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ORIGINAL PAPER

Mapping of *Fhb2* on chromosome 6BS: a gene controlling Fusarium head blight field resistance in bread wheat (*Triticum aestivum* L.)

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Abstract Fusarium head blight (FHB) is one of the most important fungal wheat diseases worldwide. Understanding the genetics of FHB resistance is key to facilitate the introgression of different FHB resistance genes into adapted wheat. The objective of this project was to study the FHB resistance QTL on chromosome 6B, quantify the phenotypic variation, and qualitatively map the resistance gene as a Mendelian factor. The FHB resistant parent BW278 (AC Domain*2/Sumai 3) was used as the source of the resistance allele. A large recombinant inbred line (RIL) mapping population was developed from the cross BW278/AC Foremost. The population segregated for three known FHB resistance QTL located on chromosomes 3BSc, 5A, and 6B. Molecular markers on chromosome 6B (WMC104, WMC397, GWM219), 5A (GWM154, GWM304, WMC415), and 3BS (WMC78, GWM566, WMC527) were amplified on approximately 1,440 $F_{2:7}$ RILs. The marker information was used to select 89 RILs that were fixed homozygous susceptible for the 3BSc and 5A FHB QTLs and were recombinant in the 6B interval. Disease response was evaluated on 89 RILs and parental checks in the greenhouse and field nurseries. Dual floret injection (DFI) was used in greenhouse trials to evaluate disease severity (DS). Macroconidial spray inoculations were used in field nurseries conducted at two locations in southern Manitoba (Carman and Glenlea) over two years 2003 and 2004, to evaluate disease incidence, disease severity, visual rating index, and Fusarium-damaged kernels. The phenotypic distribution for all five-disease infection measurements was bimodal, with lines resembling either the resistant or susceptible checks and parents. All of the four field traits for FHB resistance mapped qualitatively to a coincident position on chromosome 6BS, flanked by GWM133 and GWM644, and is named *Fhb2*. The greenhouse-DS trait mapped 2 cM distal to *Fhb2*. Qualitative mapping of *Fhb2* in wheat provides tightly linked markers that can reduce linkage drag associated with marker assisted selection of *Fhb2* and aid the pyramiding of different resistance loci for wheat improvement.

Keywords *Fusarium graminearum* · Marker-assisted selection · Microsatellite · Fusarium head blight

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Introduction

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch), has become the most serious fungal disease of small cereal grains in Manitoba, eastern Saskatchewan, and eastern Canada. FHB infection is favoured by warm humid conditions during flowering and early stages of kernel development (Gilbert and Tekauz 2000). Lightweight Fusarium-damaged kernels (FDK) may contain high concentrations of mycotoxins, such as deoxynivalenol (DON), rendering the grain unsuitable for food or feed (Gilbert and Tekauz 2000).

Producers can follow a number of management practices to help control FHB; however, the most efficacious and economical strategy to this devastating problem is to breed genetic disease resistance into adapted cultivars. Breeding for resistant cultivars is difficult given the complexity of FHB resistance, the need to screen host plants at maturity, and the large environmental effects on disease expression. Several types of FHB resistance in wheat have been proposed (Bai and Shaner 1994; Yang 1994; McMullen et al. 1997). Resistance to initial infection (Type I) is assessed as the incidence of infection in the presence of natural or augmented inoculum (e.g., spray inoculations). Resistance to spread within the spike (Type II) is most accurately assessed as the spread of infection within the spike following single/dual floret injections (SFI/DFI). Other types of resistance are not as well characterized. Disproportionate reductions in the accumulation of DON (i.e., statistically uncorrelated with Type I or II resistance) are described as Type III resistance (Mesterhazy 1995).

The most common source of genetic resistance is derived from the Chinese wheat cultivar Sumai 3 (Yang 1994; Bai and Shaner 1994; Wan et al. 1997) and resistance to FHB is quantitatively inherited (Chen 1983; Lin et al. 1992; Yang 1994; Bai et al. 2000). Problems associated with greenhouse- and Fieldbased screening for FHB resistance include dependence on the environment for symptom development, the high cost of phenotyping, and significant time and resource requirements (Yang 1994; Bai and Shaner 1994; Campbell and Lipps 1998). Development of DNA marker-based screening for the presence of resistance genes may make selection for resistance more efficient in breeding programs (Bai et al. 1999; Kolb et al. 2001).

Exploitation of molecular markers associated with FHB resistance genes has focused on Type II FHB resistance (Bai et al. 1999; Waldron et al. 1999; Anderson et al. 2001; Buestmayr et al. 2002; Zhou et al. 2002; Yang et al. 2003). Previous QTL mapping studies have revealed major Sumai 3-derived Type II FHB resistance QTL on chromosomes 3BS (Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Somers et al. 2003; Zhou et al. 2002; Yang et al. 2003), 5A (Ban and Suenaga 1998; Xu et al. 2001; Buesrstmayr et al. 2002) and 6B (Anderson et al. 2001; Yang et al. 2003). Understanding the genetics of FHB resistance and mapping the location of genes will be necessary to facilitate the introgression and pyramiding of different FHB resistance genes into adapted wheat.

To determine the map location of individual genes controlling quantitative traits, substitution lines, recombinant inbred lines (RILs) or near-isogenic lines (NILs) can be developed to isolate the gene of interest as a Mendelian factor. The objective of this research was to study the FHB resistance QTL on chromosome 6B (Yang et al. 2003) by quantifying the phenotypic variation in disease incidence (DI), disease severity (DS), visual rating index (VRI), and Fusarium-damaged kernels (FDK) and qualitatively map the resistance gene.

Materials and methods

Population, genotyping and selection of recombinants

Single-seed decent was used to develop a recombinant inbred population of 1,440 $F_{2:7}$ lines from the cross BW278 (AC Domain*2/Sumai 3, FHB resistant) and AC Foremost (HY320*5/BW553//HY320*6/7424-BW5B4, FHB susceptible). Sumai 3 was the source of FHB resistance in the population, which segregated for three known FHB resistance QTL on chromosomes 3BSc, 5A, and 6B. The QTL identified on 3BSc is located proximal to the centromere (Somers et al. 2003). The 1,440 $F_{2:7}$ recombinant inbred lines (RILs) were genotyped using microsatellite markers on chromosome 6B (WMC104, WMC397, GWM219), 5A (GWM154, GWM304, WMC415), and 3BS (WMC78, GWM566, WMC527) (Somers et al, 2004) to facilitate selection of RILs homozygous susceptible for QTL intervals on 3BSc, 5A, and recombinant for the interval on 6B carrying the FHB resistance gene (Table 1). BW278 is known to lack resistance alleles at *Fhb1* (Cuthbert et al. 2006) on 3BS near GWM493 (data not shown).

Seed from the 1,440 RILs were germinated on moist filter paper in Petri dishes for one week. The leaf tissue was harvested and lyophilized, then DNA was extracted using the Qiagen DNeasy 96 Plant Kit (Qiagen, Mississauga, ON) and quantified by fluorimetry using Hoechst 33258 stain. DNA from five plants per line was collected and bulked for initial genotyping of the 1,440 lines. Lines showing heterogeneity or heterozygosity were eliminated from the project. DNA was collected from five new plants per line for genotyping of the selected recombinant lines, and no heterogeneity or heterozygosity was detected within the families of the final selected recombinant population. Genotypic data for the population was collected using M13-tailing and fluorescent capillary electrophoresis on an ABI3100 genotyper (Applied Biosystems Inc., Foster City, CA,USA). M13-tailing required adding the M13 sequence (CACGACGTTGTAAAACGAC) to the 5′ end of the forward primer during primer

Table 1 Primer sequences, annealing temperatures, and allele sizes of markers used in the Domain*2/Sumai 3//AC Foremost population for mapping *Fhb2* in spring wheat

Marker	Forward Primer (5′→3′)	Reverse Primer (5′→3′)	Chromosome	Anneal (°C)	Allele Size Sumai	References
WMC104	TCTCCCTCATTAGAGTTGTCCA	ATGCAAGTTTAGAGCAACACCA	6B	61	153	http://www.wheat.pw.usda.gov
GWM132	TACCAAATCGAAACACATCAGG	CATATCAAGGTCTCCTTCCCC	6B	61	134	Röder et al. (1998)
CFD13	CCACTAACCAAGCTGCCATT	TTTTTGGCATTGATCTGCTG	6B	61	Null	http://wheat.pw.usda.gov
GWM518	AATCACAACAAGGCGTGACA	CAGGGTGGTGCATGCAT	6B	61	208	Röder et al. (1998)
GWM494	ATTGAACAGGAAGACATCAGGG	TTCCTGGAGCTGTCTGGC	6B	61	217	Röder et al. (1998)
GWM508	GTTATAGTAGCATATAATGGCC	GTGCTGCCATGATATTT	6B	51	156	Röder et al. (1998)
WMC398	GGAGATTGACCGAGTGGAT	CGTGAGAGCGGTTCTTTG	6B	61	173	http://wheat.pw.usda.gov
GWM133	ATCTAAACAAGACGGCGGTG	ATCTGTGACAACCGGTGAGA	6B	61	120	Röder et al. (1998)
GWM644	GTGGGTCAAGGCCAAGG	AGGAGTAGCGTGAGGGGC	6B	61	206	Röder et al. (1998)
WMC397	AGTCGTGCACCTCCATTTTG	CATTGGACATCGGAGACCTG	6B	61	175	http://wheat.pw.usda.gov
WMC756	TTCCGTGGCCTCTCGTTC	CATTGCCATCAGTCACCCTC	6B	51	231	http://wheat.pw.usda.gov
GWM88	CACTACAACTATGCGCTCGC	TCCATTGGCTTCTCTCTCAA	6B	61	248	Röder et al. (1998)
WMC179	CATGGTGGCCATGAGTGGAGGT	CATGATCTTGCGTGTGCGTAGG	6B	61	217	http://wheat.pw.usda.gov
WMC182	GTATCTCACGAGCATAACACAA	GAAAGTGTATGGATCATTAGGC	6B	61	180	http://wheat.pw.usda.gov
GWM608	ACATTGTGTGTGCGGCC	GATCCCTCTCCGCTAGAAGC	6B	61	163	Röder et al. (1998)
GWM107	ATTAATACCTGAGGGAGGTGC	GGTCTCAGGAGCAAGAACAC	6B	61	209	Röder et al. (1998)
WMC152	CTATTGGCAATCTACCAAACTG	TCTCTTCTTGCCACATATTCGT	6B	61	256	http://wheat.pw.usda.gov
BARC24	CGCCTCTTATGGACCAGCCTAT	GCGGTGAGCCATCGGGTTACAAAG	6B	51	211	http://www.scabusa.org
GWM219	GATGAGCGACACCTAGCCTC	GGGGTCCGAGTCCACAAC	6B	61	198	Röder et al. (1998)
GWM154	TCACAGAGAGAGAGGGAGGG	ATGTGTACATGTTGCCTGCA	5A	51	121	Röder et al. (1998)
GWM304	AGGAAACAGAAATATCGCGG	AGGACTGTGGGGAATGAATG	5A	61	234	Röder et al. (1998)
WMC415	AATTCGATACCTCTCACTCACG	TCAACTGCTACAACCTAGACCC	5A	61	176	http://wheat.pw.usda.gov
WMC78	AGTAAATCCTCCCTTCGGCTTC	AGCTTCTTTGCTAGTCCGTTGC	3BSc	61	285	http://wheat.pw.usda.gov
WMC527	ACCCAAGATTGGTTGCAGAA	GCTACAGAAAACCGGAGCCTAT	3BSc	61	402	http://wheat.pw.usda.gov
GWM566	TCTGTCTACCCATGGGATTTG	CTGGCTTCGAGGTAAGCAAC	3BSc	61	142	Röder et al. (1998)

synthesis (Schuelke 2000). The PCR conditions were: 24 ng DNA, 1.5 mM $MgCl_2$, 50 mM KCl, 0.8 mM dNTPs, 2 pmol reverse primer, 0.2 pmol forward primer, and 1.8 pmol M13 primer (CACGACGTTGTAAAACGAC) fluorescently labelled with 6-FAM, HEX, NED (Applied Biosystems Inc.), and 0.5 U Taq DNA polymerase (Promega, Madison, Wis.). Thermal cycling included: 94°C–2 min, 30 cycles of 95°C–1 min, (0.5°C/s to 61/51°C), 61/51°C–50 s, (0.5°C/s to 73°C), 73°C–1 min, 1 cycle 73°C–5 min. The internal molecular weight standard for the ABI3100 was Genescan 500-ROX (Applied Biosystems Inc.). Data collected by fluorescent capillary electrophoresis was first converted to a gel-like image using Genographer (available at http://www.hordeum.oscs.montana.edu/genographer).

Fusarium head blight phenotyping: greenhouse

The 89 RILs and parents from the mapping population were randomly arranged and grown in the greenhouse with supplemental lighting set for 16 h daylight. The greenhouse temperature was monitored and recorded daily and averaged 22°C during the day with a range of 18–25°C and 18°C at night with a range of 17–21°C. The inoculum used throughout the experiment was a mixture of virulent strains of *Fusarium graminearum* Schwabe (JM-6-00; EEI-23-00; RK-9-02; RK-16-02) provided by Dr. Jeannie Gilbert, Agriculture and Agri-Food Canada–Cereal Research Centre, Winnipeg, Manitoba. The inoculum was produced as described by Sung and Cook (1981). Ten single spikes from ten plants for each $F_{2:7}$ RIL and parent were inoculated to assess FHB resistance. A single primary spike on each recombinant plant was inoculated when the spike reached 50% anthesis. Each spike was inoculated by injecting a 10 µl macroconidial suspension (50,000 spores/mL) between the lemma and palea of the primary and secondary florets positioned at the inoculation point. The inoculation points on each spike were the spikelet positioned 2/3 of the way from the base of the spike and the spikelet immediately above that point. For example, the inoculation points were the adjacent 8th and 9th spikelets on a spike that had a total of 12 spikelets ($12 \times 2/3 = 8$). Following point inoculation, plants were incubated in a chamber at 100% relative humidity for 24 h and then returned to the greenhouse bench. Ratings were performed at 7, 14, and 21 days post-inoculation. Disease severity (DS) ratings were assessed by counting the number of infected spikelets directly below the inoculated florets and excluding the inoculated florets. The number of infected spikelets was only counted below the point of inoculation since infection can restrict the flow of water to distal florets and cause early senescence. The percentage of infected florets was averaged for each plant and RILs were classified as resistant or susceptible based on the bimodal distribution of ratings.

Fusarium head blight phenotyping: field trials

The field trials included 97 entries (89 RILs and 8 check varieties: AC Barrie, AC Foremost, AC Morse, AC Vista, Alsen, BW278, CDC Teal, and FHB 37), which were screened in Fusarium head blight nurseries at two locations in southern Manitoba (Glenlea and Carman) during the 2003 and 2004 field seasons. Trial entries and checks were replicated four times in a randomized complete block design. Selected checks [AC Morse (susceptible), CDC Teal (susceptible), Alsen (moderately resistant), and FHB37 (resistant)] were placed every 50 rows to monitor disease development throughout the field. Plots in Carman 2003 and 2004 consisted of a single 1 m row with 17 cm row spacings. Plots at Glenlea consisted of a single 1.5 m row in 2003 and 0.9 m row in 2004 row both with 30 cm row spacings. Sowing density was approximately 60 seeds per row. The spikes of the entire row were spray-inoculated at 50% anthesis with a 50 ml inoculum solution of virulent strains of *Fusarium graminearum* (2003 [JM-6-00, EEI-23-00, RK-9-02, RK-16-02] and 2004 [RK-16-02, MS/DS-15-03, MS/DS-3-03, EM/MB-19-03, MB/DS/DB-47-03]) using a CO_2 backpack sprayer calibrated at 30 psi. Re-inoculation of the same rows was performed two and three days following the first inoculation in the Glenlea and Carman nurseries, respectively. The inoculum solution was a suspension of 50,000 macro-conidia spores/mL in water and Tween 20. There was a difference in isolates used for the inoculation procedure from year to year, which is standard procedure to ensure current isolates are being used for testing of breeding material. The nursery at Glenlea in 2003 and 2004 was irrigated with a sprinkler system for 30 min following each inoculation to favor development of the disease. Plots at the Carman nursery were irrigated 2 h post-inoculation for 5 min every hour for 12 h. All plots at Carman were mist-irrigated on alternate days for a period of 10 days.

Disease incidence (DI-initial infection) and disease severity (DS - disease spread within the spike) of each row were rated 18–21 days post-inoculation using a 1 (resistant)to 10 (susceptible) scale. Visual rating index (VRI) was calculated ($VRI = DI \times DS$) for each line. Twenty-five spikes per row were harvested at random and stored at −20°C the day visual field ratings were performed to later verify the visual rating in the field.

Nurseries were hand harvested at the end of the season when they reached physiological maturity using a Whitecapper–offset double row thresher (Glenlea 2003) or Wintersteiger Elite combine (Carman 2003/2004, Glenlea 2004). The threshing mechanism was set at a normal setting on the combine; however, the wind speed was decreased and sieves were opened to ensure the Fusarium-damaged kernels were maintained in the harvested samples. Harvested seed samples were placed in paper bags and dried for 1 week at 36°C using a forced air system. A 50 g sample from each plot was visually assessed to attain the percentage of FDK. Fusarium-damaged kernels were identified as shriveled; lightweight and chalky white kernels with occasional pink colouration. These kernels were distinguishable from plump visually disease free kernels within a sample.

Construction of the genetic map

A total of 40 microsatellite markers on chromosome 6B (Somers et al. 2004) were screened for polymorphism between the parents of the population. Polymorphic marker primer sequences, annealing temperatures, and allele sizes are listed in Table 1. Nineteen polymorphic markers on 6B were used to genotype the RILs and create the genetic map. JoinMap, V3.0 (Biometris, Wageningen, The Netherlands, http://www.joinmap.nl) was used to determine the marker order and map distances.

Statistical analysis

Analysis of variance (ANOVA) for DI, DS, VRI, and FDK for each site and a combined analysis across the four site years were performed using the "PROC GLM" procedure of the SAS software package (SAS Institute Inc., Version 8.2). A homogeneity test was conducted to ensure the data could be combined over site years. The model statement used in the combined analysis was variables = env rep(env) entry entry × env. All factors in this statement with the exception of entry were considered to be random. The *F*-test values were considered approximate since the trait values were not normally distributed.

Results

There were 89 RILs identified from the 1,440 $F_{2:7}$ mapping population to be homozygous susceptible for FHB resistance QTL on 3BSc and 5A and recombinant near the FHB resistance 6B QTL interval. The interval distance between flanking markers WMC104 and GWM219 on 6BS was 32 cM (Fig. 2).

Fusarium head blight phenotyping

Greenhouse trials

Ten plants of each RIL and parental check were inoculated in the greenhouse using DFI on a single primary spike to assess disease severity. The range in GH-DS (1–10) infection ratings showed a bimodal distribution (Fig. 1 GH-DS). The range in GH-DS of RILs classified as resistant was 1.5–5.3 and for lines classified as susceptible was 7.6–10.0. There was very low variability (standard error: resistant RILs 4.1 ± 0.25 and susceptible RILs 9.3 ± 0.25) within the ten plants of each RIL and no overlap between the resistant and susceptible classes (Fig. 1). Resistant parental checks showed a disease severity range of 2.1–2.3 (resistant) and the susceptible check were 9.3. Darkening of the inoculation point was visible by day 7; however, disease progression was minimal by day 14 for the susceptible RILs and susceptible parental check. Disease development progressed basally from the inoculation point and there was a substantial change in infection ratings for susceptible RILs between day 14 and 21 post-inoculation. The population segregated 41 resistant to 48 susceptible plants, fitting a 1:1 *chi-square* ratio ($P < 0.10$).

Field trials

Environmental data was collected and conditions differed between the 2003 and 2004 field seasons. Mean temperature and precipitation during inoculation and prior to rating (July and August) are the most critical for infection and disease development. High amounts of precipitation and flooding in the spring of 2004 affected plant establishment at Glenlea and led to loss of one of the replicates. Despite the differences in temperature and precipitation between years, the mean infection level for all field traits did not differ between the years.

The ANOVA for combined site years indicated all sources of variation for Field-DI, -VRI, -FDK, and –DS were significant with the exception of environment for Field-DS (Table 2). The phenotypic distribution of all field traits over site years was bimodal (Fig. 1). All 89 RILs that were classified showed the same classification for all four-field traits. The population segregated 45 resistant to 44 susceptible RILs for all field traits, fitting a 1:1 *Chi-square* ratio ($P < 0.05$) (Fig. 1). The correlation between the averaged

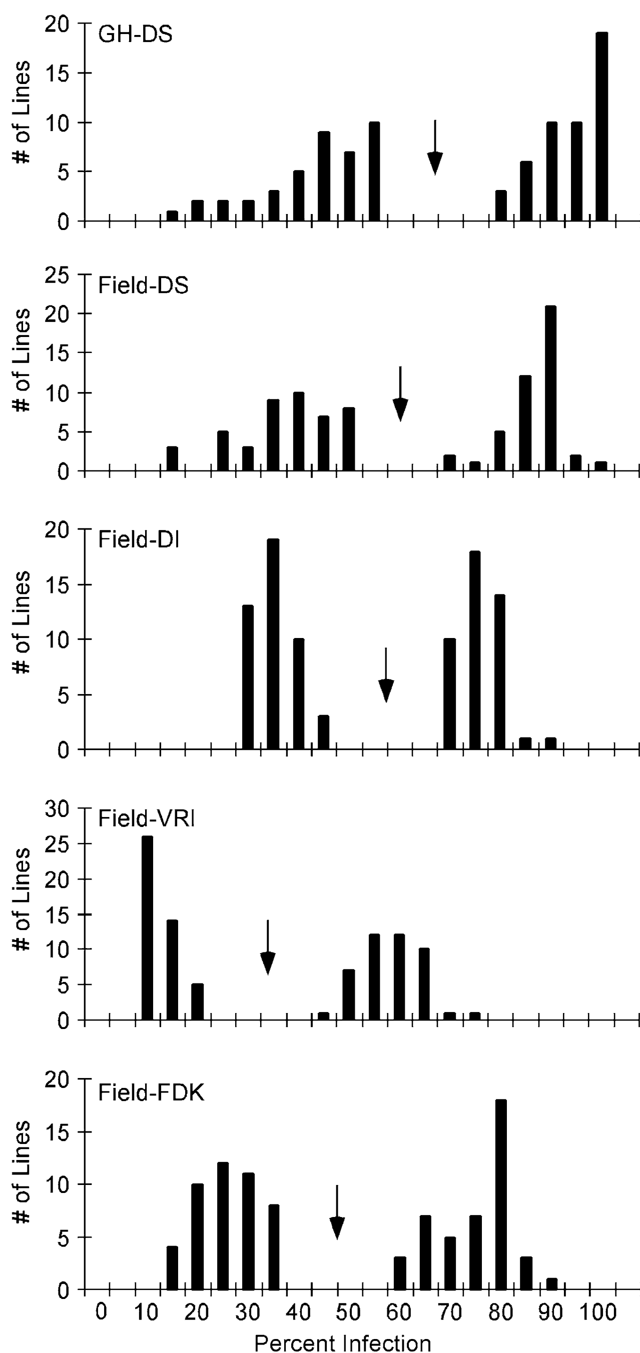

Fig. 1 Phenotypic distribution based on FHB infection of 89 $F_{2:7}$ RILs from the cross Domain*2/Sumai 3//AC Foremost. Percent infection was measured from one replicated greenhouse (GH) experiment and replicated field disease nurseries combined over four site-years. Traits included disease severity (DS), disease incidence (DI), visual rating index (VRI = DS × DI), and Fusarium damaged kernels (FDK). Resistant and susceptible parent and check lines showed infection phenotypes within the respective modes of the distributions. Small *black arrows* indicate the division point to classify lines as resistant or susceptible

greenhouse and field data measurements for the 89 RILs showed all correlations were high and statistically significant ($\alpha = 0.01$) (Table 3).

There were 12 RILs that were recombinant between the GH-DS and field traits. Four RILs were resistant for GH-DS and susceptible for field traits with a range of 47–52% and a mean rating of 50% infection. There were 8 RILs susceptible for the GH-DS rating and resistant for the field traits with a range of 78–100% and a mean rating of 89% infection. The correlation based on DS between the visual assessment of each row in the FHB field nurseries and the harvested spike analysis (25 heads) were also high: Carman 2003 ($r = 0.91$), Glenlea 2003 ($r = 0.89$), Carman 2004 ($r = 0.88$), and Glenlea 2004 ($r = 0.85$).

Genetic map

A genetic map was constructed by genotyping the 89 RILs with 19 polymorphic microsatellite markers on chromosome 6B (Table 1). The marker order was identical to the wheat consensus map (Somers et al. 2004), with the exception of markers GWM518 and CFD13; and GWM608 and WMC182, which were inverted. The total map length for the population was 32 cM (Fig. 2) compared to 42 cM on the wheat consensus map (Somers et al. 2004). The 89 RILs were classified as resistant or susceptible using five disease infection measurements (Field-DI, Field-DS, Field-VRI, Field-FDK and GH-DS). All of the four field traits mapped to a coincident genetic position on chromosome 6BS flanked by GWM133 and GWM644. This gene controlling field resistance to FHB is here named *Fhb2.* The GH-DS mapped 2 cM distal to *Fhb2* due to the presence of 12 RILs, which were recombinant between GH-DS and field traits (Fig. 2).

Discussion

There are inherent difficulties associated with phenotypic characterization of FHB due to methodological problems of inoculation and confounding effects of the environment (Andersen 1948; Hanson et al. 1950; Scott 1927). The present study decreased this variability and increased the reproducibility in phenotyping FHB resistance by focusing on variables that could be controlled. This included the development of a large RIL mapping population that segregated for a single major FHB resistance gene, multiple site-years of field phenotyping, and indoor DFI phenotyping.

The significant level of variation for all field traits (Table 2) may be due to the varying level of disease pressure for combined site years, differences in isolates used and differences in the type of irrigation system used between locations. Statistically, the most significant

Table 2 Analysis of variance for four phenotypic field variables (disease incidence, disease severity, visual rating index, and Fusarium-damaged kernels) from two field locations (Glenlea and Carman, MB) over 2 years (2003 and 2004)

Source	*df*	Mean square	***F*** value	***P*** value
Disease incidence (DI)				
Env.	3	16.6	16.0	<0.0001
Rep(env)	11	2.3	2.3	0.0102
Entry	102	61.8	59.8	<0.0001
Env × entry	304	1.7	1.6	<0.0001
Error	1,115	1.0		
Disease severity (DS)				
Env.	3	1.4	1.2	0.3118
Rep(env)	11	2.6	2.3	0.0095
Entry	102	75.6	65.1	<0.0001
Env × entry	304	2.0	1.7	<0.0001
Error	1,115	1.2		
Visual rating index (VRI)				
Env.	3	278.3	4.2	0.0055
Rep(env)	11	169.3	2.3	0.0031
Entry	102	7,445.5	113.3	<0.0001
Env × entry	304	118.9	1.8	<0.0001
Error	1,115	65.7		
Fusarium-damaged kernels (FDK)				
Env.	3	4,368.9	36.5	<0.0001
Rep(env)	11	202.0	1.7	0.0708
Entry	102	9,065.8	75.8	<0.0001
Env × entry	304	274.8	2.3	<0.0001
Error	1,115	119.6		

Table 3 Correlation coefficients (*r*) calculated using averaged values for greenhouse and field data measurements from two field locations (Glenlea and Carman, MB) over two years (2003 and 2004)

	Greenhouse/field data measurements[1]				
	Greenhouse-DS	Field-VRI	Field-DI	Field-DS	Field-FDK
Greenhouse–DS					
Field-VRI	0.75				
Field-DI	0.74	0.99			
Field-DS	0.78	0.94	0.92		
Field-FDK	0.73	0.97	0.97	0.91	

VRI Visual rating index, *DI* Disease incidence, *DS* Disease severity, *FDK* Fusarium-damaged kernels

[1] significance level $\alpha = 0.01$

factors affecting FHB ratings in the population were entry and env × entry interaction. The env × entry interaction for Field-DI, Field-DS, Field-VRI and Field-FDK compared to the entry effect is relatively small since the sum of squares for entry is more than ten fold higher than the sum of squares for the env × entry interaction (Table 2). There is no overlap in the two classes of RILs (Fig. 1) therefore the env × entry interaction is more a function of annual differences in values as opposed to overlapping phenotypic classes.

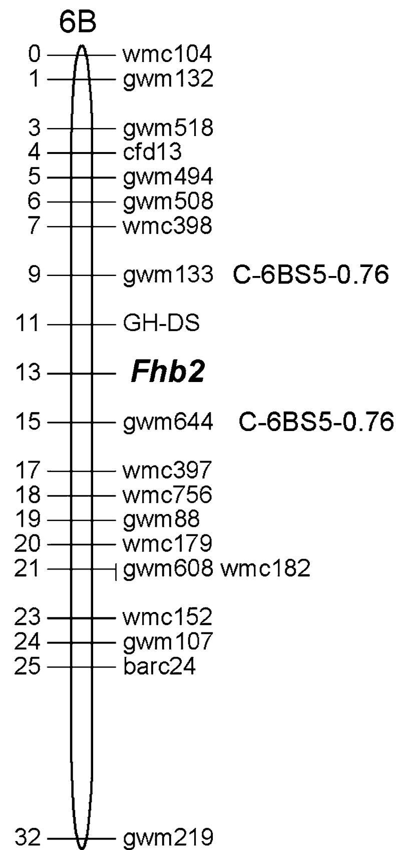

Fig. 2 Genetic map position of *Fhb2* on chromosome 6BS in the cross Domain*2/Sumai 3//AC Foremost. A population of 89 RILs recombinant for a segment of chromosome 6B was phenotyped for FHB infection symptoms and RILs were classified as resistant or susceptible based on replicated greenhouse and field disease nurseries combined over four site years. Recombination distance is shown on the left in cM and the Chinese Spring deletion bin assignment for GWM133 and GWM644 (Sourdille et al. 2004) are shown on the right

The ANOVA showed that the entries were highly significant for all field traits and using the phenotype distribution each RIL could be classified as either resistant or susceptible (Table 2; Fig. 1). The experimental design and data collection methods were effective in removing these sources of variation from the entry effects.

The DS was measured both in the greenhouse using DFI and in the field using spray inoculation to ensure consistency amongst ratings and proper characterization of the RILs. There were 12 RILs that were recombinant between the GH-DS and field traits. The overall rating results indicated the GH-DS levels were greater than the FieldDS (Fig. 1); however, the GH-DS and FieldDS were highly correlated ($r = 0.78$). The data showed all four-field measurements of FHB resistance mapped to one coincident location on chromosome

6BS, represented by *Fhb2*. The GH-DS measurement of FHB resistance mapped 2 cM distal to *Fhb2* due to the presence of the 12 recombinant lines between GH-DS and *Fhb2*.

This study provided an approach to qualitatively map the gene *Fhb2* using the Sumai 3 source of resistance in a large mapping population by collecting phenotypic data from both the field and greenhouse. The total genetic distance between the two flanking markers WMC104 and GWM219 on the wheat consensus map is 42 and 32 cM in the present population. The main difference in genetic distance between the two populations is attributed to the map distance between markers CFD13 and GWM518 in the wheat consensus map of 10 versus 1 cM in the BW278/AC Foremost map. The increased genetic distance may be due to differences in background genetics, population types, and population sizes used to create the wheat consensus map.

The distance to flanking markers surrounding *Fhb2* is 2 and 4 cM. Comparisons of physical and genetic maps of wheat indicate that most genetic recombination occurs in gene-rich, telomeric regions (Gill et al. 1996; Faris et al. 2000). *Fhb2* is shown to map to 6BS, proximal to the centromere, since the flanking markers GWM133 and GWM644 are assigned to deletion bin C-6BS5–0.76 (Sourdille et al. 2004) (Fig. 2). In the study by Yang et al. 2003, QTL analysis of chromosome 6B for FHB resistance in the population DH181 (Sumai 3 derivative)/AC Foremost was completed evaluating Type II resistance in the greenhouse using SFI. The results of the research revealed a major QTL on chromosome 6B contributes to FHB resistance. The most important microsatellite marker in the study located on 6B was GWM644 and explained 21% of the phenotypic variation in DH181(Sumai 3 derivative)/AC Foremost population. An additional study conducted by Shen et al. (2003) developed a RIL population from the cross of Ning 894037 and Alondra. Type II resistance was evaluated in the field and greenhouse using SFI. The QTL on 6B was found to be closest to marker GWM644. Based on the location of markers on the wheat consensus map, these intervals in these two studies are coincident on 6BS proximal to the centromere (Somers et al. 2004; Sourdille et al. 2004). The present results indicated there was one gene, *Fhb2*, controlling FHB field resistance on 6BS and an additional locus 2 cM distal to *Fhb2* controlling FHB Type II resistance. *Fhb2* was estimated to map within 2 cM of the QTL intervals reported by Yang et al. (2003) and Shen et al. (2003), suggesting the FHB resistance QTL on 6BS and *Fhb2* are likely coincident.

In summary, *Fhb2* was successfully mapped to 6BS and confers field resistance to FHB. The large population design with a fixed susceptible background, qualitative mapping and comparative mapping were used to attain a precise map position of *Fhb2*. *Fhb2* provides FHB field resistance as a single gene present in a susceptible background. Yang et al. (2003) reported a coefficient of determination on GH-DS of 21% for the microsatellite marker GWM644 on 6BS, which reduced FHB severity by 52%. In the present study the resistant allele on 6BS reduced FHB GH-DS by 56% when compared to the RILs carrying the susceptible allele. A more precise map location should reduce linkage drag associated with marker-assisted selection and assist with efficient and effective pyramiding of different FHB resistance genes for wheat improvement.

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