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ORIGINAL PAPER

Haplotype diversity and population structure in cultivated and wild barley evaluated for Fusarium head blight responses

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Abstract Fusarium head blight (FHB) is a threat to barley (*Hordeum vulgare* L.) production in many parts of the world. A number of barley accessions with partial resistance have been reported and used in mapping experiments to identify quantitative trait loci (QTL) associated with FHB resistance. Here, we present a set of barley germplasm that exhibits FHB resistance identified through screening a global collection of 23,255 wild (*Hordeum vulgare* ssp. *spontaneum*) and cultivated (*Hordeum vulgare* ssp. *vulgare*) accessions. Seventy-eight accessions were classified as resistant or moderately resistant. The collection of FHB resistant accessions consists of 5, 27, 46 of winter, wild and spring barley, respectively. The population structure and genetic relationships of the germplasm were investigated with 1,727 Diversity Array Technology (DArT) markers. Multiple clustering analyses suggest the presence of four subpopulations. Within cultivated barley, substructure is largely centered on spike morphology and growth habit. Analysis of molecular variance indicated highly significant genetic variance among clusters and within clusters, suggesting that the FHB resistant sources have broad genetic diversity. The haplotype diversity was characterized with DArT markers associated with the four FHB QTLs on chromosome 2H bin8, 10 and 13 and 6H bin7. In general, the wild barley accessions had distinct haplotypes from those of cultivated barley. The haplotype of the resistant source Chevron was the most prevalent in all four QTL regions, followed by those of the resistant sources Fredrickson and CIho4196. These resistant QTL haplotypes were rare in the susceptible cultivars and accessions grown in the upper Midwest USA. Some two- and six-rowed accessions were identified with high FHB resistance, but contained distinct haplotypes at FHB QTLs from known resistance sources. These germplasm warrant further genetic studies and possible incorporation into barley breeding programs.

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Introduction

Barley (*Hordeum vulgare*) is an important cereal crop used for animal feed, malting and brewing, and food. However, Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Schwein.) Petch] can seriously reduce grain yield and quality (for detailed reviews, see Bai and Shaner 2004; Choo 2005; McMullen et al. 1997; Steffenson 2003a). Grain quality is reduced primarily through lower kernel plumpness, but also the accumulation of trichothecene mycotoxins such as deoxynivalenol (DON) during infection. Extensive economic losses due to FHB outbreaks in the Upper Midwest region of the USA have been reported (Nganje et al. 2004). FHB can be managed through an integrated approach that includes the use of resistant cultivars, cultural practices that

reduce inoculum residing in residue, and fungicide application. The development of resistant cultivars is a very important component of this integrated management scheme. Therefore, identification of germplasm that carries FHB resistance is a critical activity.

A number of studies have been conducted to identify barley accessions with FHB resistance (Buerstmayr et al. 2004; Chen et al. 1991; Choo et al. 2004; Ma et al. 2009; McCallum et al. 2004; Takeda and Heta 1989; Zhou et al. 1991). Takeda and Heta (1989) screened 4,957 accessions and identified 23 with desirable levels of resistance. Evaluation of FHB resistance in 143 spring barley lines identified CIho4196 and PI 566203 with the lowest disease severity (Buerstmayr et al. 2004). Choo et al. (2004) characterized 64 barley cultivars for FHB reaction, most of which were grown in Eastern Canada, and found two cultivars (Island and AC Alberte) that exhibited resistance. A diverse collection of 77 two-rowed and 81 six-rowed barley lines were screened for FHB resistance by McCallum et al. (2004), and 18 lines with low FHB were identified. Ma et al. (2009) examined the FHB responses of 266 cultivars and breeding lines of Chinese origin. Twenty-seven lines were found more resistant than the resistant check of Zhedar 2. These limited germplasm evaluation studies identified resistance sources that have been used in several breeding and genetic studies; however, an exhaustive screening of a large worldwide barley collection has not been conducted in a systematic manner.

As a prerequisite to marker-assisted selection of resistance alleles, a number of bi-parental mapping studies were conducted to identify FHB resistance quantitative trait loci (QTL) using some of the most resistant cultivars including Chevron, Fredrickson, Zhedar 2, CIho4196 and Russia 6 (Dahleen et al. 2003; de la Peña et al. 1999; Hori et al. 2005; Horsley et al. 2006; Ma et al. 2000; Mesfin et al. 2003). These studies focused on disease severity and DON accumulation, and described QTLs contributing to low FHB severity and low DON accumulation. Many QTLs were associated with agronomic and morphological traits such as plant height, heading date, flowering type, and spike row number. Four FHB QTL regions, three on chromosome 2H and one on 6H, were consistently detected from several resistant parents and in multiple environments. The chromosome 2H bin8 QTL was found in Chevron, Fredrickson, CIho4196 and Zhedar 2 (Dahleen et al. 2003; de la Peña et al. 1999; Horsley et al. 2006; Ma et al. 2000; Mesfin et al. 2003). This QTL region also was associated with heading date with late maturing plants being associated with FHB resistance (de la Peña et al. 1999). Fine mapping of this QTL showed that heading date and FHB resistance are controlled by distinct tightly linked loci (Nduulu et al. 2007). The chromosome 2H bin10 QTL was identified in Fredrickson, CIho4196, Zhedar 2, and Russia 6, and was coincident with the *Vrs1* locus which controls inflorescence row-type (Dahleen et al. 2003; Hori et al. 2005; Horsley et al. 2006; Mesfin et al. 2003). The two-row inflorescence type was associated with lower FHB severity. Whether this association is due to tight linkage or the pleiotropic effect of *Vrs1* is unresolved. The third QTL on 2H was detected near bin13 from Fredrickson, Zhedar 2, Russia 6, and Harbin (Dahleen et al. 2003; Hori et al. 2005; Hori et al. 2006; Mesfin et al. 2003), and was in the vicinity of the *Cleistogamy1* (*Cly1*) locus which determines floret opening/closing. It was suggested that the cleistogamy trait contributed to FHB resistance, but the possibility of a tightly linked resistance gene cannot be excluded (Hori et al. 2005; Yoshida et al. 2005). Several studies have mapped the FHB QTL near 6H bin7 from Chevron, Fredrickson, and Harbin (Canci et al. 2004; Hori et al. 2006; Mesfin et al. 2003). The genetic variation explained by this QTL was generally smaller than those explained by the chromosome 2H QTLs. The chromosome 6H bin7 FHB resistant QTL was associated with high grain protein concentration (Canci et al. 2004). The presence of these major FHB QTLs in multiple parents suggested that the diverse resistant sources used may have the same FHB resistance alleles or different alleles of the same QTL. Haplotype characterization of these QTL regions can help to determine the allelic distribution in different resistance sources and aid in the choice of resistant parents for future QTL mapping studies and breeding.

The specific objectives of this study were to: (1) identify and catalog FHB resistance sources from a worldwide collection of barley germplasm, (2) investigate population structure and genetic relationships of identified resistance sources, and (3) analyze haplotype diversity of markers linked to four previously identified FHB QTLs.

Materials and methods

Plant materials and genotyping

A total of 23,255 cultivated (*Hordeum vulgare* ssp. *vulgare*) and wild (*Hordeum vulgare* ssp. *spontaneum*) barley accessions were received from seven gene banks including the USDA-ARS National Small Grains Collection (NSGC, Aberdeen, ID USA, 16,696 accessions), N. I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIR, St. Petersburg Russia, 1,979 accessions), Station federale de recherché en production vegetale de Changins (SFRSPP, Nyon Switzerland, 74 accessions), Nordic Gene Bank (NGB, Alnarp Sweden, 654 accessions), Institute for Cereal Crops Improvement (ICCI, Tel Aviv Israel, 150 accessions), International Center for Agricultural Research in the Dry Areas (ICARDA, Aleppo Syria, 318 accessions) and Plant

Table 1 Summary of sources of cultivated and wild barley germplasm screened for FHB resistance

Sources of germplasm[a]	Location of seed sources	No. of accessions screened	Location of screening trials	Years of screening trials	Inoculation method	FHB assessment method
USDA-ARS NSGC	Aberdeen, ID USA	16,696	USA, China	1999–2010	Grain spawn, foliar spray	% of infected kernels, FHB scale (1–5)
VIR	St. Petersburg, Russia	1,979	USA, China	2003–2010	Grain spawn, foliar spray	% of infected kernels, FHB scale (1–5)
SFRSPP	Nyon, Switzerland	74	USA, China	2003–2010	Grain spawn, foliar spray	% of infected kernels, FHB scale (1–5)
NGB	Alnarp, Sweden	654	USA, China	2004–2010	Grain spawn, foliar spray	% of infected kernels, FHB scale (1–5)
ICCI	Tel Aviv, Israel	150	China	2005–2010	Grain spawn	FHB scale (1–5)
ICARDA	Aleppo, Syria	318	China	2006–2010	Grain spawn	FHB scale (1–5)
PGRC	Saskatoon, Canada	3,384	USA, China	2005–2010	Grain spawn, foliar spray	% of infected kernels, FHB scale (1–5)

[a] NSGC, USDA-ARS National Small Grains Collection; VIR, N. I. Vavilov All-Russian Scientific Research Institute of Plant Industry; SFRSPP, Station federale de recherches en production vegetale de Changins; NGB, Nordic Gene Bank, now NordGen; ICCI, Institute for Cereal Crops Improvement; ICARDA, International Center for Agricultural Research in the Dry Areas; PGRC, Plant Genetic Resources of Canada

Genetic Resources of Canada (PGRC, Saskatoon Canada, 3,384 accessions) (Table 1). In addition, a bulked seed lot (25,000–32,000 seeds) of Composite Cross population CC XXX-G was used to identify FHB resistance in barley. The accessions were screened for FHB resistance in disease nurseries in the United States and/or China in one or more years (for methods, see Prom et al. 1996). Seventy-eight resistant accessions were selected from this set by comparison with the cultivar Chevron, a widely used resistant six-rowed accession. Twenty-three susceptible accessions, comprising susceptible parents from mapping studies, Upper Midwest cultivars and breeding lines, and those identified from FHB screening, were also included in the study. Taken together, the susceptible and resistant genotypes used in the present study included 74 cultivated and 27 wild barley accessions for a total of 101. The cultivated accessions were composed of 27 spring two-rowed, 42 spring six-rowed and 5 winter six-rowed types. Wild barley accessions are all two-rowed. Sources of FHB resistance used in previous linkage mapping studies were included such as Chevron, Fredrickson, Zhedar 1, Russia 6, and CIho4196.

Plants of each of the 101 genotypes were grown in the greenhouse to the two-leaf stage, i.e., about 14 days. DNA was isolated from leaf tissues using the DNeasy plant extraction kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions. DNA samples (1.6 μg) from each genotype were sent to Triticarte Pty Ltd. (http://www.triticarte.com.au) for analysis with barley DArT markers (Wenzl et al. 2004).

Fusarium head blight evaluation

The FHB severity data were compiled from trials spanning multiple years (1996–2010) and multiple locations (North Dakota and Minnesota nurseries in the USA and the Hangzhou nursery in China). Due to space limitations, the FHB screening strategy and procedures are only briefly described (for summary, see Table 1). In an initial screen for FHB resistance in cultivated and wild barley, the entire six-rowed spring barley collection (8,131 accessions) and 600 six-rowed winter barley accessions from the NSGC was conducted in the U.S. (North Dakota and Minnesota) and China (Hangzhou). In addition, 510 wild barley accessions (from NSGC and ICCI) were screened in Hangzhou China in 2000–2001 (preliminary results reported in Steffenson and Scholz 2001). In brief, each of the spring accessions was initially tested in two North Dakota nurseries (Langdon and Osnabrock) for FHB disease severity. Those that displayed less than 30–40 % severity were re-screened in the field in the USA and China. FHB nurseries were inoculated using the "grain spawn" method, except in St. Paul where the "foliar spray" method was used (Steffenson 2003a). For the grain spawn inoculation, equal amounts of two to six regional *F. graminearum* isolates were applied to autoclaved grain spawn, and when infected, the spawn was spread uniformly across plots in the field. The first inoculation was made when the flag leaves of the earliest maturing plants were expanding. One to four additional inoculations were made at regular intervals to ensure that sufficient inoculum was available for infection of later maturing accessions. Overhead irrigation was applied to plants in the morning and evening to promote FHB infection. For the foliar spray method, plants were inoculated twice with a microconidia suspension of *F. graminearum* using a backpack sprayer (Steffenson 2003a). Several local strains of *F. graminearum* were used for inoculum production in the Hangzhou environment. Disease severity was assessed on each

accession at the mid-dough stage. Disease assessments of accessions in the initial screenings done in North Dakota (1999–2000) were made by estimating the percentage (0–100 %) of infected kernels in spikes across the planted hill plots. FHB severity in subsequent screening tests in the USA was determined by arbitrarily selecting 10–20 spikes per accession across a 1-m planted row. The number of infected kernels in each spike was then counted and divided by the total number of kernels within the respective spikes. A collection of 74 Swiss barley landraces, of which 50 were two-rowed and 24 six-rowed, were evaluated in St. Paul and Crookston, Minnesota using the spray and grain spawn inoculation method, respectively (preliminary data reported in Dahl et al. 2009; Steffenson and Dahl 2003). Barley populations of Composite Cross XXX (CC XXX) were established to facilitate selection of genes governing a high incidence of natural cross pollination, where the USDA world barley collection were naturally crossed with male sterile plants (Ramage et al. 1976). As an alternative germplasm for identifying FHB resistance, we screened a bulked seed lot from one of the CC XXX populations (CC XXX-G) for reaction to FHB at Hangzhou in 1996, and single plant selections with partial resistance were subsequently evaluated at both Hangzhou and Minnesota. The 1,979 VIR accessions were evaluated for resistance in disease nurseries at Hangzhou and both St. Paul and Crookston in Minnesota (preliminary data reported in Steffenson et al. 2005). A diverse collection of wild barley (1,768 accessions) received from the gene banks of NSGC, VIR, ICCI and ICARDA were evaluated at Hangzhou from 2003 to 2008. Accessions were planted in late October to early November, inoculated using the grain spawn method in late March to early April, and scored for FHB severity in May. Disease assessment of 0.5 m single row plots were based on a 1–5 scale, where 1 was most resistant and 5 most susceptible (preliminary results reported in Dahl et al. 2009; Steffenson and Dahl 2008). In total, 21,487 cultivated two-rowed and six-rowed accessions with spring or winter habit and 1,768 wild barley accessions were collected from seven gene banks worldwide and screened for FHB resistance in one or more nurseries in China and/or Upper Midwest, USA. A number of two-rowed and six-rowed spring accessions and contemporary Upper Midwest cultivars and breeding lines were evaluated in the Minnesota nurseries from 2000 to 2010 with disease severity reported as the percentage of infected kernels within a spike (K. Smith, unpublished). Finally, 97 of the 101 accessions used in the present study were phenotyped for FHB on a 1–5 scale at the Hangzhou nursery in 2009–2010. To compare the FHB responses of different accessions over multiple years and environments, the FHB severity scores of each accession, expressed either as the percentage of infected kernels per spike or based on general 1–5 rating scale, were converted to a percentage of that of Stander, a six-rowed susceptible check, which was included in all but one trial. In a 1999 trial in the North Dakota nursery, FHB severities of barley accessions were expressed as percentages of that of Steptoe, a six-rowed susceptible accession similar to Stander in terms of FHB severity.

Population structure

DArT genotyping of the 101 barley accessions produced 2,368 polymorphic markers. The quality of each marker is indicated by a Q value where a value greater than 77 is considered good quality. After filtering out poor quality and redundant markers, a total of 1,727 non-redundant markers with a Q value greater than 77 were identified. Seven hundred and twenty-eight out of 1,727 DArT markers had no missing data. Polymorphic information content (PIC) values of each marker were calculated using the formula: $PIC = 1\text{-}\Sigma(P_i)^2$, where P_i is the frequency of the population carrying the ith allele (Botstein et al. 1980). Three clustering approaches were used to study population structure. Phylogenetic analysis was performed with the PHYLIP software suite (Felsenstein 1989) using a total of 728 DArT markers with 100 % genotyping success. In brief, the DArT marker data matrix was used as an input for the calculation of restriction fragments distance (d) matrix (Restdist) (Nei and Li 1979). The dendrogram was constructed based on the d matrix using the unweighted pair group method with arithmetic mean (UPGMA) method (Neighbor). The reliability of inferred tree was tested by bootstrapping 1,000 times. Bootstrap support numbers indicate the percentage of the number of times the partition of the genotypes into the two sets separated by that branch occurred when the data were resampled 1,000 times and were $\geq$50 % for all branches. The visualization and editing of the final consensus tree was generated using Geneious v5.4 (Drummond et al. 2011). Principal coordinate analysis (PCoA), based on the d matrix, was conducted to visualize the relationships among the genotypes (Anderson 2003). Population structure of the germplasm collection was also analyzed using the software STRUCTURE version 2.3 (Falush et al. 2003; Pritchard et al. 2000), which implements a model-based clustering method to infer the number of subpopulations K. For this purpose, thirty unlinked markers (average map distance greater than 45 cM) distributed evenly across the barley genome were used. The admixture and correlated allele frequencies model were used to test K from one to ten, with burn-in length of 10^5 and repetitions of 10^5. Nine replicate runs at different values of K which ensured statistical power for posterior probabilities estimation across runs. Once the population structure was determined, an analysis of molecular variance (AMOVA) was conducted

using Arlequin ver 3.5.1 (Excoffier and Lischer 2010) to estimate population differentiation from the DArT genotyping data. Significance tests were conducted with 16,000 permutations. For population comparisons, Nei's average number of pairwise differences was computed. The number of permutations was set to 1,000 and significance level at 0.01. Settings for exact test of population differentiation were 10^5 steps in Markov chain and 10^4 dememorization steps. The LD between pairs of DArT loci, measured as the squared allele frequency correlation coefficient (r^2), was calculated in the software TASSEL 2.1 (Bradbury et al. 2007).

DArT marker haplotype at FHB resistance QTLs

Four major FHB resistance QTL regions were analyzed for haplotype diversity. The QTLs reside in bin8, 10, and 13 on barley chromosome 2H and bin7 on chromosome 6H (for a summary of FHB QTLs, see Massman et al. 2011). Each of the QTL was detected from at least four different sources and seven different environments, thus representing robust resistance loci. The positions of DArT markers within each bin were determined based on comparisons of a barley consensus DArT map (Wenzl et al. 2006) and the barley bin map (http://barleygenomics.wsu.edu/all-chr.pdf, accessed September 2011). One of the markers was removed, if two markers were in high LD ($r^2 > 0.3$) in the same bin region.

Results

FHB responses of a worldwide collection of barley accessions

To mine barley germplasm for FHB resistance, a large worldwide collection of accessions was evaluated. A total of 21,487 cultivated barley and 1,768 wild barley accessions from seven gene banks were screened in disease nurseries in China and the USA, over a period of 12 years. The six-rowed variety Chevron was used as a resistant check in each of these trials. None of the tested accessions was immune to FHB. In fact, few exhibited any useful level of resistance. The term "resistance" as used herein actually refers to different levels of partial resistance, since individual kernels of spikes are most often completely blighted representing a "susceptible" infection response. Partial resistance to FHB is therefore manifested as reduced disease severity during the course of the epidemic (Niks et al. 2011). The percentages of accessions with levels of partial resistance comparable to that of Chevron were quite low: 1.3 % (279 accessions) for cultivated barley and 1.5 % (27) for wild barley. Due to variable phenotypic expression and complex genotype-by-environment interactions in the FHB-barley pathosystem, these putatively resistant cultivated and wild barley accessions were further evaluated in multiple environments and years, which resulted in the selection of 78 with consistent levels of partial resistance. Of these 78 accessions, 27 (34.6 %) were six-rowed and 51 (65.4 %) were two-rowed. This set of 78 partially resistant accessions was combined with 23 known susceptible accessions to represent a diverse range of FHB responses. The 23 susceptible accessions included 15 identified through the large scale FHB screening effort and 8 that are cultivars and breeding lines from the Upper Midwest. The susceptible accessions were included to assess the extent of their FHB susceptibility, as controls for phenotypic comparisons to the resistant genotypes, and for future use as mapping population parents.

Over 95 % of the resistant accessions were evaluated in at least two environments (Table 2). The mean relative FHB severity scores over all trials for each accession were reported as a percentage of the scores observed on the adapted, local six-rowed susceptible check of Stander and in one case Steptoe. None of the accessions exhibited immunity or even a high level of partial resistance to FHB. Instead, most accessions exhibited moderate to low levels of partial resistance. The lowest average disease severity found, as given by the percent of disease severity compared to Stander/Steptoe, was with Atahualpa at 24.5 % and the highest with PI383933 at 690 %. Based on the following general criteria for mean relative FHB severity, 35 accessions were classified as resistant (mean relative FHB severity $\leq$50 % of susceptible Stander/Steptoe controls), 43 as moderately resistant (50 %< mean relative FHB severity $\leq$75 %), 12 as moderately susceptible (75 %< mean relative FHB severity $\leq$100 %), and 11 as susceptible to highly susceptible (100 %< mean relative percent FHB severity). The five accessions of Atahualpa, CIho6611, AC Oxbow, Hv529, and VIR 25313 had mean relative FHB severities lower than that of the resistant checks Chevron (35.7 %) and CIho4196 (36.4 %). Within this group, one was six-rowed (CIho6611) and four were two-rowed. Among the 78 accessions classified as resistant or moderately resistant, the mean relative FHB disease severity of two-rowed barley (52.3 %) was similar to that of six-rowed barley (50.8 %). The spring, winter, and wild barley groups had mean relative FHB severities of 49.1, 50.9 and 56.4 %, respectively. The 27 wild barley accessions showed mean relative disease severities ranging from 40.7 to 73.9 %. With respect to the selected susceptible accessions, all exhibited moderately susceptible to highly susceptible FHB responses as expected. This group comprised 20 six-rowed accessions and three two-rowed accessions.

Table 2 Row-type, growth habit, origin and FHB responses of 101 barley accessions used in the analysis

Barley line[a]	Row-type	Growth habit	Origin	Source of germplasm	No. of trials	Mean relative FHB severity % ± SD[b]	FHB response
AC Oxbow	2	Spring	Canada	PGRC	3	29.1 ± 9.1	R
Atahualpa	2	Spring	Ecuador	NSGC	3	24.5 ± 11.3	R
Baronesse	2	Spring	Germany	WPB	1	50	R
CIho4196	2	Spring	China	NSGC	22	36.4 ± 26.2	R
CIho11976	2	Spring	Former Soviet Union	PGRC	11	140.1 ± 119.5	S
CIho3957	2	Spring	Georgia	PGRC	3	45.3 ± 9.6	R
Conlon	2	Spring	USA	NDSUBBP	8	84.1 ± 61.4	MS
Fredrickson	2	Spring	Japan	NSGC	8	51.7 ± 32.2	MR
Harrington	2	Spring	Canada	PGRC	3	64.4 ± 67.0	MR
Hv527	2	Spring	Switzerland	SFRSPP	16	39.5 ± 31.5	R
Hv529	2	Spring	Switzerland	SFRSPP	17	31.8 ± 22.9	R
Hv541	2	Spring	Switzerland	SFRSPP	10	56.8 ± 64.7	MR
Hv584	2	Spring	Switzerland	SFRSPP	14	46.8 ± 35.8	R
Hv707	2	Spring	Switzerland	SFRSPP	13	47.7 ± 52.8	R
Hv717	2	Spring	Switzerland	SFRSPP	12	49.5 ± 32.6	R
ICB111809	2	Spring	Turkey	ICARDA	21	190.0 ± 123.6	S
Kutahya	2	Spring	Netherlands	PGRC	6	53.6 ± 16.6	MR
Nepolegajuscij	2	Spring	Russian Federation	VIR	1	50.0	R
NGB9443	2	Spring	Denmark	NGB	8	43.0 ± 23.3	R
Russia6	2	Spring	Former Soviet Union	Takeda and Heta (1989)	0	nd	R[c]
Shenmai3	2	Spring	China	NDSUBBP	4	61.1 ± 10.6	MR
VIR16537	2	Spring	Russian Federation	VIR	10	69.6 ± 71.7	MR
VIR21084	2	Spring	Uzbekistan	VIR	14	66.0 ± 44.1	MR
VIR25313	2	Spring	Denmark	VIR	5	33.8 ± 25.4	R
Zhedar1	2	Spring	China	UMBBP	9	45.7 ± 50.4	R
Zhedar2	2	Spring	China	UMBBP	1	37.5	R
396 (CN 5317)	2	Spring	Ethiopia	PGRC	10	58.4 ± 28.0	MR
Chevron[d]	6	Spring	Switzerland	NSGC	22	35.7 ± 21.0	R
CIho14266	6	Spring	Afghanistan	VIR	2	102.8 ± 74.7	S
CIho14765	6	Spring	USA	NSGC	2	79.3 ± 59.1	MS
CIho2236	6	Spring	USA	NSGC	18	63.3 ± 52.3	MR
CIho3942	6	Spring	Ethiopia	NSGC	18	43.8 ± 44.6	R
CIho588	6	Spring	Australia	NSGC	18	40.3 ± 28.9	R
CIho6610	6	Spring	USA	NSGC	2	40.4 ± 4.1	R
CIho6611	6	Spring	USA	NSGC	3	28.4 ± 13.4	R
CIho6613	6	Spring	USA	NSGC	18	54.9 ± 43.1	MR
CIho7162	6	Spring	USA	NSGC	18	42.0 ± 26.8	R
CIho9056	6	Spring	Austria	NSGC	18	43.2 ± 29.1	R
Comp351	6	Spring	USA	NSGC	19	70.6 ± 60.7	MR
Comp355	6	Spring	USA	NSGC	19	40.4 ± 32.4	R
Hor211	6	Spring	Ukraine	NSGC	5	42.9 ± 22.8	R
Hv746	6	Spring	Switzerland	SFRSPP	16	80.3 ± 63.7	MS
Hv779	6	Spring	Switzerland	SFRSPP	17	105.3 ± 98.6	S
Lacey	6	Spring	USA	UMBBP	15	88.6 ± 40.9	MS
Legacy	6	Spring	USA	BARI	5	95.1 ± 34.5	MS
M92-301	6	Spring	USA	UMBBP	1	75.0	MR

Table 2 continued

Barley line[a]	Row-type	Growth habit	Origin	Source of germplasm	No. of trials	Mean relative FHB severity % ± SD[b]	FHB response
M98-102	6	Spring	USA	UMBBP	5	176.5 ± 97.8	S
MNBrite	6	Spring	USA	NSGC	24	71.4 ± 57.1	MR
Morex	6	Spring	USA	NSGC	2	70.5 ± 6.3	MR
PFC88209	6	Spring	Brazil	UMBBP	3	38.9 ± 7.2	R
PI328607	6	Spring	Germany	NSGC	19	40.8 ± 29.3	R
PI356765	6	Spring	Morocco	NSGC	2	83.6 ± 65.2	MS
PI361705	6	Spring	Denmark	NSGC	2	108.6 ± 29.9	S
PI371317	6	Spring	Switzerland	NSGC	20	65.2 ± 41.6	MR
PI383933	6	Spring	Japan	NSGC	16	690.8 ± 498.2	S
PI402396	6	Spring	Colombia	NSGC	2	83.6 ± 65.2	MS
PI452324	6	Spring	UK	NSGC	2	98.0 ± 32.6	MS
PI525187	6	Spring	Finland	NSGC	2	110.5 ± 14.9	S
PI566360	6	Spring	China	NSGC	2	121.1 ± 12.2	S
PI573976	6	Spring	Nepal	NSGC	20	330.5 ± 363.2	S
PI574078	6	Spring	Nepal	NSGC	2	110.5 ± 14.9	S
Rasmusson	6	Spring	USA	UMBBP	5	96.8 ± 21.4	MS
Robust	6	Spring	USA	NSGC	27	78.3 ± 30.6	MS
Stander	6	Spring	USA	NSGC	48	100	MS
Stellar	6	Spring	Sweden	NSGC	5	52.3 ± 20.8	MR
Steptoe	6	Spring	USA	NSGC	3	99.1 ± 36.2	MS
Tradition	6	Spring	USA	BARI	5	64.5 ± 37.0	MR
VIR28797	6	Spring	Austria	VIR	10	37.2 ± 38.2	R
VIR28807	6	Spring	France	VIR	10	56.3 ± 59.4	MR
CIho197	6	Winter	China	PGRC	4	53.1 ± 17.9	MR
PI565955	6	Winter	China	NSGC	4	44.0 ± 13.1	R
PI566012	6	Winter	China	NSGC	4	42.8 ± 7.7	R
PI566372	6	Winter	China	NSGC	4	59.7 ± 38.8	MR
PI566373	6	Winter	China	NSGC	4	54.9 ± 47.1	MR
ICCI-Hvs-023	2	Wild	Israel	ICCI	3	40.7 ± 4.7	R
ICCI-Hvs-038	2	Wild	Israel	ICCI	3	48.3 ± 17.9	R
ICCI-Hvs-129	2	Wild	Israel	ICCI	3	56.0 ± 31.2	MR
PI282581	2	Wild	Israel	NSGC	4	53.2 ± 13.4	MR
PI282627	2	Wild	Israel	NSGC	4	70.4 ± 45.8	MR
PI282628	2	Wild	Israel	NSGC	4	54.1 ± 25.7	MR
PI282629	2	Wild	Israel	NSGC	4	48.4 ± 14.6	R
PI282632	2	Wild	Israel	NSGC	4	53.2 ± 13.4	MR
PI282651	2	Wild	Israel	NSGC	4	54.1 ± 25.7	MR
PI466421	2	Wild	Israel	NSGC	4	48.4 ± 14.6	R
PI466423	2	Wild	Israel	NSGC	5	46.9 ± 10.9	R
PI466424	2	Wild	Israel	NSGC	4	58.6 ± 23.0	MR
PI466427	2	Wild	Israel	NSGC	4	54.1 ± 25.7	MR
PI466526	2	Wild	Israel	NSGC	4	58.9 ± 23.5	MR
PI466528	2	Wild	Israel	NSGC	4	58.9 ± 23.5	MR
PI466690	2	Wild	Iran	NSGC	4	54.1 ± 25.7	MR
W-365	2	Wild	Iraq	VIR	5	53.2 ± 7.3	MR
W-500	2	Wild	Azerbaijan	VIR	4	57.9 ± 18.1	MR
W-521	2	Wild	Azerbaijan	VIR	4	64.9 ± 36.4	MR

Table 2 continued

Barley line[a]	Row-type	Growth habit	Origin	Source of germplasm	No. of trials	Mean relative FHB severity % ± SD[b]	FHB response
W-541	2	Wild	Iran	VIR	5	64.3 ± 25.5	MR
W-543	2	Wild	Iran	VIR	5	59.4 ± 22.7	MR
W-544	2	Wild	Iran	VIR	5	69.5 ± 20.0	MR
W-714	2	Wild	Israel	VIR	5	55.4 ± 14.0	MR
W-739	2	Wild	Syria	VIR	5	73.9 ± 38.1	MR
W-742	2	Wild	Syria	VIR	3	59.4 ± 20.1	MR
WBDC046	2	Wild	Jordan	ICARDA	4	53.2 ± 13.4	MR
WBDC148	2	Wild	Iran	ICARDA	4	53.2 ± 13.4	MR

BARI Busch Agricultural Resources Inc.; *ICARDA* International Center for Agricultural Research in the Dry Areas; *ICCI* Institute for Cereal Crops Improvement; *NDSUBBP* North Dakota State University Barley Breeding Program; *NGB* Nordic Gene Bank, now NordGen; *NSGC* National Small Grains Collection; *PGRC* Plant Genetic Resources of Canada; *SFRSPP* Station federale de recherches en production vegetale de Changins; *UMBBP* University of Minnesota Barley Breeding Program; *VIR N. I.* Vavilov All-Russian Scientific Research Institute of Plant Industry; *WPB* Western Plant Breeders, now WestBred; *R* resistant, mean relative FHB severity % $\leq$ 50 %; *MR* moderately resistant, 50 %< mean relative FHB severity % $\leq$ 75 %; *MS* moderately susceptible, 75 %< mean relative FHB severity % $\leq$ 100 %; *S* susceptible, 100 %< mean relative FHB severity %

[a] accessions are ordered alphabetically within growth habit based on 728 DArT markers

[b] Expressed as percentage of infected kernels of that of Stander or Steptoe and averaged over all trials, SD standard deviation, nd no data. See methods for details

[c] Classification based on data from Takeda and Heta (1989)

[d] Disease responses of accessions shaded in gray were compared to that of Steptoe in a 1999 trial in North Dakota nursery

DArT genotyping and population structure

DArT genotyping produced 2,368 polymorphic markers form the studied germplasm. After filtering out the redundant and poor quality markers, 1,727 non-redundant polymorphic loci across the 101 barley genotypes were identified. The average genome coverage was 125 markers per chromosome (1 marker per 1.31 cM). Chromosome 2H had the highest marker density at 1 marker per 1.03 cM (162 markers), while chromosome 4H had the lowest at 1 marker per 3.10 cM (48 markers). The marker PIC values ranged from 0.039 to 0.500 (the maximal value for a dominant marker) with a mean value of 0.393. The mean PIC values for cultivated and wild barley were 0.362 and 0.342, respectively, suggesting that there may be an ascertainment bias caused by the underrepresentation of wild barley alleles on the DArT array used for our analysis (Nielsen 2000; Russell et al. 2011; Wenzl et al. 2004). Based on the consensus DArT map (Wenzl et al. 2006), 872 out of 1,727 DArT loci were assigned a map position. The mean PIC values per chromosome were similar in the range of 0.383 (5H) to 0.411 (6H).

To gain an understanding of the extent of genetic variation among the whole collection of genotypes, both distance-based and model-based clustering methods were used to explore genetic structure of the population. The principal coordinate analysis (PCoA) indicated that the first three coordinates explained 55 % of the variance. The 101 barley accessions separated into four clusters as indicated by the scatter plots of the first three principal coordinates (Fig. 1). The cultivated and wild barley accessions separated clearly along the first principal coordinate, which explained 33.1 % of the variation. Most of the wild barleys clustered in the upper right quadrant (Fig. 1a). Based on the second principal coordinate, winter barley formed a distinct cluster (lower right quadrant) from that of spring barley. The third principal coordinate axis (Fig. 1b) separated most of the spring six-rowed and spring two-rowed accessions. The majority of the spring six-rowed accessions were located in the upper left quadrant, while most of the spring two-rowed accessions were in the lower left quadrant.

To further explore the population stratification of all the accessions, the program STRUCTURE (Pritchard et al. 2000) was used to determine the number of subpopulations (k) and calculate the cluster membership coefficient of each accession. Under the admixture and correlated allele frequencies model, data were best explained by assuming four subpopulations ($k = 4$, Fig. S1), which is in agreement with the PCoA analysis. The estimated membership coefficient of each accession is listed in Table S1. The 23 susceptible accessions were partitioned into three subpopulations, which largely corresponded to two-rowed spring, and six-rowed spring, and winter barley, respectively.

Genetic relationships among barley genotypes and clusters

To further explore the genetic relationships among the genotypes, 728 DArT markers with no missing genotyping

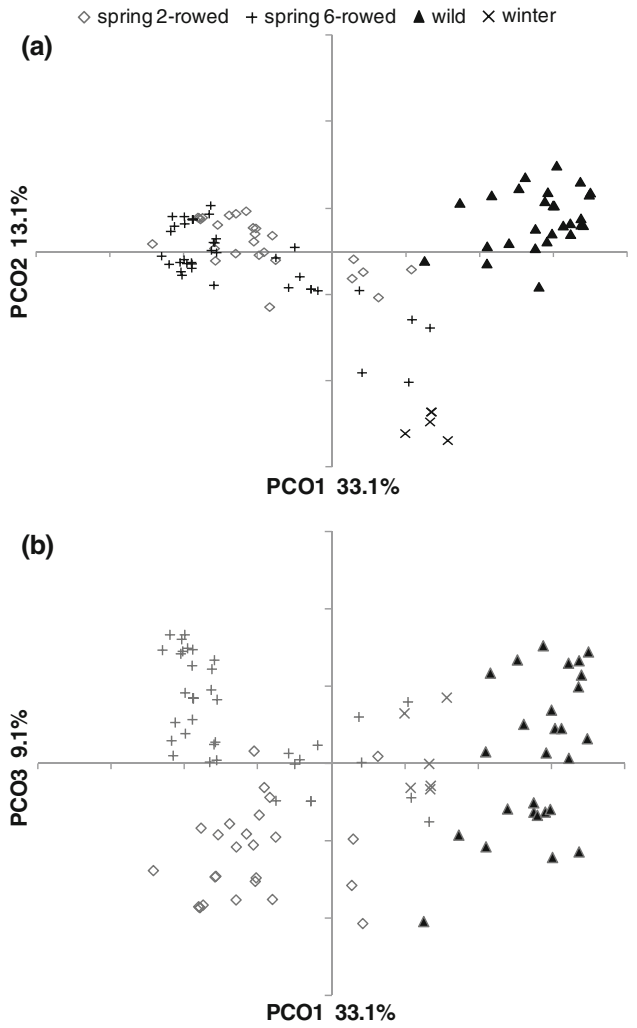


Fig. 1 Principal coordinate analysis of 101 barley accessions. Four clusters separated by **a** PCo1 versus PCo2 and **b** PCo1 versus PCo3. Percent of variation accounted for by each principal coordinate is shown along each axis

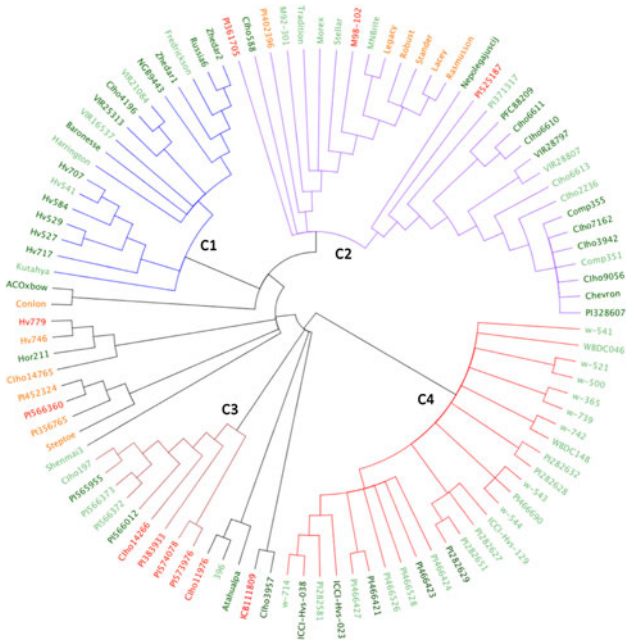


Fig. 2 Dendrogam showing the relationships of 101 barley accessions based on 728 DArT markers using the UPGMA algorithm implemented in PHYLIP. The bootstrap support percentages for all branches are greater than 50 %. Clades are colored to indicate different clusters: C1 (*blue*); C2 (*violet*); C3 (*brown*) and C4 (*red*). Each accession is colored to represent its FHB response: *green*, resistant; *light green*, moderately resistant; *orange*, moderately susceptible; *red*, susceptible

data out of the 1,727 markers were used to derive the pairwise Nei-Li genetic distance matrix (*d*). The average *d* found between two genotypes was 0.0233, with the maximum distance (0.0389) observed between Kutahya and W-714. A consensus tree was built using UPGMA clustering based on *d* to illustrate the genetic relationships among the genotypes (Fig. 2). The clustering pattern generally reflected the differences in growth habit and spike row-type. Cultivated barley and wild barley accessions were clearly separated into two groups. Four main clusters were evident. The first cluster (C1) included 18 spring two-rowed accessions, which were moderately resistant or resistant to FHB. Six Swiss landraces (Hv707, Hv541, Hv584, Hv529, Hv527 and Hv717) formed a subgroup within C1 with a mean relative FHB severity of 45.3 %. Another subgroup consisted of resistant cultivars used in FHB mapping studies (Zhedar 2, Russia 6, Fredrickson and Zhedar 1) and NGB9443 which originated from Denmark. The second cluster (C2) comprises 30 spring six-rowed accessions and one spring two-rowed accession (Nepolegajuscij). The Midwest cultivars and breeding lines (from M92-301 to Rasmusson in Fig. 2) formed a distinct subgroup, of which many were susceptible or moderately susceptible to FHB with a mean relative disease severity of 87.7 %. The other subgroup within C2 contained 17 accessions including Chevron, a widely used resistance source. All of these exhibited some FHB resistance, except PI525187. Five winter six-rowed and four spring six-rowed accessions formed cluster 3 (C3). All five winter accessions were from China and exhibited some resistance, while the four six-rowed accessions were susceptible. The fourth cluster (C4) consisted of 27 wild barley accessions, most of which were collected from the Fertile Crescent region (Israel, Syria, Iraq and Iran). The average relative FHB severity of the wild barley accessions was 56.4 %.

An analysis of molecular variance was performed based on the results of clustering analysis. The results revealed low variation among groups (5.6 %), and high variation within clusters (61.6 %) and among clusters (32.8 %) (Table S2). Pairwise genetic differences between genotypes within cluster 4 (wild barley) was greater than those within the other three clusters. C1 was most closely related to C2, while C3 was most closely related to C4 (Table S3).

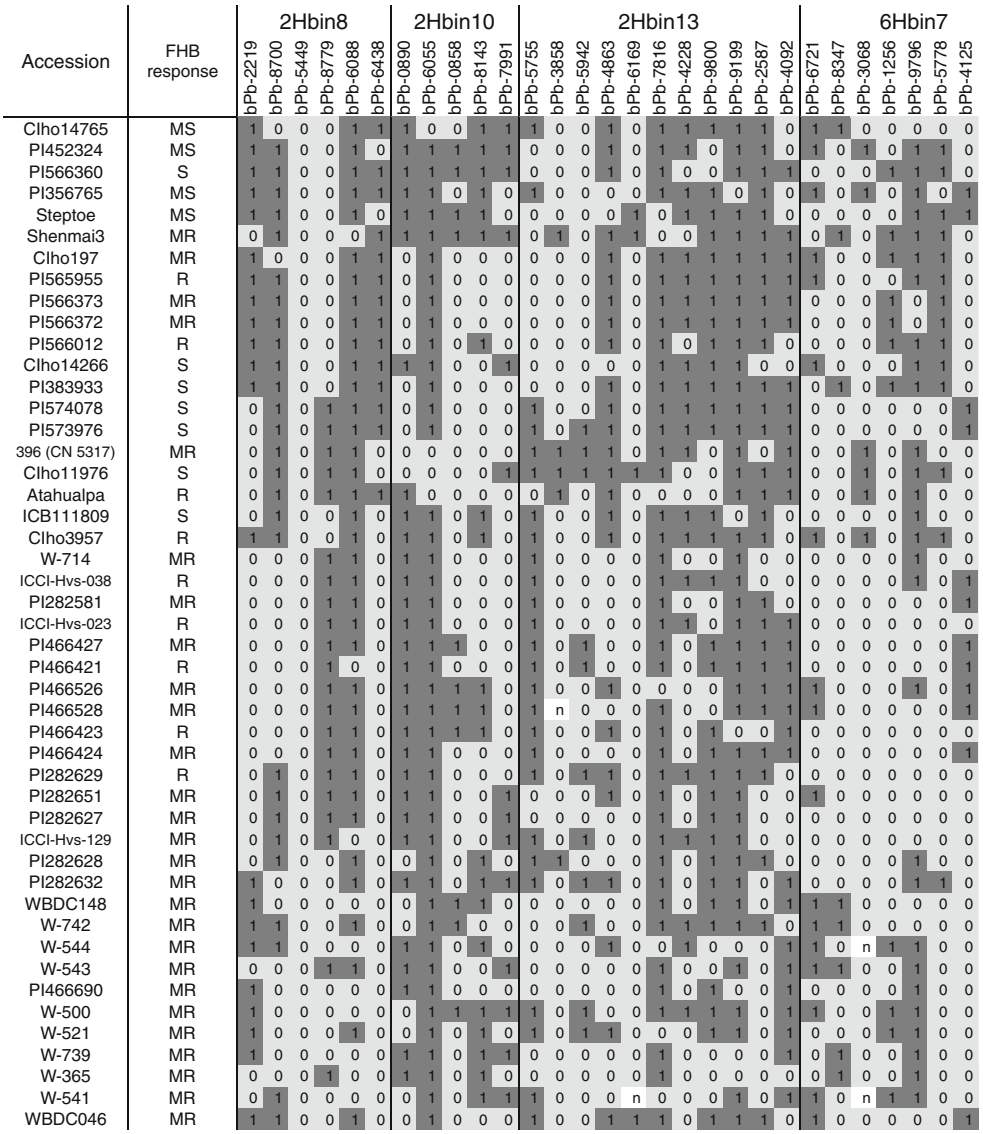
Haplotype patterns of DArT markers linked to FHB QTL regions

To determine the haplotypes near the four major QTL conferring FHB resistance on chromosome 2H bin8, bin10 and bin 13 and chromosome 6H bin7, we examined the allelic distribution at 29 DArT marker loci which were mapped within the four respective regions on chromosomes 2H and 6H (Fig. 3). The marker information used for haplotype analysis is listed in Table 3. PIC values for these 29 markers ranged from 0.19 to 0.50. The number of haplotypes for the whole sample varied from 18 for the 2H bin10 region to 60 for the 2H bin13 region. The haplotypes of wild barley showed overlap with those of cultivated barley on 2H bin8, 2H bin10 and 6H bin7, while no overlaps were identified on 2H bin13. To identify haplotype patterns which are associated with FHB resistance, major haplotypes were analyzed from each of the four QTL regions, and compared with those of resistant mapping population parents including Fredrickson, Russia 6, Zhedar 2, Chevron, and CIho4196 (Table 4). To reveal the population structure in these haplotypes, the cluster from which each accession was derived is indicated based on phylogenetic analysis (Table 5). Only accessions in haplotype 2, 8 and 9 were found in a single cluster, while accessions in other haplotypes were from different clusters indicating

Fig. 3 Haplotype diversity of twenty-nine DArT markers linked to four FHB QTL regions for 101 barley accessions. *Light gray cell* indicates the absence of a DArT marker (0); *dark gray cell* the presence of a DArT marker (1). *n*, no data. The FHB response is shown next to each accession

Accession	FHB response	2Hbin8						2Hbin10					2Hbin13											6Hbin7						
		bPb-2219	bPb-8700	bPb-5449	bPb-8779	bPb-6088	bPb-6438	bPb-0890	bPb-6055	bPb-0858	bPb-8143	bPb-7991	bPb-5755	bPb-3858	bPb-5942	bPb-4863	bPb-6169	bPb-7816	bPb-4228	bPb-9800	bPb-9199	bPb-2587	bPb-4092	bPb-6721	bPb-8347	bPb-3068	bPb-1256	bPb-9796	bPb-5778	bPb-4125
Zhedar2	R	1	1	0	0	0	0	1	1	1	1	0	0	0	1	1	1	0	1	1	1	1	0	0	0	1	0	1	0	0
Russia6	R	1	1	1	0	0	0	1	1	1	1	0	0	0	1	1	1	0	1	1	1	1	0	0	0	1	0	1	0	0
Fredrickson	MR	1	1	1	0	0	0	1	1	1	1	0	0	0	1	1	1	0	1	1	1	1	0	0	0	1	0	1	0	0
Zhedar1	R	1	1	0	0	0	0	1	1	1	1	0	0	0	1	1	1	0	1	1	1	1	0	0	0	1	0	1	0	0
NGB9443	R	1	1	0	0	0	0	1	n	1	1	1	0	1	1	1	0	1	1	0	1	1	0	0	0	1	0	1	0	1
VIR21084	MR	1	1	0	0	n	1	n	1	1	1	1	1	0	0	1	1	0	1	1	1	1	0	1	0	0	1	1	1	1
CIho4196	R	1	1	0	0	1	1	1	1	1	1	1	1	0	0	1	0	0	0	1	1	1	0	1	0	0	1	1	1	1
VIR25313	R	1	1	0	0	0	0	1	1	1	1	1	0	0	1	1	1	0	1	1	1	1	0	1	0	0	1	1	1	1
VIR16537	MR	1	1	1	0	0	0	1	1	0	1	1	1	0	0	1	1	0	1	1	1	1	0	0	1	0	1	1	0	1
Baronesse	R	1	1	0	0	1	0	1	0	0	1	1	1	0	0	1	0	1	1	1	1	1	0	0	1	0	1	1	1	1
Harrington	MR	1	1	1	0	0	0	1	1	1	1	0	1	0	0	1	1	0	1	0	0	1	1	0	1	0	1	1	0	0
Hv707	R	0	1	0	0	1	0	1	1	0	1	1	1	0	0	1	0	1	0	0	1	1	0	0	0	1	0	1	0	1
Hv541	MR	0	1	0	0	1	0	1	1	0	1	1	1	0	0	1	0	1	0	0	1	n	0	0	0	1	0	1	0	1
Hv584	R	0	1	0	0	1	0	1	0	0	1	1	1	0	0	1	1	1	0	0	1	1	0	0	0	1	0	1	0	1
Hv529	R	0	1	0	0	1	0	1	1	0	1	1	1	0	0	1	1	1	0	1	1	1	1	0	0	1	0	1	1	1
Hv527	R	1	1	1	0	1	0	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	0	0	0	1	0	1	1	1
Hv717	R	1	1	0	0	1	0	1	1	0	1	0	1	0	0	1	1	1	0	0	1	1	0	0	0	0	0	1	1	1
Kutahya	MR	1	1	0	0	0	1	1	1	0	1	1	0	1	1	1	1	1	0	0	0	1	1	1	0	1	0	1	1	1
AC Oxbow	R	1	1	0	0	1	1	1	1	0	1	0	1	0	0	1	1	0	1	1	1	1	0	0	0	1	0	1	1	1
Conlon	MS	1	0	0	0	1	0	1	1	1	1	0	1	0	0	1	0	0	n	0	1	1	0	0	0	1	0	1	1	1
Legacy	MS	1	0	0	0	1	1	0	0	0	1	1	0	1	1	1	1	1	0	0	0	1	1	1	0	0	0	1	1	0
MNBrite	MR	1	0	0	0	1	1	1	0	0	1	1	0	1	1	1	1	1	0	0	0	1	1	1	0	0	0	1	1	1
Lacey	MS	1	0	0	0	1	1	1	0	0	1	1	0	1	1	1	1	0	1	1	0	1	1	1	0	0	0	1	1	0
Rasmusson	MS	1	0	0	0	1	1	1	0	0	1	1	0	1	1	1	1	0	1	1	0	1	1	1	0	0	0	1	1	0
Stander	MS	1	0	0	0	1	1	0	0	0	1	1	1	0	0	1	1	0	1	1	0	1	1	0	0	1	1	1	1	0
Robust	MS	1	0	0	0	1	1	0	0	0	1	1	1	0	0	1	1	0	1	1	0	1	1	1	0	1	1	1	1	1
M98-102	S	1	0	0	0	1	1	1	0	0	1	1	1	0	0	1	1	0	1	1	0	1	1	1	0	0	0	1	1	0
Stellar	MR	1	0	0	0	1	1	1	1	0	1	1	1	0	0	1	1	0	1	0	0	1	1	0	0	1	1	1	1	0
Morex	MR	1	0	0	0	1	1	1	1	0	1	1	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1	1	1	0
Tradition	MR	1	0	0	0	1	1	1	0	0	1	1	1	0	0	1	1	0	1	1	0	1	1	1	0	0	0	1	1	0
M92-301	MR	1	0	0	0	1	1	0	0	0	1	1	0	1	1	1	1	1	0	0	0	1	1	1	0	0	0	1	1	0
PI402396	MS	1	0	0	0	1	1	1	0	0	1	1	1	1	0	1	0	0	1	1	1	1	1	1	0	0	0	1	1	0
PI328607	R	0	1	0	0	1	0	1	n	0	1	1	1	0	0	1	1	0	1	1	0	n	1	0	0	1	0	1	0	1
Chevron	R	0	1	0	0	1	0	1	1	0	1	1	1	0	0	1	1	0	1	1	0	1	1	0	0	1	0	1	0	1
CIho9056	R	0	1	0	0	1	0	1	0	0	1	1	1	0	0	1	1	0	1	1	0	1	1	0	0	1	0	1	0	1
Comp351	MR	0	1	0	0	1	0	1	1	0	1	1	1	0	0	1	1	0	1	1	0	1	1	0	0	1	0	1	0	1
CIho3942	R	0	1	1	0	1	0	1	0	0	1	1	1	0	0	1	1	0	1	1	0	1	1	0	0	1	0	1	0	1
CIho7162	R	0	1	0	0	1	0	1	1	0	1	1	1	0	0	1	1	0	1	1	0	1	1	0	0	1	0	1	0	1
Comp355	R	0	1	0	0	1	0	1	1	0	1	1	1	0	0	1	1	0	1	1	0	1	1	0	0	1	0	1	0	1
CIho2236	MR	0	1	1	0	1	0	1	1	0	1	1	1	0	0	1	1	0	1	1	0	1	1	0	0	1	0	1	0	1
CIho6613	MR	0	1	1	0	1	0	1	1	0	1	1	1	0	0	1	1	1	0	1	0	1	1	1	0	0	0	1	0	1
VIR28807	MR	0	1	1	0	1	0	1	1	0	1	1	1	0	0	1	1	0	1	1	0	1	1	0	0	0	0	1	0	1
VIR28797	R	0	1	1	0	1	0	1	1	0	1	1	1	0	0	1	1	0	1	1	0	1	1	1	0	0	0	1	0	1
CIho6610	R	0	1	0	0	1	0	0	1	0	1	0	1	0	0	1	1	0	1	1	0	1	1	0	0	1	0	1	1	1
CIho6611	R	0	1	n	0	1	0	0	1	0	1	0	1	0	0	1	1	0	1	1	0	1	1	0	0	1	0	1	1	1
PFC88209	R	0	1	1	0	1	1	n	1	0	1	0	0	1	1	1	1	0	1	1	0	1	0	0	0	0	0	1	0	1
PI371317	MR	0	1	0	0	1	1	0	1	0	1	1	1	0	1	1	1	1	0	1	0	1	1	0	0	1	1	1	0	1
PI525187	S	0	1	1	0	1	0	1	1	0	1	1	0	1	1	0	1	1	0	0	0	1	0	0	0	1	1	1	0	1
Nepolegajuscij	R	0	1	0	0	1	0	1	1	0	1	1	0	1	1	1	1	1	1	0	1	1	1	0	0	1	1	1	0	1
CIho588	R	0	1	0	0	0	0	0	0	0	1	1	1	0	0	1	1	0	1	1	1	n	0	0	0	1	1	1	0	1
PI361705	S	0	1	1	0	1	1	1	1	0	1	1	0	1	1	1	1	1	1	0	0	n	1	0	0	1	1	1	1	0
Hv779	S	0	1	0	0	1	1	1	1	0	1	0	1	0	1	1	1	1	0	1	1	1	0	0	0	1	1	1	1	1
Hv746	MS	0	1	0	0	1	1	1	1	0	1	0	1	0	1	1	1	1	0	1	1	1	0	0	0	1	1	1	1	1
Hor211	R	0	1	0	0	1	1	1	1	0	1	0	0	1	1	1	1	1	0	1	1	1	0	0	0	1	0	1	1	0

Fig. 3 continued



Accession	FHB response	2Hbin8						2Hbin10					2Hbin13										6Hbin7						
		bPb-2219	bPb-8700	bPb-5449	bPb-8779	bPb-6088	bPb-6438	bPb-0890	bPb-6055	bPb-0858	bPb-8143	bPb-7991	bPb-5755	bPb-3858	bPb-5942	bPb-4863	bPb-6169	bPb-7816	bPb-4228	bPb-9800	bPb-9199	bPb-2587	bPb-4092	bPb-6721	bPb-8347	bPb-3068	bPb-1256	bPb-9796	bPb-5778	bPb-4125
CIho14765	MS	1	0	0	0	1	1	1	0	0	1	1	1	0	0	1	0	1	1	1	1	1	0	1	1	0	0	0	0	0
PI452324	MS	1	1	0	0	1	0	1	1	1	1	1	0	0	0	1	0	1	1	0	1	1	0	1	0	1	0	1	1	0
PI566360	S	1	1	0	0	1	1	1	1	1	1	1	0	0	0	1	0	1	0	0	1	1	1	0	0	0	1	1	1	0
PI356765	MS	1	1	0	0	1	1	1	1	0	1	0	1	0	0	0	0	1	1	1	0	1	0	1	0	1	0	1	0	1
Steptoe	MS	1	1	0	0	1	0	1	1	1	1	0	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	1	1	1
Shenmai3	MR	0	1	0	0	0	1	1	1	1	1	1	0	1	0	1	1	0	0	1	1	1	1	0	1	0	1	1	1	0
CIho197	MR	1	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1	0	0	1	1	1	0
PI565955	R	1	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1	0	0	0	1	1	0
PI566373	MR	1	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0	1	1	1	1	1	1	0	0	0	1	0	1	0
PI566372	MR	1	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0	1	1	1	1	1	1	0	0	0	1	0	1	0
PI566012	R	1	1	0	0	1	1	0	1	0	1	0	0	0	0	1	0	1	0	1	1	1	0	0	0	0	1	1	1	0
CIho14266	S	1	1	0	0	1	1	1	1	0	0	1	0	0	0	0	0	1	1	1	1	0	0	1	0	0	0	1	1	0
PI383933	S	1	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0	1	1	1	1	1	1	0	1	0	1	1	1	0
PI574078	S	0	1	0	1	1	1	0	1	0	0	0	1	0	0	1	0	1	1	1	1	1	1	0	0	0	0	0	0	1
PI573976	S	0	1	0	1	1	1	0	1	0	0	0	1	0	1	1	0	1	1	1	1	1	1	0	0	0	0	0	0	1
396 (CN 5317)	MR	0	1	0	1	1	0	0	0	0	0	0	1	1	1	1	0	1	1	0	1	0	1	0	0	1	0	1	0	0
CIho11976	S	0	1	0	1	1	0	0	0	0	0	1	1	1	1	1	1	1	0	0	1	1	1	0	0	1	0	1	1	0
Atahualpa	R	0	1	0	1	1	1	1	0	0	0	0	0	1	0	1	0	0	0	0	1	1	1	0	0	1	0	1	0	0
ICB111809	S	0	1	0	0	1	0	1	1	0	1	0	1	0	0	1	0	1	1	1	0	1	0	0	0	0	0	1	0	0
CIho3957	R	1	1	0	0	1	0	1	1	0	1	0	1	0	0	1	0	1	1	1	1	1	0	1	0	1	0	1	1	0
W-714	MR	0	0	0	1	1	0	1	1	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0
ICCI-Hvs-038	R	0	0	0	1	1	0	1	1	0	0	0	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	0	1
PI282581	MR	0	0	0	1	1	0	1	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	1
ICCI-Hvs-023	R	0	0	0	1	1	0	1	1	0	0	0	1	0	0	0	0	1	1	0	1	1	1	0	0	0	0	0	0	0
PI466427	MR	0	0	0	1	1	0	1	1	1	0	0	1	0	1	0	0	1	0	1	1	1	1	0	0	0	0	0	0	1
PI466421	R	0	0	0	1	0	0	1	1	0	0	0	1	0	1	0	0	1	0	1	1	1	1	0	0	0	0	0	0	1
PI466526	MR	0	0	0	1	1	0	1	1	1	1	0	1	0	0	1	0	0	0	0	1	1	1	1	0	0	0	1	0	1
PI466528	MR	0	0	0	1	1	0	1	1	1	1	0	1	n	0	0	0	1	0	0	1	1	1	1	0	0	0	0	0	1
PI466423	R	0	0	0	1	1	0	1	1	1	1	0	1	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0
PI466424	MR	0	0	0	1	1	0	1	1	0	0	0	1	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0	0	1
PI282629	R	0	1	0	1	1	0	1	1	0	0	0	1	0	1	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0
PI282651	MR	0	1	0	1	1	0	1	1	0	0	1	0	0	0	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0
PI282627	MR	0	1	0	1	1	0	1	1	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0
ICCI-Hvs-129	MR	0	1	0	1	0	0	1	1	0	0	1	1	0	1	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0
PI282628	MR	0	1	0	0	1	0	0	1	0	1	0	1	1	0	0	0	1	0	1	1	1	0	0	0	0	0	0	1	0
PI282632	MR	1	0	0	0	1	0	1	1	0	1	1	1	0	1	1	0	1	0	1	1	0	1	0	0	0	0	1	1	0
WBDC148	MR	1	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	1	1	0	1	1	1	0	0	0	0	0
W-742	MR	1	1	0	0	1	0	0	1	1	0	0	0	0	1	0	0	1	1	1	1	1	0	1	1	0	0	0	0	0
W-544	MR	1	1	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	1	0	0	0	1	1	0	n	1	1	0	0
W-543	MR	0	0	0	1	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	1	1	1	0	0	1	0	0
PI466690	MR	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	1	0	0
W-500	MR	1	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	1	1	1	1	0	1	1	0	0	1	1	0	0
W-521	MR	1	0	0	0	1	0	0	1	0	1	0	1	0	1	1	0	0	0	1	1	0	1	0	0	0	1	1	0	0
W-739	MR	1	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0
W-365	MR	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0
W-541	MR	0	1	0	0	0	0	0	1	0	1	1	1	0	0	0	n	0	0	0	1	0	1	1	0	n	1	1	0	0
WBDC046	MR	1	1	0	0	1	0	0	1	0	0	0	1	0	0	1	1	1	0	1	1	1	0	1	0	0	0	0	0	1

that diverse germplasm contains similar haplotypes for the major QTLs conferring FHB resistance. An overview of the genetic relationships among accessions based on haplotype patterns in the four FHB QTL regions is shown in Fig. S2.

Twenty haplotypes were identified for six DArT markers linked to the FHB QTL on chromosome 2H bin8. Thirteen accessions shared the same haplotypes with Chevron (Haplo1, Table 4; for names of accessions, see Table 5). Except ICB111809, all the other 12 accessions displayed resistance. Three barley accessions (Harrington, Russia 6 and VIR16537) had the same haplotype as Fredrickson (Haplo2). Four accessions (Zhedar 1, VIR25313, NGB9443 and W-544) had the same haplotype as Zhedar 2 (Haplo3). The CIho4196 haplotype (Haplo4) comprised 10 accessions, of which six were resistant and four susceptible.

Eighteen haplotypes were found on chromosome 2H bin10. Three major haplotypes accounted for more than one-third of the total haplotypes in frequency (Haplo5-7). The Chevron haplotype (Haplo5) comprised 7 spring two-rowed, 12 spring six-rowed and 2 wild barley accessions. All but two accessions (PI361705 and PI525187) showed high levels of resistance, with a mean relative FHB severity of 58.0 %. The Fredrickson haplotype (Haplo6) was composed of 10 accessions, of which eight were resistant and two were susceptible (Conlon and Steptoe). The CIho4196 haplotype (haplo7) included three resistant and two susceptible accessions, with a mean relative FHB severity of 70.1 %.

Sixty DArT marker haplotypes were identified on chromosome 2H bin13, representing the most haplotype diversity for the selected four FHB QTL intervals. The QTL region had a similar interval length (centiMorgan) as

Table 3 Summary of DArT markers within four FHB QTL regions on chromosomes 2H and 6H

FHB QTL intervals	Number of haplotypes[a]	Marker position (cM)[b]	Distance to next marker (cM)	PIC
2HBin8	20/16/10	bPb-2219 (71.37)	0.71	0.50
		bPb-8700 (72.09)	5.32	0.44
		bPb-5449 (77.41)	0	0.23
		bPb-8779 (77.41)	4.29	0.33
		bPb-6088 (81.70)	0.43	0.32
		bPb-6438 (82.13)	–	0.46
2HBin10	18/13/12	bPb-0890 (86.99)	3.47	0.38
		bPb-6055 (90.46)	5.22	0.31
		bPb-0858 (95.68)	2.53	0.33
		bPb-8143 (98.21)	3.06	0.38
		bPb-7991 (101.27)	–	0.50
2HBin13	60/36/24	bPb-5755 (133.29)	1.39	0.47
		bPb-3858 (134.68)	1.88	0.31
		bPb-5942 (136.56)	1.67	0.43
		bPb-4863 (138.23)	0	0.35
		bPb-6169 (138.23)	0.80	0.50
		bPb-7816 (139.03)	0.71	0.48
		bPb-4228 (139.75)	2.02	0.49
		bPb-9800 (141.77)	4.18	0.43
		bPb-9199 (145.95)	0.39	0.47
		bPb-2587 (146.34)	0.29	0.30
		bPb-4092 (146.64)	–	0.49
6HBin7	36/28/13	bPb-6721 (72.71)	0.73	0.44
		bPb-8347 (73.44)	0.86	0.19
		bPb-3068 (74.30)	0.04	0.49
		bPb-1256 (74.34)	1.85	0.40
		bPb-9796 (76.19)	8.44	0.31
		bPb-5778 (84.64)	0.17	0.49
		bPb-4125 (84.81)	–	0.50

[a] Number of haplotypes in whole sample, cultivated barley and wild barley, respectively

[b] Based on a barley consensus DArT map (Wenzl et al. 2006)

other QTLs, but had higher marker coverage (11 markers). Chevron shared the same haplotype (Haplo8) with three susceptible accessions (e.g., Stander), suggesting that this haplotype might not be associated with resistance or other QTLs that increased susceptibility were present in susceptible accessions. The Fredrickson haplotype (Haplo9) comprised five spring two-rowed accessions and all were resistant.

Two main haplotypes for FHB resistance were evident on chromosome 6H bin7. The Chevron haplotype (Haplo10) included four spring two-rowed and eight spring six-rowed accessions, which were all resistant with an average relative disease severity of 47.9 %. Five spring two-rowed accessions (Zhedar 1, Zhedar 2, Russia 6, Atahualpa, and 396) shared the same haplotype as that of Fredrickson (Haplo11). The Chevron and Fredrickson haplotypes were very similar.

Discussion

Evaluation of barley accessions for FHB resistance

The primary aims of this study were to identify new sources of FHB resistance by systematically screening a worldwide collection of barley accessions, and to characterize their genetic relationships with known resistant accessions using molecular marker-based clustering. The number of cultivated barley accessions screened in this study (21,487) was much larger than in previous works (Buerstmayr et al. 2004; Chen et al. 1991; Choo et al. 2004; Ma et al. 2009; McCallum et al. 2004; Takeda and Heta 1989; Zhou et al. 1991), and included, in addition, a diverse sample of wild barley accessions (1,768). As in previous studies, the frequency of FHB resistance was very low in the screened germplasm, with only 305 (1.3 %) accessions

Table 4 Characteristics of haplotype patterns of known FHB resistant accessions identified from four QTL regions

No./QTL position	Haplotype	Frequency	No. of spring 2-row	No. of spring 6-row	No. of winter barley	No. of wild barley	No. of R lines	No. of S lines	Mean relative FHB severity % (range) ± SE	Representative line
Haplo1/2Hbin8	010010	0.14	6	7	0	1	13	1	56.5 (31.8–190.0) ± 10.6	Chevron
Haplo2/2Hbin8	111000	0.04	4	0	0	0	4	0	61.9 (51.7–69.6) ± 5.3	Fredrickson
Haplo3/2Hbin8	110000	0.05	4	0	0	1	5	0	45.9 (33.8–69.6) ± 6.3	Zhedar2
Haplo4/2Hbin8	110011	0.1	2	4	4	0	6	4	126.5 (29.1–690.8) ± 63.4	CIho4196
Haplo5/2Hbin10	11011	0.21	7	12	0	2	19	2	58.0 (31.8–110.5) ± 4.6	Chevron
Haplo6/2Hbin10	11110	0.1	6	1	0	3	8	2	60.8 (37.5–99.1) ± 6.5	Fredrickson
Haplo7/2Hbin10	11111	0.05	3	2	0	0	3	2	70.1 (33.8–121.1) ± 17.2	CIho4196
Haplo8/2Hbin13	10011011011	0.15	0	15	0	0	12	3	61.4 (28.4–176.5) ± 9.6	Chevron
Haplo9/2Hbin13	00111011110	0.05	5	0	0	0	5	0	42.2 (33.8–51.7) ± 4.0	Fredrickson
Haplo10/6Hbin7	0010101	0.12	4	8	0	0	12	0	47.9 (35.7–70.6) ± 3.0	Chevron
Haplo11/6Hbin7	0010100	0.06	5	0	1	0	6	0	43.6 (24.5–58.4) ± 5.4	Fredrickson

possessing potentially useful levels of partial resistance. Selected accessions were further evaluated in two or more trials, which resulted in the identification of 78 accessions that exhibited a consistent level of partial resistance. Twenty-seven of the 78 selected accessions were wild barley and may carry novel FHB resistance alleles. Two wild barley accessions (PI466423 and W-365) are currently being used in molecular mapping studies to elucidate the number, effect, and chromosomal position of FHB resistance loci (Steffenson, unpublished). Within cultivated germplasm, two-rowed Atahualpa and six-rowed CIho6611 were the two accessions with the lowest relative disease severities (24.5 and 28.4 %, respectively). Among the resistant cultivated barley, 27 were six-rowed and 24 two-rowed indicating that six-rowed barley can possess resistance levels comparable to the most resistant two-rowed barley. This finding was unexpected since two-rowed types were generally shown to have lower FHB severities than six-rowed types in several previous studies (Buerstmayr et al. 2004; Choo et al. 2004; Takeda and Heta 1989; Zhou et al. 1991). The identification of resistant six-rowed germplasm is important for barley breeding programs in the Upper Midwest, since this row type predominates in the region. It should be noted that certain agronomic traits, such as heading date and plant height, may influence the development of FHB (for a detailed review, see Steffenson 2003a). Some of the identified resistant sources were also late heading, and this could have contributed to the lower levels of FHB.

Genetic relationships in sources of FHB resistance

Previous studies have assessed barley genetic diversity in germplasm collections (Comadran et al. 2009; Zhang et al. 2009). Zhang et al. (2009) reported 942 polymorphic markers for 170 Canadian barley cultivars. Comadran et al. (2009) reported 1,130 bi-allelic markers for 192 accessions of barley landraces and cultivars that represent germplasm grown in the Mediterranean basin. Our study revealed a total of 1,727 polymorphic DArT markers for 101 accessions including cultivated and wild barley. Barley populations are known to be highly structured based on inflorescence type and growth habit (Rostoks et al. 2006; Zhang et al. 2009). Rostoks et al. (2006) reported that growth habit (spring vs. winter) was a major determinant of population structure in 102 northern European barley cultivars. The inflorescence type (two-rowed vs. six-rowed) was found to account for the population stratification in 170 Canadian barley cultivars (Zhang et al. 2009). Population structure of the present germplasm collection was queried using three different approaches, which generated similar clustering patterns. Four clusters were identified that roughly corresponded to spring two-rowed, spring six-rowed, winter, and wild barley accessions. The cluster

Table 5 Accessions associated with the major haplotypes identified within the four QTL regions

No.	QTL	Lines
Haplo1	2H bin8	Chevron (C2[a]), CIho6610 (C2), CIho7162 (C2), CIho9056 (C2), Comp351 (C2), Comp355 (C2), PI328607 (C2), Nepolegajuscij (C2), Hv529 (C1), Hv541 (C1), Hv584 (C1), Hv707 (C1), PI282628 (C4), ICB111809[b]
Haplo2	2H bin8	Fredrickson (C1), Harrington (C1), Russia6 (C1), VIR16537 (C1)
Haplo3	2H bin8	Zhedar2 (C1), Zhedar1 (C1), NGB9443 (C1), VIR25313 (C1), W-544 (C4)
Haplo4	2H bin8	CIho4196 (C1), AC Oxbow, PI565955 (C3), PI566012 (C3), PI566372 (C3), PI566373 (C3), CIho14266[b] (C3), PI356765[b], PI383933[b] (C3), PI566360[b]
Haplo5	2H bin10	Chevron (C2), CIho2236 (C2), CIho6613 (C2), CIho7162 (C2), Comp351 (C2), Comp355 (C2), Morex (C2), PI361705[b] (C2), PI525187[b] (C2), Stellar (C2), VIR28797 (C2), VIR28807 (C2), Neplegajuscij (C2), Hv527 (C1), Hv529 (C1), Hv541 (C1), Hv707 (C1), Kutahya (C1), VIR16537 (C1), PI282632 (C4), W-739 (C4)
Haplo6	2H bin10	Fredrickson (C1), Zhedar1 (C1), Zhedar2 (C1), Russia6 (C1), Harrington (C1), Conlon[b], Steptoe[b], PI466423 (C4), PI466526 (C4), PI466528 (C4)
Haplo7	2H bin10	CIho4196 (C1), VIR25313 (C1), Shenmai3, PI452324[b], PI566360[b]
Haplo8	2H bin13	Chevron (C2), CIho2236 (C2), CIho3942 (C2), CIho6610 (C2), CIho6611 (C2), CIho7162 (C2), CIho9056 (C2), Comp351 (C2), Comp355 (C2), VIR28807 (C2), VIR28797 (C2), Tradition (C2), Stander[b] (C2), Robust[b] (C2), M98-102[b] (C2)
Haplo9	2H bin13	Fredrickson (C1), Zhedar1 (C1), Zhedar2 (C1), Russia6 (C1), VIR25313 (C1)
Haplo10	6H bin7	Chevron (C2), CIho2236 (C2), CIho3942 (C2), CIho7162 (C2), CIho9056 (C2), Comp351 (C2), Comp355 (C2), PI328607 (C2), NGB9443 (C1), Hv541 (C1), Hv584 (C1), Hv707 (C1)
Haplo11	6Hbin7	Fredrickson (C1), Zhedar1 (C1), Zhedar2 (C1), Russia6 (C1), Atahualpa, 396

[a] Indicates the cluster to which each accession belongs based on Fig. 2

[b] Indicates susceptible accessions

analyses allowed comparison of genetic variation among accessions with mapped and unmapped FHB resistance loci. Resistant two-rowed mapping parents Fredrickson, Zhedar 2, Russia 6, and CIho4196 were placed in the C1 cluster together with 14 other two-rowed resistant accessions (Fig. 2). Resistant six-rowed Chevron and 21 other resistant accessions were clustered in C2. Previous QTL mapping studies for FHB resistance identified the same set of QTLs, namely 2H bin8, 10, 13 and 6H bin7, from different resistant sources aforementioned (Dahleen et al. 2003; de la Peña et al. 1999; Hori et al. 2005; Horsley et al. 2006; Ma et al. 2000; Mesfin et al. 2003). This suggests that the resistant accessions in C1 and C2 could carry the same FHB resistance QTLs. Potentially different sources of resistance might be present in accessions not clustered in C1 and C2, which includes AC Oxbow, Hor211, Shenmai 3, Atahualpa, CIho3957, five winter accessions, and the wild barley accessions. However, prediction of new resistance sources based on their genetic dissimilarity from known resistant accessions should be applied with caution. For example, Wingbermuehle et al. (2004) evaluated five potential new sources for their genetic relationships with resistant cultivars already utilized in linkage mapping studies, and showed that Atahualpa and Hor211 were the most dissimilar from Chevron and Fredrickson. Two of the six known FHB resistance QTLs were identified by selective genotyping from populations derived from Atahualpa. However, none of the six QTLs were associated with variation for FHB severity in populations derived from resistance source Hor211. Therefore, the resistant accessions identified in our study that did not cluster with the major sources of resistance that have been previously mapped might possess new resistance loci.

Haplotypes at previously identified FHB resistance QTLs potentially carry novel resistance alleles

The haplotype patterns of molecular markers linked to disease resistance QTLs may prove useful in predicting whether an accession carries known or novel resistance alleles (McCartney et al. 2004; Yu et al. 2006). Using simple sequence repeat (SSR) markers linked to six FHB QTLs, McCartney et al. (2004) characterized the haplotypes of 79 resistant and susceptible wheat accessions, and identified a number of non-Asian resistance sources carrying potentially novel resistance genes. The assumption underlying these studies is that accessions with the same haplotype pattern spanning a resistance QTL likely have the same or similar alleles of the QTL. In contrast, if an accession has a different haplotype pattern from that of a known resistant line, they likely have different alleles of the QTL or unique loci (Bai et al. 2003; McCartney et al. 2004).

In our study, 78 FHB resistant accessions were identified which included 73 unmapped and 5 mapped FHB

resistance sources namely Chevron, Fredrickson, CIho 4196, Zhedar 2 and Russia 6. Comparison of haplotypes in FHB QTL intervals among these resistant accessions may help to identify potentially novel resistance alleles. Haplotype patterns of 29 markers associated with four FHB resistance QTLs (2H bin8, 10, 13 and 6H bin7) were analyzed to gain a better understanding of the QTLs in diverse accessions. The QTL regions were previously identified in mapping populations with Chevron and Fredrickson as parents (Canci et al. 2004; de la Peña et al. 1999; Ma et al. 2000; Mesfin et al. 2003). A number of other QTL mapping studies using different resistant parents (CIho4196, Zhedar 2, Russia 6 and Harbin) have identified many of the same QTLs (Dahleen et al. 2003; Hori et al. 2005; Hori et al. 2006; Horsley et al. 2006). Thus, the four QTL intervals represent robust FHB resistance loci. Our results suggest that many of the resistant wild barley accessions, predominantly of Israeli origin, had haplotypes distinct from those of resistant cultivated accessions indicating that they could carry novel FHB resistance genes. Among cultivated barley accessions, the Chevron haplotypes on 2H bin8, 10, 13 and 6H bin7 were the highest in frequency, followed by the Fredrickson haplotype and the CIho4196 haplotype. Ten of the resistant spring six-rowed accessions (PI328607, CIho9056, CIho3942, CIho7162, CIho2236, CIho6613, Comp351, Comp355, VIR28807, and VIR28797) had identical or very similar haplotypes to that of Chevron at all four QTLs indicating that they could have the same FHB resistance alleles. Resistant spring two-rowed accessions (Hv707, Hv541, Hv584, Hv529, Hv527, VIR16537, Kutahya, and Nepolegajuscij) shared the same haplotype as that of Chevron on at least one of the QTLs on 2H bin8, bin10, and 6H bin7 suggesting that they might carry one or more Chevron resistance QTLs. PI328607, CIho9056, VIR28807, VIR28797, Kutahya, and the Hv accessions had geographic origins from or near central Europe. Three accessions (CIho7162, CIho2236, and CIho6613) were collected or developed in the Upper Midwest, and their Chevron FHB resistance alleles could be derived from European lines carrying the Chevron FHB QTLs. Composite Cross XXX was developed from natural crosses between the USDA world barley collection and a male sterile line (Ramage et al. 1976). Two Composite Cross selections, Comp351 and Comp355, were chosen based on their consistent FHB resistance (Steffenson 2003b, unpublished results). On the basis of phenotype, marker, and haplotype data of the present study, the two Comp lines are most likely derived from Chevron as they carry all four Chevron FHB resistance QTL alleles. The fact that these Comp lines were selected from a bulked seed lot of over 25,000 seeds from our FHB screening suggests that our evaluation strategy was successful in identifying accessions with moderate levels of partial resistance. Chevron is interesting because it possesses distinct bright kernels even under induced FHB and kernel discoloration (caused by *Cochliobolus sativus*) epidemics (Canci et al. 2004; de la Peña et al. 1999; Rasmusson et al. 1999). This phenotype is apparently easy to select under different environments, and is the likely reason that different resistance sources carrying the Chevron haplotypes were repeatedly identified from the screening trials. Interestingly, three of the wild barley accessions had the same haplotypes as those of Chevron. The haplotype of PI282628 was identical to Chevron on 2H bin8 and similar on 2H bin10. PI282632 and VIR W-739 had the Chevron haplotype on 2H bin10. The results suggest that these wild barley accessions could carry the Chevron FHB resistance alleles on these QTL regions. Previously, PFC88209 and Hor211 were suggested to contain novel genes for FHB resistance (Wingbermuehle et al. 2004), and our results agree with this conclusion. Clustering analysis suggests that PFC88209 is closely related to CIho6610 and CIho6611. While the haplotypes of CIho6610 and CIho6611 were the same or similar to those of Chevron, the PFC88209 haplotypes were quite different suggesting that FHB resistance of PFC88209 might be contributed by yet unmapped QTL regions. The haplotypes of FHB resistant accession Hor211 were identical or similar to Hv779 and Hv746, two susceptible six-rowed accessions, indicating that novel resistance QTLs could be present in Hor211. According to the haplotype data, the Chevron FHB resistance QTLs were rare in Midwest barley cultivars and breeding lines. This is surprising given that Chevron was used as a source of stem rust resistance (contributing the gene *Rpg1*) and kernel discoloration resistance in the early and middle part of the last century (Steffenson 1992; Steffenson 2003a).

Characterization of the Fredrickson haplotypes on four QTL regions revealed that Zhedar 1, Zhedar 2, and Russia 6 had the same or very similar haplotypes at the FHB resistance QTLs. This was not unexpected, since QTL mapping studies utilizing Zhedar 2 and Russia 6 identified QTL regions coincident with those identified from Fredrickson (Dahleen et al. 2003; Hori et al. 2005; Mesfin et al. 2003). Two closely related two-rowed accessions (NGB9443 and VIR16537) had combined FHB QTL haplotypes from Fredrickson, Zhedar 2, and Chevron. For example, NGB9443 had the Zhedar 2 haplotype on 2H bin8 and Chevron haplotype on 6H bin7. VIR16537 had the Fredrickson haplotype on 2H bin8 and Chevron haplotype on 2H bin10, respectively. Harrington, a two-rowed malting barley cultivar from Canada, had the Fredrickson haplotypes on 2H bin8 and bin10. Wild barley accessions, PI466423, PI466526, and PI466528, had the Fredrickson QTL haplotype on 2H bin10, while W-544 had a similar Fredrickson QTL haplotype on 2H bin8. These accessions

might possess the Fredrickson FHB QTLs on chromosome 2H bin8 and 10. Interestingly, four six-rowed winter barley accessions shared the same CIho4196 haplotype on chromosome 2H bin8 suggesting that their FHB resistance might be contributed by the CIho4196 on 2H bin8. The CIho4196 QTL haplotype on 2H bin8 was different from that of Fredrickson and Chevron, thereby representing a possible different allele at the QTL.

As molecular marker haplotypes are only approximations of the underlying FHB resistance QTL, they can only predict if an accession has the same or different QTL as the known resistance source with variable accuracy. Factors influencing the prediction include the non-precise locations of QTLs and the marker distance from the QTLs (Yu et al. 2006). For example, two six-rowed accessions (PI452324 and PI566360) had the CIho4196 haplotype on chromosome 2H bin10, but showed high FHB susceptibility. Another example is Atahualpa, the most resistant line in our collection. It had an identical haplotype with Fredrickson on 6H bin7, and this QTL was identified in populations derived from Fredrickson and Atahualpa in a mapping study (Wingbermuehle et al. 2004). The Atahualpa and Fredrickson haplotypes were quite different on chromosome 2H bin10. However, Beaubien et al. (2004) identified a major FHB QTL on 2H bin10 using Atahualpa as a resistant parent indicating that Atahualpa may have a different allele at the 2H bin10 QTL. It should be noted that the 2H bin10 region contains the *Vrs1* allele which controls spike morphology, and the two-rowed spike type may be less conducive to disease development and be responsible, in part, for the association of FHB resistance at this region.

Sources with potentially new QTLs for FHB resistance

The present study evaluated the FHB disease responses of 23,255 barley accessions, and identified 78 that were classified as resistant or moderately resistant. Clustering analysis and haplotype diversity at DArT markers linked to four robust FHB resistance QTLs revealed the relationships of resistance between mapped and unmapped resistant barley accessions. Chevron QTL haplotypes were the most prevalent in our germplasm, and were present in some of the two-rowed accessions as well. Fredrickson QTL haplotypes were almost exclusively shared by two-rowed accessions only. The CIho4196 haplotypes were present in both two-rowed and six-rowed accessions. In addition to identifying accessions with QTL haplotypes the same as or similar to those of Chevron, Fredrickson, and CIho4196, a number of other sources with potentially novel alleles were identified based on their different haplotype patterns from those of known resistant accessions in the four FHB QTL regions, which included Baronesse, Kutahya, PFC88209, CIho588, Hor211, and CIho3957. These resistant sources could be utilized in future linkage mapping studies. With the exception of seven wild barley accessions which shared FHB QTL haplotypes with those of Chevron, Fredrickson, and Zhedar 2 (Tables 4 and 5), 20 wild barley accessions had distinct haplotype patterns in FHB QTL regions from those of mapped, well-known resistance sources, suggesting that they could carry new resistance QTLs. Studies should be advanced to position these loci in mapping populations. The potential new FHB resistance alleles present in cultivated and wild barley could be exploited in breeding programs to enhance levels of FHB resistance.

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