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Fine mapping *Fhb1*, a major gene controlling fusarium head blight resistance in bread wheat (*Triticum aestivum* L.)

Abstract A major fusarium head blight (FHB) resistance gene *Fhb1* (syn. *Qfhs.ndsu-3BS*) was fine mapped on the distal segment of chromosome 3BS of spring wheat (*Triticum aestivum* L.) as a Mendelian factor. FHB resistant parents, Sumai 3 and Nyubai, were used as sources of this gene. Two mapping populations were developed to facilitate segregation of *Qfhs.ndsu-3BS* in either a fixed resistant (Sumai 3*5/Thatcher) (S/T) or fixed susceptible (HC374/3*98B69-L47) (HC/98) genetic background (HC374 = Wuhan1/Nyubai) for Type II resistance. Type II resistance (disease spread within the spike) was phenotyped in the greenhouse using single floret injections with a mixture of macro-conidia of three virulent strains of *Fusarium graminearum*. Due to the limited heterogeneity in the genetic background of the crosses and based on the spread of infection, fixed recombinants in the interval between molecular markers XGWM533 and XGWM493 on 3BS could be assigned to discrete "resistant" and "susceptible" classes. The phenotypic distribution was bimodal with progeny clearly resembling either the resistant or susceptible parent. Marker order for the two maps was identical with the exception of marker STS-3BS 142, which was not polymorphic in the HC/98 population. The major gene *Fhb1* was successfully fine mapped on chromosome 3BS in the same location in the two populations within a 1.27-cM interval (S/T) and a 6.05-cM interval (HC/98). Fine mapping of *Fhb1* in wheat provides tightly linked markers that can reduce linkage drag associated with marker-assisted selection of *Fhb1* and assist in the isolation, sequencing and functional identification of the underlying resistance gene.

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Introduction

Fusarium head blight (FHB), also known as scab, is a disease of small grain cereals and is caused by several species of *Fusarium*. *Fusarium graminearum* Schw. (*Giberella zeae* Schw. and Petch) is the *Fusarium* species that primarily causes FHB in wheat in eastern Saskatchewan, Manitoba, eastern Canada, and the United States (Gilbert and Tekauz 2000). FHB infection is favored by warm humid conditions during flowering and early stages of kernel development (Gilbert and Tekauz 2000). This fungal disease reduces yield and grain quality through shrivelled kernels, and contaminates the grain with mycotoxins (such as deoxynivalenol) rendering it unsuitable for food or feed (Gilbert and Tekauz 2000).

Several types of resistance have been identified in spring wheat (Schroeder and Christensen 1963). 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 assessed as the spread of infection within the spike following single floret injections (SFI). Other types of resistance are not as well established. 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).

Fusarium head blight resistance is polygenic (Bai and Shaner 1994) and expression of resistance is highly influenced by the environment. Together, these factors make it very challenging to reproduce phenotypic results. Therefore, researchers have attempted to perform genetic analysis of FHB resistance through chromosomal manipulation (e.g., intervarietal substitutions)

or through the mapping of quantitative trait loci based on high-density genetic maps.

Molecular mapping and marker-assisted selection (MAS) are innovative tools that have been used in research programs to aid in the manipulation and pyramiding of several resistance genes in a short period of time. Molecular markers have been linked to QTL associated with various types of FHB resistance, particularly in Sumai 3 (Bai et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Yang et al. 2003, 2005; Zhou et al. 2002). Other sources of FHB resistance include: Ning derivatives, Wangshuibai, Frontana, Wuhan and Nyubai (Zhou et al. 2002; Buerstmayr et al. 2002; Somers et al. 2003; Steiner et al. 2004; Han et al. 2005). The most prominent QTL for FHB resistance have been associated with a specific type of resistance: Type II resistance on chromosome 3BS (Waldron et al. 1999; Anderson et al. 2001; Bai et al. 1999; Ban 2000), and 6B (Anderson et al. 2001; Yang et al. 2003); Type 1 resistance on 3A (Steiner et al. 2004), and 5A (Buerstmayr et al. 2002).

Extensive efforts have been made to map QTL for FHB resistance in the Chinese wheat cultivar Sumai 3, a source of FHB resistance which has been used successfully worldwide. A major QTL designated *Qfhs.ndsu-3BS* was originally mapped by RFLP analysis (Waldron et al. 1999) and verified later by several research groups (Bai et al. 1999; Anderson et al. 2001; Zhou et al. 2002; Somers et al. 2003; Yang et al. 2003, 2005). *Qfhs.ndsu-3BS* is located on chromosome 3BS between microsatellite markers GWM493 and GWM533 (Anderson et al. 2001). Liu and Anderson (2003) increased the marker density in this chromosome region using sequence-tagged site (STS) markers developed from wheat expressed sequence tags near *Qfhs.ndsu-3BS*, which facilitated the fine mapping of the resistance gene.

To determine the map location of individual genes controlling quantitative traits, substitution lines or near-isogenic lines can be developed to isolate the gene of interest as a Mendelian factor. The objective of this research was to isolate and fine map *Fhb1* using STS markers in both a fixed resistant (Sumai 3) and fixed susceptible (98B69-L47) genetic background.

Materials and methods

Mapping populations

Two large fine mapping populations were developed at Agriculture and Agri-Food Canada containing different sources of Type II FHB resistance, Sumai 3 and HC374 (Wuhan1/Nyubai). The pedigrees of the two mapping populations (Fig. 1) were (Sumai 3*5/Thatcher) and (HC374/3*98B69-L47) where 98B69-L47 = Augusta/ HWAlpha//3*Superb. The populations are identified in this study as (S/T) and (HC/98), respectively.

The S/T mapping population was developed using a backcrossing program, each time selecting the BCF_1
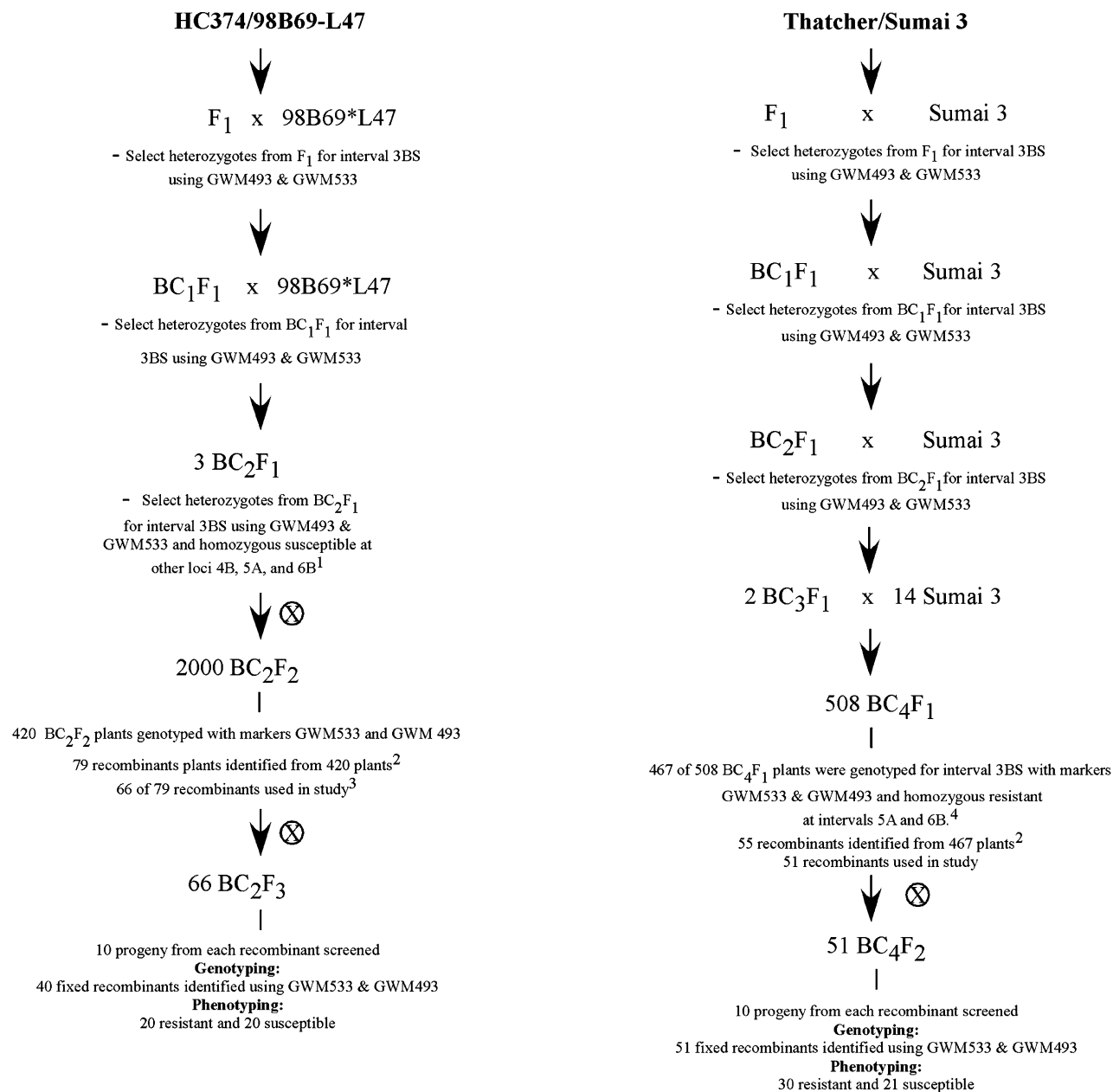
plants that were heterozygous for microsatellite marker GWM493 and GWM533 previously identified to flank *Qfhs.ndsu.3BS* (Liu and Anderson 2003). Two BC_3F_1 plants were pollinated by 14 Sumai 3 plants to generate 508 BC_4F_1 seeds. A subset of 467 plants was genotyped using GWM533 and GWM493 to select recombinant plants. The BC_4F_1 recombinant plants were self-pollinated and ten BC_4F_2 progeny were genotyped with GWM533 and GWM493 to select fixed recombinant plants in the 3BS marker interval, i.e., plants that showed a recombination event in the interval and were homozygous for both microsatellite markers. BC_4F_1 recombinant plants were also genotyped with markers from 5A (BARC117, GWM293, GWM304, and WMC705) and 6B (BARC146, GWM508, GWM191, GWM608, GWM644, WMC397, and WMC398) to verify the plants were homozygous resistant for other reported FHB resistance QTLs for Type II resistance (Somers et al. 2003; Yang et al. 2003).

The HC/98 population is a derivative from an ongoing molecular breeding program at Agriculture and Agri-Food Canada to introgress FHB resistance into spring wheat. Three BC_2F_1 plants were selected to be heterozygous between markers GWM533 and GWM493 on 3BS, and homozygous susceptible for the following markers at other reported FHB resistance QTLs for Type II resistance 4B (WMC710, WMC238, GWM149), 5A (GWM154, GWM304), and 6B (WMC494, GWM644, GWM219) (Somers et al. 2003; Yang et al. 2003). The three plants were self-pollinated to generate a large population of approximately 2,000 BC_2F_2 seeds. A subset of 420 BC_2F_2 plants was genotyped using GWM533 and GWM493 to select recombinant plants. The recombinant plants were self-pollinated and ten progeny from each recombinant plant were genotyped with GWM533 and GWM493 to select fixed recombinant plants in the 3BS marker interval (Fig. 1).

Thirty non-recombinant, homozygous plants carrying the resistant parental allele and 30 non-recombinant, homozygous plants carrying the susceptible parental allele from both populations were selected genotypically using markers GWM533 and GWM493 and were used as checks for phenotyping.

Genotyping and selection of recombinants

The populations were germinated and leaf tissue was harvested and lyophilized for DNA extraction with the Qiagen DNeasy 96 Plant Kit (Qiagen, Mississauga, Ont., Canada). DNA was quantified by fluorimetry using Hoechst 33258 stain. Genotypic data for the two populations 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 (CAC-GACGTTGTAAAACGAC) to the 5′ end of the forward primer during primer synthesis (Schuelke 2000).

HC374/98B69-L47

↓

F_1 x   98B69*L47

- Select heterozygotes from F_1 for interval 3BS
using GWM493 & GWM533

↓

BC_1F_1 x   98B69*L47

- Select heterozygotes from BC_1F_1 for interval

3BS using GWM493 & GWM533

↓

3 BC_2F_1

- Select heterozygotes from BC_2F_1
for interval 3BS using GWM493 &
GWM533 and homozygous susceptible at
other loci 4B, 5A, and 6B[1]

↓ ⊗

2000 BC_2F_2

|

420 BC_2F_2 plants genotyped with markers GWM533 and GWM 493

79 recombinants plants identified from 420 plants[2]
66 of 79 recombinants used in study[3]

↓ ⊗

66 BC_2F_3

|

10 progeny from each recombinant screened
Genotyping:
40 fixed recombinants identified using GWM533 & GWM493
Phenotyping:
20 resistant and 20 susceptible

Thatcher/Sumai 3

↓

F_1 x   Sumai 3

- Select heterozygotes from F_1 for interval 3BS
using GWM493 & GWM533

↓

BC_1F_1 x   Sumai 3

- Select heterozygotes from BC_1F_1 for interval 3BS
using GWM493 & GWM533

↓

BC_2F_1 x   Sumai 3

- Select heterozygotes from BC_2F_1 for interval 3BS
using GWM493 & GWM533

↓

2 BC_3F_1 x   14 Sumai 3

↓

508 BC_4F_1

|

467 of 508 BC_4F_1 plants were genotyped for interval 3BS with markers
GWM533 & GWM493 and homozygous resistant
at intervals 5A and 6B.[4]
55 recombinants identified from 467 plants[2]
51 recombinants used in study

↓ ⊗

51 BC_4F_2

|

10 progeny from each recombinant screened
Genotyping:
51 fixed recombinants identified using GWM533 & GWM493
Phenotyping:
30 resistant and 21 susceptible

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[1]Three BC_2F_1 plants were selected to be heterozygous between markers GWM533 and GWM493 on 3BS, and homozygous susceptible for the following markers at other reported FHB resistance QTLs for Type II resistance 4B (WMC710, WMC238, GWM149), 5A (GWM154, GWM304), and 6B (WMC494, GWM644, GWM219) (Somers et al. 2003; Yang et al. 2003)

[2]Recombinants were selected through genotyping using two flanking markers (GWM533 and GWM493) around Qfhs.ndsu-3BS determined by Anderson et al. 2001.

[3]Thirteen plants were lost due to pests in the greenhouse prior to selection of fixed recombinants. Twenty six plants did not have complete genotyping data and resulted in the inability to select a fixed recombinant.

[4]BC_4F_1 recombinant plants were genotyped with markers from 5A (BARC117, GWM293, GWM304, and WMC705) and 6B (BARC146, GWM508, GWM191, GWM608, GWM644, WMC397, andWMC398) to verify the plants were homozygous resistant for other reported FHB resistance QTLs for Type II resistance (Somers et al. 2003; Yang et al. 2003).

Fig. 1 Development of two large mapping populations for fine mapping *Fhb1* on the distal end of chromosome 3BS in spring wheat crosses: Sumai 3*5/Thatcher (*left*) and HC374/3*98B69-L47 (*right*)

The PCR conditions were 24 ng DNA, 1.5 mM MgCl₂, 50 mM KCl, 0.8 mM dNTPs, 2 pmol reverse primer, 0.2 pmol forward primer, and 1.8 pmol M13 primer (CACGACGTTGTAAAACGAC) fluorescently labeled with 6-FAM, HEX, NED (Applied Biosystems Inc., Foster City, CA, USA), and 0.5 U Taq DNA polymerase (Promega, Madison, WI, USA). 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., Foster City, CA, USA). 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

Fixed recombinant, and non-recombinant resistant and susceptible check plants from each mapping population were phenotyped using SFI in the greenhouse. The inoculum used throughout the phenotyping process was a mixture of three virulent strains of *F. graminearum* Schwabe (EEI #20/6, EEI #23/6, and JM #6) provided by Dr Jeannie Gilbert, Agriculture and Agri-Food Canada, Cereal Research Centre. The inoculum was produced as described by Sung and Cook (1981). Recombinant lines from each mapping population were grown in the greenhouse at an average daytime temperature of 23°C and a night-time temperature of 16°C. Four or five spikes per recombinant plant were inoculated when each spike reached 50% anthesis. Each spike was inoculated by injecting a 10 μl macro-conidial suspension (50,000 spores/ml) between the lemma and palea of a floret positioned at the inoculation point. To determine the inoculation point on each spike, the total number of spikelets per spike was multiplied by two-thirds (i.e., total number of spikelets = $12 \times \frac{2}{3} = 8$). The inoculation point was the eighth and ninth spikelet from the base of the spike on opposite sides of the spike in this example (Fig. 2). Primary or secondary florets were inoculated but not the tertiary floret. Following point inoculation, a 10×5 cm clear, Bitran S Series (Fisher Scientific, Atlanta, GA, USA) liquid tight specimen bag was placed over the spike to increase the humidity around the spike. Moisture from transpiration was visible in the bag covering the spike within 30 min of placement over the spike. The bags were left on the spikes for an incubation period of 48 h. Plants remained on the bench in the greenhouse and ratings were performed at 7, 14, and 21 days post-inoculation. Ratings were assessed by counting the number of infected florets directly below the inoculated florets and excluding the inoculated florets. The percentage of infected florets was averaged for each plant and plants were classified as resistant or susceptible. Resistant and susceptible classes were determined based on the bimodal distribution of ratings.

The inoculation procedure was repeated on five progeny plants derived from the original fixed recombinant plants in both populations to verify FHB ratings and classify resistant and susceptible plants. Four or five spikes per progeny plant were phenotyped and the average percentage of infected florets was recorded as previously described.

Construction of the genetic map

A total of 15 markers on chromosome 3BS including STS (Liu and Anderson 2003) and microsatellite markers (Somers et al. 2004) were screened for polymorphism between the parents of the two populations. Polymorphic marker primer sequences, annealing temperatures, and allele sizes are listed in Table 1. The polymorphic markers were used to genotype the fixed recombinant plants in
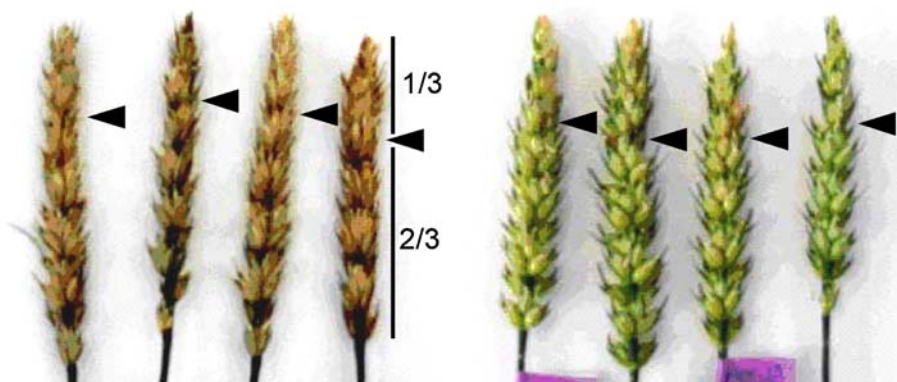

Fig. 2 Infection phenotype 21 days post-inoculation in four spikes from a susceptible plant (*left*) and four spikes from a resistant plant (*right*). A pair of florets on opposite sides of the spike (*arrowheads*), were injected with *Fusarium graminearum*. The inoculation point was set at 2/3 of the distance from the base of the spike. The spike was enclosed in a polypropylene bag for 48 h post-inoculation

Table 1 Primer sequences, annealing temperatures, and allele sizes of markers used in the Sumai 3*5/Thatcher (S/T) and HC374*3/98B69-L47 (HC/98) populations for fine mapping *Fhb1* in spring wheat

Marker	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Anneal (°C)	Allele size Nyubai/Sumai	References
BARC133	AGCGCTCGAAAAGTCAG	GGCAGGTCCAACTCCAG	51	125/125	http://www.scabusa.org
GWM493	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	61	197/195	Röder et al. (1998)
GWM533	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGGAAAAGCC	61	139/141	Röder et al. (1998)
STS3B-66	AGTCAGGCGAAGAGCGATAA	AGCACTGCACAATGAGCATC	55	192/192	Liu and Anderson (2003)
STS3B-80	AGAAGAAGGAAGCCCCTCTG	GCCATGTCTTTTGTGCCTTT	55	Null/null	Liu and Anderson (2003)
STS3B-138	CAAGATCAAGAAGGCCAAGC	AGGTACACCCCGTTCTCGAT	55	355/355	Liu and Anderson (2003)
STS3B-142	CGAGTACTACCTCGGCAAGC	CATAGAATGCCCCGAAACTG	50	156/156	Liu and Anderson (2003)
STS3B-163	TTCATGGACGAGTACGACGA	AAGGTTGCCATTGCTCTCAC	55	470/468	Liu and Anderson (2003)

both populations. JoinMap, version 3.0 (Biometris, Wageningen, The Netherlands; http://www.joinmap.nl) was used to determine the marker order and map distances.

Results

Genotyping

There were 79 recombinant plants identified in the HC/98 population (BC_2F_2) and 55 recombinant plants in the S/T population (BC_4F_1) using flanking markers GWM533 and GWM493. The HC/98 population was reduced from 79 to 66 recombinant plants due to pests in the greenhouse. Genotypic data was fully characterized for 51 of the 55 S/T BC_4F_2 families and 40 of the 66 HC/98 BC_2F_3 families (Fig. 1). The interval distance between GWM533 and GWM493 was 10.63 and 11.05 cM in the S/T population and HC/98 populations, respectively.

Phenotyping

Four or five spikes per plant for fixed recombinants and non-recombinant resistant and susceptible checks were inoculated in the greenhouse using SFI to assess Type II resistance. The range in infection ratings for S/T fixed recombinants was 0–5% (resistant) and 70–100% (susceptible); HC/98 fixed recombinants 5–25% (resistant) and 90–100% (susceptible); and for non-recombinant checks 0–8% (resistant) and 83–100% (susceptible) (Fig. 3). There were no intermediate ratings, all inoculated spikes showed either a clear resistant or susceptible infection phenotype and thus all plants could be clearly classified (Fig. 2). Darkening of the inoculation point was visible by day 7, and susceptible fixed recombinant and check plants had 83–100% infected spikes by day 14. There was no change in infection ratings between day 14 and 21 post-inoculation. Disease development progressed basally from the inoculation point of the spike and was similar in both populations according to ratings completed 7, 14, and 21 days post-inoculation.

The S/T population segregated 30 resistant to 21 susceptible plants, fitting a 1:1 chi-square ratio ($P > 0.10$). The HC/98 population segregated 20 resistant to 20 susceptible plants, fitting a 1:1 chi-square ratio ($P > 0.975$). Progeny plants derived from each initial fixed recombinant showed infection ratings to be the same as the previous generation.

Genetic map

The two maps were constructed with three microsatellite and five STS markers. Marker order was identical on each map, with the exception of marker, STS3BS-142, which was not polymorphic in the HC/98 population. Allele size for three of the eight markers was 2 bp smaller in Sumai 3 than in HC374 (Table 1). The total map length for the two populations was 10.63 cM (S/T) and 11.05 cM (HC/98) (Fig. 4). The major gene *Fhb1* was successfully mapped to syntenic intervals in the two populations flanked by STS3B-80 and STS3B-142 (S/T) and STS3B-80 and STS3B-66 (HC/98). *Fhb1* was mapped to a 1.27- and 6.05-cM interval in the S/T and HC/98 populations, respectively (Fig. 4).

Discussion

There are inherent difficulties associated with phenotypic characterization of FHB due to methodological problems of inoculation, the variability of the fungus, and confounding effects of the environment (Andersen 1948; Hanson et al. 1950; Scott 1927). Researchers have had great difficulty achieving reliable and reproducible FHB infection data. Reproducible phenotypic data is essential to create a reliable fine map of QTL candidate genes. The present study successfully decreased the variability in phenotyping FHB resistance and increased the reproducibility by focusing on variables that could be managed through the development of two large mapping populations with selected fixed resistant and fixed susceptible backgrounds, use of a mixture of isolates of the pathogen, proper inoculation techniques, multiple
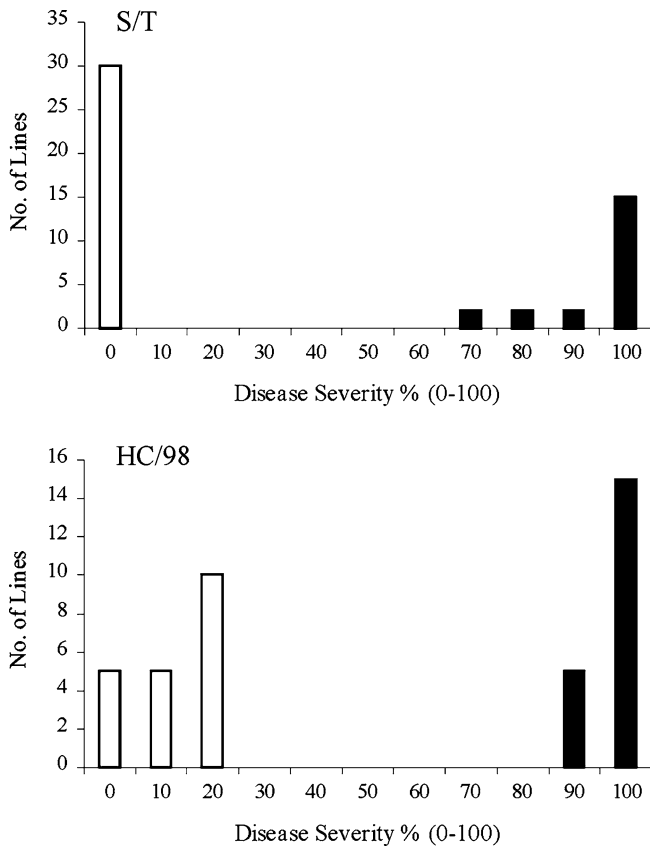
Fig. 3 Phenotypic distribution of Type II resistance to fusarium head blight in fixed recombinant plants measured by single floret injection. The data was collected in a greenhouse for the two crosses Sumai 3*5/Thatcher (S/T) and HC374/3*98B69-L47 (HC/98) and is represented as disease severity (percentage of infected florets). Resistant and susceptible parent and check lines showed infection phenotypes within the respective resistant (*open square*) and susceptible (*filled square*) modes of the distribution
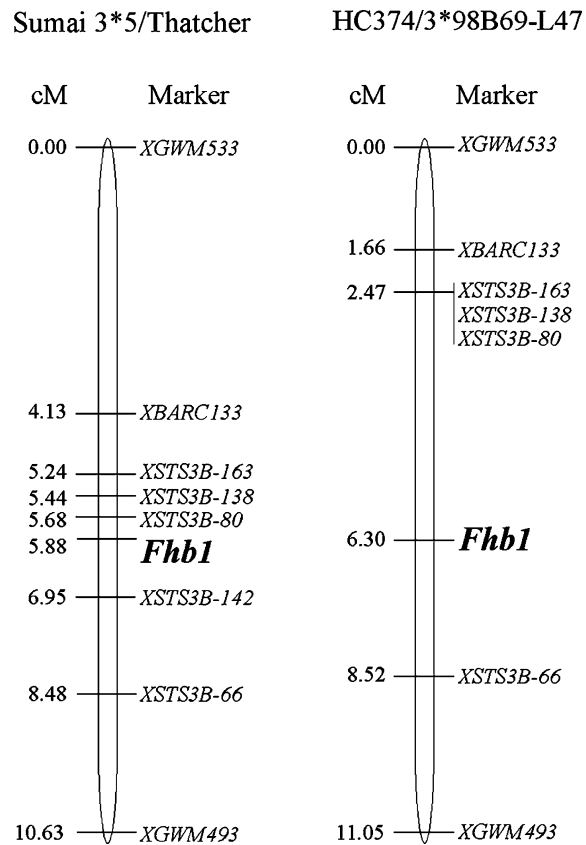
Fig. 4 Fine map of the major fusarium head blight (FHB) resistance gene, *Fhb1*, in two large bread wheat populations containing different sources of FHB resistance—Sumai 3 (*left*) and HC374 (Wuhan1/Nyubai) (*right*). The crosses of the populations were Sumai 3*5/Thatcher (S/T) and HC374*3/98B69-L47 (HC/98)

replication and progeny testing, and a temperature monitored indoor environment.

The SFI phenotyping completed in a greenhouse during this study showed similarity and consistency of infection rating within plants and generations. The use of four or five spikes per fixed recombinant plant provided replication and allowed the plants to be classified as resistant or susceptible (Fig. 2). There was a high level of confidence and reproducibility in the phenotypic data based on the consistency in infection ratings of the non-recombinant resistant and susceptible checks from both populations [LSD ($\alpha = 0.05$) S/T = 4.36; HC/98 = 8.02] and the similarity in infection data between the fixed recombinant plants and progeny testing.

Resistance to FHB is a complicated quantitative trait; however, the limitation of the spread of symptoms in a spike is a major component of resistance and may be controlled by a few major genes (Gu 1983; Bai and Xiao 1989; Bai et al. 1990). In this study, a coefficient of determination (R^2) was calculated for both populations with an ANOVA based on the percentage infection of the initial fixed recombinants. The R^2 value for both

populations was 0.99 and indicated there was a major effect of *Fhb1* on infection. The major effect of *Fhb1* on Type II resistance was clearly evident in the susceptible fixed recombinant plants of the S/T population even in a fixed resistant background. Although Sumai 3 alleles were present at FHB QTLs on 5A and 6B in the S/T population, 21 out of 51 plants homozygous for Thatcher alleles at *Fhb1* were still fully susceptible to spread of the disease (Fig. 1). In the HC/98 population, 98B69-L47 alleles were present at the 5A and 6B locus. Phenotypic data from the study demonstrated that 20 of the 40 recombinant plants were fully susceptible when Nyubai alleles were not present at *Fhb1*, and 20 of the 40 recombinant plants were fully resistant when Nyubai alleles were present. When the infection range for phenotypic data is analyzed, the degree of resistance in phenotypic data is greater in the S/T fixed recombinant plants (0–5%) versus the HC/98 fixed recombinant plants (5–25%) and non-recombinant resistant check plants (0–8%) (Fig. 3). These data from both populations suggests that *Fhb1* is functionally essential to provide Type II resistance and that *Fhb1* is an additive gene relative to other resistance loci. For example, Yang et al. (2003) detected strong Type II resistance derived

from the 6B locus of Sumai 3 ($R^2 = 0.21$) in a spring wheat cross (HY368/Sumai3//AC Foremost), yet this level of Type II resistance is not apparent in the present study when the Sumai 3 alleles of *Fhb1* are substituted for Thatcher alleles.

This study provided a novel approach to fine map the gene *Fhb1* from two different sources of FHB resistance (Sumai 3 and Nyubai) in two independent, large populations. The Sumai 3 and Nyubai sources of resistance had different allele sizes for three of the eight markers suggesting the sources of resistance present in the populations were genetically diverse. The two sources of FHB resistance mapped to syntenic marker intervals in independent populations (Fig. 4). Identification of original recombinant plants showed the interval distance between the two flanking markers GWM493 and GWM533 was 10.63 and 11.05 cM in the S/T and HC/98 populations, respectively. Comparative mapping between the present populations and Liu and Anderson (2003) showed the same marker order; however, the GWM493 to GWM533 distance was 6.8 map units based on results from Liu et al. (2003). The difference in genetic distance between GWM493 and GWM533 could be due to the difference in population size and structure. The increased recombination surrounding *Fhb1* in the HC/98 population compared to the S/T population could be due to population size, genetics of the two different FHB resistance sources of *Fhb1*, and/or the difference between the generations that were phenotyped and genotyped in this study.

The interval distance surrounding *Fhb1* indicated there was a higher recombination frequency in the HC/98 population compared to the S/T population immediately distal to *Fhb1* (Fig. 4). Comparisons of physical and genetic maps of wheat indicate that most genetic recombination occurs in gene-rich regions (Gill et al. 1996; Faris et al. 2000). In the study by Liu and Anderson (2003), QTL analysis of chromosome 3BS for FHB resistance in the Sumai 3 × Stoa population was completed using two greenhouse FHB evaluations. The present results indicate the *Fhb1* gene maps to a similar position as the peak of *Qfhs.ndsu-3BS* identified by Liu and Anderson (2003). However, Liu and Anderson (2003) indicate due to the small population size used, the exact order of markers and precise QTL position could not be determined.

In summary, *Fhb1* was successfully fine mapped to the same location in two populations with two genetically diverse sources of FHB resistance in spring wheat using an innovative strategy. This strategy combined unique population design to fix resistant and susceptible backgrounds, large population sizes, and the use of microsatellite markers to select fixed recombinants, robust phenotypic data, good marker density, and comparative mapping to attain a precise map position of *Fhb1*. This study suggests *Fhb1* is functionally essential to provide Type II resistance and *Fhb1* is an additive gene relative to other resistance loci. This information should aid plant breeders worldwide to reduce linkage drag associated with MAS and assist in the cloning of the functional resistance gene.

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