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ORIGINAL PAPER

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Haplotype diversity at fusarium head blight resistance QTLs in wheat

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Abstract Fusarium head blight (FHB) reduces grain yield and quality in common and durum wheat. Host FHB resistance is an effective control measure that is achieved by stacking multiple resistance genes into a wheat line. Therefore, breeders would benefit from knowing which resistance sources carry different resistance genes. A diverse collection of FHB-resistant and -susceptible wheat lines was characterized with microsatellite markers linked to FHB resistance quantitative trait loci (QTLs) on chromosomes 2DL, 3BS (distal to the centromere), 3BSc (proximal to the centromere), 4B, 5AS and 6BS identified in wheat lines Maringa, Sumai 3 and Wuhan 1. Putative Sumai 3 QTLs were commonly observed in advanced breeding lines, whereas putative Maringa and Wuhan 1 QTLs were relatively rare. Marker data suggested the 3BS, 3BSc and 5AS QTLs in the Brazilian cv. Maringa were derived from Asian germplasm and not from Frontana or other Brazilian lines. Haplotype diversity was reduced near the 5AS QTL, which might impact the deployment of this QTL. Finally, Brazilian germplasm was not closely related to other resistance sources and might be useful for pyramiding with Asian wheat-derived FHB resistance.

Introduction

Fusarium head blight (FHB) is a devastating fungal disease of common wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L. var. *durum*) worldwide. Several species of *Fusarium* can cause FHB; however, *F. graminearum*, teleomorph *Gibberella zeae* (Schwein.) Petch is the most prevalent in Manitoba, Canada (Gilbert et al. 1998, 1999). FHB reduces wheat yields and changes milling, baking and pasta-making properties (Dexter et al. 1996, 1997). In addition, infected kernels contain mycotoxins, such as deoxynivalenol, which can make grain unfit for human or animal consumption (McMullen et al. 1997). The use of host resistance is an economically and environmentally sound method of FHB control.

Sources of FHB resistance have been identified in (1) spring wheats from Asia, including Sumai 3 and its derivatives; (2) spring wheats from Brazil; and (3) winter wheats from Europe (Snijders 1994). Wheat breeding programs around the globe have relied heavily on Sumai 3-derived FHB resistance. The utilization of additional resistance sources in breeding programs is needed to diversify the genetic basis of FHB resistance in elite wheat germplasm and increase the level of FHB resistance through stacking of resistance genes.

A number of QTL mapping studies have analyzed the genetic control of FHB resistance. A major QTL for FHB resistance in Sumai 3 (Funo/Taiwan Wheat) and its derivatives mapped to the distal end of chromosome 3BS (Anderson et al. 2001; Buerstmayer et al. 2002; Waldron et al. 1999; Zhou et al. 2002). Additional FHB resistance QTLs in Sumai 3 and its derivatives have also been mapped to chromosomes 5AS (Buerstmayer et al. 2002), 6AS (Anderson et al. 2001) and 6BS (Anderson et al. 2001; Yang et al. 2003). FHB resistance in Wuhan 1 (unknown pedigree) mapped to 2DL and 4B (Somers et al. 2003b). In the Brazilian cv. Maringa (Frontana/Kenya 58// PG 1), FHB resistance QTLs mapped to the distal end of 3BS, the proximal end of 3BS, near the centromere, (termed "3BSc" throughout the text) and 5AS (Somers et al. 2003b). The 3BS QTLs in Sumai 3 and Maringa and the 5AS QTLs in CM-82036 (Sumai 3/Thornbird-S) and Maringa were mapped to the same intervals and might be allelic (Anderson et al. 2001; Buerstmayer et al. 2002; Somers et al. 2003b).

Disease-resistance genes can often be differentiated using differential isolates of the pathogen, the chromosomal location in the host genome, allelism tests or DNA-based markers. Physiological races in *Fusarium* spp. have not been identified to date, so FHB resistance genes cannot be distinguished by different pathogen isolates (van Eeuwijk et al. 1995). QTL mapping studies can differentiate resistance genes based on the location in the wheat genome, but are time consuming and expensive. Thus, mapping studies should be restricted to resistant lines that have a high probability of carrying novel resistance genes. Allelism tests can differentiate resistance genes in gene-for-gene pathosystems. However, they are not as likely to

work for FHB resistance genes since subtle phenotypic differences might be difficult to detect.

DNA-based markers can be used to assess genetic diversity across an entire genome or at specific chromosome regions. Bai et al. (2003) and Liu and Anderson (2003) used markers, mapping near the 3BS FHB resistance QTL identified in Sumai 3, to identify wheat lines that putatively carry the 3BS QTL. This approach makes use of previous mapping information and has the potential to rapidly differentiate germplasm with different FHB resistance genes.

The objectives of this study were to (1) compare the microsatellite marker haplotypes of FHB-resistant wheat lines with those of Sumai 3, Maringa and Wuhan 1 at known FHB resistance QTLs on chromosomes 2DL, 3BS, 3BSc, 4B, 5AS and 6BS and (2) identify FHB-resistant wheat lines with putatively novel FHB resistance genes.

Materials and methods

Plant material

A diverse collection of wheat germplasm, consisting of 79 lines with varying levels of FHB resistance, was used in this study (Table 1). The FHB resistance amongst these lines was derived from wheats from Asia, Europe and South America. Susceptible Canadian bread wheats from a number of marketing classes were also included for comparison. FHB reaction was evaluated in field epiphytotic nurseries in Ottawa, Ontario in 1999 and 2000. The experimental protocol was described previously (Somers et al. 2003b). Briefly, the plots were double rows, 1 m in length, with 17-cm row spacing. The seeding rate was 6 g/plot, and each wheat line was replicated four times. Plots were spray inoculated with a backpack sprayer at 50% anthesis. Inoculum consisted of a mixture of *F. graminearum* isolates at 50,000 spores/ml; 60 ml was applied per plot. This treatment was repeated 2 days later. A misting system maintained high humidity to aid FHB-symptom development. Disease ratings were made 21 days post inoculation with a 0–9 FHB rating scale (Xue et al. 2004). FHB reaction types 0–2 were considered resistant, 2–5 were considered moderately resistant, 5–7 were considered moderately susceptible and 7–9 were considered susceptible. Leaf and seed tissue was collected from a single individual for each wheat line. DNA was then extracted with the DNeasy 96 Plant Kit (Qiagen, Mississauga, Ontario) and was quantified by fluorometry using Hoechst 33258 stain.

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PCR amplification

BARC, gwm and wmc microsatellite markers mapping near FHB resistance QTLs identified in Maringa (chromosomes 3BS, 3BSc and 5AS), Sumai 3 (chromosomes 3BS, 5AS and 6BS) and Wuhan 1 (chromosomes 2DL and 4B) were analyzed. Markers with simple banding patterns and high polymorphic information content (PIC) values were selected for this study. PIC values were calculated with the following formula (Botstein et al. 1980):

$$\mathrm{PIC}_i = 1 - \sum_{j=1}^{n} p_{ij}^2 \quad (1)$$

Where n is the number of marker alleles for marker i and p_{ij} is the frequency of the jth allele for marker i. Microsatellite primer sequences for the BARC and gwm markers were obtained from the USA Wheat and Barley Scab Initiative Web site (http://www.scabusa.org/pdfs/BARC_SSRs_011101.html) and Röder et al. (1998), respectively. Table 2 presents the forward and reverse primer sequences and annealing temperatures of the 41 microsatellite markers used in the study.

PCR reactions were performed in 10 µl volumes and included 25 ng of template DNA, 0.5 U of *Taq* DNA polymerase (Gibco/BRL, Mississauga, Ontario), 1× PCR buffer (Applied Biosystems, Foster City, Calif.), 1.5 mM $MgCl_2$, 200 µM each dNTP, 20 µM forward primer, 180 µM 6-FAM/HEX/NED-labelled M13 primer (5′→3′ CACGACGTTGTAAAACGAC, Applied Biosystems) and 200 µM reverse primer. All forward microsatellite primers were modified to contain a 5′, 19-nucleotide M13 tail (5′→3′, CACGACGTTGTAAAACGAC) (Schuelke 2000). The reaction mixture was denatured at 94°C for 2 min; followed by 30 cycles of 95°C for 1 min, 49°C/58°C for 50 s, 73°C for 1 min; with a final extension step of 73°C for 5 min. PCR amplicons were resolved in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) with GeneScan software and GeneScan-500 ROX as an internal size standard (Applied Biosystems).

Cluster analysis

NTSYSpc version 2.11a (Exeter Software, Setauket, N.Y.) was used for cluster analysis. The SIMQUAL module was used to calculate similarity coefficients between wheat lines. The SAHN module was used for cluster analysis with the unweighted pair group method with arithmetic mean (UPGMA).

Results

Table 1 presents the reaction of the wheat lines to *F. graminearum* infection. Forty-nine wheat lines were classified as resistant, 19 were moderately resistant, four were moderately susceptible and seven were susceptible. The moderately susceptible and susceptible wheat lines were of Canadian origin and are primarily registered cultivars. The only exception was Funo, an Italian line that was moderately susceptible under these conditions. The remaining wheat lines were classified as resistant or moderately resistant.

The microsatellite markers had PIC values ranging from 0.25 to 0.89 (mean = 0.69) and detected 2 to 20 alleles (mean = 8.3) among the 79 wheat lines (Table 3). A total of 76 haplotypes were detected with 41 microsatellite markers. The following lines had the same haplotype: Aso Zairai and Aso Zairai II (based on 40 markers), Nyuubai

and Yanggangfangzhu (based on 41 markers) and Funo and Wuhan 2-37E (based on 39 markers). Cluster analysis of 41 microsatellite markers, mapping near six FHB resistance QTLs, grouped wheat lines with similar microsatellite marker haplotypes (Fig. 1). The germplasm tended to cluster based on the country of origin, resulting in five major clusters. The first cluster consisted of Japanese lines, a few Chinese lines, JC-1, JC-3, JC-4, JC-6 and the Brazilian cv. Maringa. The second major cluster was mainly composed of Canadian spring wheat germplasm. The third cluster consisted of mainly Chinese germplasm, including Sumai 3 and Wuhan 1. The fourth and fifth clusters were predominantly of Brazilian germplasm. The CIMMYT germplasm was distributed amongst clusters two, three and five. The accession 14-3-C (Siberia, Russia) was not closely related to the other *T. aestivum* lines, and the artificial amphiploid L960 was the most divergent line.

The 5AS locus had relatively few haplotypes relative to the other five chromosome regions (Table 3). The PIC values of the microsatellite markers on 5AS were similar to the values for the other microsatellite markers (Table 3). The genetic interval interrogated on chromosome 5AS was similar in length to the 3BS interval, which had nearly twice as many haplotypes as the 5AS region (Table 3). The relative haplotype diversity for the six intervals was also compared using the number of haplotypes/cM (Table 3). This statistic suggested the haplotype diversity at the 5AS FHB resistance QTL was similar to other intervals. However, the number of haplotypes/cM might not be a better measure of diversity than the absolute number of haplotypes because the number of haplotypes cannot exceed the number of wheat lines tested regardless of the chromosome interval length (in centiMorgans). The most common haplotype at the 5AS region in this study was present among the Canadian wheat lines (Fig. 2). Other predominant haplotypes included the Sumai 3 haplotype, a haplotype common to Brazilian lines and a haplotype common to Funo, four Chinese lines and two CIMMYT lines.

Figure 2 presents the allele distribution at 41 microsatellite loci, grouped according to chromosome interval, among the 79 wheat lines. The order of the wheat lines in Fig. 2 reflects the output from the cluster analysis of the 41 microsatellite loci (Fig. 1). Microsatellite loci were ordered based on a consensus map of wheat generated using JoinMap V2.0 with mapping data from the crosses W7984/Opata 85, Wuhan 1/Maringa and RL4452/AC Domain (Somers et al. 2003a). This microsatellite locus order is also reflected in Table 3, where the distance (in centiMorgans) between adjacent microsatellite loci is indicated. In Fig. 2, the alleles linked to known FHB resistance QTLs were colour coded to aid in the interpretation of the haplotype structure at these six loci. Microsatellite amplicons of the same size as Maringa amplicons at 3BS, 3BSc and 5AS were coded yellow, microsatellite amplicons of the same size as Sumai 3 amplicons at 3BS, 5AS and 6BS were coded red, and microsatellite amplicons of the same size as Wuhan 1 amplicons at 2DL and 4B were coded blue. The Maringa and Sumai 3 amplicons of the markers, BARC075, BARC117, gwm293, gwm304 and gwm415, were indistinguishable, so they were represented by orange-coloured cells.

Three wheat lines, Itou Komugi, Nyuubai and Yanggangfangzhu, had the same haplotype as Maringa at the 3BS locus (Fig. 2). Four or more putative Maringa 3BS alleles were present in the wheat lines JC-1, JC-3, JC-4, JC-6 and Wangshuibai at six microsatellite loci. Nyuubai and Yanggangfangzhu were the only wheat lines that had the same haplotype as Maringa at the 3BSc locus (Fig. 2). Nyuubai and Yanggangfangzhu were also the only wheat lines that had the same haplotype as Maringa at the 5AS locus (Fig. 2). Four and five putative Maringa 5AS alleles were present in the wheat lines Itou Komugi and Shou Komugi II, respectively, at six microsatellite loci. A number of additional lines had alleles that were common to both Maringa and Sumai 3, since four of the six microsatellite markers near the 5AS locus did not differentiate Maringa and Sumai 3. The haplotypes of Maringa and Frontana were compared to determine whether Frontana, a parent of Maringa, contributed the 3BS, 3BSc or 5AS FHB resistance QTLs to Maringa. Frontana and Maringa had two alleles in common at 20 microsatellite loci from the 3BS, 3BSc and 5AS FHB resistance QTL regions combined (Fig. 2).

Six wheat lines, 93FHB37, CM-82036, ND2710, 894037, WF1 and WF4, had the same haplotype as Sumai 3 at the 3BS locus (Fig. 2). The following wheat lines have four or five putative Sumai 3 3BS alleles at six microsatellite loci: Asakaze Komugi, Aso Zairai, Aso Zairai II, Fu 5125, HY644, Kikuchi, Shou Komugi II and WF2. Eight lines, Abura Komugi, JC-1, JC-3, JC-4, JC-6, ND2710, N 8026 and 894037, had the same haplotype as CM-82036 and Sumai 3 at the 5AS locus (Fig. 2). In addition, the following wheat lines had four or five putative Sumai 3 5AS alleles at six microsatellite loci: 983 222, Asakaze Komugi, Aso Zairai, Aso Zairai II, Fanshanxiaomai, Kikuchi, Shiro Nankin, Sotome, Sotome A and Zairai Yuubou. The Sumai 3 haplotype at the 6BS locus was identified in six lines, JC-1, JC-3, JC-4, JC-6, Kooperatorka and ND2710 (Fig. 2). The wheat lines, 894037, N 8026 and Zairai Yuubou, had six or seven putative Sumai 3 6BS alleles at eight microsatellite loci. The haplotypes of Sumai 3 and Funo were compared to determine whether Funo, a parent of Sumai 3, contributed the 3BS, 5AS, or 6BS FHB resistance QTLs to Sumai 3. Funo and Sumai 3 had five alleles in common at 20 microsatellite loci from the 3BS, 5AS and 6BS FHB-resistance QTL regions combined (Fig. 2).

No wheat lines had the same haplotype as Wuhan 1 at the 2DL locus (Fig. 2). However, the following wheat lines had four or five 2DL alleles in common with Wuhan 1 at six microsatellite loci: 920292, 93FHB37, BRS 119, CEP 14, CEP 24, Fu 5125, Funo, Huapei 32-2, HY644, KS 10-2, KS 24-1, Mianyang 96-12, Ning 991069, Ning 962424, WF2, WF4 and Wuhan 2-37E. Two wheat lines, N 8026 and 920292, had the same haplotype as Wuhan 1

Table 1 Origin, pedigree and fusarium head blight (FHB) reaction of 79 wheat lines

Wheat line	Source	Pedigree	FHB reaction[a]
BR 32	Brazil	IAS 60/Indus//IAS 62/3/Ald′s/4/IAS 59	MR
BR 34	Brazil	ALZ 110/2*IAS 54//F 5530	MR
BR 38	Brazil	IAS 55*4/Agent//IAS 55*4/Ci 14123	MR
BR 43	Brazil	PF 833007/Jacui	MR
BRS 119	Brazil	8PF 82252/BR 35//IAPAR 17/PF 8550	MR
BRS 120	Brazil	PF 83899/PF 813//F27141	MR
BRS 177	Brazil	PF 83899/PF 813//F27141	R
BRS 179	Brazil	BR 35/PF 8596/3/PF 772003*2/PF813//PF 83899	R
CEP 14	Brazil	Pel 72380/Arthur 71	MR
CEP 24	Brazil	BR 3/CEP7887//CEP7775/CEP 11	MR
Frontana	Brazil	Fronteira/Mentana	R
Fundacep 29	Brazil	Br 23/CEP 8423//Buc′s′	R
Maringa	Brazil	Frontana/Kenya 58//PG 1	R
OR-1	Brazil	EMBRAPA 27/Bagula′s′	MR
93FHB37	Canada	HY611/Ning 8331	R
AC Barrie	Canada	Neepawa/Columbus//BW90	MS
AC Domain	Canada	BW83/ND585	S
AC Foremost	Canada	HY320*5/BW553//HY320*6/7424-BW5B4	S
AC Intrepid	Canada	Laura/RL4596//CDC Teal	S
AC Karma	Canada	HY320*5/BW553//HY358/3/HY358//7915-QX76B2	S
Glenlea	Canada	Pembina*2/Bage//CB100	S
HY644	Canada	A16//Alpha*4/BgBSR/3/Sceptre/Ning 8331	R
KS 10-2	Canada	7E-7D Translocation from *Agropyron elongatum*	MS
KS 24-1	Canada	7E-7D Translocation from *A. elongatum*	MS
L960	Canada	Artificial amphiploid (AABBDD) of *Triticum carthlicum/Aegilops squarrosa*	MR
McKenzie	Canada	Columbus/Amidon	S
Superb	Canada	Grandin*2/AC Domain	S
WF1	Canada	SD3055//93FHB37/Grandin	MR
WF2	Canada	93FHB37/AC Majestic	MR
WF4	Canada	93FHB37/AC Majestic	MR
894037	China	Somaclonal variant of Yangmai 3	R
920292	China	Yangmai 3//C.S./*Roegneria kamoji*	R
983 222	China	Derivative of wheat/Elymus	R
Emai 14	China	Fan 6/Yanda 72-629	R
Fanshanxiaomai	China	Landrace	MR
Fu 5125	China	Fufan 904/Ning8017	R
Huapei 32-2	China	Unknown	R
JC-1	China	Unknown	MR
JC-3	China	Unknown	R
JC-4	China	Unknown	MR
JC-6	China	Unknown	R
Mianyang 96-12	China	Unknown	R
N 8O26	China	Aurora/Sumai 3//Yangmei 2	R
Ning 962424	China	Somaclonal variant of Yangmai 158	R
Ning 991069	China	Yangmai 158/yang 8992//894013	R
Sumai 3	China	Funo/Taiwan Wheat	R
Wangshuibai	China	Landrace from Jiangsu	R
Wuanmai 38	China	Yanzhong//Wuanmai	R
Wuhan 1	China	Unknown	R
Wuhan 2-37E	China	Unknown	R
7990-244B	CIMMYT	Translocation from rye	R
CIMMYT 1	CIMMYT	Sha3/Catbird	R
CIMMYT 11	CIMMYT	80456/Yangmai 5	R
CIMMYT 2000 3	CIMMYT	Jian 85.11//Suzhou 7906/Ning 5249	MR

[a] *R* Resistant, *S* susceptible, *MR* moderately resistant, *MS* moderately susceptible

Table 1 (continued)

Wheat line	Source	Pedigree	FHB reaction[a]
CIMMYT 2000 5/1/1	CIMMYT	Jian 85.11//Suzhou 7906/Ning 5259	MR
CM-82036	CIMMYT	Sumai 3/Thornbird-S	R
Funo	Italy	Duecentodieci/Demiano	MS
Abura Komugi	Japan	JGB99-12, accession no. 23516, unknown pedigree	R
Asakaze Komugi	Japan	Hiyoku-komugi/Shirogane-komugi	R
Aso Zairai	Japan	JGB99-18, accession no. 23521, unknown pedigree	R
Aso Zairai II	Japan	JGB99-16, accession no. 23524, unknown pedigree	R
Chile	Japan	JGB99-20, accession no. 26869, unknown pedigree	R
Itou Komugi	Japan	JGB99-23, accession no. 23647, unknown pedigree	MR
Kagoshima	Japan	JGB99-25, accession no. 23542, unknown pedigree	R
Kikuchi	Japan	JGB99-28, accession no. 23546, unknown pedigree	R
Nyuubai	Japan	JGB99-36, accession no. 22957, unknown pedigree	R
Shiro Nankin	Japan	JGB99-58, accession no. 23277, unknown pedigree	R
Shou Komugi II	Japan	JGB99-61, accession no. 23653, unknown pedigree	R
Soba Komugi IB	Japan	JGB99-1, accession no. 23662, unknown pedigree	R
Soba Komugi IC	Japan	JGB99-2, accession no. 23665, unknown pedigree	R
Sotome	Japan	JGB99-62, accession no. 23595, unknown pedigree	R
Sotome A	Japan	JGB99-63, accession no. 23660, unknown pedigree	R
Yanggangfangzhu	Japan	Landrace	R
Zairai Yuubou	Japan	JGB99-70, accession no. 22130, unknown pedigree	R
14-3-C	Siberia	Unknown	R
Kooperatorka	Ukraine	Selection of Krymka	R
Novokrymka	Ukraine	Novokrymka-258/Kooperatorka	R
Ernie	USA	PI584525 Pike/3/Stoddard/Blueboy//Stoddard/D1707	R
ND2710	USA	ND2603/Grandin	R

at the 4B locus (Fig. 2). The wheat lines Cimmyt 11, Cimmyt 2000 5/1/1 and L960 have five putative Wuhan 1 4B alleles at seven microsatellite loci.

Maringa and Sumai 3 had similar haplotypes at the 3BS and 5AS FHB resistance chromosome regions. At the 3BS locus, Maringa and Sumai 3 had the same alleles for the marker BARC075 (Fig. 2). The size of the Maringa and Sumai 3 amplicons for the five other markers were similar, given the large range and diversity of amplicon sizes for these markers (Table 3). The Maringa and Sumai 3 amplicons differed by 2 bp for each of BARC147, gwm389, gwm493 and gwm533 and differed by 4 bp for wmc754 (Fig. 2). Additionally, the Maringa and Sumai 3 amplicons were at one extreme of the amplicon size range for the markers, BARC147, gwm493, gwm533 and wmc754, whereas the Brazilian and Canadian germplasm were at the other extreme (Fig. 2). At the 5AS locus, the alleles for markers BARC117, gwm293, gwm304 and gwm415 were identical for Maringa and Sumai 3 (Fig. 2). The Maringa and Sumai 3 amplicons differed by 2 bp for the remaining markers, gwm129 and wmc705. The Maringa and Sumai 3 amplicons were at one extreme of the amplicon size range for wmc705, whereas the Brazilian and Canadian germplasm were at the other extreme (Fig. 2). The amplicon size range and diversity for gwm129 was relatively small. Wheat lines, such as Aso Zairai, Aso Zairai II, JC-6, Kagoshima, Kikuchi, Shou Komugi II and Zairai Yuubou, have varying combinations of putative Maringa and Sumai 3 alleles at the 3BS locus (Fig. 2). Pedigrees are not available for the Japanese accessions so the source of their FHB resistance is unknown.

Figures 1 and 2 identify a number of wheat lines with putatively novel FHB resistance. The fourth and fifth clusters of wheat lines from the UPGMA cluster analysis consisted of Brazilian germplasm and the wheat lines CIMMYT 1, Wuanmai 38, Emai 14, 7990-244B, Ernie and Huapei 32-2 (Fig. 1). These lines had very few alleles in common with Maringa, Sumai 3, or Wuhan 1 near the six FHB resistance QTLs included in this study (Fig. 2). The only exceptions were the lines BRS 119, CEP 14, CEP 24 and Huapei 32-2, which shared four of six alleles with Wuhan 1 at the 2DL locus. Novokrymka, Chile and 14-3-C were not closely related to the other wheat lines (Fig. 1) and did not have haplotypes similar to Maringa, Sumai 3, or Wuhan 1 (Fig. 2).

Discussion

The present study is a thorough analysis of allele diversity of microsatellite markers linked to multiple FHB-resistance QTLs across a diverse collection of FHB-resistant and -susceptible wheat germplasm. This study illustrates the utility of microsatellite markers to identify wheat lines likely carrying the same FHB-resistance QTLs and wheat lines with potentially novel resistance. Wheat lines with the same haplotype spanning an FHB resistance QTL likely carry that FHB resistance QTL. Wheat lines with a number of alleles in common with an FHB resistance

Table 2 Primer sequences and annealing temperatures of 41 microsatellite markers linked to FHB resistance QTLs

Marker	Forward primer (5′→3′)	Reverse primer (5′→3′)	Annealing temperature (°C)
BARC020	GCGATCCACACTTTGCCTCTTTTACA	GCGATGTCGGTTTTCAGCCTTTT	49
BARC075	AGGGTTACAGTTTGCTCTTTTAC	CCCGACGACCTATCTATACTTCTCTA	49
BARC117	TCATGCGTGCTAAGTGCTAA	GAGGGCAGGAAAAAGTGACT	49
BARC147	GCGCCATTTATTCATGTTCCTCAT	CCGCTTCACATGCAATCCGTTGAT	49
BARC228	CCCTCCTCTCTTTAGCCATCC	GCACGTACTATTCGCCTTCACTTA	49
gwm113	ATTCGAGGTTAGGAGGAAGAGG	GAGGGTCGGCCTATAAGACC	58
gwm129	TCAGTGGGCAAGCTACACAG	AAAACTTAGTAGCCGCGT	49
gwm157	GTCGTCGCGGTAAGCTTG	GAGTGAACACACGAGGCTTG	58
gwm165	TGCAGTGGTCAGATGTTTCC	CTTTTCTTTCAGATTGCGCC	49
gwm219	GATGAGCGACACCTAGCCTC	GGGGTCCGAGTCCACAAC	58
gwm285	ATGACCCTTCTGCCAAACAC	ATCGACCGGGATCTAGCC	58
gwm293	TACTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	58
gwm304	AGGAAACAGAAATATCGCGG	AGGACTGTGGGGAATGAATG	58
gwm389	ATCATGTCGATCTCCTTGACG	TGCCATGCACATTAGCAGAT	58
gwm415	GATCTCCCATGTCCGCC	CGACAGTCGTCACTTGCCTA	58
gwm493	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	58
gwm508	GTTATAGTAGCATATAATGGCC	GTGCTGCCATGATATTT	49
gwm513	ATCCGTAGCACCTACTGGTCA	GGTCTGTTCATGCCACATTG	58
gwm518	AATCACAACAAGGCGTGACA	CAGGGTGGTGCATGCAT	58
gwm533	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	58
gwm539	CTGCTCTAAGATTCATGCAACC	GAGGCTTGTGCCCTCTGTAG	58
wmc048	GAGGGTTCTGAAATGTTTTGCC	ACGTGCTAGGGAGGTATCTTGC	58
wmc078	AGTAAATCCTCCCTTCGGCTTC	AGCTTCTTTGCTAGTCCGTTGC	58
wmc105	AATGTCATGCGTGTAGTAGCCA	AAGCGCACTTAACAGAAGAGGG	49
wmc144	GGACACCAATCCAACATGAACA	AAGGATAGTTGGGTGGTGCTGA	58
wmc152	CTATTGGCAATCTACCAAACTG	TCTCTTCTTGCCACATATTCGT	58
wmc231	CATGGCGAGGAGCTCGGTGGTC	GTGGAGCACAGGCGGAGCAAGG	58
wmc238	TCTTCCTGCTTACCCAAACACA	TACTGGGGGATCGTGGATGACA	58
wmc245	GCTCAGATCATCCACCAACTTC	AGATGCTCTGGGAGAGTCCTTA	58
wmc307	GTTTGAAGACCAAGCTCCTCCT	ACCATAACCTCTCAAGAACCCA	58
wmc397	AGTCGTGCACCTCCATTTTG	CATTGGACATCGGAGACCTG	58
wmc398	GGAGATTGACCGAGTGGAT	CGTGAGAGCGGTTCTTTG	58
wmc418	AGAGCAGCAAGTTGTGTAGCCA	TGAAGCTATTGCCAGCACGAG	58
wmc494	GGATCGAGTCTCAAGTCTACAA	AGAAGGAACAAGCAACATCATA	58
wmc601	ACAGAGGCATATGCAAAGGAGG	CTTGTCTCTTTATCGAGGGTGG	58
wmc612	GAGGTCAGTACCCGGAGA	CCACCCCAATTCAAAAAG	58
wmc625	CACAGACCTCAACCTCTTCTT	AGTACTGTTCACAGCAGACGA	58
wmc705	GGTTGGGCTCCTGTCTGTGAA	TCTTGCACCTTCCCATGCTCT	58
wmc710	GTAAGAAGGCAGCACGTATGAA	TAAGCATTCCCAATCACTCTCA	49
wmc754	ATCCACATGAACCTCAACTTATGG	GGCATTGTTGTTGTACTGCAGTC	58
wmc777	GCCATCAAGCGGATCAACT	GTAGCGCCCTGTTTCACCTC	58

haplotype might have a similar FHB resistance gene. Based on these assumptions and the microsatellite data in this study, the FHB resistance QTLs from Sumai 3 were the most widely deployed QTLs. This was expected since Sumai 3 has been used widely as a source of FHB resistance around the world. The Maringa 3BS QTL and the 5AS QTL, to a lesser extent, were also commonly present based on the marker data. The 3BSc QTL from Maringa and 2DL and 4B QTLs from Wuhan 1 were relatively rare in this germplasm collection based on the marker data. Thus, these FHB resistance QTLs might be useful for complementing the resistance already present in advanced breeding material.

The Canadian breeding lines 93FHB37, HY644, WF1, WF2 and WF4 likely carry the Sumai 3 QTL on 3BS and might also carry a Wuhan 1-like QTL on 2DL. These lines do not appear to carry the 5AS and 6BS QTLs from Sumai 3. The wheat line ND2710 (ND2603/Grandin, developed in North Dakota, USA) appears to carry the Sumai 3 QTLs on chromosomes 3BS, 5AS and 6BS. This line might be a useful parent for introgressing 5AS and 6BS QTLs into Canadian germplasm, since this line is better adapted to

Table 3 Data summary of the six FHB resistance intervals and the 41 microsatellite markers. *PIC* Polymorphic information content

Chromosome interval	Number of haplotypes	Number of haplotypes/centiMorgan	Marker	Distance to next marker (cM)[a]	Number of alleles	PIC	Amplicon size range (bp)
2DL	57	2.4	wmc144	0	3	0.61	156–160
			wmc245	2	3	0.28	164–168
			wmc601	4	18	0.84	227–282
			gwm157	8	3	0.45	119–123
			BARC228	10	6	0.35	190–200
			gwm539	-	20	0.85	144–212
3BS	55	3.7	BARC075	1	4	0.50	122–129
			gwm389	5	8	0.79	134–159
			gwm533	1	3	0.61	133–160
			BARC147	5	7	0.53	123–167
			gwm493	3	9	0.80	157–215
			wmc754	-	14	0.88	155–202
3BSc	56	2.7	wmc078	5	9	0.83	248–281
			wmc231	2	6	0.71	247–269
			wmc612	0	16	0.89	262–320
			wmc777	1	12	0.77	111–168
			wmc625	2	11	0.80	86–147
			gwm285	4	14	0.86	236–269
			wmc307	7	4	0.71	163–169
			wmc418	-	6	0.63	280–290
4B	56	2.2	wmc710	16	13	0.83	108–164
			gwm165	2	11	0.76	258–282
			wmc238	2	8	0.85	238–254
			gwm113	1	3	0.25	166–170
			wmc048	1	7	0.80	205–217
			BARC020	3	5	0.65	190–208
			gwm513	-	6	0.76	159–169
5AS	33	2.5	gwm304	4	10	0.81	215–241
			BARC117	1	3	0.53	235–243
			gwm129	0	4	0.62	237–243
			wmc705	2	15	0.83	144–198
			gwm415	6	4	0.46	145–151
			gwm293	-	9	0.77	208–222
6BS	61	1.9	gwm518	2	16	0.88	168–219
			wmc494	3	13	0.86	223–274
			gwm508	4	2	0.47	200
			wmc398	4	6	0.71	159–198
			wmc105	0	11	0.86	341–382
			wmc397	9	7	0.80	172–184
			wmc152	10	5	0.60	265–294
			gwm219	-	12	0.78	165–209

[a]Based on a consensus wheat map (Somers et al. 2003a)

Canadian growing conditions than Sumai 3. Wheat breeders should also consider introgressing the 4B FHB resistance QTL from Wuhan 1 into adapted germplasm. This FHB resistance QTL reduces field FHB infection (Somers et al. 2003b) and is rare among FHB-resistant germplasm, based on the present marker data.

Anderson et al. (2001) did not report an FHB resistance QTL on 5AS in the cross ND2603/Butte 86 when evaluated for type II resistance. However, ND2710 (ND2603/Grandin) presumably inherited the Sumai 3 5AS FHB resistance QTL through ND2603 (Sumai 3/Wheaton), based on the microsatellite data presented in this study. The 5AS resistance QTL was likely missed in ND2603 because this QTL appears to have a larger effect on type I FHB resistance than on type II (Buerstmayr et al. 2002, 2003). The 5AS QTL could have also been missed in ND2603 because QTL analysis tends to underestimate the number of QTLs controlling a quantitative trait (Asíns 2002). Alternatively, Butte 86 could have an FHB resistance QTL on 5AS such that the 5AS locus was not

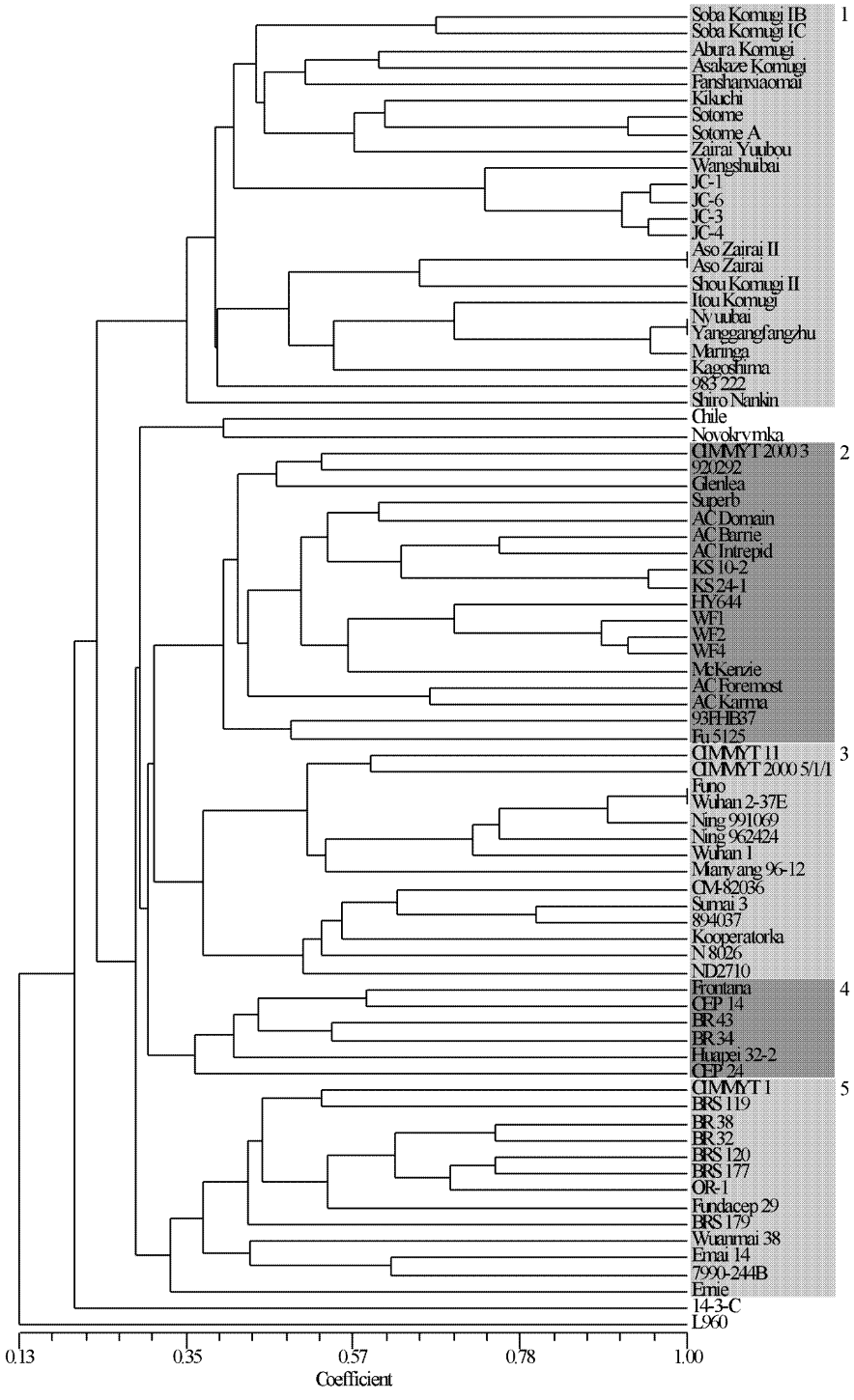

Fig. 1 Dendrogram resulting from the genetic distance matrix for a diverse collection of wheat lines differing in reaction to fusarium head blight (FHB), using the unweighted pair group method with arithmetic mean as the clustering method. The similarities were calculated from data from 41 microsatellite markers across six chromosome regions associated with FHB resistance. *Shaded areas* identify major clusters

segregating in the ND2603/Butte 86 population. Wiersma et al. (1996) reported that Butte 86 possesses some FHB resistance and is moderately susceptible to FHB.

Few haplotypes were identified with the microsatellite markers at the 5AS locus relative to the other intervals. The large number of lines with the Sumai 3 haplotype in this germplasm collection was not surprising and suggests the importance of this FHB-resistance QTL in breeding for FHB resistance. However, the prevalence of the Canadian (AC Barrie, AC Domain, AC Foremost, AC Intrepid, AC Karma, CIMMYT 2000 3, Glenlea, HY644, McKenzie, Superb, WF1, WF2, WF4) and Brazilian (BR 32, BR 38, BRS 119, BRS 120, BRS 177, CEP 24, Fundacep 29, OR-1) haplotypes at the 5AS locus was not expected. Many of the Canadian lines with this haplotype are susceptible to FHB, so the Canadian haplotype is not likely associated with FHB resistance. A number of important agronomic traits map to chromosome 5AL, such as earliness, vernalization requirement, frost tolerance, spike length, 1,000-kernel weight and drought stress (Börner et al.

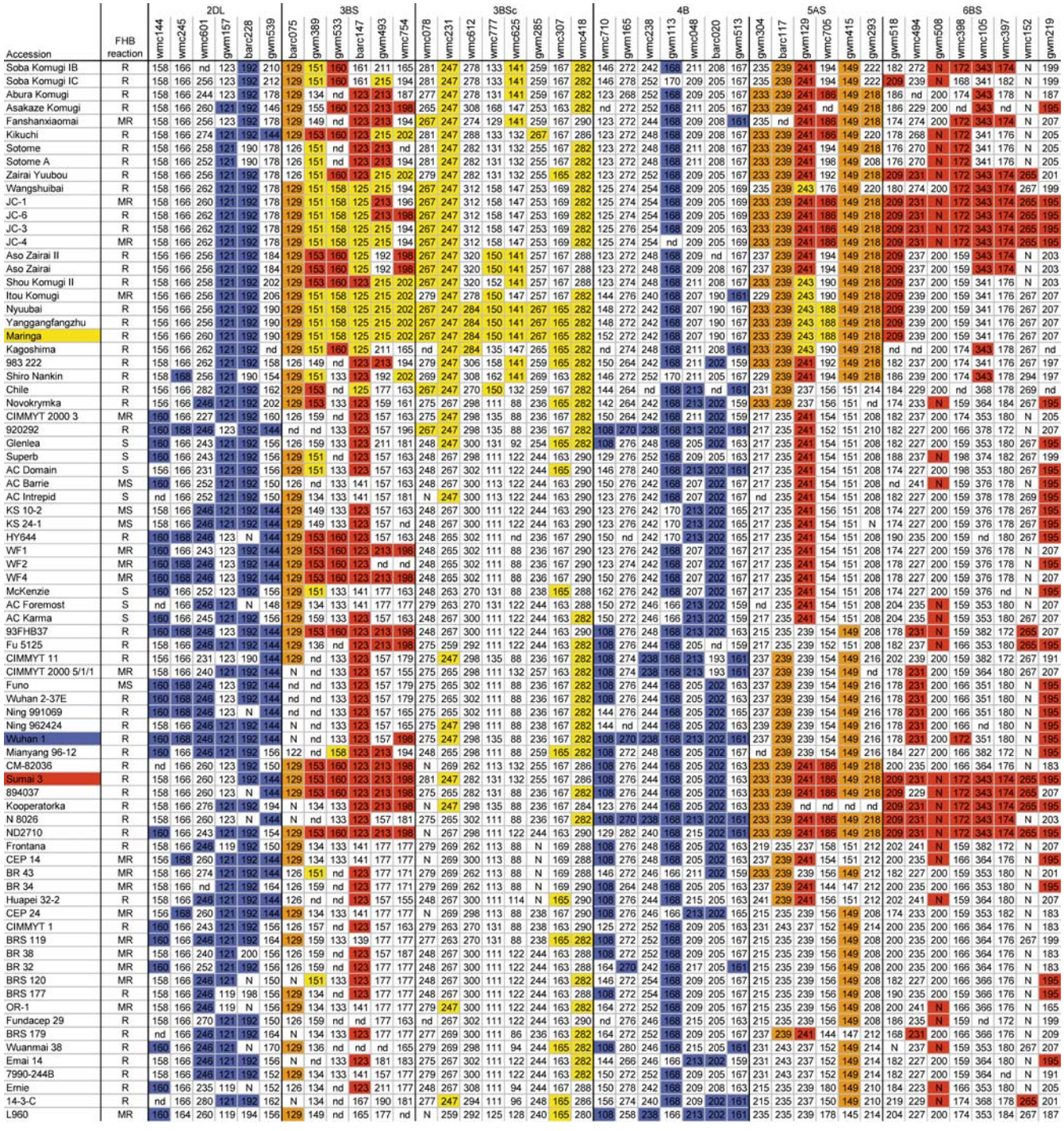

Fig. 2 Microsatellite allele distribution in a diverse collection of wheat lines differing in reaction to FHB. Numeric values are the amplicon size (in bp) for the respective marker and wheat line: *N* null allele, *nd* no data. Colour coding: *yellow cells* identify accessions with alleles the same size as Maringa linked to the 3BS, 3BSc and 5AS FHB resistance QTLs; *red cells* identify accessions with alleles the same size as Sumai 3 linked to 3BS, 5AS and 6BS QTLs; *blue cells* identify accessions with alleles the same size as Wuhan 1 linked to 2DL and 4B QTLs. *Orange cells* indicate that the Maringa and Sumai 3 amplicons were the same size for respective microsatellite marker

2002; Galiba et al. 1995; Quarrie et al. 1994; Snape et al. 1985). Important alleles for agronomic traits on 5AL could limit genetic diversity on the entire chromosome because of selection in breeding programs and genetic linkage. However, preliminary data indicate that microsatellite loci across 5AL in Canadian bread wheat cultivars show considerable variability relative to the region on 5AS analyzed in this study (data not shown). Therefore, the lack of allelic variability at the 5AS microsatellite loci in this study is not likely the result of highly conserved genes

on 5AL. A highly conserved allele combination on chromosome 5AS seems more likely. Recently, the chromosome arm 5AS was reported to have a positive effect on the expression of the glutenin gene *Glu-D1-2* (Wanous et al. 2003). Selection for quality in wheat breeding programs might have reduced the allelic variation at microsatellite loci on 5AS in this study. The utilization of the Maringa and Sumai 3 FHB resistance QTLs on 5AS might be hindered in Brazilian, Canadian and possibly other wheat breeding programs if the Brazilian and Canadian 5AS haplotypes are conserved because of important genes for adaptation or quality in this interval.

Funo, a parent of Sumai 3, is not likely the source of FHB resistance in Sumai 3 found on chromosome 3BS, 5AS and 6BS, based on the microsatellite marker data in this study. In a similar study, Bai et al. (2003) reported marker data indicating that Taiwan Wheat was most likely the source of the FHB resistance on chromosome 3BS. Somers et al. (2003b) suggested that the resistance in Maringa is likely from Frontana, because Frontana is a known source of FHB resistance (van Ginkel et al. 1996) and Frontana is in Maringa's pedigree. The microsatellite marker data collected in this study showed that Frontana did not contribute the FHB resistance mapped to chromosome regions 3BS, 3BSc and 5AS in Maringa. Frontana is reported to have FHB resistance genes that are independent of the resistance genes in Ning 7840 (Aurora/Anhui11//Sumai 3) (van Ginkel et al. 1996). Frontana and the other Brazilian wheat lines had little similarity to the Maringa, Sumai 3 and Wuhan 1 FHB resistance QTLs included in this study, based on microsatellite marker data. Therefore, Frontana or another Brazilian wheat line should be considered for a QTL mapping study of FHB resistance in wheat.

Maringa and Sumai 3 had similar haplotypes at the 5AS locus and the 3BS locus to a lesser extent. This similarity was unexpected, given their divergent pedigrees. Five of 12 microsatellite loci near the 3BS and 5AS FHB resistance QTLs were the same between Maringa and Sumai 3. The differences in the microsatellite alleles at the remaining seven loci were small, typically 2 bp and never greater than 4 bp, relative to the range in amplicon size for most of the microsatellite markers at the 3BS and 5AS FHB resistance QTL regions. These small changes could have resulted from mutation of common ancestral sequences for each of the 3BS and 5AS FHB resistance genes and subsequent recombination. This hypothesis would suggest that Maringa and Sumai 3 carry the same FHB resistance genes at the 3BS and 5AS FHB resistance QTLs. Interestingly, the Japanese accessions Aso Zairai, Aso Zairai II, Kagoshima, Kikuchi, Shiro Nankin, Shou Komugi II, Soba Komugi IC and Zairai Yuubou had various combinations of putative Maringa and Sumai 3 microsatellite alleles near the 3BS FHB resistance QTL. These accessions all have some FHB resistance, which might be derived from a resistance gene on 3BS.

The observed similarity between Wuhan 1 and L960 (*Triticum carthlicum*/*Aegilops squarrosa*) at the 4B microsatellite loci was also unexpected. L960 is a synthetic bread wheat that has resistance derived from *T. carthlicum*. If L960 were shown to have a 4B FHB resistance QTL, this would suggest that Wuhan 1 has a *T. carthlicum*-like FHB resistance allele on 4B, or vice versa. A QTL mapping study of FHB resistance in L960 would be useful to verify the presence of the 4B FHB resistance QTL in L960.

Fine-scale mapping studies are needed to better resolve the location of FHB resistance QTLs to improve the accuracy of marker-assisted selection of FHB resistance and to introgress FHB resistance QTLs with as little linkage drag as possible. Fine-scale mapping of FHB resistance might be accomplished using populations that segregate for one FHB resistance QTL and map the resistance as a Mendelian trait. Alternatively, QTL validation studies in breeding populations might also be of use. For instance, the FHB-resistant Canadian wheat line HY644 has a recombination event near the 3BS QTL between BARC147 and gwm493 (Fig. 2). Breeding populations, developed with HY644 as a parent, could be assessed with markers mapping near the 3BS FHB resistance QTL, in addition to the routine FHB resistance phenotyping that is conducted in breeding program. The presence or absence of the QTL in such breeding populations would provide additional genetic evidence of location of the QTL on the chromosome.

Analysis of microsatellite loci linked to six FHB resistance QTLs provided insight into the genetic similarity of a diverse collection of FHB-resistant germplasm. In general, Chinese and Japanese resistant wheat lines were closely related. The resistant Brazilian cv. Maringa showed similarity to Asian lines and not Brazilian lines. A number of FHB-resistant wheat lines, predominantly of Brazilian origin, were related to each other but were distinct from the Asian sources of FHB resistance based on the marker data. Other wheat lines, such as Novokrymka, Chile and 14-3-C, were not closely related to the Asian or South American germplasm and might be novel sources of FHB resistance. Therefore, non-Asian sources of FHB resistance should carry novel resistance genes that could be useful for FHB resistance gene stacking in wheat breeding programs and further genetic analysis.

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