Research

Mapping the A Genome for QTL Conditioning Resistance to Fusarium Head Blight in a Wheat Population with *Triticum timopheevii* Background

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Abstract

Development and use of resistant wheat cultivars is the most practical and economical approach for the control of Fusarium head blight (FHB). In the present study, a population of recombinant inbred lines derived from the cross between 'AC Brio' (a Canadian bread wheat cultivar moderately susceptible to FHB) and 'TC 67' (an FHB-resistant cultivar derived from *Triticum timopheevii*) was used to map quantitative trait loci (QTL) for FHB resistance using microsatellite molecular markers. Multiple interval mapping detected several QTL for FHB resistance on the chromosomes 5AL and 6A. The QTL detected in the marker interval of *cfd6.1-barc48* on chromosome 5AL explained 10.9, 5.2, and 7.8% of phenotypic variation for disease incidence (type I resistance), disease severity (a combination of type I and type II resistance), and *Fusarium*-damaged kernels (FDK) (type IV resistance) under field conditions, respectively. The second QTL mapped

to 5AL, in the marker interval of *cfd39-cfa2185*, explained 19.4 and 20.6% of phenotypic variation for FDK under field conditions and disease severity in the greenhouse (type II resistance), respectively. The QTL located on chromosome 6A conferred resistance to disease incidence and severity under field conditions and to disease severity in the greenhouse, explaining 6.8 to 11.8% of phenotypic variation for these traits. Several QTL for agronomic traits were also mapped in this study, including one and two QTL to the chromosomes 2A and 5AL, respectively, all for plant height, and two QTL to chromosome 6A for plant height and flowering date, respectively. The 5AL QTL for FHB resistance mapped in the marker interval of *cfd39-cfa2185* in the present study is a novel QTL that originated from *T. timopheevii* and is reported here for the first time. Further validation of this QTL is required for wheat breeding programs to enhance resistance levels to FHB.

Fusarium head blight (FHB) is one of the most destructive diseases of wheat worldwide. The disease is caused mainly by *Fusarium graminearum* sensu lato but other species, including *F. culmorum* and *F. avenaceum*, are also important in some wheat-growing regions (Parry et al. 1995). Infection occurs primarily during flowering and may lead to severe yield reduction and decreased grain quality (Bai and Shaner 1994). Development of resistant cultivars is an important tool for mitigation of FHB (Yang et al. 2005). The Chinese FHB-resistant wheat 'Sumai 3' and its derivatives, including 'Ning7840', have been successfully used as a resistant parent in many wheat breeding programs worldwide (Bai and Shaner 2004). Other sources of resistance, including 'Shinchunaga', 'Nobeokabouzu', and 'Nyu Bai' from Japan; 'Frontana' and 'Encruzilhada' from Brazil; and 'Ernie' and 'Freedom' from the United States have also been used in breeding programs in several countries (Bai and Shaner 2004). Since 2000, several FHB-resistant wheat cultivars such as 'Steele-ND' (Mergoum et al. 2005), 'ND 2710' (Frohberg et al. 2004), 'Glenn' (Mergoum et al. 2006), and 'VA04W-433' and 'VA04W-474' (Chen et al. 2012) from the United States; 'TC 67' (Cao et al. 2009) and 'Waskada' (Fox et al. 2009) from Canada; and 'Morvarid' from Iran (A. Malihipour, unpublished) were released and grown on a commercial scale. However, breeding wheat for resistance to FHB

is difficult because of the polygenic nature of resistance, complex genetic inheritance, genotype–environment interactions, and the high cost of phenotyping (Bai and Shaner 1994; McMullen et al. 1997; Somers et al. 2003; Yang et al. 2005).

Five types of resistance to FHB have been proposed: resistance to initial infection or type I resistance (Schroeder and Christensen 1963); resistance to spread of infection within the spike or type II resistance (Schroeder and Christensen 1963); ability to prevent synthesis of deoxynivalenol (DON) or promote degradation of the produced DON (Miller et al. 1985) and tolerance of high DON concentrations (Wang and Miller 1988), which are referred together as resistance to toxin accumulation, or type III resistance in more recent literature (Mesterházy et al. 1999); resistance to kernel infection or type IV resistance (Mesterházy 1995; Mesterházy et al. 1999); and disease tolerance (Mesterházy 1989, 1995; Mesterházy et al. 1999). Type I resistance is determined by spraying a spore suspension onto flowering spikes and measuring percentage of diseased spikes, which is commonly referred to as disease incidence (Buerstmayr et al. 2009). Type II resistance, which is determined based on the proportion of diseased spikelets (frequently referred to as disease severity) from central-floret inoculation (Bai et al. 1999; Waldron et al. 1999), has been extensively studied in wheat, because it appears to be more stable and less affected by nongenetic factors (Bai and Shaner 1994). However, disease severity assessed under spray-inoculated field experiments is used as a measure of overall FHB resistance (Buerstmayr et al. 2009), because both type I and type II resistance are considered. Type III resistance is determined by assessing DON accumulation in kernels using different methods such as chromatography or enzyme-linked immunosorbent assay (ELISA). Finally, type IV resistance is often measured by determining the proportion of *Fusarium*-damaged kernels (FDK).

Resistance to FHB in wheat is quantitatively inherited and the underlying quantitative trait loci (QTL) are distributed throughout the wheat genome. Six QTL conferring resistance to FHB have been formally named as genes *Fhb1*, *Fhb2*, *Fhb3*, *Fhb4*, *Fhb5*, and

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Fhb6, *Fhb1*, present on chromosome 3BS from Sumai 3 and its derivatives (Liu et al. 2006), is the most consistent gene with the largest effect on type II resistance identified to date (Buerstmayr et al. 2009). This gene also reduces disease incidence (Chen et al. 2006; Yang et al. 2005), disease severity (Buerstmayr et al. 2003a; Jia et al. 2005; Mardi et al. 2005; Szabó-Hevér et al. 2014), FDK (Suzuki et al. 2012; Szabó-Hevér et al. 2014; Yang et al. 2005), and DON accumulation (Jiang et al. 2007; Lemmens et al. 2005; Somers et al. 2003; Suzuki et al. 2012; Szabó-Hevér et al. 2014; Yu et al. 2008; Zhou et al. 2002). *Fhb1* is now extensively used in wheat breeding programs due to its stable major effect on type II resistance across different genetic backgrounds (Buerstmayr et al. 2009). Several QTL specifically associated with resistance to fungal penetration (type I resistance) such as *Qfhs.ifa-5A* from 'CM-82036' (Buerstmayr et al. 2003a,b), *Qfhi.nau-4B* and *Qfhi.nau-5A* from 'Wangshuibai' (Lin et al. 2006), a major QTL mapped to chromosome 3A from Frontana (Steiner et al. 2004), and a few QTL from the Sumai 3-derived line 'DH181' (Yang et al. 2005) have also been reported. These QTL have a strong effect on resistance expression.

Several wheat-related species such as *Elymus humidus* (Ohwi et Sakamoto) Osada, *E. racemifer* (Steud.) Tzvel., *Roegneria kamoji*, and *Leymus racemosus* (Lam.) Tzvel. are potential donors of FHB resistance genes (Chen et al. 2005; Oliver et al. 2005). However, the resistance found in related species is usually associated with undesirable traits which are difficult to remove from the progeny (Bai and Shaner 2004). Special chromosome manipulation is needed to introgress FHB resistance genes into wheat from distantly related species (Cai et al. 2005). Resistance to FHB has also been identified in tetraploid wheat species such as wild emmer wheat (*Triticum turgidum* L. subsp. *dicoccoides* (Körn ex Asch. et Graebn.) Thell.) (Oliver et al. 2007), cultivated emmer wheat (*T. turgidum* L. subsp. *dicoccum* (Schränk ex Schübl.) Thell.), and Persian wheat (*T. turgidum* L. subsp. *carthlicum* (Nevski) A. Löve et D. Löve) (Cai et al. 2005; Oliver et al. 2008; Somers et al. 2006). *T. timopheevii* (Zhuk.) Zhuk., which is another tetraploid wheat, has a high level of resistance to FHB (Cao et al. 2009). The FHB resistance present in *T. timopheevii*, which may be of novel resistance, is not clearly understood yet. Mapping and tagging of FHB resistance available in wheat cultivars with a background of *T. timopheevii* may be of great interest for use in wheat breeding programs. The objectives of this study were to (i) map FHB resistance QTL in a population of wheat derived from *T. timopheevii*, (ii) detect simple sequence repeat (SSR) molecular markers linked to these QTL in this population, and (iii) determine the novelty of these QTL in comparison with other available resistance sources.

Materials and Methods

Plant materials. In an attempt to enhance FHB resistance in bread wheat, several wheat-related wild species, including a number of *T. timopheevii* ($2n = 4x = 28$, AAGG) lines, were screened for FHB resistance (Cao et al. 2009). An FHB-resistant accession of *T. timopheevii*, PI 343447, was crossed to the susceptible spring wheat 'Crocus' (*T. aestivum*, $2n = 6x = 42$, AABBDD). The resulting F_1 plants were backcrossed to Crocus to produce BC_1F_1 plants ($2n = 6x = 42$, AABBDD). A population of 1,500 F_2 plants was established and 535 BC_1F_7 lines were developed using single-seed descent (SSD). In total, 100 lines were selected on the basis of plant fertility and agronomic traits, and were evaluated for reaction to FHB in the greenhouse and field FHB nursery. TC 67 was selected from this population on the basis of its superior FHB reaction and reasonable agronomic traits (Cao et al. 2009). Later, the moderately susceptible wheat 'Brio' (*T. aestivum*, $2n = 6x = 42$, AABBDD), with the pedigree of 'Columbus'/'S68147'/'Laval19'/Columbus, was crossed to TC 67. An F_7 mapping population consisting of 230 recombinant inbred lines (RIL) developed using SSD from the cross of Brio and TC 67 was used in this study.

Fusarium isolates and inoculum production. A mixture of *F. graminearum* isolates (40/04, 71/04, 98/04, 136/04, MSDS 3/03, and EMMB 19/03) stored at the Cereal Research Centre (CRC), Winnipeg, Manitoba, Canada, was used for the field inoculations in

Carman, Manitoba. The same isolates were used in Glenlea, Manitoba, with the exception that the last two isolates were replaced with two other isolates (M1-04-5 and M3-04-3). For inoculum production, actively growing cultures of the isolate on potato dextrose agar (PDA) were blended, added to liquid carboxymethyl cellulose sodium culture media (Sigma-Aldrich, St. Louis), and incubated under aeration for 5 to 7 days at room temperature. The culture media was passed through a cheesecloth and the suspension was diluted to 5×10^4 macroconidia/ml before the field inoculations.

For the greenhouse studies, a mixture of four highly aggressive isolates (M6-04-4, M9-04-6, M1-04-1, and M8-04-3) was used. The method used by Afshari-Azad (1992) was modified as follows and used for the greenhouse inoculum production: 5 g of blended straw from wheat or barley was added to 125 ml of tap water in a 250-ml flask and autoclaved two times with a 24-h interval, a small plug of PDA (Difco, Sparks, MD) containing the fungal isolate was added to the culture, and the culture was shaken for 96 h at 120 rpm at room temperature. Like the field inoculations, the culture was passed through a cheesecloth and the suspension was diluted for inoculations.

Field experiments. The mapping population and the parental lines were evaluated for FHB in spray-inoculated field trials conducted in two locations, Carman and Glenlea, Manitoba, Canada, for 2 years (2006 and 2007). The experimental design was a randomized complete block design in both locations in 2006 and a 16-by-15 lattice design in 2007, each with three replications. Plots consisted of rows 1 m (Carman) or 1.5 m (Glenlea) in length, with 30-cm row spacing. Sowing density was approximately 5 g of seed per plot. Sowing dates were 29 to 30 May and 5 June in Carman and Glenlea, respectively, in 2006, and 9 May in both locations in 2007. Standard procedures were used to maintain the nurseries, and control weeds and insects. 'Roblin' wheat was used as the susceptible check to monitor disease level in the nurseries.

Plots were spray inoculated individually when 50% of the plants had reached anthesis with a conidial suspension of the pathogen isolates (5×10^4 macroconidia/ml) using a CO₂-powered backpack sprayer till runoff of the inoculum on the plants. The inoculation procedure was repeated 2 or 3 days later. Nurseries were mist irrigated 5 min/h (Carman) for 12 h or sprinkler irrigated for 1 h (Glenlea) after inoculation. After the first day of inoculation, the irrigation system was operated two to three times a day, until the disease developed in the nurseries.

Three weeks after inoculation, the RIL were scored for disease incidence and severity. Disease incidence was determined as the percentage of diseased spikes per plot and disease severity according to a scale of 0 to 100% for the visually infected spikelets on a whole-plot basis. To score the RIL for FDK, rows were sickle harvested at maturity and threshed using a Wintersteiger Nursery Master Elite combine (Wintersteiger AG, Ried, Austria). The threshing mechanism was set at a normal setting on the combine; however, the wind speed was decreased and sieves were opened to ensure that FDK were retained in the harvested samples. A wheat head thresher (Precision Machine Co. Inc., Lincoln, NE) later was used to thresh wheat samples, which were not well threshed. FDK were assessed by counting the visually damaged kernels in three random subsamples of 100 grains from each plot.

Data of agronomic traits, including flowering date and plant height, were also collected under field conditions. Flowering date was measured as the number of days from seeding to 50% anthesis of the plot and plant height as the distance from soil surface to top of the spikes minus awns at growth stages 65 and 91, respectively, according to Zadoks et al. (1974).

Greenhouse experiments. The RIL were evaluated for resistance to fungal spread within the spike (disease severity) following single-floret inoculation in the greenhouses in Winnipeg in 2007. The experimental layout was a randomized complete block design with three replicates, and each pot (16 by 13 by 13 cm³) was an experimental plot. The greenhouse was maintained under conditions of 16 h of light and 8 h of darkness. The temperature of the greenhouse averaged 24°C during the day, with a range of 18 to 28°C, and 18°C at night, with a range of 16 to 20°C. Wheat plants were treated 3 weeks after seeding with a mixture of propiconazole + spinosad

to control powdery mildew and insects. As in the field experiments, Roblin was used as the susceptible check.

The RIL varied in flowering date. As spikes reached 50% anthesis, they were inoculated using a micropipette to inject 10^4 of conidial suspension of *F. graminearum* isolates (5×10^4 macroconidia/ml) into the floret in a spikelet positioned one-third of the way from the top of the spike. At least five spikes in each pot (replication) were inoculated, and the spikes were covered with a glassine bag (20 by 5 cm²; Seedburo Equipment Co., Chicago) for 48 h to maintain constant high humidity. Disease severity in the inoculated spikes was measured as the percentage of diseased spikelets per spike 21 days after inoculation. The greenhouse experiment was conducted twice.

Measurements were also taken for plant height and flowering date. Plant height was measured as mentioned for the field data and flowering date as the average number of days from seeding date to anthesis for the first five early-emerging spikes.

Marker analysis. In total, 323 SSR primer combinations stored at the CRC, including *barc* (Song et al. 2002, 2005), *cfa*, *cfb* (Guyomarc'h et al. 2002; Sourdille et al. 2003), *gdm*, *gwm* (Pestsova et al. 2000; Röder et al. 1998), and *wmc* (Gupta et al. 2002), were used for marker analysis. We used these markers, all from the 'A' genome, because, most likely, the resistance introgressed from *T. timopheevii* ($2n = 4x = 28$, AAGG) into TC 67 was expected to be on this genome. They covered a total length of 1,778 centimorgans (cM) with an average distance of 2.77 cM between the markers based on the microsatellite consensus map for bread wheat (Schuelke 2000; Somers et al. 2004). For polymerase chain reaction (PCR) amplification, forward primers tailed with a 19-bp fluorescent-labeled M13 primer (5'-CACGACGTTGTAAACGAC) at the 5' end were used. A universal fluorescent-labeled M13 primer homologous to the same sequence added to each forward primer was also added to the PCR (Schuelke 2000; Somers et al. 2004). Reverse primers used in the reaction were normal. The PCR was performed in a 10- μ l volume, containing 5 μ l of template DNA at 10 ng/ μ l; 1.5 mM MgCl₂; 0.8 mM each dNTP (Invitrogen, Carlsbad, CA); forward primer at 0.02 pmol/ μ l; reverse primer at 0.2 pmol/ μ l (Invitrogen); M13 primer fluorescently labeled with FAM, HEX, or NED at 1.8 pmol/ μ l (Applied Biosystems, Foster City, CA); 1 $\times$ PCR buffer; and *Taq* DNA polymerase at 1 unit/ μ l. The PCR products were amplified in a PTC-200 thermal cycler (MJ Research, Waltham, MA) with the following cycling program: (i) 94°C for 2 min (initial denaturing step); (ii) 31 cycles of 95°C for 1 min (for DNA denaturation), 0.5°C/s to 51 or 61°C, 51 or 61°C for 30 s (for primer annealing), 0.5°C/s to 73°C, and 73°C for 1 min (for primer extension); (iii) 73°C for 5 min (for final extension); and (iv) 4°C to hold the program. PCR products were multiplexed by combining 0.5 μ l of FAM-, 0.6 μ l of HEX-, and 0.5 μ l of NED-labeled products with 5.0 μ l of a 4% mixture of GeneScan 500-ROX (Applied Biosystems) in Hi-Di formamide (Applied Biosystems). The multiplex was denatured for 10 min at 95°C and quickly chilled on ice for 5 min. The denatured sample was loaded on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) and fragment analysis was performed with GeneScan 3.7.1 (Applied Biosystems). Data collected by fluorescent capillary electrophoresis was converted to a gel-like image using Genographer 2.0 (Benham et al. 1999) (freely available at <http://sourceforge.net/projects/genographer>). The images were formatted using Canvas 11 (ACD Systems of America Inc., Fort Lauderdale, FL) and the final images were printed and scored manually.

All primers were screened for polymorphism on the two parents, and those which produced polymorphic fragments between the parental samples were used to genotype the entire mapping population. The χ^2 test was employed to estimate segregation distortion for the markers in the population.

The linkage maps for marker data were created using JoinMap V 3.0 (Van Ooijen and Voorrips 2001). A Kosambi map function (Kosambi 1943) was applied to calculate the distance between the ordered markers. Linkage groups were established using a minimum logarithm of odds (LOD) threshold of 2.0. The maps were compared with the wheat consensus SSR map (Schuelke 2000; Somers et al.

2004) available in the GrainGenes database (<http://wheat.pw.usda.gov/ggpages/maps.shtml>).

Analysis of field and greenhouse data. Data collected from the six environments (two field locations in 2 years plus two greenhouse experiments) were used for data analysis. Combined data from four environments were used for estimating correlations and analyses of variance (ANOVA) under field conditions. For the greenhouse experiments, a combined data set of disease severity obtained from two experiments was used for the analysis. Before conducting ANOVA, the field and greenhouse data sets were tested for normality using PROC UNIVARIATE of SAS. In the event that the residuals of the dependent variables did not follow a normal distribution, an arcsine transformation was applied to data. ANOVA were performed using PROC GLM based on a randomized complete block design. Genotype effect in the statistical model was considered fixed, while the other effects were considered random. Broad-sense heritabilities for the RIL were estimated from ANOVA (Hallauer and Miranda 1981) using the formulae $h^2 = \sigma_G^2 / [\sigma_G^2 + (\sigma_e^2/r)]$ for the single-location or greenhouse, $h^2 = \sigma_G^2 / [\sigma_G^2 + (\sigma_{GL}^2/l) + (\sigma_e^2/r)]$ for the combined data of two locations or greenhouses, and $h^2 = \sigma_G^2 / [\sigma_G^2 + (\sigma_{GL}^2/l) + (\sigma_{GY}^2/y) + (\sigma_{GYL}^2/yl) + (\sigma_e^2/ryl)]$ for the combined data of two locations in 2 years, where σ_G^2 is the genotypic variance, σ_{GL}^2 is genotype–location variance, σ_{GY}^2 is genotype–year variance, σ_{GYL}^2 is genotype–location–year variance, σ_e^2 is residual variance, r is the number of replications (blocks), l is the number of locations, and y is the number of years. All statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Raleigh, NC).

QTL mapping. Before QTL analysis, data transformations were applied to phenotypic data if necessary. QTL analysis was conducted using the computer program QGene V 4.3.10 (Joehanes and Nelson 2008). The single-trait multiple IM option of this software was performed to detect genomic regions on linkage groups associated with phenotypic means. A LOD threshold of 2 was used to declare a QTL significant. However, the significance was determined based on a permutation test of $n = 1,000$ (Churchill and Doerge 1994). For each QTL, the marker, the percentage of phenotypic variation explained, and the additive effects were estimated. The single-marker regression option of QGene was also applied to detect association between single markers and phenotypic data.

Results

FHB resistance. Results from the ANOVA using the combined field data of two locations in 2 years showed significant differences among the RIL and significant location–genotype interaction ($P \leq 0.05$) for disease incidence and FDK but not for disease severity (data not shown). On the other hand, the interactions of year–genotype and year–location–genotype were significant ($P \leq 0.05$) for all FHB resistance traits studied (data not shown). For the greenhouse data of disease severity, ANOVA showed significant differences ($P \leq 0.01$) among the RIL as well (data not shown).

Based on the FHB traits measured, 'AC Brio' wheat was more susceptible than TC 67 to FHB (Table 1). A high range of variation was observed among the RIL for disease incidence, severity, and FDK under field conditions and for disease severity in the greenhouse (Table 1). The frequency distributions for all traits were continuous and showed transgressive segregation (Fig. 1).

Broad-sense heritability, which measures the proportion of the phenotypic variance that is due to genetic effects, was highest for disease severity in the greenhouse and lowest for disease severity under field conditions. Intermediate heritability values were observed for disease incidence and FDK under field conditions (Table 1).

Correlations among FHB resistance traits. All correlation coefficients among the FHB resistance traits estimated using the field and greenhouse data were positive and significant ($P \leq 0.01$). The correlation coefficient observed between disease incidence and severity under field conditions was 0.58, which is relatively high. However, the correlation coefficients observed between these traits and FDK were in a range of 0.29 to 0.32, which are not very high. Finally,

the correlations between disease severity measured in the greenhouse and the traits in the field were also relatively low, with correlation coefficients in a range of 0.32 to 0.35.

Plant height and flowering date. High variation in plant height and flowering date was observed among the genotypes, and the frequency distributions of both traits were continuous. ANOVA also indicated significant differences among the genotypes for both traits

(data not shown). The detailed phenotypic characterization of agronomic traits for the population was previously published (Malhipour et al. 2016).

Genetic linkage maps. Of the 323 SSR primer combinations screened on the parental DNA samples, 83 primer combinations amplified 96 polymorphic marker fragments. Those primers were used to evaluate the mapping population. Seven markers were discarded

Table 1. Parents values, population mean, ranges of recombinant inbred lines (RIL), and heritabilities for disease incidence, disease severity, and *Fusarium*-damaged kernels (FDK) using data collected from 230 recombinant inbred lines from the cross AC Brio/TC 67 under field and greenhouse conditions

Trait	Parent value		Population mean	RIL range	Heritability
	AC Brio	TC 67			
Field					
Disease incidence (%)	43.5	24.3	37.6	14.4–64.5	0.60
Disease severity (%)	42.1	41.3	50.6	27.0–75.5	0.47
FDK (%)	7.7	3.1	8.9	1.8–24.1	0.67
Greenhouse					
Disease severity (%)	34.9	4.9	34.5	4.8–94.9	0.96

Fig. 1. Frequency distributions using data collected from 230 recombinant inbred lines from the cross AC Brio/TC 67. Field data for disease incidence, disease severity, and *Fusarium*-damaged kernels (FDK) were collected from two locations in 2 years and disease severity in the greenhouse from two experiments.

due to distorted segregation ratios, and the genotypic data of 89 markers from the mapping population were used to construct the genetic linkage maps. The genetic map, which was subsequently used for QTL analysis, consisted of 17 linkage groups and comprised, in total, a length of 298.8 cM. The A genome was the most represented genome, with nine linkage groups, 55 molecular markers, and a length of 210.9 cM. Even though the SSR markers used in this study were originally from the A genome, the primer combinations of some of them amplified polymorphic marker fragments on the 'B' genome. As a result, one linkage group with four molecular markers and a total length of 21.9 cM was assigned to the B genome. However, no marker or linkage group was located to the 'D' genome. In the present study, seven linkage groups consisting of 18 markers could not be assigned clearly to one specific chromosome. In addition, 12 markers remained unlinked. The average distance between markers in the linkage groups was 3.9 cM in this investigation.

QTL mapping. Multiple interval mapping using QGene detected two major QTL on chromosome arm 5AL and another one on chromosome 6A for resistance to FHB (Table 2; Fig. 2). Another minor QTL for FHB resistance was also identified in the present study (data not shown). On the other hand, several QTL for agronomic traits were mapped, including one and two minor QTL for plant height on chromosomes 2A and 5A, respectively, and a major QTL for plant height and a minor one for flowering date, both mapped to chromosome 6A (Table 2; Fig. 2).

The 5AL QTL detected in the marker interval of *cf6.1-barc48* (closest marker *cf6.1*) was associated with disease incidence (type I resistance) under field conditions. This QTL accounted for 10.9% of phenotypic variation for this trait, with an additive effect of -0.04. This QTL also had small effects on disease severity (type I + type II resistance) and FDK (type IV resistance) under field conditions. The second QTL mapped to this chromosome, in the marker interval of *cf6.1-cfa2185* (closest marker *cfa2185*), was associated with FDK in the field and disease severity in the greenhouse, explaining 19.4 and 20.6% of phenotypic variation for these traits, respectively. The additive effects of this QTL were -0.04 and -0.13, respectively. The two 5AL QTL were approximately 30 cM apart on the chromosome and the alleles from TC 67 were associated with resistance. In addition, the closest markers to either of these QTL shared the same pattern and size of alleles in TC 67 and its resistant parent, *T. timopheevii* (data not shown). The QTL mapped in the marker interval

of *gwm132.1-wmc621* (closest marker *wmc621*) on chromosome 6A was associated with disease severity in the greenhouse, explaining 11.8% of phenotypic variation, with an additive effect of 0.08. This genomic region was associated with resistance to disease incidence and severity under field conditions, accounting for 6.8 and 10.1% of phenotypic variation for these traits, respectively. The allele from AC Brio in this chromosome region contributed to reduced disease incidence and severity under field conditions. Pattern and size of alleles for the closest markers to either of the 6A QTL were identical in AC Brio and the susceptible parent of TC 67, Crocus (data not shown). In the present study, a minor QTL associated with disease severity in the greenhouse, linked to the marker *barc154.1*, was also detected (data not shown). However, the linkage group associated with this QTL could not be assigned to a chromosome.

The 2A QTL detected in the marker interval of *gwm275-gwm95* (closest marker *gwm95*) explained 5.8% of phenotypic variation for plant height using field data, with an additive effect of -0.01. For this QTL, the TC 67 allele reduced plant height even though this is the tall parent in the cross. The 5AL QTL detected in the marker interval of *cfa2185-cfa2141* (closest marker *cfa2141*) explained 6.4% of phenotypic variation for plant height using field data, with an additive effect of 0.01. Similarly, the QTL in the marker interval of *gwm666.1-barc151* (closest marker *gwm666.1*) from this chromosome accounted for 7.0% of phenotypic variation for plant height using greenhouse data, with an additive effect of 0.01. For both of these QTL, the alleles for tall stature came from resistant parent TC 67. Two QTL for agronomic traits were also mapped to chromosome 6A, of which the QTL detected in the marker interval of *cf6.1-wmc748* (closest marker *cf6.1*) explained 9.9% of phenotypic variation for plant height, with an additive effect of 0.02. The second 6A QTL, detected in the marker interval of *wmc621-cf6.2* (closest marker *wmc621*), accounted for 5.5% of phenotypic variation for flowering date, with an additive effect of 0.01. The alleles for tall stature and late flowering for these QTL, respectively, were from the resistant parent TC 67.

The combined QTL in the marker intervals of *cf6.1-barc48* from 5AL and *gwm132.1-wmc621* from 6A explained 17.7% of phenotypic variation for disease incidence under field conditions or type I resistance. These QTL also explained 15.3% of phenotypic variation for disease severity under field conditions, which represents a combination of type I and type II or overall field resistance. In addition, the QTL in the marker intervals of *cf6.1-barc48* and *cf6.1-cfa2185* from chromosome 5AL together explained 27.2% of

Table 2. Quantitative trait loci (QTL) analysis of Fusarium head blight (FHB) resistance by multiple interval mapping for disease incidence, disease severity, and Fusarium-damaged kernels (FDK) using data collected from 230 recombinant inbred lines from the cross AC Brio/TC 67 under field and greenhouse conditions

Trait, chromosome	Marker interval	Closest marker	Trait ^a	LOD thrsh ^b	LOD	R ² (%)	Additive effect ^c	Donor parent ^d
FHB resistance								
5AL	<i>cf6.1-barc48</i>	<i>cf6.1</i>	DI (F)	2.13	5.75	10.9	-0.04	TC 67
5AL	<i>cf6.1-barc48</i>	<i>cf6.1</i>	DS (F)	2.09	2.65	5.2	-0.02	TC 67
5AL	<i>cf6.1-barc48</i>	<i>cf6.1</i>	FDK (F)	2.09	4.03	7.8	-0.02	TC 67
5AL	<i>cf6.1-cfa2185</i>	<i>cfa2185</i>	FDK (F)	2.09	10.75	19.4	-0.04	TC 67
5AL	<i>cf6.1-cfa2185</i>	<i>cfa2185</i>	DS (G)	2.18	11.50	20.6	-0.13	TC 67
6A	<i>gwm132.1-wmc621</i>	<i>gwm132.1</i>	DI (F)	1.52	3.50	6.8	+0.03	AC Brio
6A	<i>gwm132.1-wmc621</i>	<i>gwm132.1</i>	DS (F)	1.57	5.32	10.1	+0.03	AC Brio
6A	<i>gwm132.1-wmc621</i>	<i>wmc621</i>	DS (G)	1.49	6.26	11.8	+0.08	AC Brio
Agronomic								
2A	<i>gwm275-gwm95</i>	<i>gwm95</i>	PH (F)	1.79	3.00	5.8	-0.01	AC Brio
5AL	<i>cfa2185-cfa2141</i>	<i>cfa2141</i>	PH (F)	2.02	3.29	6.4	+0.01	TC 67
5AL	<i>gwm666.1-barc151</i>	<i>gwm666.1</i>	PH (G)	2.04	3.61	7.0	+0.01	TC 67
6A	<i>cf6.1-wmc748</i>	<i>cf6.1</i>	PH (G)	1.46	5.19	9.9	+0.02	TC 67
6A	<i>wmc621-cf6.2</i>	<i>wmc621</i>	FD (G)	1.34	2.85	5.5	+0.01	TC 67

^a Abbreviations: DI = disease incidence, DS = Disease severity, F indicates field data based on combined data of two locations in 2 years, G refers to the greenhouse data, PH = plant height, and FD = flowering date.

^b Logarithm of odds (LOD) threshold, estimation using a permutation test of $n = 1,000$ at $P < 0.05$.

^c Negative values for the additive effects of Fusarium head blight QTL indicate that the TC 67 allele reduces the average trait values relative to the AC Brio allele. Positive values for the additive effects of agronomic trait QTL indicate that the TC 67 allele increases the average trait values relative to the AC Brio allele.

^d Alleles from the donor parent confers lower disease incidence and disease severity, fewer FDK, taller plants, and later flowering date under field conditions or in the greenhouse.

phenotypic variation for FDK or type IV resistance. Furthermore, the 5AL QTL in the marker interval of *cfd39-cfa2185* and the 6A QTL in the marker interval of *gwm132.1-wmc621* together explained 32.4% of the phenotypic variation for disease severity in the greenhouse. On the other hand, the QTL conferring agronomic traits mapped to the chromosomes 2A, 5AL, and 6A all together explained 29.1% of phenotypic variation for plant height, whereas the only QTL from 6A conferring flowering date accounted for 5.5% of phenotypic variation for this trait.

Discussion

Transgressive segregation was observed among the RIL for all traits measured. Presence of transgressive segregation in this study indicates that neither of the parents carries a full complement of resistance QTL or genes. It also suggests that improvements in FHB resistance can be made by combining resistance genes from different parents (Somers et al. 2003). However, there were very few transgressive segregants with lower disease severity than the resistant parent in the greenhouse study. This suggests that identifying recombinants that are better than the best parent for all traits may be difficult. Transgressive segregation is a common phenomenon in FHB resistance and has been discussed in published literature (Bai and Shaner 1994; Buerstmayr et al. 2000; Ghavami et al. 2011; Malla et al. 2010; Somers et al. 2006). In addition, reports have indicated that resistance alleles sometimes contributed to transgressive segregation by a susceptible parent (Liu et al. 2005; Snijders 1990; Waldron et al. 1999). Obtaining transgressive segregants from a cross between a resistant and a susceptible variety is beneficial for breeders.

The moderate to high heritability of traits measured in different environments showed that there was high genetic variability for the traits facilitating QTL detection. By better expression of genetic effects, uniform environmental conditions in the greenhouse were conducive to a high value of heritability for the resistance trait. In contrast, the interaction effects of year–genotype or year–location–genotype might have contributed to reduce the values of heritability for some traits under field conditions. Despite the high influence of environment on expression of the resistance traits, QTL could be consistently identified.

The correlation observed between disease incidence measured under field conditions and disease severity assessed in the greenhouse was not high. This was to be expected because each trait represented a specific type of resistance (i.e., type I and type II, respectively). In contrast, the relatively high positive correlation coefficient observed between disease incidence and severity under field conditions demonstrates that these measurements are interrelated under field conditions. Our findings are consistent with the results of Steiner et al. (2004), who observed a high positive correlation between FHB incidence and severity. However, the correlation coefficients observed between these traits and FDK were not very high. Because FDK is a measure of type IV resistance (Mesterházy 1995; Mesterházy et al. 1999), it is possible that there is different genetic control for FDK than for disease incidence or severity under field conditions.

Chromosome 5A has been found to be involved in FHB resistance in widely diverse wheat germplasm, and QTL from this chromosome have been reported for type I and type II resistance, low FDK, and low DON content (Buerstmayr et al. 2009). In the present study, two stable QTL conferring resistance to FHB were mapped to the chromosome arm 5AL; both conferred resistance to more than one resistance trait. The QTL detected in the marker interval of *cfd39-cfa2185* showed the highest effects on disease severity in the greenhouse (type II resistance) and FDK under field conditions (type IV resistance). The effect of a genomic region on more than one trait may be attributed to the presence of a pleiotropic QTL conferring resistance to different traits, or to the presence of more than one overlapping QTL in one position. There are several reports discussing pleiotropic effects of a QTL on more than one trait or the presence of overlapping QTL in wheat populations. In their study of a population of ‘W14’/Pioneer Brand 2684’, Chen et al. (2006) detected a 5AS QTL which explained phenotypic variation for FHB incidence and severity, DON accumulation, and FDK. A QTL on chromosome 5A for reduced DON accumulation and type II resistance was also reported in the cross

of Wangshuibai/‘Annong 8455’ (Ma et al. 2006b). In addition, in a population of wheat from the cross Frontana/‘Remus’, Szabó-Hevér et al. (2012) identified a QTL on chromosome 5A which conferred resistance to both disease severity and FDK. Therefore, there should be at least one QTL in each position of the 5AL conferring resistance to one or more FHB resistance traits in the population we studied. However, the chance of the presence of more than one overlapping QTL in one genomic region is not impossible.

Because the pattern and size of the closest markers to either of the 5AL QTL were identical in TC 67 and its resistant parent *T. timopheevii*, both of these QTL should have come from *T. timopheevii*. To date, no QTL for resistance to FHB has been reported from *T. timopheevii*. In addition, while most 5A QTL for FHB resistance reported from other wheat species are located in the range of 30 to 90 cM (Buerstmayr et al. 2009) and another QTL in the range of 146 to 167 cM (Buerstmayr et al. 2011), the QTL detected in the marker interval of *cfd39-cfa2185* in this investigation was located in a range of 103 to 142 cM, based on microsatellite consensus map (Schuelke 2000; Somers et al. 2004). Therefore, we assume that this QTL differs from the previously reported QTL at this chromosome. We conclude that the QTL mapped to this marker interval from the chromosome arm 5AL is a novel QTL. However, the novelty of the 5AL QTL in the marker interval of *cfd6.1-barc48* cannot be proved using the available data.

In the present study, another QTL for FHB resistance was mapped in the marker interval of *gwm132.1-wmc621* to chromosome 6A, with minor effects on disease incidence (field) and major effects on disease severity (field and greenhouse). It is possible that there is only one pleiotropic QTL present in this marker position conferring resistance to several traits. Chromosome 6A is not widely used in FHB resistance in wheat. However, there are a few reports indicating a role of this chromosome in increasing type II resistance and lowering disease severity in wheat (Anderson et al. 2001; Buerstmayr et al. 2012; Ma et al. 2006a; Schmolke et al. 2005). It is not clear from our data if there is association between the QTL detected in this population and those previously reported. However, it is the first time that a 6A QTL with an effect on disease incidence is reported. The closest markers to either of the 6A QTL which came from the moderately susceptible parent AC Brio shared a common allele with the susceptible parent of TC 67, Crocus. It is possible that this similarity is due to genetic relatedness between these hexaploid wheat cultivars, because both Crocus and AC Brio have a common background of Columbus.

Several QTL for plant height on the chromosomes 2A, 5A, and 6A and a QTL for flowering date on chromosome 6A were also detected in the present investigation. There are many reduced height (*Rht*) genes identified for reduction of plant height in wheat such as *Rht-B1b* (*Rht1*), *Rht-D1b* (*Rht2*), and *Rht8* (Worland et al. 1998). In addition, all wheat chromosomes carry genes for flowering date, including vernalization response (*Vrn*), photoperiod response (*Ppd*), and ‘‘earliness per se’’ (*Eps*) genes (Snape et al. 2001). However, plant height and heading date may be controlled by different QTL in different crops (Börner et al. 2002; Shindo et al. 2003; Xu et al. 2005; Zhang et al. 2009). Several QTL for plant height have been previously reported from several chromosomes, including 2A, 5A, and 6A (Buerstmayr et al. 2011; Griffiths et al. 2009, 2012; Liu et al. 2011; Peng et al. 2011). QTL for flowering date have also been reported from chromosome 6A (Kamran et al. 2013). However, there is no evidence for the association between the QTL for plant height and flowering date detected in the present study and the QTL previously reported. In addition, even though plant height and flowering date both showed negative associations with disease incidence and severity or positive associations with FDK under field conditions (Malihipour et al. 2016), the association between these agronomic QTL and the FHB QTL detected in the present population cannot be determined. Detailed mapping of the FHB resistance QTL as well as the linked QTL determining important agronomic characteristics is necessary in order to make significant progress in FHB resistance (Chrpová et al. 2011).

Mapping QTL using microsatellite markers in the present population detected several QTL for FHB resistance, of which two QTL from chromosome 5AL and two from chromosome 6A all were stable QTL with major effects on FHB resistance. The QTL mapped in the marker

interval of *cfb39-cfa2185* from chromosome 5AL is a novel QTL originating from *T. timopheevii*, which is reported here for the first time. The QTL mapped in the present study along with other well-known QTL in wheat breeding programs should be useful for enhancing the level of resistance to FHB in wheat cultivars. *T. timopheevii* is considered a source of resistance not only for FHB but also for several important diseases of wheat such as common root rot (Bailey et al. 1993), stem

rust (Allard and Shands 1954), leaf rust (Leonova et al. 2007), powdery mildew (Peusha et al. 1995), and *Septoria nodorum* blotch (Ma and Hughes 1995). Several genes for resistance to leaf rust have been transferred to common wheat from diverse accessions of *T. timopheevii*; of these, *Lr18* (Dyck and Samborski 1968) and *Lr50* (Brown-Guedira et al. 2003) are noteworthy. Therefore, transfer and use of *T. timopheevii* QTL in wheat germplasm may enhance

Fig. 2. Linkage maps and logarithm of odds curves after multiple interval mapping using data collected from 230 recombinant inbred lines from the cross AC Brio/TC 67. Field data were collected from two locations in 2 years and the greenhouse from two experiments.

resistance to other diseases as well. However, the negative correlation between FHB and undesirable agronomic traits such as plant height (Malihpour et al. 2016) may impede breeding wheat for resistance to FHB using TC 67 QTL. In addition, the FHB resistance QTL and their linked markers must be validated by examining the segregation of markers with QTL in different genetic backgrounds.

Even though the QTL detected in the present study explained a high proportion of phenotypic variation for FHB resistance traits, there are gaps between the proportion of phenotypic variation that is potentially due to genetic effects and the amount of phenotypic variation explained by the genetic factors (QTL). One possible explanation is incomplete coverage of our genetic map from the A and B genomes, which may hamper the detection of one or several QTL with strong effects. In addition, genetic factors affecting FHB resistance may be present on the D genome, which was not mapped in this study. Even though a few minor QTL were detected in the present population, the effect of undetected ones may also be mentioned. Detection and use of the QTL not identified yet from this population may be of great importance to complete the study.

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