barrie and 5602HR to moderately resistant cultivars Alsen and ND2710 or better. In addition to FHB response, lines were selected that showed adequate to superior response for leaf rust, stem rust, leaf spot, and agronomic traits relative to maturity, plant height, lodging, and propensity for seed shelling (kernel exposure). The criteria were applied relative to the check cultivars AC Elsa (Clarke et al. 1997), AC Barrie (McCaig et al. 1996), Alsen (Frohberg et al. 2006), ND2710 (Frohberg et al. 2004), and 5602HR (Syngenta 2014) that were grown in the nurseries along with the populations. The phenological stage at the time of assessment for FHB was approximately Zadoks scale 83. The criterion for retaining lines on the basis of maturity was as early as or earlier than Alsen. Plant height and straw strength were selected within the range of Alsen to AC Barrie. Exposure of the kernel was similar to AC Barrie and less than Alsen. The lines were selected based on leaf rust resistance within the range of response of cultivars AC Elsa, Alsen, 5602HR, and ND2710 or better, and stem rust resistance within the range of response of cultivars AC Elsa, Alsen, and ND2010 or better. The leaf and stem rust races were the prevalent races of the region as described by DePauw et al. (2011). Selection against the worst leaf spot disease response was based on natural infection. Lines that were selected near PLP that simultaneously met criteria of resistance to FHB, other diseases, and the agronomic traits were simply documented as 'retain.'

Lines of the Alsen/Superb, Alsen/Prodigy, Alsen/Waskada, and Somerset/Alsen populations were not grown at PLP, but rather were rated for FHB response in an FHB nursery near Carman, MB (Table 1). Alsen/Helios, BW796/Alsen, and Carberry/AC Cadillac populations, in addition to field selection at PLP, were rated at Carman in the same year. Lines of populations AC Barrie/Alsen, BW796/ND744, BW796/ND3085, Infinity/ND3085, Infinity/ND744, and Alsen/Helios retained based on field selection at PLP were subsequently rated for response to FHB in the nursery near Carman. The Carberry/AC Cadillac was additionally rated at PLP in 2010 and 2011. Lines that met the criteria of resistance to FHB with an index of 41% or less depending on disease pressure based on the Carman results were retained.

At PLP, a six row cassette Row XL, Wintersteiger planter was used to plant 50 seeds per line in 1 m long rows with 30 cm between rows and 0.5 m between passes. The left row of each set of six rows was planted to either a control genotype or a disease spreader. The disease spreader was a mixture of equal proportions of seeds of wheat genotypes highly susceptible to FHB, leaf and stem rust. The other five rows were planted with experimental lines.

F. graminearum infested corn grain (FHB colonized corn kernels) was applied to the PLP nursery. The infested corn grain was prepared

Table 1. Doubled haploid populations^a and years of evaluation for Fusarium head blight response at Portage la Prairie (PLP) and Carman disease nurseries in Canada

Population ^b	Number of DH lines produced	Year 1 ^c	Location	Year 2	Location	Year 3	Location
AC Barrie/Alsen	1,635	2003	PLP	2004	Carman		
Alsen/Superb	701	2004	Carman				
Alsen/Prodigy	1,179	2004	Carman				
BW796/ND3085	816	2007	PLP	2008	Carman		
BW796/ND744	796	2007	PLP	2008	Carman		
Infinity/ND3085	790	2007	PLP	2008	Carman		
Infinity/ND744	422	2007	PLP	2008	Carman		
Alsen/Helios	710	2007	Carman, PLP	2008	Carman		
Alsen/Waskada	792	2008	Carman				
BW796/Alsen	658	2008	Carman, PLP				
Infinity/Glenn	415	2008	PLP				
Somerset/Glenn	717	2008	PLP				
Somerset/Alsen	631	2008	Carman				
Carberry/AC Cadillac	814	2009	Carman, PLP	2010	PLP	2011	PLP

^a For the numbers of lines evaluated in each population, see Table 4 for PLP and Table 5 for Carman.

^b Parent names provided according to cross rule.

^c Refers to the year in which a population was tested in respective locations.

by pouring dry corn into a tub then adding tap water to a level of 10 to 15 cm above the corn. The following morning (after 16 h of imbibition), the tubs were drained and covered with two layers of industrial strength aluminum foil beneath a plastic lid. The corn was autoclaved and allowed to cool overnight and the disinfested corn was removed the following day. Prior to corn preparation the four *F. graminearum* isolates M9-07-1 (3-ADON), M7-07-1 (3-ADON), M1-07-1 (15-ADON), and M3-07-2 (15-ADON) described by Gilbert and Woods (2006) were cultured on potato dextrose agar (PDA) plates. Isolates were subcultured to colonize the entire Petri dish by keeping them under fluorescent lighting at room temperature to get good culture growth. Using aseptic techniques in a laminar flow hood, three to five plates of the four *F. graminearum* isolate cultures were blended per tub of disinfested corn in 150 ml sterile water with 0.2 g of streptomycin sulfate. After inoculation, the corn medium was then recovered and allowed to incubate for 2 to 3 weeks. Following the incubation period of 2 weeks, the corn kernel inoculum was thinly spread in the greenhouse to dry for 3 to 8 days depending on the amount of corn, kernel water content, green house temperature, and humidity to allow fungal infestation. After the corn dried, it was packed in mesh bags and stored in a cold room at about 0°C until it was required.

The PLP nursery was infested by spreading the *F. graminearum*-colonized corn grain (Gilbert and Woods 2006) between wheat rows at a rate of 20 g m⁻². Infestation was done at the end of tillering of the wheat plants, which was approximately 2 to 3 weeks prior to flowering (Zadoks stage 58). An overhead low pressure irrigator applied about 12 mm of water three times per week to maintain high humidity that contributed to formation of dew on the spikes. Between 22 and 25 days after the majority of the lines had completed anthesis and a differential response to FHB was expressed in the controls, assessment of the lines relative to the controls was performed. AC Elsa, distributed throughout the nursery, was used as the susceptible check to monitor the quality of the inoculum and the level of expression of disease. Two additional assessments were made, with 2 to 3 days between assessments. Generally, lines of the populations grown near PLP were retained or discarded based on visual assessment of criteria described above. Lines which did not meet the selection criteria described above were noted and discarded from the further consideration.

The nursery at Carman consisted of 50 seeds planted in 1 m long rows, 30 cm apart. The same controls as used at PLP were grown and repeated in a manner similar to that at PLP. The inoculation of the Carman nursery entailed spraying (at 50% anthesis) a macroconidial suspension (Gilbert and Woods 2006) of an equal mixture of M9-07-1 (3-ADON), M7-07-1 (3-ADON), M1-07-1 (15-ADON), and M3-07-2 (15-ADON) (Gilbert et al. 2002) representing the

predominant chemotypes of *F. graminearum*. A CO₂ powered backpack sprayer calibrated at 2 kPa was used for spraying the inoculum at a rate of 50 ml per 1 m of row. Reinoculation of the same rows was performed 4 days after the first inoculation. The inoculum was a suspension of 50,000 conidia ml⁻¹ in water and 0.2 ml Tween 20 per 100 ml inoculum. The nurseries were mist irrigated the evening of, and the morning after each inoculation. Rating was done 18 to 21 days after the first inoculation. As with the PLP nurseries, AC Elsa, distributed throughout the nursery, was used as susceptible check to monitor the quality of the inoculum and the level of expression of disease. FHB incidence was assessed as number of spikes affected by FHB over the total number of spikes in the row multiplied by 100. Severity was the visual percentage estimate of necrotic spike tissue across symptomatic spikes in the row. A visual index (% incidence × % severity/100) was calculated (Gilbert and Woods 2006). The FHB data collected at Carman not only was used to make line selections within the populations but allowed single point quantitative trait marker locus analysis.

Genotyping. Table 2 shows molecular markers used in characterizing D₁ seed of lines of the DH populations, DNA fragment sizes, and polymorphism between population parents. Five microsatellite markers (*Xgwm389*, *Xgwm533*, *Xsts3B-163*, *Xsts3B-66*, and *Xgwm493*) associated with *Fhb1* (*Qfhs.ndsu-3BS*) and three markers (*Xwmc150*, *Xwm304*, and *Xgwm595*) associated with *Fhb5* (*Qfhs.ifa-5A*) (Anderson et al. 2001; Buerstmayr et al. 2003; Cuthbert et al. 2006) were tested on the parents. In some cases, no marker information was generated, for example if no amplification occurred in the parents. Polymorphic markers were applied to lines of the populations. Genotyping was performed prior to phenotypic selection in the Alsen/Waskada, BW796/Alsen, Somerset/Alsen, Alsen/Helios, BW796/ND3085, BW796/ND744, Infinity/ND3085, and Infinity/ND744 populations, whereas genotyping followed limited phenotypic selection for height, maturity, and straw strength in the Alsen/Superb, Alsen/Prodigy, AC Barrie/Alsen, Infinity/Glenn, Somerset/Glenn, and Carberry/AC Cadillac populations. Extraction, amplification, and visualization of DNA were performed as described by Knox et al. (2014).

Statistical analysis. Statistical analyses were performed using SAS program version 9.3 (SAS Institute, Cary, NC, U.S.A.). The χ^2 test to investigate the change in the allele frequencies from before selection to after selection of parental-type molecular variants was performed with SAS PROC FREQ on lines within each population and to determine if distortion from random segregation existed prior to selection for FHB resistance and other traits. Lines were classified as R (retained) and D (discarded) following PLP nursery selections (no data were collected) within the breeding program for FHB and other traits previously

Table 2. Polymorphism between doubled haploid population parents and DNA fragment sizes of microsatellite markers associated with 3BS (*Qfhs.ndsu-3BS*) and 5A (*Qfhs.ifa-5A*) Fusarium head blight resistance quantitative trait loci

Population ^a	Chromosome 3BS					Chromosome 5A		
	<i>Xgwm493</i>	<i>Xgwm533</i>	<i>Xsts3B-163</i>	<i>Xsts3B-66</i>	<i>Xgwm389</i>	<i>Xgwm304</i>	<i>Xgwm595</i>	<i>Xwmc150</i>
<i>Alsen/Superb</i>	Mono ^b	150/125 ^c	NA ^d	NA	Mono	225/200	NA	160/175
<i>Alsen/Prodigy</i>	200/150	150/125	NA	NA	150/175	225/200	NA	Mono
<i>AC Barrie/Alsen</i>	Mono	125/150	NA	NA	175/150	200/225	NA	175/160
<i>BW796/ND3085</i>	150/200	NA	474/476	NA	NA	NA	NA	NA
<i>BW796/ND744</i>	150/200	NA	474/476	NA	NA	NA	NA	NA
<i>Infinity/ND3085</i>	150/200	Mono	474/476	Mono	140/130	200/225	200/150	170/160
<i>Infinity/ND744</i>	150/200	125/150	474/476	Mono	140/130	200/225	200/150	NA
<i>Alsen/Waskada</i>	Mono	150/125	476/474	Mono	NA	NA	NA	NA
<i>Alsen/Helios</i>	Mono	150/125	476/474	175/180	130/140	225/200	150/200	NA
<i>Infinity/Glenn</i>	150/175	NA	NA	NA	NA	NA	NA	NA
<i>BW796/Alsen</i>	Mono	125/150	476/478	NA	NA	NA	NA	NA
<i>Somerset/Glenn</i>	150/175	Mono	NA	NA	NA	NA	NA	NA
<i>Somerset/Alsen</i>	Mono	125/150	476/478	NA	NA	NA	NA	NA
<i>Carberry/AC Cadillac</i>	NA	150/125	NA	NA	Mono	Mono	150/200	Mono

^a Italicized parental names have Sumai 3 in genealogy.

^b Mono indicates the marker was monomorphic between DH parents.

^c Parental type fragment size in base pairs paralleling the cross configuration of female/male parent.

^d NA, not available.

described. The lines were further classified as M+, having the parental molecular variant associated with the FHB resistant phenotype, and M–, having the parental molecular variant associated with the FHB susceptible phenotype. Within either the R-group or the D-group, the ratio of M+/M– after selection was expected not to change from the ratio before selection for populations with preselection data listed in Table 3. Therefore, the null hypothesis of no effect of selection for FHB resistance at each marker locus was tested against actual ratios of M+/M– prior to selection where the preselection marker data were available, and against a 1:1 random segregation ratio of the M+/M– where marker data prior to selection was not available. Mean FHB incidence, severity, and visual index of lines selected at the PLP nursery and subsequently evaluated at the Carman nursery were classified by marker molecular variant such that the RM+ group were compared with the RM– group using the Student *t* test.

Table 3. Marker, population, counts of lines with resistant (M+) and susceptible (M–) parent molecular variants, and χ^2 value and significance of deviations from a 1:1 segregation for eight doubled haploid populations tested with markers prior to phenotypic selection

Marker	Population	M+ count	M– count	χ^2 ^a
<i>Xsts3B-163</i>	Alsen/Waskada	402	459	3.77
	BW796/Alsen	306	345	2.34
	Somerset/Alsen	200	253	6.20*
<i>Xsts3B-66</i>	Alsen/Helios	312	375	5.78*
<i>Xgwm493</i>	BW796/ND3085	400	408	0.08
	BW796/ND744	391	402	0.15
	Infinity/ND3085	377	413	1.64
	Infinity/ND744	214	214	0.00

^a An asterisk (*) indicates significance at the $P \leq 0.05$ level.

χ^2 analysis was performed for each marker-population combination, but as well, classes were pooled for χ^2 analysis if populations utilized the same Sumai 3-derived parent or the markers were marking the same locus.

Results

Table 3 shows results of the χ^2 test on counts of D₁ lines for molecular variants M+/M– in populations tested with three *Fhb1* markers prior to selection. All but Infinity/ND744 had a deficit in M+. The χ^2 of lines in most crosses was not significantly different from a monogenic 1:1 random segregation between M+ and M–. Exceptions to the 1:1 segregation were Somerset/Alsen tested with *Xsts3B-163* and Alsen/Helios tested with *Xsts3B-66*.

Table 4 shows the χ^2 test on the frequency of lines retained or discarded after selection in the PLP nursery. Generally the line count for the M+ variant was higher than the count for the M– variant in the retained group. In contrast, the discarded group generally showed a lower count for the M+ variant and a higher count for M–. Significant deviations between M+ and M– were observed for populations derived from FHB resistance sources ND3085 and ND744 for 3BS marker *Xgwm493*, with M+ being favored in the retained category and M– being elevated in the discarded category. Only the retained group of lines from the Carberry/AC Cadillac population grown in 2010 showed a significant deviation in segregation for the 3BS marker *Xgwm533* with an elevated frequency of the M+ variant from the parent contributing resistance, Carberry. Pooled results of populations derived from ND3085 tested to *Xgwm493*, or pooled across populations for the marker *Xgwm493*, also showed a significant effect on line selection with M+ favored in the retained group and M– favored in the discarded group. However, the *Xgwm493* marker effect on line selection was not significant in the Glenn-derived populations. The effect of *Xsts3B-163* and *Xsts3B-66* markers on line

Table 4. Number of lines discarded or retained based on FHB incidence and severity of doubled haploid populations grown at Portage la Prairie, MB, in different years and subdivided by marker molecular variant M+ and M– are reported. χ^2 test results of deviation from a 1:1 ratio and actual segregation (see Table 3 for counts) of lines classified as individuals with *Fhb1* and *Fhb5* QTL molecular marker variants (M+) and without (M–) are provided for multiple markers.

Marker	Population	Year	Ratio	Discarded ^a			Retained ^a		
				M+	M–	χ ²	M+	M–	χ ²
Chromosome 3BS									
<i>Xgwm533</i>	AC Barrie/Alsen ^{b,h}	2003	1:1	7	7	0	3	3	0
	Carberry/AC Cadillac ^{b,h}	2009	1:1	59	71	1.1	50	38	1.6
	Carberry/AC Cadillac	2010	1:1	16	29	3.8	19	5	8.2**
	Carberry/AC Cadillac	2011	1:1	76	98	2.8	56	38	3.4
	<i>Xgwm533</i> (pooled) ^b		1:1	66	78	1.0	53	41	1.5
<i>Xsts3B-163</i>	BW796/Alsen ^{c,i}	2008	1:1.13	7	17	3.1	8	6	0.6
<i>Xsts3B-66</i>	Alsen/Helios ^{c,i}	2007	1:1.20	5	2	1.9	2	3	0.1
<i>Xgwm493</i>	BW796/ND3085 ^{d,g,i}	2007	1:1.02	59	97	8.5**	119	49	30.6***
	Infinity/ND3085 ^{d,g,i}	2007	1:1.10	45	81	7.3**	50	40	2.2
	ND3085 (pooled) ^d		1:1.06	104	178	15.5***	169	89	29.5***
	BW796/ND744 ^{g,i}	2007	1:1.03	27	75	21.3***	97	62	8.7**
	Infinity/ND744 ^{f,h}	2007	1:1	21	35	3.5	16	4	7.2**
	Infinity/Glenn ^{e,f,h}	2008	1:1	9	15	1.5	4	7	0.8
	Somerset/Glenn ^{e,f,h}	2008	1:1	25	29	0.3	16	13	0.3
	Glenn (pooled) ^e		1:1	34	44	1.3	20	20	0
	<i>Xgwm493</i> (pooled) ^f		1:1	55	79	4.3*	36	24	2.4
	<i>Xgwm493</i> (pooled) ^g		1:1.02	131	253	29.1***	266	151	42.4***
Alsen (3BS pooled) ^c			1:1.16	12	19	0.7	10	9	0.3
Chromosome 3BS (pooled) ^h			1:1	121	157	4.7*	89	65	3.7
Chromosome 3BS (pooled) ⁱ			1:1.09	143	272	29.5***	276	160	42.1***
Chromosome 5A									
<i>Xwmc150</i>	AC Barrie/Alsen ^j	2003	1:1	8	7	0.1	3	4	0.1
<i>Xgwm304</i>	AC Barrie/Alsen	2003	1:1	5	11	2.3	5	2	1.3
<i>Xgwm595</i>	Carberry/AC Cadillac ^j	2009	1:1	64	62	0.0	45	41	0.2
	Carberry/AC Cadillac	2010	1:1	47	58	1.2	69	39	8.3**
	Carberry/AC Cadillac	2011	1:1	22	30	1.2	13	9	0.7
Chromosome 5A (pooled) ^j			1:1	72	69	0.1	48	45	0.1

^a Frequency of lines carrying favorable and unfavorable marker variants of FHB resistance in the retained and discarded categories. Population names followed by letters in superscript (letters b to j) indicate which marker data were used in the combined χ^2 analysis and each letter designates which markers or marker-locus combinations were pooled. *, **, and *** indicate significance at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

selection was not significant in two Alsen-derived populations. Pooling results over all populations and 3BS markers showed M+ to be significantly higher than M- in the retained group and higher M- than M+ in the discarded group for data analyzed using actual segregation ratio before selection; and higher M- than M+ in the discarded group with a 1:1 segregation ratio.

A significant deviation from a 1:1 random segregation ratio between M+ and M- was observed for the population derived from the Carberry source of FHB resistance for the 5A marker *Xgwm595*, with M+ being favored over M- in the retained category, but deviation was not significant for the discarded group (Table 4). Markers

Xwmc150 and *Xgwm304* did not have a significant effect on selection of lines of the AC Barrie/Alsen population at PLP. Upon pooling results across populations for markers polymorphic for 5A, the effect was not significant in both the retained and discarded categories.

Table 5 shows the χ^2 test on the frequency of lines retained or discarded after selection in the Carman nursery. Of the populations Alsen/Superb, Alsen/Prodigy, Alsen/Waskada, Somerset/Alsen, BW796/Alsen, Alsen/Helios, and Carberry/AC Cadillac not previously selected at PLP, only Alsen/Helios and BW796/Alsen for the 3BS markers and Alsen/Superb for the 5A markers generated a segregation distortion at Carman.

Table 5. χ^2 test results of deviations from a 1:1 or actual segregation ratio of lines classified as individuals with (M+) and without (M-) *Fhb1* and *Fhb5* QTL molecular marker variants in the DH populations grown at Carman, MB in different years

				Discarded ^a			Retained ^a		
Marker	Population	Year	Ratio	M+	M−	χ ²	M+	M−	χ ²
Chromosome 3BS									
<i>Xgwm389</i>	Alsen/Prodigy ^{b,c,j}	2004	1:1	8	6	0.3	26	18	1.5
	AC Barrie/Alsen ^{b,c,j}	2004	1:1	4	8	1.3	23	18	0.6
	Alsen/Helios ^{b,c,j}	2007	1:1	19	26	1.1	19	15	0.5
	Alsen (pooled) ^b		1:1	31	40	1.1	68	51	2.4
	Infinity/ND744 ^{c,j}	2008	1:1	9	6	0.6	3	3	0
<i>Xgwm533</i>	<i>Xgwm389</i> (pooled) ^c		1:1	40	46	0.5	71	54	2.3
	AC Barrie/Alsen ^{d,e}	2004	1:1	1	9	6.4*	11	16	0.9
	Alsen/Helios ^{d,e}	2007	1:1	8	20	5.1*	10	7	0.5
	Alsen/Helios	2008	1:1	3	2	0.2	8	13	1.2
	Alsen (pooled) ^d		1:1	9	29	10.5*	21	23	0.1
	Carberry/AC Cadillac ^{e,j}	2009	1:1	19	19	0	13	8	1.2
	<i>Xgwm533</i> (pooled) ^e		1:1	28	48	5.3*	34	31	0.1
<i>Xsts3B-163</i>	Alsen/Waskada ^{f,k}	2008	1:1.14	12	19	0.8	21	24	0.0
	BW796/Alsen ^{f,k}	2008	1:1.13	22	52	8.9**	30	17	5.3*
	Somerset/Alsen ^{f,k}	2008	1:1.26	1	6	2.5	26	34	0.0
	<i>Xsts3B-163</i> / Alsen / (pooled) ^f		1:1.18	35	77	9.8***	77	75	1.3
<i>Xsts3B-66</i>	Alsen/Helios ^k	2007	1:1.2	23	23	0.4	19	15	1.5
	Alsen/Helios	2008	1:1.2	8	11	0.1	45	53	0.0
<i>Xgwm493</i>	Alsen/Prodigy ^h	2004	1:1	6	9	0.6	22	22	0.0
	BW796/ND3085 ^{g,i,j,k}	2008	1:1.02	26	14	3.8*	41	7	24.8***
	Infinity/ND3085 ^g	2008	1:1.10	7	8	0.2	13	6	2.0
	ND3085 (pooled) ^g		1:1.06	33	22	1.8	54	13	23.6***
	BW796/ND744 ^{i,k}	2008	1:1.03	3	6	0.9	13	0	13.4***
	Infinity/ND744 ^h	2008	1:1	13	6	2.6	2	3	0.2
	<i>Xgwm493</i> (pooled) ^h		1:1	19	15	0.5	24	25	0.0
	<i>Xgwm493</i> (pooled) ⁱ		1:1.03	29	20	1.9	54	7	37.4***
Chromosome 3BS (pooled) ^j			1:1	59	65	0.3	84	62	3.3
Chromosome 3BS (pooled) ^k			1:1.06	94	128	2.6	163	103	19.4***
Chromosome 5A									
<i>Xwmc150</i>	AC Barrie/Alsen ^{t,u,y,z}	2004	1:1	6	4	0.4	27	11	6.7**
	Alsen/Superb ^{t,u,y,z}	2004	1:1	1	1	0	9	7	0.3
	Alsen (pooled) ^t		1:1	7	5	0.3	36	18	6.0*
	Infinity/ND3085 ^{u,z}	2008	1:1	2	13	8.1**	5	8	0.7
	<i>Xwmc150</i> (pooled) ^u		1:1	9	18	3.2	41	26	3.4
<i>Xgwm304</i>	Alsen/Superb ^{v,w}	2004	1:1	1	1	0	4	12	4.0*
	Alsen/Prodigy ^{v,w,y,z}	2004	1:1	6	9	0.6	23	21	0.1
	AC Barrie/Alsen ^{v,w}	2004	1:1	7	20	6.3*	25	32	0.9
	Alsen/Helios ^{v,w,y,z}	2007	1:1	17	20	0.2	12	18	1.2
	Alsen (pooled) ^v		1:1	31	50	4.5*	64	83	2.5
	Infinity/ND3085 ^w	2008	1:1	2	13	8.1**	5	8	0.7
	Infinity/ND744 ^{w,z}	2008	1:1	2	13	8.1**	1	2	0.3
	<i>Xgwm304</i> (pooled) ^w		1:1	35	76	15.1**	70	93	3.2
<i>Xgwm595</i>	Alsen/Helios ^x	2007	1:1	14	17	0.3	13	9	0.7
	Alsen/Helios	2008	1:1	1	4	1.8	12	8	0.8
	Infinity/ND3085 ^x	2008	1:1	5	5	0	10	2	5.3*
	Carberry/AC Cadillac ^{x,z}	2009	1:1	14	25	3.1	14	8	1.6
	<i>Xgwm595</i> (pooled) ^x		1:1	33	47	2.5	37	19	5.8*
Alsen (3BS pooled) ^y			1:1	30	34	0.3	71	57	1.5
Chromosome 5A (pooled) ^z			1:1	48	85	10.3**	91	75	1.5

^a Frequency of lines carrying favorable and unfavorable marker variants of FHB resistance in the retained and discarded categories. Population names followed by letters in superscript (letters b to k, and t to z) indicate which marker data were used in the combined χ^2 analysis and each letter designates which markers or marker-locus combinations were pooled. *, **, and *** indicate significance at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Significant deviations between the frequencies of M+ and M– were observed for populations AC Barrie/Alsen, Alsen/ Helios, and BW796/Alsen. The FHB source of resistance was Alsen, with M– being elevated in the discarded category for 3BS markers *Xgwm533* and *Xsts3B-163*. For the same populations the differences were not significant between M+ and M– within the retained category for *Xgwm533*, but were significant for *Xsts3B-163*. Pooled results across populations polymorphic for *Xgwm533* and *Xsts3B-163* were significant with the M– variant being elevated in the discarded category. Of the populations tested with *Xgwm493*, BW796/ND744 and BW796/ND3085 produced a significant marker effect on selection with M+ favored in the retained group and for the same marker M+ was significantly elevated when the results were pooled by resistant parent ND3085. No significant distortion between M+ and M– lines was associated with markers *Xgwm389* and *Xsts3B-66*. Based on the overall variation in the lines across markers, the 3BS chromosome had a highly significant effect with a larger number of lines carrying M+ than lines with M– in retained group for populations with distortion in M+/M– ratio before selection, but the effects were not significant for populations pooled and tested against a 1:1 theoretical random segregation ratio.

The M– group displayed a significantly elevated number of lines in the discard category at Carman for ND3085 with 5A markers *Xwmc150* and *Xgwm304*, and Alsen and ND744 with *Xgwm304* (Table 5). The effect of increased M+ types in the retained group was significant for Alsen with *Xwmc150* in AC Barrie/Alsen population and *Xwmc304* in Alsen/Superb population, and similarly for ND3085 with *Xgwm595* in the population Infinity/ND3085. Markers *Xgwm304* and *Xgwm595* showed significant effects on selection when pooled across populations, with *Xgwm304* showing a reduction in M+ in the discard category whereas *Xgwm595* showed an increase in M+ in the retained category. Based on the overall variation in lines

across all markers, the 5A chromosome had a significant effect on selection with fewer lines with M+ being discarded than lines with M–. The molecular variant from the resistant parent, M+, for 5A markers was elevated in the retained category but the effect was not significant.

The 3BS FHB resistance locus in ND3085 and ND744 significantly influenced line selection both in PLP and Carman environments (Tables 4 and 5). The 3BS marker, *Xgwm493*, was effective in all four populations derived from ND3085 and ND744 evaluated at PLP in 2007. But in the 2008 Carman nursery, the 3BS locus of ND3085 and ND744 assessed with *Xgwm493* was significant in only two of the four populations. The 3BS locus in Carberry, as determined by the *Xgwm533* marker, influenced line selection at the PLP 2010 nursery, but not at Carman in 2009. The 5A Carberry locus affected line selection at PLP only, whereas the 5A locus in Alsen affected selection only at Carman. The Alsen loci associated with the 3BS markers *Xgwm533* and *Xsts3B-163* and 5A marker *Xgwm304* were significant at Carman, but not significant at PLP.

The *t* test results on lines selected in the PLP nursery and grown in the Carman nursery and evaluated for FHB incidence and severity are shown in Table 6. The 3BS marker *Xgwm493* was significantly associated with the reductions in FHB severity and index from ND3085 and ND744 and incidence from ND3085. Pooled over populations, the 3BS markers were significantly associated with reduced FHB incidence, severity, and index. The 5A marker *Xgwm304* was significantly associated with reduced FHB severity from the Alsen parent, but no association was detected for *Xwmc150*.

Discussion

The results showed that different markers were polymorphic in different populations. The increase in frequency of the M+ favorable molecular marker variant when selection was applied in the PLP

Table 6. Mean, standard deviation, and *t*-statistic of Fusarium head blight scores of doubled haploid lines classified based on resistant (M+) and susceptible (M–) molecular marker variants for populations selected at the PLP nursery and subsequently grown at Carman, MB

Parameter	Marker	Population	No. lines		Mean $\pm$ S.E. ^a		<i>t</i> -value ^b
			M+	M–	M+	M–	
Chromosome 3BS							
Incidence	<i>Xgwm389</i>	AC Barrie/Alsen	4	2	57.5 $\pm$ 4.3	65.0 $\pm$ 0.0	–1.7
	<i>Xgwm533</i>	AC Barrie/Alsen	10	10	49.5 $\pm$ 4.3	49.5 $\pm$ 6.2	0.0
	<i>Xgwm493</i>	BW796/ND3085	67	21	56.2 $\pm$ 3.3	72.9 $\pm$ 4.0	–3.2**
	<i>Xgwm493</i>	BW796/ND744	16	6	66.9 $\pm$ 5.6	80.0 $\pm$ 3.2	–2.0
	<i>Xgwm493</i>	Infinity/ND3085	30	19	55.8 $\pm$ 4.2	66.8 $\pm$ 4.2	–1.9
Pooled			127	58	58.0 $\pm$ 2.2	67.3 $\pm$ 2.5	–3.1**
Severity	<i>Xgwm389</i>	AC Barrie/Alsen	4	2	23.7 $\pm$ 4.7	30.0 $\pm$ 5.0	–0.9
	<i>Xgwm533</i>	AC Barrie/Alsen	10	10	23.0 $\pm$ 2.4	25.5 $\pm$ 2.7	–0.7
	<i>Xgwm493</i>	BW796/ND3085	67	21	31.9 $\pm$ 2.4	47.4 $\pm$ 3.9	–3.4**
	<i>Xgwm493</i>	BW796/ND744	16	6	23.4 $\pm$ 3.3	55.0 $\pm$ 8.4	–3.5*
	<i>Xgwm493</i>	Infinity/ND3085	30	19	24.7 $\pm$ 2.5	32.6 $\pm$ 3.4	–1.9
Pooled			127	58	28.1 $\pm$ 1.5	39.0 $\pm$ 2.4	–3.8**
Index	<i>Xgwm389</i>	AC Barrie/Alsen	4	2	14.2 $\pm$ 3.4	19.5 $\pm$ 3.2	–0.9
	<i>Xgwm533</i>	AC Barrie/Alsen	10	10	12.0 $\pm$ 1.9	13.7 $\pm$ 2.8	–0.5
	<i>Xgwm493</i>	BW796/ND3085	67	21	21.2 $\pm$ 2.3	35.0 $\pm$ 3.8	–3.1**
	<i>Xgwm493</i>	BW796/ND744	16	6	15.5 $\pm$ 2.4	43.2 $\pm$ 5.9	–4.3**
	<i>Xgwm493</i>	Infinity/ND3085	30	19	15.3 $\pm$ 2.1	22.4 $\pm$ 2.7	–2.1
Pooled			127	58	18.2 $\pm$ 1.4	27.5 $\pm$ 2.2	–3.6***
Chromosome 5A							
Incidence	<i>Xwmc150</i>	AC Barrie/Alsen	11	10	50.4 $\pm$ 6.0	49.5 $\pm$ 5.4	0.1
	<i>Xgwm304</i>	AC Barrie/Alsen	10	13	47.5 $\pm$ 5.2	53.1 $\pm$ 5.3	–0.7
Pooled			21	23	49.0 $\pm$ 3.9	51.5 $\pm$ 3.7	–0.4
Severity	<i>Xwmc150</i>	AC Barrie/Alsen	11	10	26.8 $\pm$ 2.6	25.5 $\pm$ 2.7	0.3
	<i>Xgwm304</i>	AC Barrie/Alsen	10	13	20.5 $\pm$ 1.7	29.6 $\pm$ 2.4	2.8*
Pooled			21	23	23.8 $\pm$ 1.7	27.8 $\pm$ 1.7	–1.6
Index	<i>Xwmc150</i>	AC Barrie/Alsen	11	10	14.7 $\pm$ 2.8	13.1 $\pm$ 2.4	0.4
	<i>Xgwm304</i>	AC Barrie/Alsen	10	13	10.3 $\pm$ 1.7	16.6 $\pm$ 2.6	–2.0
Pooled			21	23	12.6 $\pm$ 1.7	15.1 $\pm$ 1.8	–1.0

^a Data are means $\pm$ standard error.

^b * and ** indicate significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

and Carman nurseries indicated that selection affected the representation of molecular variants at loci of interest. Molecular variant frequencies showed no significant distortion from a 1:1 random segregation prior to phenotypic assessment for FHB in six of the eight populations.

Considering results from the PLP nurseries, the significant increase in the favorable molecular variant (M+) of *Xgwm493* derived from ND3085 and ND744 resistant parents in the retained group suggested these parents carry the resistant allele for *Fhb1*. The influence of the *Fhb1* locus from these two lines was emphasized by a reduction in the favorable molecular variant in the discard group. The contribution of ND3085 and ND744 of *Fhb1* to FHB resistance was further supported with the Carman segregation results showing an elevated proportion of the ND3085 and ND744 molecular variant in the retained group. The *t* test of Carman disease index rating means partitioned by *Xgwm493* molecular variants are consistent with ND3085 and ND744 possessing the *Fhb1* resistant allele. The significant differences in segregation distortion for *Xgwm493* pooled over populations at both PLP and Carman affirmed this marker's association with *Fhb1*.

The *Xgwm533* segregation results at PLP suggested that Carberry contributed the resistant allele for *Fhb1* and affirmed resistance to FHB on 3BS is linked to *Xgwm533*. The segregation distortion at the *Xgwm533* locus in the discarded group from selection within the two populations AC Barrie/Alsen and Alsen/Helios at Carman demonstrated a contribution of *Fhb1* resistance from Alsen. Previous studies have shown that the SSR markers *Xgwm493* and *Xgwm533* are closely linked with *Fhb1* (Buerstmayr et al. 2003; Cuthbert et al. 2006; McCartney et al. 2004).

The *Xsts3B-163* results from Carman suggested that Alsen contributed the resistant allele for *Fhb1* and affirmed resistance to FHB on 3BS is linked to *Xsts3B-163*. No conclusions could be made about the relationship of markers *Xsts3B-66* and *Xgwm389* to FHB resistance in the present study; however, other studies (Anderson et al. 2001; Cuthbert et al. 2006) have indicated that these two markers are linked to *Fhb1*. The lack of significant distortion between M+ and M- lines when tested with markers *Xgwm389* and *Xsts3B-66* indicated these markers may not be as closely associated with the genetic factors controlling FHB resistance on chromosome 3BS.

The *Xgwm595* results suggested that Carberry contributed the resistant allele for *Fhb5* and affirmed resistance to FHB on chromosome 5A is linked to *Xgwm595*. No conclusions could be made about the status of *Fhb5* in Alsen based on the *Xgwm595* marker or about the relationship of markers *Xwmc150* and *Xgwm304* to FHB resistance using the PLP results. However, results of *Xwmc150* and *Xgwm304* for Carman suggested the presence of the *Fhb5* resistance allele in Alsen. The Carman *Xgwm304* results suggest ND3085 and ND744 also contribute *Fhb5* resistance.

A large proportion of lines in the retained group lacking *Fhb1* and *Fhb5* resistance marker molecular variants indicate either disease escapes or the involvement of other FHB loci in resistance expression during selection. Other FHB loci contributing to resistance are expected to be present in the populations given that Alsen, Carberry, Glenn, ND3085, and ND744 not only have Sumai 3 but also Frontana, which is known for its FHB resistance, in their genealogy (IWIS). Sumai 3 could be contributing QTL other than those on 3BS and 5A (Anderson et al. 2001; Buerstmayr et al. 2003; Waldron et al. 1999), and Frontana could contribute yet other QTL (Steiner et al. 2004). The less resistant parent in each of the populations under study also could contribute QTL for FHB resistance. For example, Frontana is in the pedigree of the more susceptible parents: Infinity, Somerset, AC Barrie, AC Cadillac, BW796, Helios, Prodigy, Superb, and Waskada (IWIS). Waskada also has Sumai 3 in its distant pedigree (Fox et al. 2009).

The observation that some population-marker combinations showed no response to selection at the targeted loci is possibly a result of small effect alleles interacting with the environment with a range of expression. It is also possible that there are unidentified FHB QTL in the parents affecting line selection between nurseries. Consequently, FHB resistance can be achieved in breeding lines by

expression of other major or minor QTL in the parents without sole reliance on *Fhb1* and *Fhb5*.

Environment played a role in the expression of resistance. For example, the 3BS (*Fhb1*) locus associated with *Xgwm493* in ND3085 and ND744 was more effective at PLP than Carman. Alsen expressed *Fhb1* resistance in the BW796/Alsen, AC Barrie/Alsen, and Alsen/Helios populations in the Carman environment with the *Xgwm533* and *Xsts3B-163* markers, but Alsen did not express in all the populations tested at PLP. In contrast, Carberry expressed *Fhb1* at PLP but not at Carman. With the *Fhb5* locus, none of the resistant parents showed consistency across nurseries. However, considering the wide range of phenotypic variances (24.8 to 60%) explained by *Fhb1* and *Fhb5* QTL (Anderson et al. 2001; Buerstmayr et al. 2002, 2003) and the stability of these QTL in different populations (Buerstmayr et al. 2009), marker assisted selection using these major QTL should be feasible in accelerating the development of resistant cultivars.

The substantial variation from genetic and environmental factors and their interaction on disease development make it difficult to score resistance, which is a major reason for using DNA markers in breeding. For example, there is no certainty that a particular resistance locus was transferred from Sumai 3 to progenies such as Carberry in the absence of genetic analysis. Here, the significant effects of markers found linked to either one or the two major QTL located on 3BS (*Fhb1*) and 5A (*Fhb5*) wheat chromosomes provided evidence that resistance at these loci were transferred to DH progenies from Carberry, Alsen, ND3085, and ND744. Although the resistant parents ND744 and Glenn are sister lines (Mergoum et al. 2005), the 3BS locus from ND744 significantly affected line selection but that of Glenn did not. Unfortunately, none of the markers used in this study to test the effect of the 5A locus were polymorphic in Glenn. It is possible the population size in this study was too small to show an effect with Glenn. Alternatively, Glenn may not have inherited the resistance allele from Sumai 3 for *Fhb1*. Epistatic genes with suppression effects on the resistance locus in Glenn-derived populations cannot be ruled out. Genes that increase disease susceptibility by suppressing other resistance genes in wheat have been reported in different studies (Garvin et al. 2009; Ghavami et al. 2011; Rudd et al. 2001; Stack et al. 2002). Hence, further study should be conducted using a larger population size to determine the status of *Fhb1* in Glenn and alternative markers with polymorphism would be needed to test the effect of *Fhb5*.

The low number of significant *t* tests for the Carman FHB data indicated that selection for FHB resistance by phenotypic data alone was only marginally effective in selecting lines with the elevated proportion of FHB resistant marker molecular variants. Notably, sample sizes after selection and division by marker and population were small relative to the small differences they had to detect.

The results of this study demonstrated that, like Sumai 3, Alsen, Carberry, ND3085, and ND744 possess *Fhb1* and *Fhb5*. There was no evidence of Glenn having *Fhb1*, and *Fhb5* could not be tested. This study validates the application of *Xgwm493* and *Xgwm533* in marker assisted selection of *Fhb1*, and *Xgwm304*, *Xwmc150*, and *Xgwm595* in selection of *Fhb5* in populations derived from Alsen, Carberry, ND3085, and ND744 to enhance FHB resistance in wheat. The study further validates the association of the markers with genetic factors controlling resistance.

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