



# Genetic analyses of native Fusarium head blight resistance in two spring wheat populations identifies QTL near the *B1*, *Ppd-D1*, *Rht-1*, *Vrn-1*, *Fhb1*, *Fhb2*, and *Fhb5* loci

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## Abstract

**Key message** QTL analyses of two bi-parental mapping populations with AC Barrie as a parent revealed numerous FHB-resistance QTL unique to each population and uncovered novel variation near *Fhb1*.

**Abstract** Fusarium head blight (FHB) is a destructive disease of wheat worldwide, leading to severe yield and quality losses. The genetic basis of native FHB resistance was examined in two populations: a recombinant inbred line population from the cross Cutler/AC Barrie and a doubled haploid (DH) population from the cross AC Barrie/Reeder. Numerous QTL were detected among the two mapping populations with many being cross-specific. Photoperiod insensitivity at *Ppd-D1* and dwarfing at *Rht-B1* and *Rht-D1* was associated with increased FHB susceptibility. Anthesis date QTL at or near the *Vrn-A1* and *Vrn-B1* loci co-located with major FHB-resistance QTL in the AC Barrie/Reeder population. The loci were epistatic for both traits, such that DH lines with both late alleles were considerably later to anthesis and had reduced FHB symptoms (i.e., responsible for the epistatic interaction). Interestingly, AC Barrie contributed FHB resistance near the *Fhb1* locus in the Cutler population and susceptibility in the Reeder population. Analyses of the *Fhb1* candidate genes *PFT* and *TaHRC* confirmed that AC Barrie, Cutler, and Reeder do not carry the Sumai-3 *Fhb1* gene. Resistance QTL were also detected at the expected locations of *Fhb2* and *Fhb5*. The native FHB-resistance QTL detected near *Fhb1*, *Fhb2*, and *Fhb5* do not appear to be as effective as *Fhb1*, *Fhb2*, and *Fhb5* from Sumai-3. The presence of awns segregated at the *B1* awn inhibitor locus in both populations, but was only associated with FHB resistance in the Cutler/AC Barrie population suggesting linkage caused the association rather than pleiotropy.

## Abbreviations

ANOVA Analysis of variance  
BLAST Basic local alignment search tool

BLUPs Best linear unbiased predictors  
C/B Cutler/AC Barrie  
DH Doubled haploid  
ICIM Inclusive composite interval mapping  
IM Interval mapping  
LOD Logarithm of odds  
META-R Multi-environment trial analysis with R  
PVE Phenotypic variation explained

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QTL	Quantitative trait loci
B/R	AC Barrie/Reeder
RIL	Recombinant inbred line
SNP	Single-nucleotide polymorphism

## Introduction

Fusarium head blight (FHB), primarily caused by the fungus *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schwein.) Petch), is one of the most damaging diseases of wheat worldwide, leading to severe yield and quality losses (Gilbert and Tekauz 2000). The pathogen produces harmful mycotoxins such as deoxynivalenol (DON) during infection, and consumption of DON-contaminated grain is harmful to human and animal health (Ferrigo et al. 2016). In Canada, serious outbreaks of FHB were first reported in eastern Canada in 1980 and in Manitoba in 1993 (Gilbert and Haber 2013). Despite the significant efforts to develop more tolerant cultivars and agronomic techniques to manage the disease, FHB has become more widespread in western Canada and elsewhere over the last several years (Gilbert and Tekauz 2000; Gilbert and Haber 2013). In 2016, farmers confronted potentially the worst FHB damage in most wheat growing areas of the western Canada, particularly in the province of Saskatchewan (Canadian Grain Commission 2019).

The development of FHB resistant wheat cultivars is recognized as the most practical and effective solution for minimizing FHB damage in wheat (Bai and Shaner 2004; Gilbert and Haber 2013). Resistance to FHB in wheat is quantitatively inherited, and symptom expression is often modulated by environmental factors (Miedaner et al. 2001; Buerstmayr et al. 2012). The genetic basis of FHB resistance in wheat has been studied extensively, and more than 100 QTL associated with FHB resistance have been reported on the 21 wheat chromosomes (Buerstmayr et al. 2009; Liu et al. 2009; Löffler et al. 2009; Giancaspro et al. 2016). Although several mechanisms of FHB resistance have been proposed, the commonly studied types are resistance to initial infection (type I) and resistance to spread of infection within the spike (type II) (Schroeder and Christensen 1963; Bai and Shaner 2004; Buerstmayr et al. 2009). The other types include resistance to accumulation of mycotoxins such as deoxynivalenol (DON) (type III), resistance to kernel infection (type IV), and yield reduction (type V) (Mesterházy 1995). *Fhb1* (*QFhs.ndsu-3BS*) on chromosome arm 3BS, derived from Sumai-3 and its derivative Ning 7840, was described as the strongest and best-validated FHB-resistance QTL and is primarily associated with type II resistance (Buerstmayr et al. 2002; Liu et al. 2006, 2008). Despite significant efforts to identify the candidate gene for *Fhb1* and define its biological mechanism through genomic and transcriptome-based

analyses, functional validation of candidate genes remains challenging due to genome size and the polyploid nature of wheat (Schweiger et al. 2013, 2016; Xiao et al. 2013). Recently, *Fhb1* region was sequenced and a pore-forming toxin-like (*PFT*) gene was reported as the gene responsible for FHB resistance at the *Fhb1* locus (Rawat et al. 2016; He et al. 2018; Su et al. 2018). Subsequently, another gene encoding a putative histidine-rich calcium-binding protein (*TaHRC*) was proposed as the gene for *Fhb1* (Schweiger et al. 2016; Su et al. 2018, 2019; Li et al. 2019).

Despite extensive work on the inheritance of FHB resistance and disease-related morphological traits (Bai and Shaner 2004; Miedaner et al. 2006; Buerstmayr et al. 2009), little is understood about the genetic basis of native FHB resistance in Canadian spring wheat (i.e., FHB resistance not introduced from Sumai-3 and other Asian spring wheats). Breeding for FHB resistance in wheat is often hampered by a poor understanding of the genetics of resistance, particularly native FHB resistance. Fully exploiting the FHB resistance in Sumai-3 and related Asian germplasm requires a thorough understanding of the genetic basis of FHB resistance of not only the donor (e.g., Sumai-3) but also the recipient (i.e., local elite) germplasm. This study aims to enhance our understanding of the FHB resistance in the latter group of germplasm. AC Barrie is a hard red spring wheat cultivar in the Canada Western Red Spring marketing class that possesses an intermediate level of FHB resistance and an important parent for many Canadian cultivars. The Canadian cultivar Neepawa was the main contributor of the genetics to AC Barrie (see the pedigrees in Materials and Methods), but Mexican and American germplasm is also present in its pedigree.

The objective of the present study was to examine the genetic basis of FHB resistance in the Cutler/AC Barrie (C/B) recombinant inbred line (RIL) and AC Barrie/Reeder (B/R) doubled haploid (DH) populations.

## Materials and methods

### Plant populations

Two mapping populations were developed to study FHB resistance from the spring wheat line AC Barrie. The first was a RIL population of 212 lines from the cross Cutler/AC Barrie (C/B). AC Barrie (pedigree: Neepawa/Columbus/BW90) (McCaig et al. 1996) is a hard red spring wheat that possesses an intermediate level of FHB resistance and is photoperiod sensitive. Columbus has the pedigree Neepawa\*6/RL4137, and BW90 has the complex pedigree BW15/BW38//RL4359/RL4353 (where BW15 = Manitou/Tobari-66, BW38 = Sonora-64/Tezanos-Pintos-Precoz//Neepawa, RL4359 = Sonora-64/Tezanos-Pintos-Precoz//

Neepawa, and RL4353 = WORLD-SEEDS-1809/Neepawa//Era/Park). Cutler (pedigree: Ciano'S'/4/Sonora-64/Yaqui-50E5//Gaboto/3/Inia'S') (Briggs et al. 1992) is a Canadian spring wheat derived from CIMMYT germplasm that is susceptible to FHB and photoperiod insensitive. The second mapping population was a DH population of 188 lines developed from the cross AC Barrie/Reeder (B/R), where Reeder (PI-613586) is a moderately susceptible American spring wheat developed by North Dakota State University (pedigree: IAS20\*4/H567.71//Stoa/3/ND674) (Oelke and Kolmer 2004).

### Phenotyping and statistical analysis

The C/B RIL population and parents were phenotyped in eight environments (i.e., field nurseries) over 2013 and 2014 (Carman MB 2013 and 2014, Winnipeg MB 2013, Morden MB 2014, Charlottetown PEI 2013 and 2014, and Ottawa ON 2013 and 2014). The check lines were Shaw, Neepawa, AC Morse, AC Vista, CDC Teal, Roblin, AC Cora, 5602 HR, 93FHB37, and ND2710. Entries were grown in an alpha lattice design with 12 incomplete blocks and three replicates per environment. The B/R DH population and parents were phenotyped in eight environments over 2015 and 2016 (Carman MB 2015 and 2016, Morden MB 2015 and 2016, Charlottetown PEI 2015 and 2016, and Ottawa ON 2015 and 2016). The check lines were Shaw, Neepawa, CDC Teal, Roblin, 93FHB37, and ND2710. Field experiments were arranged in an alpha lattice design with 12 incomplete blocks and three replicates per environment. The experimental plot was a single one metre row.

Plant height, anthesis date, FHB incidence, and FHB severity data were collected. Plant height was measured from the soil surface to the top of main tiller spikes (excluding awns if present). Anthesis date was recorded when 50% of the main tillers in the row had begun anthesis. Plant height data were not collected in Charlottetown PEI nurseries in 2013, 2014, and 2015. Anthesis date was not collected in any of the Charlottetown PEI nurseries.

The FHB nurseries in all locations were inoculated with *F. graminearum* isolates as described in McCartney et al. (2016). The Carman and Winnipeg FHB nurseries were spray inoculated twice (on the recorded anthesis date and 2–3 days later) with backpack sprayers. In PEI, plots were spray inoculated three times per week over the course of flowering in the trial with standard pesticide sprayer. Inoculum consisted of a mixture of *F. graminearum* isolates prepared to 50,000 macroconidia per ml in these nurseries. Carman nurseries were intermittently mist irrigated overnight. PEI nurseries were intermittently mist irrigated day and night. The Winnipeg nursery was irrigated with sprinkler heads to maintain wet soil to promote a humid micro-environment. The Morden and Ottawa FHB nurseries were

inoculated with *F. graminearum*-infected corn and barley kernels on the soil surface. The infested grain was spread in plots about six weeks after seeding. Plots were irrigated with sprinklers to maintain wet soil to promote a humid micro-environment.

FHB incidence was the percentage of spikes with visual FHB symptoms. FHB severity was the percentage of the spike with visual FHB symptoms, when only considering diseased spikes. FHB incidence and severity data were converted into FHB visual rating index (VRI),  $VRI = (FHB \text{ incidence} \times FHB \text{ severity}) / 100$ . Deoxynivalenol (DON) content was determined (Sinha et al. 1995) on the C/B RIL population from grain samples harvested utilizing low air speeds to prevent loss of Fusarium-damaged kernels.

The software multi-environment trial analysis with R for Windows (META-R) (Alvarado et al. 2017) was used for analysis of variance (ANOVA) and to calculate best linear unbiased predictors (BLUPs) for plant height, anthesis date, FHB incidence, FHB severity, and DON content. Genotypes, environment, replicates, and blocks were considered random. Heritability was also calculated using META-R.

### Genotyping

Genomic DNA was extracted from lyophilized leaf tissue with the DNeasy 96 Plant Kit (Qiagen, Toronto, Canada) and quantified using PicoGreen stain (Molecular Probes, Inc., Eugene, Oregon, USA). Single-nucleotide polymorphism (SNP) markers were genotyped on the RIL and DH populations and parents using the Illumina Infinium 90 K wheat SNP beadchip (Illumina, San Diego, CA) (Cavanagh et al. 2013; Wang et al. 2014). The raw data were analyzed with genotyping module of GenomeStudio V2011.1 software (Illumina, San Diego, USA) using default clustering parameters.

### Construction of genetic maps

A total of 195 RILs and 176 DHs were used to calculate genetic linkage maps of the C/B RIL and B/R DH populations, and mapping QTL for FHB resistance and disease-related morphological traits. Before constructing genetic maps, SNPs were filtered by excluding those with poor quality data. All SNPs that were monomorphic between the two parents and those with > 10% missing data were excluded from linkage analysis. Each marker was tested for deviation from the expected 1:1 ratio using Chi-squared test. Markers showing significant ( $p < 0.01$ ) segregation distortions were discarded. Markers were placed into preliminary linkage bins using the BIN module in QTL IciMapping version 4.0.6.0 (Li et al. 2007). A single marker with the least missing data was selected from each linkage bin and was used for linkage analysis with MapDisto version 1.7.7 (Lorieux

2012). A minimum logarithm of the odds (LOD) score of 4.0 and maximum recombination fraction of 0.2 were used to identify linkage groups. Loci were ordered using a combination of the “AutoMap”, “Order sequence”, and “Compare all order” functions. The best order of markers was generated using both “Auto Check Inversions” and “Auto Ripple” commands. The “Branch and Bound II” and “Seriation II” ordering methods were used in combination with the sum of adjacent recombination fractions (SARF) and count of crossover events (COUNT) as fitting criteria. For each linkage group, the shortest linkage map was selected from the linkage map solutions generated using these different mapping algorithms and criteria. Recombination fractions were converted into map distances using the Kosambi mapping function (Kosambi 1943). Linkage groups were assigned to chromosomes based on existing high-density SNP maps of wheat (Maccaferri et al. 2014; Wang et al. 2014).

DNA markers and candidate genes were located on IWGSC Chinese Spring RefSeq v1.0 (Appels et al. 2018) by basic local alignment search tool (BLAST). The candidate genes included the dwarfing genes *Rht-B1* and *Rht-D1* (Peng et al. 1999), the photoperiod sensitivity gene *Ppd-D1* (Beales et al. 2007), the vernalization genes *Vrn-A1* and *Vrn-B1* (Yan et al. 2003), and the *Fhb1* candidate genes *PFT* (Rawat et al. 2016) and *TaHRC* (Li et al. 2019; Su et al. 2019).

### QTL analysis

QTL analysis was conducted with interval mapping (IM) and inclusive composite interval mapping (ICIM) using QTL Ici-Mapping version 4.1.0.0 (Li et al. 2007, 2008). For all traits, QTL analyses were carried out using BLUPs (i.e., adjusted means) for test entries in each individual environment and pooled over all environments. Analysis for additive-effect QTL was conducted with 0.1 cM steps, and the 5% LOD significance threshold was calculated with 10,000 permutations. The LOD significance threshold was 3.11 for both IM and ICIM for the C/B RIL population and 3.09 for the B/R DH population for additive-effect QTL analysis based upon permutation analyses. Additive-effect QTL were declared when the LOD score exceeded the LOD significance threshold in two or more environments, or one or more environments plus the pooled dataset, based upon IM or ICIM. For these QTL, QTL statistics were also reported for additional environments in which the LOD score exceeded 2.5.

Analysis for epistatic QTL was conducted with 2.0 cM steps and a default LOD significance threshold of 3.5. The proportion of phenotypic variance explained by single QTL was determined by the square of the partial correlation coefficient ( $r^2$ ). For anthesis date and plant height, epistatic QTL were reported when the LOD exceeded 3.5 in four or more environments, or three environments plus the pooled dataset,

based upon IM or ICIM. For FHB resistance, epistatic QTL were reported when the LOD exceeded 3.5 in seven or more combinations of environment (individual environments or pooled dataset) by traits (VRI, FHB incidence, FHB severity, and DON content).

### Characterization of *Fhb1* candidate genes in parental lines

A panel of twenty wheat cultivars varying in FHB resistance, including AC Barrie, Cutler, and Reeder, were included in this analysis to characterize two recently reported *Fhb1* candidate genes, pore-forming toxin like (*PFT*) gene and histidine-rich calcium-binding protein (*TaHRC*) gene (He et al. 2018; Su et al. 2018, 2019; Li et al. 2019). Some of the cultivars in the panel are known to have *Fhb1* or high levels of FHB resistance, and others were randomly selected cultivars with unknown FHB resistance (Supplementary Table S13). For the characterization of *Fhb1* candidate gene *PFT*, three pairs of primers covering the entire *PFT* open reading frame (ORF) and part of the promoter region (P1F/P1R, P2F/P2R, and P3F/P3R) were used (He et al. 2018). Genomic DNA was extracted as previously described in the genotyping section. PCR reactions were carried out in a final volume of 50  $\mu$ l containing 100 ng of total genomic DNA and 0.2  $\mu$ M each primer using HotStarTaq Master Mix Kit (Qiagen, Toronto, Canada). PCR reactions were performed using the following conditions: an initial denaturation of 15 min at 95  $^{\circ}$ C, followed by 35 cycles at 94  $^{\circ}$ C for 30 s, 54  $^{\circ}$ C for 30 s, 72  $^{\circ}$ C for 1.30 min, and a final extension of 10 min at 72  $^{\circ}$ C. Three independent PCR reactions were performed for each genotype. PCR products from each reaction/genotype were separated on a 1% agarose gel to verify amplicon specificity.

Characterization of the *TaHRC* gene in the same wheat panel was performed using the gene specific primer pair TaHRC-GSM-F/TaHRC-GSM-R designed to capture the deletion polymorphism between the two *TaHRC* alleles (*TaHRC-R* and *TaHRC-S*) (Su et al. 2018). PCR reactions were performed using the following conditions: an initial denaturation of 15 min at 95  $^{\circ}$ C, followed by 35 cycles at 94  $^{\circ}$ C for 30 s, 54  $^{\circ}$ C for 30 s, 72  $^{\circ}$ C for 2 min, and a final extension of 10 min at 72  $^{\circ}$ C. Four independent PCR reactions were performed for each genotype, and aliquots of pooled PCR products were separated by agarose gel (1%) electrophoresis to verify amplicon specificity. PCR amplicons were purified with QIAquick PCR Purification Kit according to the manufacturer’s instructions (Qiagen, Toronto, Canada) and were sequenced with Sanger sequencing (National Research Council, Canada). DNA sequence trace files from the ABI 3730xl Genetic Analyzer were processed using PHRED (Ewing et al. 1998) and assembled with CAP3 (Huang and Madan 1999) as

implemented in the internal data pipeline called SOOMOS v0.6 (T. Banks, personal communication). Sequence alignments were performed with ClustalW (Thompson et al. 1994).

## Results

### Phenotypic analyses

The frequency distributions of pooled datasets for anthesis date, plant height, FHB incidence, FHB severity, VRI, and DON content in the C/B RIL and B/R DH populations are illustrated in Fig. 1a, b, respectively. FHB resistance traits and related morphological traits were approximately normally distributed in the C/B RIL population. Cutler flowered 3 days earlier and was 14 cm shorter than AC Barrie. The average plant height among the 195 RILs was 86 cm, and average anthesis date was Julian date 198. Heritability estimates were 0.92 for anthesis date, 0.86 for plant height, 0.86 for incidence, 0.81 for severity, 0.84 for VRI, and 0.82 for DON in C/B RIL population (Table 1). There was some transgressive segregation for plant height and FHB resistance in the B/R cross, but most DHs were within the means of the parents. Plant height was essentially bi-modal in the B/R population. Reeder flowered 2 days earlier and was 14 cm shorter than AC Barrie. The average plant height among the 176 DHs was 80 cm, and average anthesis date was Julian date 203 (Fig. 1a, b, Supplementary Table S1 and S7). Heritability estimates were 0.94 for anthesis date, 0.97 for plant height, 0.87 for incidence, 0.84 for severity, and 0.89 for VRI in B/R DH population (Table 2).

The resistant checks 93FHB37 and ND2710 had the lowest FHB incidence, severity, VRI, and DON content in the FHB field nurseries in both RIL and DH populations (Supplementary Tables S1 and S7). AC Morse, AC Vista, CDC Teal, and Roblin of C/B RIL experiments, and Shaw, CDC Teal, and Roblin of B/R DH experiments were the most susceptible checks to FHB based upon FHB incidence, severity, VRI, and DON content. Correlation analysis revealed a significant positive correlation between plant height and anthesis date, and a negative correlation between plant height and FHB symptoms (incidence, severity, VRI, and DON) in both the C/B and B/R populations (Tables 3, 4). Anthesis date and FHB symptoms (incidence, severity, and VRI) were negatively correlated in the B/R DH population (Table 4). In the C/B RIL population, anthesis date was negatively correlated with incidence and VRI, but not with severity or DON content (Table 3). Analysis of variance showed significant ( $p < 0.001$ ) differences among genotypes for all traits (Tables 1, 2, Supplementary Tables S2 and S8).

### Linkage maps

Two high-density whole genome linkage maps were developed using 90 K wheat Infinium SNPs tested on the C/B RIL and B/R DH populations. A total of 10,178 SNPs were mapped in the C/B population. The C/B linkage map consisted of 26 linkage groups and spanned 2438 cM (Supplementary Table S5). The number of linkage bins varied from 18 on chromosome 5D to 229 on chromosome 5B, with an average of 70 linkage bins per linkage group. The B/R linkage map consisted of 10,878 SNPs. The total map length across the 28 linkage groups was 2845 cM (Supplementary Table S11). The *B1* awn gene was mapped onto chromosome arm 5AL of both populations (Supplementary Table S5 and S11). In some instances, a chromosome consisted of two linkage groups (e.g., linkage groups 2A.1 and 2A.2, 5A.1 and 5A.2, 5D.1 and 5D.2, 6D.1 and 6D.2, and 7B.1 and 7B.2) in the C/B RIL population.

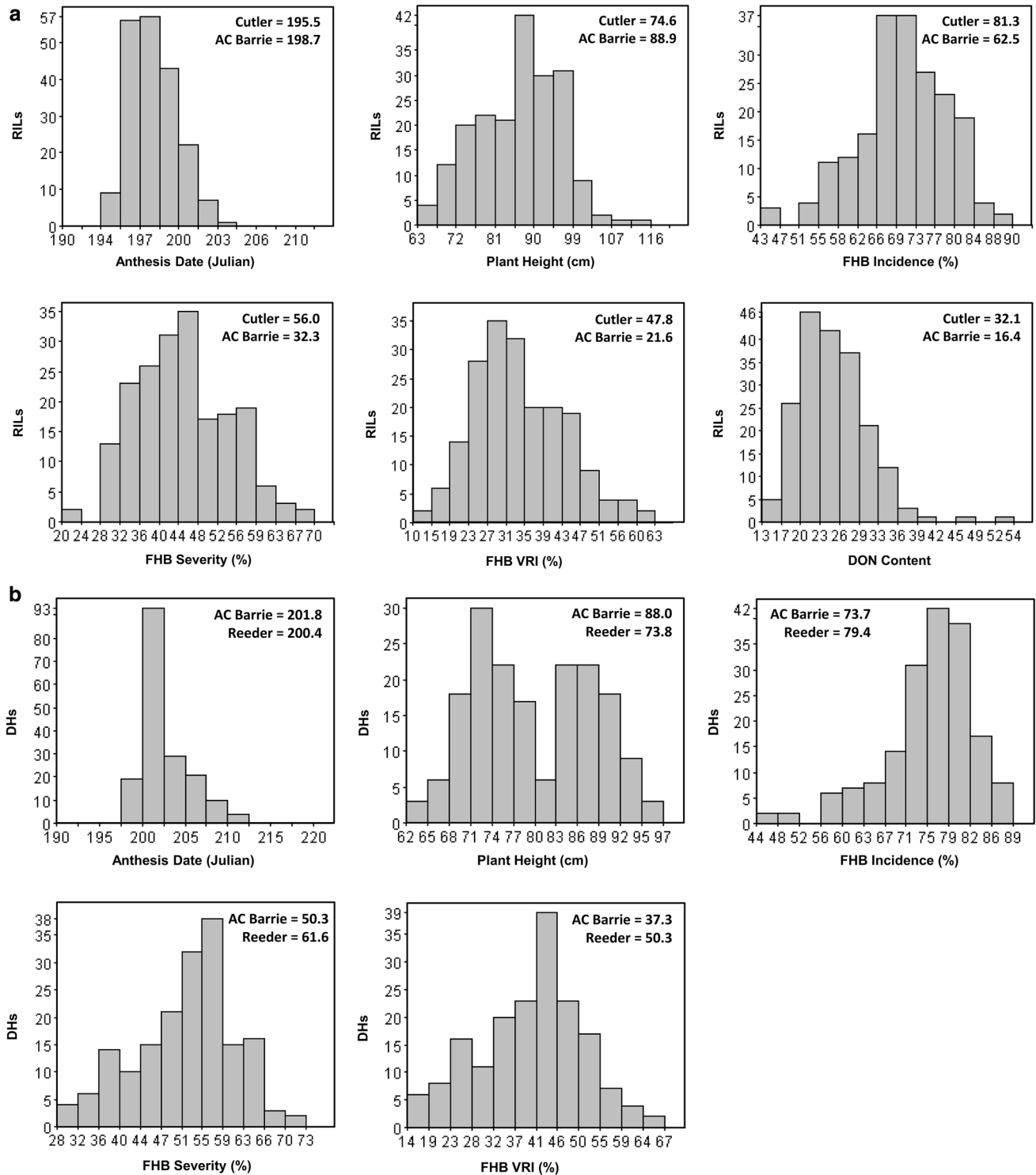
### Identification of QTL for morphological traits

In order to differentiate the QTL detected in each population, the abbreviation ‘*mcb*’ was included in the names of QTL detected in C/B population, whereas ‘*mbr*’ identified QTL in B/R population.

### Anthesis date

Four additive-effect QTL for anthesis date were identified in the C/B RIL population (Table 5). The QTL were located on chromosomes 2D, 4A, and 7D and were named *QAnth.mcb-2D*, *QAnth.mcb-4A.1*, *QAnth.mcb-4A.2*, and *QAnth.mcb-7D*. AC Barrie alleles delayed anthesis at *QAnth.mcb-2D* and *Q1Anth.mcb-4A.1*, and *QAnth.mcb-7D*, and Cutler allele delayed anthesis at *QAnth.mcb-4A.2*. These results are consistent with Cutler being earlier flowering than AC Barrie. *QAnth.mcb-2D* maps to the expected location of photoperiod sensitivity gene *Ppd-D1*. Three digenic epistatic interactions were identified between loci on chromosomes 1A and 3B, 1B and 3A, and 4A and 7A (Supplementary Table S6). The additive-effect QTL *QAnth.mcb-4A.1* was coincident with an epistatic QTL for anthesis date involving chromosome 7A.

Three additive-effect QTL for anthesis date were identified in the B/R DH population (Table 6). The QTL were located on chromosomes 5A, 5B, and 6B and were named *QAnth.mbr-5A*, *QAnth.mbr-5B*, and *QAnth.mbr-6B*. The AC Barrie allele delayed anthesis for *QAnth.mbr-5B*, while the Reeder allele delayed anthesis for *QAnth.mbr-5A* and *QAnth.mbr-6B*. *QAnth.mbr-5A* and *QAnth.mbr-5B* map to the expected locations of *Vrn-A1* and *Vrn-B1*, respectively. Analysis for epistatic QTL identified five additional QTL for anthesis date (Supplementary Table S12). The additive-effect QTL *QAnth.mbr-5A* and *QAnth.mbr-5B* were



**Fig. 1 a** Frequency distribution of best linear unbiased predictors (BLUPs) of pooled data of anthesis date, plant height, FHB incidence, FHB severity, FHB visual rating index, and DON content in the Cutler/AC Barrie RIL population from eight environments over 2013 and 2014. Means of the parents are indicated. **b** Frequency dis-

tribution of best linear unbiased predictors (BLUPs) of pooled data of anthesis date, plant height, FHB incidence, FHB severity, and FHB visual rating index in the AC Barrie/Reeder DH population from eight environments over 2015 and 2016. Means of the parents are indicated

**Table 1** ANOVA statistical analysis of anthesis date (DA), plant height (HGHT), FHB incidence (INC), FHB severity (SEV), FHB visual rating index (VRI), and DON content (DON) in the Cutler/AC Barrie RIL population grown in eight environments over 2013 and 2014

Source of Variation	DA	HGHT	INC	SEV	VRI	DON
Genotype ( $\hat{\sigma}_G^2$ )	3.47***	71.84***	68.62***	73.08***	86.44***	32.30***
Environment ( $\hat{\sigma}_E^2$ )	71.05***	123.90***	131.17***	249.32***	216.49***	186.46***
G × E <sup>a</sup> ( $\hat{\sigma}_{GE}^2$ )	1.40***	11.25***	60.40***	110.79***	109.21***	33.32***
Residual ( $\hat{\sigma}^2$ )	1.27	181.76	79.73	76.99	78.01	51.98
Grand Mean	197.50	86.07	69.40	43.87	32.92	24.78
LSD <sup>b</sup>	1.07	6.45	6.14	7.40	7.49	4.89
CV (%) <sup>c</sup>	0.57	15.66	12.87	20.00	26.83	29.10
Heritability	0.92	0.86	0.86	0.81	0.84	0.82

<sup>a</sup>Genotype × environment<sup>b</sup>Least significant difference ( $P=0.05$ )<sup>c</sup>Coefficient of variation\*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ **Table 2** ANOVA statistical analysis of anthesis date (DA), plant height (HGHT), FHB incidence (INC), FHB severity (SEV), and FHB visual rating index (VRI) in the AC Barrie/Reeder DH population grown in eight environments over 2015 and 2016

Source of Variation	DA	HGHT	INC	SEV	VRI
Genotype ( $\hat{\sigma}_G^2$ )	10.00***	72.31***	88.70***	106.37***	142.88***
Environment ( $\hat{\sigma}_E^2$ )	39.01***	85.58***	146.88***	160.73***	136.35***
G × E <sup>a</sup> ( $\hat{\sigma}_{GE}^2$ )	1.81***	11.28***	67.75***	119.10***	99.31***
Residual ( $\hat{\sigma}^2$ )	6.29	19.96	113.36	119.96	109.64
Grand Mean	202.65	80.38	73.98	51.28	39.53
LSD <sup>b</sup>	1.63	3.34	6.83	8.21	7.89
CV (%) <sup>c</sup>	1.24	5.56	14.39	21.36	26.49
Heritability	0.94	0.97	0.87	0.84	0.89

<sup>a</sup>Genotype × environment<sup>b</sup>Least significant difference ( $P=0.05$ )<sup>c</sup>Coefficient of variation\*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ **Table 3** Correlation coefficients ( $r$ ) among traits assessed in the Cutler/AC Barrie RIL population and corresponding statistical significance

Traits <sup>a</sup>	HGHT	DA	INC	SEV	VRI
DA	0.28***				
INC	− 0.67***	− 0.37***			
SEV	− 0.48***	− 0.11 ns	0.85***		
VRI	− 0.59***	− 0.20**	0.92***	0.98***	
DON	− 0.45***	− 0.12 ns	0.61***	0.69***	0.69***

<sup>a</sup>DA, anthesis date; HGHT, plant height; INC, FHB incidence; SEV, FHB severity; VRI, FHB visual rating index; DON, DON content\*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ ; ns  $P \geq 0.05$ 

consistently epistatic to each other, having large LOD scores for both QIME (i.e., interval mapping) and QICE (i.e., inclusive composite interval mapping) epistasis modules. Box plots show this interaction (Fig. 2a). Other epistatic interactions were identified among loci on chromosomes 1A and 7A, 1B and 1D, 2A and 7A, and 3B and 7A.

**Table 4** Correlation coefficients ( $r$ ) among traits assessed in the AC Barrie/Reeder DH population and corresponding statistical significance

Traits <sup>a</sup>	HGHT	DA	INC	SEV
DA	0.20**			
INC	− 0.39***	− 0.82***		
SEV	− 0.30***	− 0.79***	0.88***	
VRI	− 0.36***	− 0.81***	0.92***	0.98***

<sup>a</sup>DA, anthesis date; HGHT, plant height; INC, FHB incidence; SEV, FHB severity; VRI, FHB visual rating index\*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ 

## Plant height

Three additive-effect QTL for plant height were identified in the C/B RIL population (Table 5). These QTL were located on chromosomes 2D, 4D, and 5A and were named *QHT.mcb-2D*, *QHT.mcb-4D* and *QHT.mcb-5A*. The AC Barrie allele increased plant height for each of these QTL, which is consistent with AC Barrie being 14 cm taller than Cutler in field tests. Of the

**Table 5** Additive-effect QTL detected for anthesis date, plant height, FHB incidence, FHB severity, FHB visual rating index, and DON content in the Cutler/AC Barrie (C/B) RIL population

Trait/QTL	Location	Chr <sup>c</sup>	IM <sup>a</sup>				ICIM <sup>b</sup>			
			Pos <sup>d</sup>	LOD <sup>e</sup>	PVE <sup>f</sup>	Add <sup>g</sup>	Pos	LOD	PVE	Add
Anthesis date										
<i>QAnth.mcb-2D</i>	DA_Ott_2014 <sup>h</sup>	2D	3.3	8.60	19.14	− 1.54	3.4	9.74	20.26	− 1.60
	DA_POOL	2D	3.2	7.31	15.45	− 0.77	3.8	9.15	18.47	− 0.81
	DA_Wpg_2013	2D	4.0	6.79	15.96	− 0.54	7.6	10.25	15.90	− 0.56
	DA_Mor_2014	2D	2.2	5.65	13.59	− 0.79	3.1	7.17	14.56	− 0.83
	DA_Ott_2013	2D	3.9	5.50	11.94	− 0.69	4.4	7.45	14.37	− 0.73
	DA_Car_2014	2D	2.0	4.62	8.29	− 0.59	3.1	5.97	12.62	− 0.64
<i>QAnth.mcb-4A.1</i>	DA_Ott_2013	4A					44.9	3.77	6.17	− 0.48
	DA_Car_2014	4A	44.9	2.59	4.28	− 0.43	44.9	3.55	6.31	− 0.46
	DA_Ott_2014	4A					45.0	2.80	4.84	− 0.79
<i>QAnth.mcb-4A.2</i>	DA_Ott_2013	4A	114.3	3.66	7.15	0.52	114.3	4.76	7.77	0.54
	DA_Mor_2014	4A	113.1	4.01	8.94	0.64	114.3	4.59	7.83	0.61
	DA_POOL	4A	113.8	3.00	6.22	0.48	114.1	3.26	5.45	0.44
	DA_Car_2014	4A					114.0	2.70	4.73	0.40
<i>QAnth.mcb-7D</i>	DA_Wpg_2013	7D	22.6	2.73	6.65	− 0.34	24.3	4.19	6.20	− 0.34
	DA_Mor_2014	7D	33.4	2.63	5.85	− 0.52	33.4	3.47	5.89	− 0.53
	DA_POOL	7D					25.9	2.93	4.78	− 0.42
	DA_Car_2014	7D	21.0	2.96	6.15	− 0.50				
Plant height										
<i>QHt.mcb-2D</i>	HGHT_Wpg_2013	2D	8.2	2.99	5.97	− 2.02	8.2	5.99	8.23	− 2.29
	HGHT_Car_2014	2D	17.2	2.54	7.52	− 2.91	9.0	4.92	4.96	− 2.38
	HGHT_Car_2013	2D					8.2	3.67	5.24	− 1.85
	HGHT_POOL	2D					7.7	3.64	4.41	− 1.91
<i>QHt.mcb-4D</i>	HGHT_POOL	4D	29.3	19.46	35.00	− 5.30	29.3	22.51	37.87	− 5.57
	HGHT_Car_2014	4D	29.2	15.82	26.99	− 5.57	29.1	22.35	28.58	− 5.71
	HGHT_Ott_2013	4D	29.8	17.67	33.75	− 4.69	29.9	19.73	33.99	− 4.79
	HGHT_Wpg_2013	4D	29.6	16.57	31.32	− 4.61	29.6	18.38	31.73	− 4.47
	HGHT_Car_2013	4D	29.2	14.22	25.48	− 4.15	29.2	16.11	28.41	− 4.28
	HGHT_Ott_2014	4D	29.2	23.86	42.02	− 7.74	30.5	10.29	42.02	− 4.66
	HGHT_Mor_2014	4D	29.3	4.34	7.78	− 1.75	29.3	4.34	7.78	− 1.75
<i>QHt.mcb-5A</i>	HGHT_Car_2014	5A.2					32.5	7.97	8.08	− 3.01
	HGHT_Ott_2013	5A.2					32.5	3.80	5.06	− 1.83
FHB resistance										
<i>QFhb.mcb-1B</i>	DON_Wpg_2013	1B	96.3	4.05	6.36	1.29	96.2	6.58	11.99	1.52
	DON_POOL	1B	106.2	3.72	8.03	1.37	106.1	5.29	8.65	1.49
	DON_Ott_2014	1B	106.2	4.36	8.33	1.86	106.1	4.09	8.79	1.69
	SEV_Wpg_2013	1B	113.0	2.69	7.38	1.07	113.0	3.87	7.07	1.20
	DON_Wpg_2013	1B	108.1	3.38	5.73	1.22				
	SEV_POOL	1B					106.1	2.76	3.60	1.44
	DON_Car_2013	1B					99.2	2.73	4.97	2.81
	SEV_Car_2014	1B					109.3	2.63	4.87	3.73
<i>QFhb.mcb-2A</i>	VRI_Car_2013	2A.2					0.0	5.16	7.37	− 5.32
	VRI_Car_2014	2A.2					4.7	3.66	5.06	− 3.59
	SEV_Car_2013	2A.2					0.0	3.17	4.77	− 4.31
	DON_Car_2014	2A.2					0.2	2.64	4.93	− 0.76
<i>QFhb.mcb-2B</i>	SEV_Mor_2014	2B	91.4	4.99	9.29	3.59	91.4	6.77	10.35	3.51
	VRI_Car_2014	2B					86.5	5.63	7.92	4.55
	SEV_Car_2014	2B					90.1	5.58	9.61	5.30



**Table 5** (continued)

Trait/QTL	Location	Chr <sup>c</sup>	IM <sup>a</sup>				ICIM <sup>b</sup>			
			Pos <sup>d</sup>	LOD <sup>e</sup>	PVE <sup>f</sup>	Add <sup>g</sup>	Pos	LOD	PVE	Add
<i>QFhb.mcb-2D.1</i>	SEV_Car_2013	2B	93.0	2.70	4.71	4.68	91.6	4.98	7.73	5.50
	VRI_Car_2013	2B					91.6	4.27	6.07	4.84
	DON_Car_2014	2B					92.0	3.85	7.31	0.92
	SEV_POOL	2B					84.5	3.50	4.67	1.66
	VRI_Mor_2014	2B	80.0	2.87	4.13	3.71	92.7	3.44	5.00	3.32
	VRI_POOL	2B					83.1	3.17	4.38	1.73
	DON_Mor_2014	2B					84.2	3.11	5.76	2.26
	INC_PEI_2014	2D	4.5	4.29	9.58	2.88	10.1	7.67	12.00	3.22
	INC_POOL	2D	8.2	4.28	7.49	2.29	8.2	6.54	9.08	2.22
	VRI_PEI_2014	2D	4.1	3.93	8.48	2.97	10.1	6.09	10.09	3.00
	INC_Car_2013	2D	8.2	2.74	4.99	2.28	8.2	4.46	6.34	2.47
	VRI_POOL	2D	8.2	2.56	3.67	2.04	8.2	4.20	5.88	1.99
	INC_Wpg_2013	2D	8.4	3.34	6.24	2.51	8.1	3.67	7.43	2.38
	INC_Car_2014	2D					8.2	3.64	5.71	2.67
	INC_PEI_2013	2D	8.2	2.93	4.88	2.06	8.2	3.52	6.22	1.89
	VRI_Wpg_2013	2D	8.2	3.19	7.42	1.42	8.2	3.51	7.41	1.44
	INC_Ott_2014	2D					8.2	3.42	5.21	3.51
DON_Car_2014	2D	8.2	2.72	6.25	0.83	8.4	3.20	6.13	0.85	
VRI_PEI_2013	2D					8.2	3.18	5.63	1.57	
VRI_Car_2014	2D	8.2	2.65	4.17	3.97	8.2	3.15	4.29	3.34	
SEV_PEI_2014	2D	12.0	3.45	7.24	2.73	10.1	2.94	6.41	2.20	
DON_Ott_2014	2D	5.2	3.03	6.67	1.68	6.9	2.60	5.75	1.38	
<i>QFhb.mcb-2D.2</i>	DON_Wpg_2013	2D					66.0	3.45	6.04	1.08
	SEV_Wpg_2013	2D					68.2	3.17	6.60	1.17
	INC_Mor_2014	2D	66.0	3.12	6.35	2.75	66.0	2.95	5.68	2.45
	INC_Wpg_2013	2D	64.1	3.62	6.69	2.57	64.1	2.85	5.73	2.07
	INC_POOL	2D					66.0	2.59	3.42	1.35
<i>QFhb.mcb-3B</i>	INC_PEI_2013	3B	7.6	9.78	15.05	3.56	7.6	9.69	18.43	3.20
	VRI_PEI_2013	3B	7.6	9.63	14.36	3.16	7.6	9.21	17.56	2.72
	SEV_POOL	3B	7.6	4.71	6.41	2.40	7.4	6.75	9.57	2.34
	SEV_Car_2013	3B	9.4	3.72	6.42	5.43	9.4	5.71	8.85	5.81
	VRI_POOL	3B	7.6	5.07	7.06	2.78	7.5	5.63	8.19	2.31
	VRI_Car_2013	3B	7.4	3.53	5.53	5.45	9.4	4.35	6.15	4.80
	VRI_PEI_2014	3B	5.3	3.71	7.24	2.72	6.3	4.09	6.86	2.45
	SEV_PEI_2014	3B	5.4	3.69	7.13	2.70	5.9	3.82	8.79	2.55
	VRI_Ott_2014	3B	7.6	3.34	5.97	5.16	7.4	3.51	5.56	4.48
	INC_POOL	3B	7.6	4.40	7.69	2.28	7.4	3.33	4.53	1.55
	SEV_Ott_2014	3B	7.5	3.18	5.74	4.84	7.4	3.30	5.61	4.27
INC_Car_2014	3B	7.6	3.79	5.72	3.27	0.0	3.29	5.11	2.51	
<i>QFhb.mcb-4A</i>	SEV_PEI_2013	3B	7.6	4.91	7.86	1.31				
	DON_PEI_2013	4A	38.8	4.73	9.11	1.70	37.7	4.92	10.68	1.64
	VRI_PEI_2014	4A					44.9	4.05	6.54	2.42
<i>QFhb.mcb-4D</i>	INC_PEI_2014	4A					44.9	3.52	5.24	2.14
	DON_Ott_2013	4D	29.5	15.76	28.65	2.21	29.7	17.12	28.99	2.24
	VRI_Ott_2014	4D	30.1	13.37	23.21	10.32	29.6	13.93	26.84	9.97
	INC_Ott_2013	4D	29.8	12.79	13.67	4.24	29.8	12.79	26.09	4.24
	INC_Ott_2014	4D	32.9	12.38	25.17	7.45	30.1	13.50	25.34	7.72
SEV_Ott_2014	4D	30.2	11.14	19.60	9.04	29.6	11.43	22.90	8.74	

**Table 5** (continued)

Trait/QTL	Location	Chr <sup>c</sup>	IM <sup>a</sup>				ICIM <sup>b</sup>			
			Pos <sup>d</sup>	LOD <sup>e</sup>	PVE <sup>f</sup>	Add <sup>g</sup>	Pos	LOD	PVE	Add
	VRI_POOL	4D	30.3	10.99	15.56	4.18	30.3	12.80	21.19	3.75
	INC_POOL	4D	30.2	10.94	19.53	3.68	29.9	14.34	23.47	3.56
	VRI_Mor_2014	4D	30.1	9.51	13.79	6.79	29.9	10.59	18.33	6.38
	SEV_POOL	4D	30.3	8.64	12.23	3.35	29.9	10.52	16.40	3.09
	VRI_Ott_2013	4D	30.2	8.56	12.75	2.97	30.3	8.93	20.99	2.94
	INC_Mor_2014	4D	29.3	8.54	17.80	4.63	29.3	8.36	18.47	4.44
	INC_Ott_2013	4D	40.5	8.20	8.44	3.34				
	INC_Car_2013	4D	29.8	8.06	15.80	4.05	29.9	11.67	19.50	4.31
	DON_POOL	4D	29.2	7.99	18.82	2.11	29.3	9.71	18.30	2.18
	INC_Ott_2013	4D	46.5	7.87	8.13	3.24				
	VRI_POOL	4D	44.7	7.18	9.93	3.30				
	VRI_Mor_2014	4D	44.4	6.76	9.39	5.56				
	SEV_Ott_2013	4D	28.7	6.56	14.66	3.46	27.7	7.14	13.99	3.41
	INC_Car_2014	4D	29.3	6.54	10.77	4.54	29.5	7.84	14.06	4.17
	SEV_POOL	4D	44.8	6.25	8.48	2.76				
	DON_Mor_2014	4D	28.1	5.86	12.56	3.44	27.8	6.07	12.87	3.33
	VRI_Ott_2013	4D	46.3	5.52	8.20	2.36				
	VRI_Car_2013	4D	29.8	5.47	9.03	7.05	29.2	6.01	9.72	6.11
	VRI_Car_2014	4D	29.7	4.94	8.36	5.60	29.8	7.43	11.35	5.41
	SEV_Mor_2014	4D	30.5	4.87	9.98	3.71	30.7	4.40	7.03	2.89
	VRI_Car_2014	4D	45.0	4.56	7.05	5.09				
	SEV_Car_2014	4D	44.9	4.30	6.62	5.44				
	DON_Car_2013	4D	30.0	4.01	9.04	3.73	29.7	4.97	10.28	4.07
	SEV_Car_2014	4D	29.5	3.85	6.59	5.48	29.0	4.22	7.72	4.73
	SEV_Car_2013	4D	29.8	3.80	7.27	5.84	29.7	4.15	7.33	5.34
	INC_Car_2014	4D	45.1	3.77	5.69	3.27				
	DON_Wpg_2013	4D	32.1	3.52	6.21	1.28	30.8	4.35	8.04	1.26
	VRI_PEL_2013	4D	31.4	3.01	4.84	1.85	31.0	2.54	4.65	1.42
	INC_PEL_2013	4D	31.4	2.74	4.59	1.98				
	DON_Ott_2014	4D					37.3	2.82	5.98	1.40
<i>QFhb.mcb-5A.1</i>	INC_POOL	5A.1	52.8	2.62	7.45	-2.24	43.7	4.58	6.19	-1.81
	INC_Car_2013	5A.1	51.6	3.14	8.35	-2.90	43.6	4.43	6.28	-2.42
	INC_Car_2014	5A.1	51.0	2.66	6.23	-3.41	43.6	4.08	6.41	-2.79
	SEV_Car_2014	5A.1					43.6	2.79	4.62	-3.63
	VRI_POOL	5A.1					44.1	2.72	3.74	-1.56
	VRI_Car_2014	5A.1	48.4	2.53	5.43	-4.46				
<i>QFhb.mcb-5A.2</i>	SEV_Mor_2014	5A.2	43.9	4.18	7.88	-3.26	44.0	6.39	9.74	-3.36
	SEV_Car_2014	5A.2	43.9	4.62	7.04	-5.60	44.5	5.79	10.10	-5.35
	VRI_Car_2014	5A.2	43.9	4.19	6.49	-4.87	45.8	5.53	7.76	-4.42
	VRI_Mor_2014	5A.2	43.9	4.07	5.71	-4.32	44.0	4.33	6.36	-3.72
	SEV_POOL	5A.2					45.4	3.79	5.07	-1.70
	INC_Car_2014	5A.2	43.9	3.25	4.95	-3.04	45.8	3.74	5.86	-2.66
	VRI_Ott_2014	5A.2	45.7	2.53	4.58	-4.52	45.7	2.95	4.51	-4.03
	SEV_Ott_2014	5A.2					45.7	2.77	4.52	-3.84
<i>QFhb.mcb-5B</i>	SEV_Mor_2014	5B	82.7	3.57	6.76	3.02				
	SEV_POOL	5B	81.9	3.09	4.28	1.96				
	VRI_Mor_2014	5B	81.9	2.99	4.23	3.72				
	VRI_POOL	5B	81.9	2.83	4.05	2.11				

**Table 5** (continued)

Trait/QTL	Location	Chr <sup>c</sup>	IM <sup>a</sup>				ICIM <sup>b</sup>			
			Pos <sup>d</sup>	LOD <sup>e</sup>	PVE <sup>f</sup>	Add <sup>g</sup>	Pos	LOD	PVE	Add
<i>QFhb.mcb-6B</i>	INC_POOL	5B	70.9	2.50	4.49	1.75				
	DON_PEI_2013	5B					72.9	3.30	7.05	1.33
	SEV_Ott_2013	5B					78.6	2.97	5.15	2.06
	VRI_Car_2014	6B					33.9	4.71	6.69	4.23
	VRI_Mor_2014	6B					35.5	4.64	7.81	4.26
	SEV_Mor_2014	6B	37.2	2.55	5.83	2.91	35.3	4.48	7.46	3.04
	SEV_POOL	6B					31.6	4.28	5.69	1.85
	SEV_Car_2013	6B					31.5	3.66	5.54	4.71
	SEV_Wpg_2013	6B					31.5	3.58	6.53	1.18
	SEV_Car_2014	6B					31.6	3.24	5.40	4.01
VRI_Car_2013	6B					31.6	3.02	4.22	4.08	

<sup>a</sup>IM, interval mapping; <sup>b</sup>ICIM, inclusive composite interval mapping; <sup>c</sup>Chr, Chromosome; <sup>d</sup>Pos, position on linkage group (cM); <sup>e</sup>LOD, peak LOD score; LOD threshold (IM), 3.11; LOD threshold (ICIM), 3.11; <sup>f</sup>PVE, phenotypic variation explained ( $r^2$ ; %); <sup>g</sup>Add, additive effect of allele substitution. A positive number indicated that the ‘Cutler’ allele increased the respective quantitative trait, and vice-versa; <sup>h</sup>DA, Anthesis Date (Julian Date); HGHT, Plant Height (cm); INC, FHB Incidence (%); SEV, FHB Severity (%); VRI, FHB Visual Rating Index; DON, DON content; POOL, pooled dataset; Car, Carman; Mor, Morden; Ott, Ottawa; PEI, Prince Edward Island; Wpg, Winnipeg

three QTL, *QHt.mcb-4D* was consistently detected over environments and the explained phenotypic variation ranged from 7.7% to 42.0% and is consistent with the location of *Rht-D1*. *QHt.mcb-2D* overlapped with *QAnth.mcb-2D* (i.e., *Ppd-D1*). Three epistatic QTL interactions were detected between loci on chromosomes 1A and 1D, 1A and 4B, and 3A and 4A (Supplementary Table S6). These interactions were consistently identified by IM, but only the epistatic interaction between 3A and 4A was identified by the epistasis module of ICIM.

Four additive-effect QTL for plant height were identified in the B/R DH population (Table 6). These QTL were located on chromosome 2B, 4A, 4B, and 7A and named *QHt.mbr-2B*, *QHt.mbr-4A*, *QHt.mbr-4B*, and *QHt.mbr-7A*. The AC Barrie allele at loci *QHt.mbr-2B* and *QHt.mbr-4B* increased plant height, while the Reeder allele at *QHt.mbr-4A* and *QHt.mbr-7A* increased plant height. The location of *QHt.mbr-4B* is consistent with the location of *Rht-B1*. *QHt.mbr-4B* had a much larger effect than the other QTL, which explains why AC Barrie was 14 cm taller than Reeder in field tests. Seven epistatic QTL interactions were detected among loci on chromosomes 1A and 2A, 2B and 5B, 2B and 7A, 2D and 6B, 3A and 7A, 4B and 5A, and 4B and 6D (Supplementary Table S12). Six of these interactions were consistently identified by IM, but only the epistatic interaction between 2B and 7A was identified by the ICIM epistasis module.

## Identification of QTL for FHB resistance

### C/B RIL population

Twelve additive-effect QTL for FHB resistance (FHB incidence, FHB severity, FHB visual rating index, and DON content) were identified using IM and ICIM analysis with the additive effect module of QTL IciMapping (Table 5). AC Barrie alleles contributed FHB resistance at nine of these QTL, which is consistent with AC Barrie being more FHB resistant than Cutler. Many of the FHB-resistance QTL were consistently detected by both IM and ICIM. However, FHB-resistance QTL *QFhb.mcb-2A* and *QFhb.mcb-4A* were less consistently detected across environments. AC Barrie contributed resistance alleles at *QFhb.mcb-3B* (chromosome 3B at 7.6 cM) and *QFhb.mcb-6B* (chromosome 6B at 33.9 cM), which are the expected locations of *Fhb1* and *Fhb2*, respectively. Similarly, *QFhb.mcb-5A.1* mapped to the expected location of *Fhb5*.

FHB-resistance QTL on chromosomes 2D, 4A, 4D, and 5A were coincident for two or more traits. *QFhb.mcb-2D.1* mapped near the distal end of chromosome arm 2DS and coincided with QTL for anthesis date and plant height. Likewise, *QFhb.mcb-4A* was associated with the anthesis

**Table 6** Additive-effect QTL detected for anthesis date, plant height, FHB incidence, FHB severity, and FHB visual rating index AC Barrie/Reeder (B/R) DH population

Trait/QTL	Location	Chr <sup>c</sup>	IM <sup>a</sup>				ICIM <sup>b</sup>			
			Pos <sup>d</sup>	LOD <sup>e</sup>	PVE <sup>f</sup>	Add <sup>g</sup>	Pos	LOD	PVE	Add
Anthesis date										
<i>QAnth.mbr-5A</i>	DA_POOL <sup>h</sup>	5A	142.5	5.18	16.93	− 1.12	136.7	17.53	18.96	− 1.38
	DA_Car_2015	5A	141.2	6.40	19.94	− 1.28	136.7	12.68	18.72	− 1.43
	DA_Ott_2015	5A	146.5	6.13	19.27	− 1.26	153.3	11.61	16.29	− 1.31
	DA_Mor_2015	5A	143.2	2.92	10.17	− 0.81	136.7	10.12	11.15	− 0.97
	DA_Car_2016	5A	142.3	3.83	12.81	− 1.15	136.7	10.11	13.66	− 1.36
	DA_Ott_2016	5A	142.0	3.24	9.36	− 1.04	136.7	8.60	13.08	− 1.31
	DA_Mor_2016	5A					136.7	7.98	10.70	− 0.93
<i>QAnth.mbr-5B</i>	DA_POOL	5B	114.5	16.96	40.33	1.73	114.5	29.52	37.84	1.95
	DA_Mor_2015	5B	114.5	15.85	37.81	1.56	115.0	27.65	38.77	1.80
	DA_Mor_2016	5B	114.5	15.98	34.24	1.54	114.5	21.89	35.69	1.70
	DA_Car_2016	5B	114.5	14.72	35.91	1.92	114.5	21.65	34.17	2.15
	DA_Ott_2015	5B	116.8	10.56	27.71	1.50	116.8	18.46	28.58	1.73
	DA_Car_2015	5B	114.5	9.94	26.06	1.46	114.4	16.77	26.21	1.69
	DA_Ott_2016	5B	114.5	8.89	20.39	1.53	114.5	13.57	22.07	1.70
<i>QAnth.mbr-6B</i>	DA_Ott_2015	6B					128.4	3.53	4.45	− 0.69
	DA_Mor_2015	6B					131.8	3.16	3.31	− 0.53
Plant height										
<i>QHt.mbr-2B</i>	HGHT_POOL	2B					92.0	5.55	2.79	1.41
	HGHT_Car_2015	2B					92.0	4.58	2.22	1.43
	HGHT_Car_2016	2B					86.4	4.11	2.44	1.64
	HGHT_PEI_2016	2B					86.4	2.77	5.06	1.10
	HGHT_Ott_2016	2B					46.4	2.64	3.02	1.73
<i>QHt.mbr-4A</i>	HGHT_Car_2015	4A.1					40.0	6.32	3.12	− 1.71
	HGHT_POOL	4A.1					38.9	3.77	1.85	− 1.15
<i>QHt.mbr-4B</i>	HGHT_Mor_2016	4B	53.6	55.27	76.45	7.45	53.5	60.53	76.46	7.36
	HGHT_POOL	4B	53.6	52.08	48.54	7.18	53.5	62.29	73.41	7.30
	HGHT_Ott_2015	4B	53.6	50.58	67.37	7.22	53.5	51.67	73.40	7.14
	HGHT_Car_2016	4B	53.6	50.48	49.64	9.16	53.5	57.01	74.00	9.13
	HGHT_Car_2015	4B	53.1	48.98	47.78	8.13	53.4	62.59	72.48	8.28
	HGHT_Mor_2015	4B	53.6	35.85	60.89	6.42	53.5	35.79	60.94	6.42
	HGHT_Ott_2016	4B	54.1	23.41	31.71	6.73	53.5	28.81	42.96	6.47
	HGHT_PEI_2016	4B	52.6	17.11	19.73	3.22	59.3	16.47	36.46	3.00
<i>QHt.mbr-7A</i>	HGHT_Mor_2016	7A					0.5	5.18	2.88	− 1.42
	HGHT_Car_2015	7A					0.5	3.86	1.86	− 1.31
	HGHT_Car_2016	7A					0.0	3.05	1.78	− 1.41
FHB resistance										
<i>QFhb.mbr-1B</i>	VRi_Car_2016	1B	35.9	4.55	9.36	− 2.88	51.7	10.15	12.18	− 3.22
	SEV_Car_2016	1B	35.9	5.08	10.18	− 3.10	35.9	7.41	11.17	− 3.12
	VRi_Mor_2015	1B	51.7	2.95	5.28	− 3.16	51.7	5.11	6.91	− 3.12
	VRi_POOL	1B					43.7	4.16	2.89	− 2.09
	INC_POOL	1B					51.1	4.02	3.96	− 1.74
	SEV_POOL	1B					42.5	3.62	2.79	− 1.71
	INC_Mor_2015	1B					51.2	3.14	2.88	− 2.65
	SEV_Mor_2015	1B	38.2	3.43	7.04	− 2.02	38.2	2.99	7.33	− 1.75
	SEV_Mor_2016	1B	43.7	2.90	7.98	− 3.66	43.7	2.92	6.88	− 3.41
	SEV_Mor_2015	1B	51.7	2.90	6.00	− 1.86				
	VRi_Mor_2015	1B	38.2	2.80	5.01	− 3.07				

**Table 6** (continued)

Trait/QTL	Location	Chr <sup>c</sup>	IM <sup>a</sup>				ICIM <sup>b</sup>				
			Pos <sup>d</sup>	LOD <sup>e</sup>	PVE <sup>f</sup>	Add <sup>g</sup>	Pos	LOD	PVE	Add	
<i>QFhb.mbr-2B</i>	VRI_POOL	2B	43.9	2.66	6.53	– 2.90	42.3	7.34	5.32	– 2.86	
	SEV_POOL	2B					42.3	6.68	5.37	– 2.39	
	VRI_Car_2016	2B	67.1	3.57	7.43	– 2.60	67.1	5.35	6.01	– 2.29	
	SEV_Car_2016	2B	67.1	2.81	5.79	– 2.37	70.4	4.78	6.95	– 2.48	
	SEV_Ott_2016	2B					55.0	4.22	4.44	– 3.74	
	VRI_PEI_2016	2B	40.0	2.79	7.62	– 1.92	42.3	4.11	4.33	– 1.69	
	INC_POOL	2B					42.3	4.04	3.99	– 1.76	
	VRI_Ott_2016	2B					55.0	3.98	4.18	– 4.02	
	INC_Car_2016	2B					68.3	3.84	7.24	– 2.46	
	VRI_PEI_2015	2B	38.4	2.86	8.17	– 2.03	41.6	3.84	6.15	– 1.80	
	INC_PEI_2015	2B	40.0	3.02	4.49	– 1.72	42.3	3.65	6.80	– 1.57	
	SEV_PEI_2016	2B	40.4	2.52	5.47	– 1.76	42.3	3.63	3.78	– 1.54	
	SEV_PEI_2015	2B					39.5	3.43	5.38	– 2.01	
	VRI_Car_2015	2B					55.0	3.19	3.66	– 3.44	
	INC_PEI_2016	2B	40.3	2.84	6.67	– 0.59	40.5	3.13	5.51	– 0.51	
	<i>QFhb.mbr-2D</i>	INC_Mor_2016	2B					42.3	3.08	4.53	– 3.07
SEV_Ott_2015		2B					55.0	3.05	2.85	– 3.87	
INC_Mor_2015		2B					42.3	2.59	2.36	– 2.42	
INC_POOL		2D					93.9	4.99	5.57	– 2.07	
VRI_Car_2016		2D					84.7	4.93	5.51	– 2.18	
SEV_POOL		2D					84.7	4.61	3.60	– 1.96	
VRI_POOL		2D					84.7	4.54	3.16	– 2.20	
INC_Mor_2015		2D					84.6	4.46	4.16	– 3.21	
VRI_Mor_2015		2D					85.8	4.26	5.68	– 2.85	
SEV_PEI_2016		2D					89.3	3.75	4.03	– 1.58	
VRI_Car_2015		2D					85.8	3.31	3.80	– 3.51	
VRI_PEI_2016		2D					90.6	3.13	3.31	– 1.46	
SEV_Car_2016		2D					82.4	2.66	3.76	– 1.84	
INC_Ott_2016		2D					82.4	2.64	0.39	– 1.93	
<i>QFhb.mbr-3B</i>		INC_PEI_2016	3B	5.1	4.20	9.02	0.68	5.1	6.54	11.07	0.72
		VRI_PEI_2015	3B	6.8	4.04	10.67	2.29	6.8	5.79	9.41	2.20
	SEV_PEI_2015	3B					6.8	4.90	7.38	2.33	
	VRI_PEI_2016	3B					6.8	4.88	5.19	1.83	
	SEV_PEI_2016	3B					6.8	3.86	4.03	1.57	
	INC_PEI_2015	3B	6.8	3.48	4.88	1.77	6.8	3.36	6.27	1.49	
	SEV_Mor_2016	3B					0.5	2.61	6.13	3.21	
<i>QFhb.mbr-4B</i>	VRI_POOL	4B	53.6	2.56	6.00	– 2.77	53.5	6.37	4.57	– 2.64	
	SEV_Ott_2016	4B	54.2	5.58	13.39	– 6.24	53.5	6.16	6.63	– 4.58	
	VRI_Ott_2016	4B	54.0	5.12	11.35	– 6.58	53.5	5.59	5.98	– 4.82	
	INC_POOL	4B	53.6	3.28	7.67	– 2.32	53.5	5.55	5.60	– 2.08	
	INC_Car_2015	4B	60.5	4.88	12.05	– 3.51	60.4	5.42	12.36	– 3.55	
	VRI_Ott_2015	4B	53.6	2.50	6.72	– 5.44	53.5	2.86	3.04	– 4.08	
	VRI_PEI_2015	4B					70.2	2.71	4.22	– 1.48	
	SEV_PEI_2015	4B					41.8	2.53	3.68	– 1.70	
	INC_Car_2016	4B	53.6	2.58	6.39	– 2.16					
<i>QFhb.mbr-5A</i>	VRI_POOL	5A	141.3	5.40	14.18	4.23	136.7	21.62	19.17	5.37	
	SEV_POOL	5A	142.4	5.85	18.67	3.68	136.7	20.49	19.95	4.57	
	INC_Mor_2015	5A	139.9	6.84	15.59	5.87	136.7	19.70	22.75	7.44	

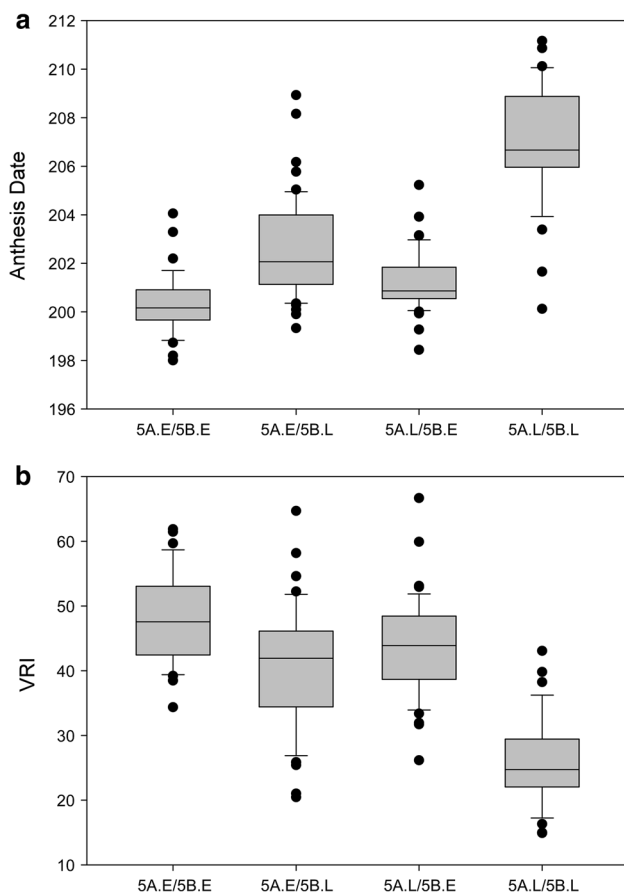
**Table 6** (continued)

Trait/QTL	Location	Chr <sup>c</sup>	IM <sup>a</sup>				ICIM <sup>b</sup>			
			Pos <sup>d</sup>	LOD <sup>e</sup>	PVE <sup>f</sup>	Add <sup>g</sup>	Pos	LOD	PVE	Add
	VRI_PEI_2016	5A					136.7	13.79	16.62	3.27
	INC_POOL	5A	140.2	4.30	11.46	2.82	136.7	13.60	15.29	3.41
	SEV_Ott_2015	5A	143.4	4.73	15.75	7.53	136.7	13.52	14.61	8.72
	SEV_PEI_2016	5A					136.7	12.93	15.35	3.08
	VRI_Ott_2015	5A	143.3	4.63	14.80	8.03	136.7	11.40	13.51	8.55
	VRI_Car_2015	5A	139.7	3.84	12.54	5.46	136.7	11.01	14.07	6.70
	VRI_Ott_2016	5A	141.8	3.56	8.98	5.81	136.7	10.00	11.33	6.59
	SEV_Ott_2016	5A	141.3	3.40	9.10	5.11	136.7	9.77	11.04	5.87
	SEV_PEI_2015	5A	141.4	4.34	13.86	2.86	136.7	9.23	14.75	3.30
	VRI_Mor_2015	5A	141.3	4.48	8.71	4.06	136.7	8.95	12.75	4.24
	SEV_Car_2015	5A	141.4	4.30	14.07	6.95	136.7	8.58	12.65	7.36
	VRI_PEI_2015	5A	139.0	3.25	9.34	2.14	136.7	7.53	12.53	2.54
	INC_Mor_2016	5A	137.9	2.92	7.49	3.71	136.7	7.33	11.43	4.82
	VRI_Car_2016	5A					146.7	7.25	9.09	2.78
	INC_Ott_2016	5A					135.6	6.69	1.04	3.12
	SEV_Car_2016	5A					145.7	6.61	10.73	3.06
	INC_Ott_2015	5A	143.2	3.25	9.24	4.71	135.6	6.19	7.00	5.03
	INC_PEI_2016	5A	139.1	2.90	6.92	0.59	136.7	5.71	9.60	0.67
	VRI_PEI_2016	5A	141.0	2.70	7.98	1.94				
	INC_PEI_2015	5A					136.7	3.95	7.43	1.63
	INC_Car_2016	5A					135.1	2.58	4.74	1.97
<i>QFhb.mbr-5B</i>	VRI_POOL	5B	115.6	10.48	22.17	− 5.29	114.5	28.47	27.84	− 6.47
	SEV_Ott_2015	5B	114.5	13.86	33.74	− 11.00	114.5	26.46	34.39	− 13.36
	SEV_POOL	5B	114.5	9.34	24.40	− 4.21	114.5	25.24	26.30	− 5.24
	VRI_Ott_2015	5B	114.5	13.86	32.25	− 11.84	114.5	24.99	35.90	− 13.92
	INC_Mor_2015	5B	114.5	9.75	19.30	− 6.52	115.0	21.85	25.69	− 7.89
	VRI_Ott_2016	5B	118.9	8.85	18.84	− 8.40	115.6	21.00	27.72	− 10.30
	SEV_Ott_2016	5B	115.6	8.50	19.01	− 7.37	115.6	20.76	27.30	− 9.23
	INC_POOL	5B	115.6	9.79	21.05	− 3.81	115.6	19.62	23.97	− 4.27
	VRI_Car_2015	5B	114.5	9.24	23.92	− 7.54	114.5	17.81	25.03	− 8.94
	INC_Ott_2015	5B	114.5	5.44	12.31	− 5.43	114.5	15.68	20.21	− 8.53
	SEV_Car_2015	5B	114.5	7.79	19.93	− 8.27	114.5	13.90	22.08	− 9.72
	SEV_PEI_2016	5B	121.3	7.03	13.64	− 2.74	120.9	13.40	16.11	− 3.15
	VRI_PEI_2016	5B	121.3	6.34	15.50	− 2.70	120.9	12.53	14.89	− 3.09
	INC_Mor_2016	5B	115.6	6.59	15.49	− 5.33	115.6	11.80	19.51	− 6.29
	VRI_Mor_2015	5B	114.4	5.01	8.72	− 4.06	115.0	11.74	17.23	− 4.92
	INC_Ott_2016	5B	118.6	6.00	14.02	− 3.54	116.3	10.99	1.81	− 4.11
	INC_PEI_2015	5B	120.5	5.78	8.07	− 2.28	120.2	7.81	15.61	− 2.36
	VRI_PEI_2015	5B	119.7	4.73	12.42	− 2.47	119.8	7.67	12.86	− 2.57
	SEV_PEI_2015	5B	119.6	3.71	10.62	− 2.50	119.6	7.34	11.40	− 2.90
	VRI_Car_2016	5B					115.1	6.38	7.25	− 2.48
	INC_Car_2016	5B	114.5	3.35	8.22	− 2.44	114.5	5.18	9.87	− 2.84
	SEV_Car_2016	5B					115.1	4.68	6.78	− 2.43
	INC_PEI_2016	5B					115.6	4.27	7.04	− 0.57
	INC_Car_2015	5B	118.8	2.79	7.17	− 2.67	116.8	3.22	7.11	− 2.67
	INC_PEI_2015	5B	128.8	4.76	6.58	− 2.06				
<i>QFhb.mbr-7A</i>	SEV_POOL	7A					11.5	5.12	4.03	2.05
	VRI_Ott_2016	7A					10.0	4.37	4.69	4.24

**Table 6** (continued)

Trait/QTL	Location	Chr <sup>c</sup>	IM <sup>a</sup>				ICIM <sup>b</sup>			
			Pos <sup>d</sup>	LOD <sup>e</sup>	PVE <sup>f</sup>	Add <sup>g</sup>	Pos	LOD	PVE	Add
<i>QFhb.mbr-7D</i>	SEV_Ott_2016	7A					10.7	4.35	4.66	3.82
	VRI_POOL	7A					11.5	3.68	2.54	1.96
	SEV_Ott_2015	7A					17.8	3.47	3.27	4.13
	VRI_Ott_2015	7A					11.5	2.78	2.93	3.98
	INC_Mor_2016	7D.2	69.1	3.05	7.52	– 3.73				
	INC_Mor_2015	7D.2	69.1	3.01	6.50	– 3.80				
	INC_POOL	7D.2	69.1	2.80	6.58	– 2.14				
	INC_Ott_2015	7D.2	69.1	2.62	6.14	– 3.84				

<sup>a</sup>IM, interval mapping; <sup>b</sup>ICIM, inclusive composite interval mapping; <sup>c</sup>Chr, Chromosome; <sup>d</sup>Pos, position on linkage group (cM). <sup>e</sup>LOD, peak LOD score; LOD threshold (IM), 3.09; LOD threshold (ICIM), 3.09; <sup>f</sup>PVE, phenotypic variation explained ( $r^2$ , %); <sup>g</sup>Add, additive effect of allele substitution. A positive number indicated that the ‘AC Barrie’ allele increased the respective quantitative trait, and vice-versa; <sup>h</sup>DA, Anthesis Date (Julian Date); HGHT, Plant Height (cm); INC, FHB Incidence (%); SEV, FHB Severity (%); VRI, FHB Visual Rating Index; POOL, pooled dataset; Car, Carman; Mor, Morden; Ott, Ottawa; PEI, Prince Edward Island



**Fig. 2** Box plots illustrating the epistatic interactions on chromosomes 5A (wsnp\_Ex\_c7266\_12475249) and 5B (RAC875\_rep\_c97153\_66) in the AC Barrie/Reeder (B/R) DH population for **a** anthesis date and **b** FHB visual rating index (VRI). E, early allele; L, late allele

date QTL *QAnth.mcb-4A.1*. At the plant height QTL *QHt.mcb-4D* (i.e., *Rht-D1*), the reduced height allele was consistently associated with increased FHB damage. Finally, *QFhb.mcb-5A.2* co-located with the *B1* awn inhibitor gene.

Additional QTL for FHB resistance were identified by digenic epistasis QTL analysis. Five digenic epistatic interactions were identified between loci on chromosomes 1A and 2A, 1D and 3B, 3A and 7D, 4A and 4B, and 4A and 7A. Epistatic interaction between 4A and 4B was also reported as additive-effect QTL for FHB resistance on chromosome 4A (*QFhb.mcb-4A*) (Supplementary Table S6).

### B/R DH population

IM and ICIM analyses identified nine additive-effect QTL controlling FHB resistance in the B/R DH population (Table 6). AC Barrie contributed resistance alleles at six of these QTL, which is consistent with AC Barrie being generally more resistant to FHB than Reeder. However, Reeder alleles contributed two major FHB-resistance QTL on chromosome 3B (*QFhb.mbr-3B* at 5.1 cM) at the expected location of *Fhb1*, and on chromosome 5A (*QFhb.mbr-5A*) at ~137 cM (Table 6). Despite the strong effect of *QFhb.mbr-5A*, this QTL was not located at the expected location of *Fhb5* and was not associated with the presence or absence of awns. The *B1* awn inhibitor mapped to 208.6 cM in the B/R population. The third QTL from Reeder was detected only by ICIM on chromosome 7A (*QFhb.mbr-7A*), and its effect was lower and less consistent than the effects found for the QTL regions on chromosomes 3B and 5A. The most prominent resistance QTL from AC Barrie was found on chromosome 5B (*QFhb.mbr-5B*) explaining up to 36% of the phenotypic variation. This 5B QTL was consistently detected in all the experiments. The resistance QTL *QFhb.*

*mbr-4B* coincided with the plant height QTL *QHt.mbr-4B* mapped at 54 cM (i.e., *Rht-B1*). Additional QTL for FHB resistance were identified by digenic epistasis QTL analysis. Five digenic epistatic interactions were identified between loci on chromosomes 1A and 5B, 1D and 7B, 2A.2 and 7A, 2B and 5A, and 5A and 5B. Epistatic interaction between 5A and 5B was also detected as additive-effect QTL for FHB resistance (*QFhb.mbr-5A* and *QFhb.mbr-5B*) (Supplementary Table S12), at the same location as the epistatic interaction between the anthesis date QTL *QAnth.mbr-5A* and *QAnth.mbr-5B*. DH lines with the late anthesis allele at *QAnth.mbr-5A* and *QAnth.mbr-5B* interaction were considerably later to anthesis than the three other genotypic classes (Fig. 2a) and had reduced VRI (Fig. 2b), FHB incidence, and FHB severity (data not shown).

### Comparison of mapping populations

Three FHB-resistance QTL on chromosomes 2D, 3B, and 5B were in common between the C/B RIL and B/R DH populations. QTL on chromosomes 2D (*QFhb.mcb-2D.2* and *QFhb.mbr-2D*) and 5B (*QFhb.mcb-5B* and *QFhb.mbr-5B*) had resistant alleles derived from AC Barrie in both populations. However, contradictory results were obtained for the QTL on chromosome 3B at the expected location of *Fhb1*. AC Barrie contributed FHB resistance at the *Fhb1* locus in the C/B RIL population and susceptibility in the B/R DH population. This result is suggestive of an allelic series in which the Cutler allele was the most susceptible, the AC Barrie allele was intermediate, and the Reeder allele was most resistant.

Cutler contributed FHB resistance at *QFhb.mcb-5A.2*, which mapped to the location of the *B1* awn inhibitor gene in the C/B RIL population, while there was no FHB-resistance QTL detected at the *B1* locus in the B/R DH population despite segregation of the presence/absence of awns at the *B1* locus in the B/R DH population.

### Characterization of *Fhb1* candidate genes in parental lines

*PFT* and *TaHRC* genes were previously reported as candidate genes that confer FHB resistance at the *Fhb1* locus (Rawat et al. 2016; Su et al. 2018, 2019; Li et al. 2019). Characterization of *PFT* and *TaHRC* was carried out in a panel of 20 wheat genotypes varying in FHB resistance including the parents AC Barrie, Cutler, and Reeder. Among the tested wheat genotypes, the complete *PFT* gene was amplified in five genotypes (DH181, FL62R1, Nyubai, Sumai-3, Wangshuibai), but not in the remaining 15 genotypes (including the three parents AC Barrie, Cutler, and Reeder) (Fig. 3a). The PCR product of *TaHRC*-GSM-F/*TaHRC*-GSM-R from Sumai-3 is shorter than that from Chinese Spring due to

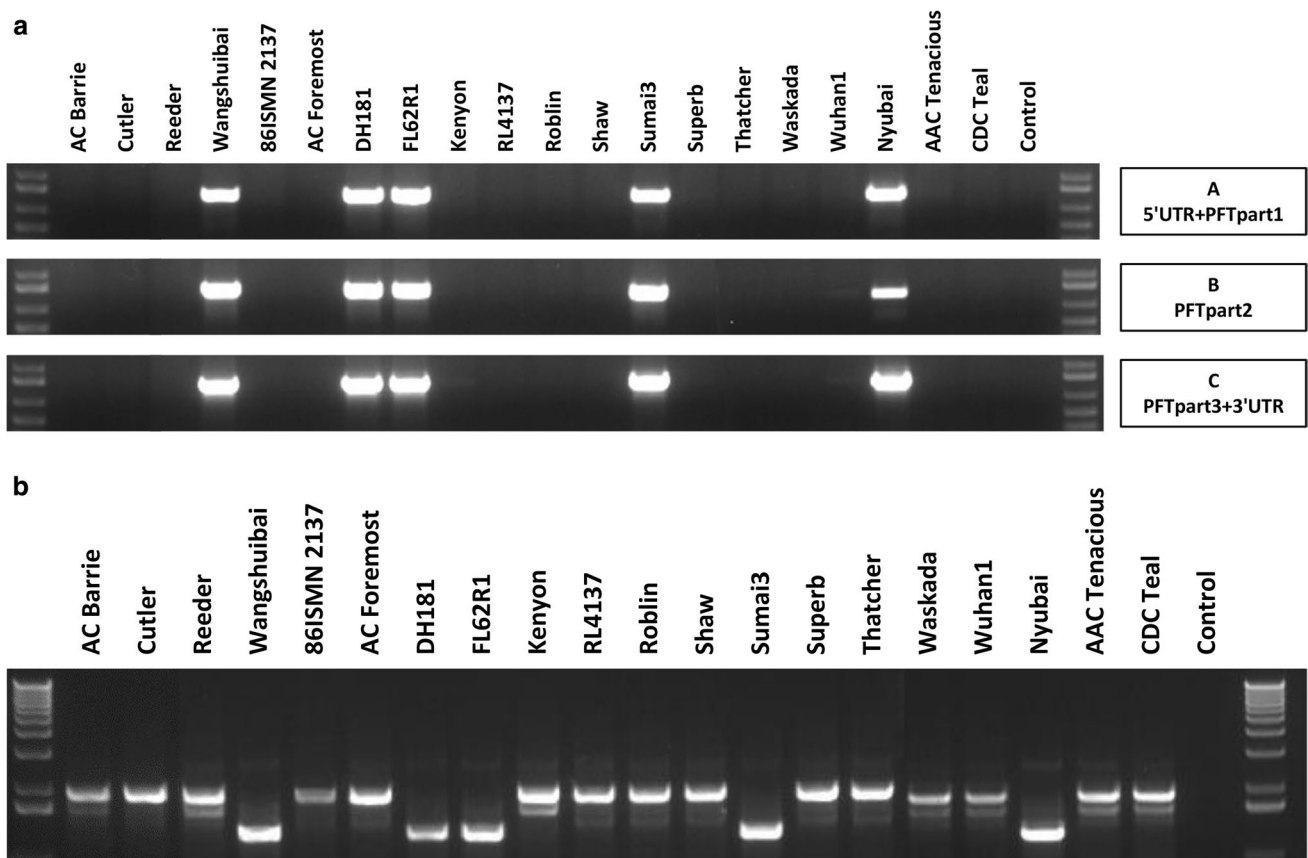
the large deletion in Sumai-3 (Su et al. 2018, 2019). The same amplification pattern was observed between genotypes carrying Sumai-3 alleles and non-Sumai-3 alleles in the germplasm tested (Fig. 3b). Sequence alignments of *TaHRC* among 20 wheat genotypes clearly showed DNA polymorphism between resistant and susceptible genotypes and agreed with the PCR amplification pattern in Fig. 3b (Supplementary Figure S1). The *Fhb1*-resistant haplotypes (Hap\_Ning) (Su et al. 2019) showed identical sequences and carried a large deletion (25 bp) including the start codon (ATG), a 22 bp sequence downstream of the original ATG in the predicted ORF region of *TaHRC* (*TaHRC-R*), whereas the other three haplotypes (Hap\_CS, Hap\_Funo, and Hap\_Clark) (Su et al. 2019) carried complete ORF (*TaHRC-S*) despite the other sequence variations such as SNPs and indels among the haplotypes (Supplementary Figure S1, Supplementary Table S13, 14). Amino acid substitution from arginine (R) to cysteine (C) at residue 169 in the deduced amino acid sequence placed Cutler and Reeder into the Hap\_Funo haplotype, and AC Barrie into the Hap\_Clark haplotype as shown in the Supplementary Table S13.

### Discussion

The genetic basis of native FHB resistance is not well understood in Canada and other parts of the world. This lack of information may limit progress in breeding FHB-resistant cultivars. Knowledge of the FHB-resistance QTL present in local germplasm allows strategic introgression of QTL from exotic wheat lines. Without this knowledge, breeders may introgress FHB resistance alleles already present in the local germplasm or even the recurrent parent, which would provide limited or no opportunity for enhancing FHB resistance in the targeted germplasm. To explore this issue, the genetic basis of FHB resistance in Canadian spring wheat cultivar AC Barrie was characterized in two mapping populations derived from crosses to the Canadian cultivar Cutler and the American cultivar Reeder.

Genetic characterization of FHB resistance is challenging due to the complexity of the disease phenotyping and the underlying genetic basis of resistance. Resistance to FHB in wheat is controlled by polygenes that usually have moderate to small effects and are often influenced by environmental factors (Miedaner et al. 2001; Buerstmayr et al. 2012). QTL analysis in the present study identified 12 QTL controlling FHB resistance in the C/B RIL population on chromosomes 1B, 2A, 2B, 2D, 3B, 4A, 4D, 5A, 5B, and 6B, and nine QTL controlling FHB resistance in the B/R DH population on chromosomes 1B, 2B, 2D, 3B, 4B, 5A, 5B, 7A, and 7D (Tables 5, 6). AC Barrie contributed resistant alleles at most of these loci in both populations, which is consistent with AC Barrie being more resistant to FHB





**Fig. 3** **a** Characterization of the pore-forming toxin like (*PFT*) gene in 24 selected wheat cultivars including three parents AC Barrie, Cutler, and Reeder. Three pairs of primers were used for identification of the *PFT*; a, b, and c indicate that PCR products amplified with primer pairs P1F/P1R, P2F/P2R, and P3F/P3R, UTR—untranslated region (He et al. 2018). **b** Characterization of the histidine-rich calcium-

binding protein (*TaHRC*) gene in 24 selected wheat cultivars including three parents AC Barrie, Cutler, and Reeder using the gene specific primer pair TaHRC-GSM-F/TaHRC-GSM-R designed to capture the deletion polymorphism between the two *TaHRC* alleles (Su et al. 2018)

in the field nurseries. Only three of these QTL have common map locations despite AC Barrie being a parent of each population: *QFhb.mcb-2D.2* and *QFhb.mbr-2D*, *QFhb.mcb-3B* and *QFhb.mbr-3B*, and *QFhb.mcb-5B* and *QFhb.mbr-5B*. The QTL *QFhb.mcb-2B* and *QFhb.mbr-2B* may also have an underlying gene in common since the location of *QFhb.mbr-2B* was somewhat inconsistent, ranging from 39 to 70 cM on the B/R chromosome 2B linkage map. The QTL peaks at position 67 to 70 cM in the B/R population may coincide with *QFhb.mcb-2B* in the C/B population.

FHB-resistance QTL co-located with major genes affecting plant height in the C/B and B/R populations. There was a strong association between the dwarfing alleles at *Rht-B1* and *Rht-D1* and increased FHB susceptibility in the B/R (*QFhb.mbr-4B*) and C/B (*QFhb.mcb-4D*) populations, respectively. These associations have been reported in past studies for *Rht-1* loci and *Rht8* (Draeger et al. 2007; Handa et al. 2008; Holzapfel et al. 2008; Srinivasachary et al. 2008, 2009). *QFhb.mcb-4D* was the largest

effect QTL in the C/B population, while *QFhb.mbr-4B* was an intermediate QTL in the B/R population. This relative ranking of *Rht-D1b* and *Rht-D1b* associated FHB susceptibility is consistent with previous studies. Oddly, the additive effects suggest that *Qht.mcb-4D* had less of an impact on plant height in the C/B population than *Qht.mbr-4B* in the B/R population, despite the strong impact of *QFhb.mcb-4D* on FHB resistance. Cumulatively, these loci and the *Ppd-D1* locus (outlined below) explained the negative correlation between plant height and the FHB traits (INC, SEV, VRI, DON) in both populations.

QTL affecting anthesis date also co-located with QTL for FHB resistance resulting in the negative correlation between anthesis date and the FHB traits (INC, SEV, VRI, DON) in both populations. Cutler carries the photoperiod insensitivity allele *Ppd-D1a*, which was associated with increased FHB susceptibility in the C/B RIL population (*QFhb.mcb-2D.1*) and reduced plant height allele (*Qht.mcb-2D*). The *Ppd-D1* locus had a similar effect in the

Kenyon/86ISMN 2137 RIL population (McCartney et al. 2016).

In the B/R DH population, two major anthesis date QTL were identified (*QAnth.mbr-5A* and *QAnth.mbr-5B*), which co-located with two major QTL for FHB resistance (*QFhb.mbr-5A* and *QFhb.mbr-5B*). In both cases, earlier anthesis was associated with increased FHB susceptibility. *QAnth.mbr-5A* mapped to ~ 136 cM on chromosome 5A, but LOD peaks were occasionally as far away at 146.7 cM based on ICIM. LOD peaks for *QAnth.mbr-5A* ranged from 139.0 to 143.4 cM based on IM. The *Vrn-A1* locus (587.416 Mbp in Chinese Spring RefSeq v1.0) is located in the interval from 136.8 to 152.7 cM (approximately 570.939 to 608.604 Mbp in Chinese Spring RefSeq v1.0) based upon BLAST locations of the *Vrn-A1* gene and the 90 K Infinium SNPs in IWGSC Chinese Spring reference genome sequence (Appels et al. 2018). AC Barrie and Reeder have the same haplotype based upon 90 K Infinium SNPs in this interval (data not shown), which explains the 16 cM interval (136.8 to 152.7 cM) in the chromosome 5A map with no polymorphic markers and suggests that AC Barrie and Reeder carry the same spring habit allele at the *Vrn-A1* locus. The haplotype data suggests that variation at the *Vrn-A1* locus is not responsible for the variation in anthesis date and FHB resistance at this region of chromosome 5A; however, the map location of *QAnth.mbr-5A* does not exclude the *Vrn-A1* locus as a candidate. Cutler and AC Barrie are reported to carry *Vrn-A1a*, *vrn-B1*, and *vrn-D1* (i.e., spring habit at *Vrn-A1* and winter alleles at *Vrn-B1* and *Vrn-D1* loci) (Iqbal et al. 2006; Chen et al. 2016; Perez-Lara et al. 2016), while Reeder carries *Vrn-A1*, *Vrn-B1*, and *vrn-D1* (i.e., spring habit at *Vrn-A1* and *Vrn-B1* loci, and winter habit at *Vrn-D1*) (Sherman et al. 2004; Blake et al. 2009, 2011). *QAnth.mbr-5B* maps to ~ 115 cM on chromosome 5B. The 90 K Infinium SNPs at 114.5 to 115.0 cM on chromosome 5B flank the *Vrn-B1* locus based on the BLAST locations of the *Vrn-B1* gene and the 90 K Infinium SNPs. AC Barrie and Reeder are polymorphic for many SNPs in this region of chromosome 5B. These results, combined with the previous reports of the spring habit allele at *Vrn-B1* in Reeder, suggest that variation at the *Vrn-B1* locus is responsible for the variation in anthesis date and FHB resistance at this region of chromosome 5B. The four genotypic classes defined by *QAnth.mbr-5A* or *QAnth.mbr-5B* consisted of spring habit plants, although the DH lines with both late alleles were 4.9 days later to anthesis than the population mean and the VRI of these DH lines was 38% lower than the population mean (Fig. 2). There was no statistically significant segregation distortion at either *QAnth.mbr-5A* or *QAnth.mbr-5B*, but there were fewer DH lines with both late alleles than the other genotypic classes. Epistasis was reported between these loci for both anthesis date and FHB resistance (Table S12, Fig. 2). The 5B QTL region had a slightly

larger effect on both anthesis date and FHB susceptibility than the 5A QTL region. Wheat researchers may wish to explore the merit of the wheat lines carrying both late alleles given the reduction in FHB symptoms observed in this study. This reduction in FHB damage may be due to less favorable environmental conditions for *F. graminearum* infection later in the growing season.

In the C/B RIL population, the FHB-resistance QTL *QFhb.mcb-5B* mapped to the location of the *Vrn-B1* locus, or slightly proximal, with AC Barrie carrying the resistant allele. However, in this population, no QTL for anthesis date was identified at the *Vrn-B1* locus despite many markers mapping to that region of the chromosome 5B linkage map. In the C/B population, *Vrn-A1* is located in the gap that separates linkage groups 5A.1 and 5A.2, which is consistent with Cutler and AC Barrie carrying the same *Vrn-A1* allele. *QFhb.mcb-5B* was a relatively weak FHB-resistance QTL in the C/B RIL population in comparison with *QFhb.mbr-5B* in the B/R DH population. In the Kenyon/86ISMN 2137 RIL population, an anthesis date QTL were detected at the *Vrn-B1* locus and an FHB-resistance QTL was detected about 10–30 cM distal of the anthesis date QTL (McCartney et al. 2016). Kenyon is closely related to AC Barrie and both share the same 90 K Infinium SNP haplotype at *Vrn-B1*. Additional research is needed to determine whether the FHB resistance in this genomic region is the result of the *Vrn-B1* locus and/or linked gene(s).

The *Fhb1* region of chromosome 3B showed very interesting QTL analysis results. FHB-resistance QTL were located at the expected location of *Fhb1* (Cuthbert et al. 2006; Liu et al. 2008; Rawat et al. 2016; Bai et al. 2018; He et al. 2018) in both populations. These QTL were not consistently detected in all FHB nurseries as would be expected for *Fhb1* from Sumai-3. The detection of FHB resistance near the expected location of *Fhb1* was surprising given that AC Barrie and Reeder do not have clear ancestry to Asian spring wheats such as Sumai-3. AC Barrie had the resistant allele in the C/B RIL population, but had the susceptible allele in the B/R DH population (i.e., the Reeder allele was more resistant than the AC Barrie allele). This contrasted with AC Barrie carrying the resistant alleles for the two other QTL segregating in both populations (i.e., *QFhb.mcb-2D.2* = *QFhb.mbr-2D*, and *QFhb.mcb-5B* = *QFhb.mbr-5B*). Additional experiments were undertaken to explore these findings at the *Fhb1* locus. Three primer pairs for the *Fhb1* candidate gene *PFT* failed to amplify a PCR product in AC Barrie, Cutler, and Reeder, while strongly amplifying products in positive controls (Sumai-3, Wangshuibai, Nyubai, DH181, and FL62R1). This suggested that *PFT* had no role in the FHB resistance segregating in these populations. Subsequently, the *Fhb1* candidate gene *TaHRC* gene was sequenced in the parents of the mapping populations and control genotypes. This revealed that AC Barrie had

the Hap\_Clark haplotype, while Cutler and Reeder had the Hap\_Funo haplotype using the haplotype nomenclature in Su et al. (2019). Reeder and Cutler had the most and least resistant alleles at the *Fhb1* locus in this study, respectively, but had the same *TaHRC* haplotype. Also, the Hap\_Clark and Hap\_Funo *TaHRC* haplotypes are not typical of *Fhb1* carriers. Therefore, variation in the *PFT* and *TaHRC* genes did not explain the variation observed at the *Fhb1* locus in the C/B RIL and B/R DH populations, as expected based on pedigree. These results suggest that at least one additional gene is involved in FHB resistance near the *Fhb1* locus.

AC Barrie contributed FHB resistance on chromosome 6B in the C/B RIL population, while Cutler contributed FHB resistance on chromosome 5A. *QFhb.mcb-6B* coincides with the expected location of the major FHB-resistance gene *Fhb2* (Cuthbert et al. 2007). *Fhb2* also originates from Sumai-3 and related Asian germplasm, so the identification of an FHB-resistance QTL in this genetic region from AC Barrie was unexpected. However, FHB resistance has been detected in the *Fhb2* region in non-Asian germplasm, such as the Swiss cultivar Arina (Draeger et al. 2007; Semagn et al. 2007), the French cultivar Apache (Holzapfel et al. 2008), the American cultivar Patton (Bonin and Kolb 2009), and *T. carthlicum* Blackbird (Somers et al. 2006). The relative effectiveness of each QTL allele remains to be determined. Similarly, *QFhb.mcb-5A.1* maps to the expected location of *Fhb5* in Wangshuibai, CM-82036, Frontana, and other wheats (Steiner et al. 2004, 2019; Xue et al. 2011; Buerstmayr et al. 2018). However, *QFhb.mcb-5A.1* was significant in two of eight FHB nurseries in this study, suggesting this QTL is not as strong as *Fhb5* from Sumai-3. The detection of native FHB-resistance QTL at the locations of *Fhb2* and *Fhb5* in AC Barrie and Cutler underlines the importance of understanding the genetic basis of FHB resistance in local breeding germplasm.

A QTL for FHB resistance (*QFhb.mcb-5A.2*) was detected at the *B1* awn inhibitor locus in the C/B RIL population. RIL lines with the awned allele had improved FHB resistance in this population. This contrasts with observations made in wheat breeding germplasm in which awned genotypes were more susceptible to FHB (Mesterházy 1995). Note that the B/R DH population also segregated for awns but no QTL for FHB resistance was detected at the *B1* locus. The results of Mesterházy (1995) may have been complicated by population structure that was not accounted for (i.e., potential false positive) or due to a linked gene. If the *B1* locus has no effect on FHB resistance, then *QFhb.mcb-5A.2* in the C/B population must have been due to a linked gene. This possibility would explain why an FHB-resistance QTL was not detected at the *B1* locus in B/R DH population despite segregation for awns at the locus.

FHB-resistance QTL on chromosome 2B have also been detected in spring wheat but have received little attention

due to their minor effect and occasional occurrence in spring wheat (Zhang et al. 2018). Zhang et al. (2018) identified a locus from chromosome 2BL originating from the Canadian spring wheat cultivar Stettler providing type II resistance. The 2B QTL from Stettler was flanked by SNP markers that overlap with SNP markers spanning the 2B QTL from AC Barrie (C/B RIL population) identified in the present study. AC Barrie also contributed a major QTL for FHB resistance in the B/R DH population, but the location of the QTL peak varied in different field trials and different measures of FHB symptoms. It is not clear if the FHB-resistance QTL on chromosome 2B are the same in the two AC Barrie mapping populations. A 2BL QTL was also detected from the Brazilian spring wheat cultivar Frontana (Steiner et al. 2004), which is in the distant pedigree of AC Barrie.

A number of other minor FHB-resistance QTL were identified in these populations. AC Barrie contributed FHB-resistance QTL on chromosome 1B in the C/B (*QFhb.mcb-1B*) and B/R (*QFhb.mbr-1B*) populations. These QTL mapped to different locations, and each was detected in multiple field tests. The FHB-resistance QTL *QFhb.mcb-2D.2* and *QFhb.mbr-2D* derived from AC Barrie in both RIL and DH populations mapped to the similar location as FHB-resistance QTL derived from Wuhan-1 (Somers et al. 2003; Hu et al. 2019). AC Barrie contributed the resistance alleles at *QFhb.mcb-4A* and *QFhb.mbr-7D*, Cutler at *QFhb.mcb-2A*, and Reeder at *QFhb.mbr-7A*.

In summary, only part of the FHB resistance can be attributed to putatively identical resistance QTL among individual populations. While the two populations shared three or four QTL in common, several other genomic regions controlling FHB resistance were identified that were unique to each population. FHB resistance is very complex and regulated by many different genomic regions with major- or minor-effect QTL, even in wheat cultivars with intermediate levels of resistance. These results indicate that a complete understanding of the FHB resistance in a wheat genotype cannot be determined with a single bi-parental mapping population. A full understanding of the FHB resistance of a highly resistant wheat may require a nested association mapping (NAM) population (Yu et al. 2008), in which the FHB resistant wheat is the common parent crossed to genetically diverse wheats.

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**Author contributions statement** DT developed the linkage maps, conducted QTL analysis, characterized candidate genes, interpreted

the data, and co-wrote the manuscript. AB, BB, GF, AF, JG, MH, DM, RM, BM, DS, MI, CP, and AN designed and conducted the field experiments and generated Fusarium head blight data. CM planned and organized the study, participated in the analysis and interpretation of data, and co-wrote the manuscript. All authors contributed to and approved the final manuscript.

**Availability of data and material** Data supporting the current study can be obtained by contacting the corresponding author ([curt.mccartney@canada.ca](mailto:curt.mccartney@canada.ca)).

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Human and animal rights** This article does not contain any studies with human participants or animals performed by any of the authors.

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