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Exploiting selective genotyping to study genetic diversity of resistance to Fusarium head blight in barley

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Abstract Numerous barley cultivars from around the world have been identified as potential sources of Fusarium head blight (FHB) resistance genes. All of these cultivars exhibit partial resistance, and several mapping studies have shown that resistance to FHB is controlled by multiple genes. Successful development of barley cultivars with high levels of FHB resistance will require combining genes from multiple sources. We characterized five potential new sources of FHB resistance ('AC Oxbow', 'Atahualpa', 'HOR211', 'PFC88209', and 'Zhedar#1') to determine if they contain new FHB resistance genes. Cluster analysis, using a set of 80 SSR markers distributed throughout the genome, showed that most of the new sources of resistance were not similar to three cultivars that have been used in previous FHB mapping studies ('Chevron', 'Frederickson', and 'Gobernadora'), with 'Atahualpa' and 'HOR211' being the most dissimilar. By selective genotyping, we determined whether markers linked to six known FHB resistance quantitative trait loci (QTLs), discovered in other genotypes, explained variation for resistance in advanced breeding populations created from the new sources of resistance. Markers linked to four of the six known QTLs were associated with FHB severity in at least one of the populations. However, none of the six QTL regions were associated with variation for FHB severity in populations derived from crosses that utilized sources of resistance HOR211 or PFC88209. Selective genotyping is an efficient method for breeders to utilize current QTL information about disease resistance to search for new resistance genes.

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Introduction

Fusarium head blight (FHB), caused primarily by the pathogen *Fusarium graminearum* Schwabe (telomorph *Gibberella zeae*), is the most destructive disease presently affecting barley (*Hordeum vulgare* L.) in North America (McMullen et al. 1997) and has resulted in substantial economic losses in the Upper Midwest since 1993 (Windels 2000; Nganje et al. 2001). Breeding resistant cultivars could be an effective strategy to manage FHB in barley, but unfortunately this strategy faces significant challenges. All barley genotypes investigated so far express only partial resistance to FHB. Further, several genetic mapping studies have shown that resistance to FHB and to the accumulation of the mycotoxin deoxynivalenol (DON) that is produced by the pathogen are conditioned by many genes distributed throughout the genome (Kolb et al. 2001). In addition, quantitative trait loci (QTLs) associated with resistance are often inconsistently detected among environments and are usually associated with agronomic and morphological traits such as late heading, tall plant height, lax spike, and two-rowed spike (Steffenson 2002). This has led several researchers to conclude that most QTLs for FHB resistance result from the pleiotropic effect of morphological or developmental genes, and consequently that the "function" of FHB resistance is primarily related to plant morphology or "form" (Zhu et al. 1999; Ma et al. 2000).

To date, more than 100 potential sources of FHB resistance have been identified (reviewed by Steffenson 2002). For the most part, the degree of relatedness among these sources of resistance is not known since in many cases pedigree information is missing or incomplete. Thus, it is not possible to accurately predict whether these sources contain the same or different FHB resistance genes. Belina et al. (2002) examined the genetic diversity of a set of spring six-rowed barley accessions with partial resistance to FHB and found that they were a relatively diverse group that was genetically distinct from current Midwest six-rowed varieties. Despite the apparent diversity, genetic mapping studies of FHB in barley have

identified many of the same QTL regions (de la Peña et al. 1999; Zhu et al. 1999; Ma et al. 2000; Mesfin et al. 2003). Conducting genetic studies to investigate all of the potential sources of resistance would be laborious and expensive; therefore, methods are needed that will identify those sources of resistance that are most likely to harbor resistance alleles at new loci.

Selective genotyping is an alternative approach to linkage mapping that reduces the number of individuals that must be genotyped to detect a QTL by using only individuals at the extremes of the distribution for the quantitative trait of interest (Lebowitz et al. 1987). Darvasi and Soller (1992) showed that genotyping individuals only from the upper and lower 25% tails of the phenotypic distribution was nearly as efficient in detecting QTLs as genotyping the entire population. However, selective genotyping has not been widely adopted, possibly due to distorted segregation in the production of linkage maps (Matinez 1996), the biased estimates of the effects of linked QTLs (Lin and Ritland 1996), and the constraint of being able to study only a single trait at a time. Despite these limitations, selective genotyping has been used to conduct QTL analyses (Foolad et al. 1997) and confirm the results of bulked segregant analyses (Prasad et al. 1999; Roy et al. 1999).

Selective genotyping could also be a powerful tool to determine if novel genes are responsible for phenotypic variation in a population. Selective genotyping in unmapped populations—using markers discovered in mapping populations that are linked to QTLs for a trait of interest—could be used to determine if allelic variation at known loci is associated with phenotypic variation for the trait. This approach, if employed in breeding populations, where large amounts of phenotypic data are collected routinely, could provide important information on the genetic diversity for specific traits, and guide researchers to focus on those populations that offer the greatest potential for discovering new genes.

The serious nature of the current FHB epidemic requires an accelerated and efficient approach to combining multiple genes for resistance in new barley cultivars. Future breeding and genetics studies need to build on the current understanding of the genetics of FHB resistance and identify new genes for resistance that are not associated with plant form. Genetic studies in advanced breeding populations, in which segregation for undesirable morphological traits has been reduced or eliminated, should enable detection of QTLs for disease resistance that are not coincident with morphological traits that are correlated to FHB resistance. To test this approach, we investigated nine advanced breeding populations tracing to five unmapped sources of FHB resistance to determine if they contained new FHB resistance genes. This research entailed three specific objectives: (1) describe the genetic diversity among potential sources of FHB resistance relative to sources that have been used in previous mapping studies, (2) compare the results of selective genotyping to traditional QTL mapping of FHB using the previously mapped ‘Chevron’ × ‘M69’ population, and (3) use selective genotyping to determine whether markers linked to known FHB QTLs explain significant variation for FHB resistance in breeding populations derived from new sources of resistance.

Table 1 Cultivars and elite breeding lines used in genetic diversity study

Cultivar or line	Origin	Spike type	Hull type	Susceptible/resistant
Chevron	Switzerland	6-Row	Covered	Resistant
Frederickson	Japan	2-Row	Covered	Resistant
Gobernadora	Mexico	2-Row	Covered	Resistant
AC Oxbow	Canada	2-Row	Covered	Resistant
Atahualpa	Ecuador	2-Row	Hull-less	Resistant
CIho4196	China	2-Row	Covered	Resistant
HOR211	Ukraine	6-Row	Hull-less	Resistant
Kitchin	USA	2-Row	Covered	Resistant
PFC88209	Brazil	6-Row	Covered	Resistant
Zhedar#1	China	2-Row	Covered	Resistant
Excel	USA	6-Row	Covered	Susceptible
Foster	USA	6-Row	Covered	Susceptible
Lacey	USA	6-Row	Covered	Susceptible
M100	USA	6-Row	Covered	Susceptible
M104	USA	6-Row	Covered	Susceptible
M105	USA	6-Row	Covered	Susceptible
M81	USA	6-Row	Covered	Susceptible
M84	USA	6-Row	Covered	Susceptible
MNBrite	USA	6-Row	Covered	Susceptible[a]
Robust	USA	6-Row	Covered	Susceptible
Stander	USA	6-Row	Covered	Susceptible

[a]Has partial resistance to Fusarium head blight (FHB) derived from ‘Chevron’ (Canci et al. 2003)

Materials and methods

Cultivars and advanced breeding populations

Twenty-one cultivars or elite breeding lines were used in the genetic diversity study and the development of breeding populations (Table 1). They included three FHB-resistant lines from previous QTL studies ('Chevron', 'Frederickson', and 'Gobernadora'), two FHB-resistant lines from ongoing QTL studies ('CIho4196' and 'Zhedar#1'), five FHB-resistant cultivars used to develop advanced breeding populations ('AC Oxbow', 'Atahualpa', 'HOR211', 'Kitchin', and 'PFC88209') and 11 susceptible cultivars or elite breeding lines.

For the selective genotyping study, we evaluated nine populations developed from crosses between elite malting breeding lines and FHB-resistant breeding lines that trace back to mapped ('Frederickson') and unmapped ('AC Oxbow', 'Atahualpa', 'HOR211', 'PFC88209', and 'Zhedar#1') sources of resistance. The populations were advanced by single-seed decent to the F_4 generation and bulked one generation to produce seed for field testing. Population sizes ranged from 38 to 100 $F_{4:5}$ lines (Table 2). Populations were evaluated in Crookston, Morris, and St. Paul, Minn., USA from 2000 to 2002 (Table 2). Populations were planted in one-row plots, 1.8 m long, and spaced 30 cm apart in a randomized complete block design with two replications at each location. Data were collected for heading date (HD), FHB severity, and DON concentration.

Disease and mycotoxin assessments

Disease data were obtained by artificial inoculation as described by Mesfin et al. (2003). The nurseries in Crookston and Morris were inoculated by dispersing a grain-spawn inoculum consisting of maize (*Zea mays* L.) kernels colonized by 10–15 different *F. graminearum* isolates. The St. Paul nurseries were artificially inoculated with macroconidial suspensions, applied using backpack sprayers several days after heading. Heading date was quantified as the number of days after planting when 50% the heads in a plot extended halfway or more out of the boot. Nurseries were mist irrigated to promote disease. Mist irrigation at St. Paul began immediately after spray inoculations and continued for at least 12 days. For grain-spawn inoculations, mist irrigation started approximately 2 weeks before anthesis and continued until the hard-dough stage. Approximately 14 days after inoculation, FHB severity was visually estimated as the percentage of infected kernels using a sample of ten spikes per plot.

DON concentration was determined on harvested grain samples from between 10 and 12 genotypes with the highest or lowest FHB severity from each population. Samples were analyzed using a gas chromatography/mass spectrometry technique following the methodology of Tacke and Casper (1996).

Evaluation of selective genotyping on previously mapped population

To test the sensitivity of the selective genotyping approach, we evaluated a mapping population ('Chevron'/'M69'), using published QTL information for FHB severity and a complete set of marker genotype data (de la Peña et al. 1999). In the original 'Chevron'/'M69' study, ten QTL regions for FHB resistance were identified by composite interval mapping (CIM). Using the markers that flank the ten QTL peaks identified with CIM, we conducted single-marker regression with the entire population (101 lines) to test marker–trait associations and estimate percentage of the variation explained by the marker locus (R^2). We selected eight individuals from each phenotypic extreme (or tail) of the population, based on FHB severity in each of the environments tested. To detect the presence of a QTL, we conducted *t*-tests (described below) of the 'Chevron' marker–allele frequencies in the upper and lower tails of the population for each flanking marker of the ten FHB QTL regions reported by de la Peña et al. (1999).

Table 2 Nine advanced breeding populations derived from six Fusarium head blight (FHB)-resistant sources used in selective genotyping

Pedigree of resistant parent[a]	Population			Evaluation	
	Parents	Size	Name	Year	Location(s)[b]
Frederickson/Stander//M81	**MAS2-54**×Lacey	91	Pop 1	2001	CR, SP
Atahualpa/M81//M81	**FEG4-98**×Excel	38	Pop 2	2001	CR, SP
Atahualpa/M81//M81	**FEG4-98**×M104	44	Pop 3	2001	CR, MO
AC Oxbow/M100	**FEG14-119**×Lacey	64	Pop 4	2001	CR, SP
HOR211/Lacey//Lacey	–	100	Pop 5	2000	SP
HOR211/Lacey//Lacey	**FEG39-03**×Lacey	81	Pop 6	2002	SP, CR
PFC88209/Lacey	**FEG29-94**×M96-106	60	Pop 7	2002	SP, CR
Zhedar#1/Stander//Foster/3/M84	**FEG2-26**/Lacey	73	Pop 8	2001	CR, SP
Atahualpa/M81//M81	**FEG4-98**×FEG2-26	63	Pop 9	2001	CR, SP
Zhedar#1/Stander//Foster/3/M84					

[a]Resistant parent is in *boldface*
[b]*CR* Crookston, Minn.; *SP* St. Paul, Minn.; *MO* Morris, Minn.

Screening breeding populations using selection genotyping

Individuals at the phenotypic extremes for FHB severity were used for selective genotyping analysis from nine advanced breeding populations. We selected both the resistant and susceptible tails for all populations except population (Pop) 5, where we selected only the resistant tail. Selection was based on one-location means in 2000 and two-location means for populations evaluated in 2001 and 2002. Each tail represented between 8% and 21% of the total population. In 2001 and 2002, 10 to 12 individuals were originally selected on the basis of FHB severity for each phenotypic extreme and were narrowed to eight individuals, each based on selection for low DON concentration. To determine if HD were affected by selection for FHB severity, we conducted one-tailed *t*-tests to compare the population mean HD to the high and low selected tails of the population.

DNA markers

Barley genomic DNA was isolated as described by Sambrook et al. (1989). PCR amplification was done using the procedures of Ramsay et al. (2000), and PCR products were separated on a LI-COR IR^2 DNA Analyzer (LI-COR, Lincoln, Neb., USA). Eighty SSR markers were screened on the 21 cultivars or elite breeding lines to assess genetic diversity. These SSR markers were selected based on linkage to previously identified FHB QTL (de la Peña et al. 1999; Zhu et al. 1999; Ma et al. 2000; Mesfin et al. 2003) and even distribution throughout the barley genome (Ramsay et al. 2000; Macaulay et al. 2001). For selective genotyping, 14 SSR markers linked to the six most significant QTL presently known for FHB resistance in barley were evaluated on the phenotypic extremes of the advanced breeding populations (Table 3).

Data analysis

From SSR marker data, a similarity matrix was calculated using the simple matching coefficient (Sokal and Sneeth 1963) in the NTSYSpc, version 2.1, software program (Rohlf 1993). NTSYS was used to perform a sequential, agglomerative, hierarchical, nested cluster analysis. The cluster diagram was constructed using the unweighted pair-group method, arithmetic average algorithm.

Using Proc GLM (SAS Institute 1985), analyses of variance were performed for each advanced breeding population with lines and replications as sources of variation for individual environments. In addition, we conducted analyses across environments to determine if there were significant line × environment interaction. The FHB severity value for each line was calculated as the mean across environments when disease was assessed in more than one environment.

To determine if allelic variation at selected marker loci were associated with phenotypic variation for FHB severity in the breeding population, we conducted *t*-tests comparing the frequency of the resistant-parent allele for each SSR marker between the phenotypic extremes of each population. The null hypothesis assumes that marker allele frequencies are P=0.50 in either tail; therefore, a *t*-test between the marker allele frequency in the resistant tail (P_r) and the susceptible tail (P_s) was conducted by calculating:

Table 3 Markers associated with six quantitative trait loci (QTLs) for FHB resistance previously identified in barley and the other traits associated with that locus

Region	Marker name	Chromosome no.	BIN[a]	Mapping populations	Other traits[b]
QTL1	ABC306	2	8	Chevron/M69, Chevron/Stander	DON, HD
	Bmac0093	2	8	Frederickson/Stander, Stander/MNS93, M92-299/M81	DON, KD, HD
QTL2	MWG503	2	11	Gobernadora/CMB643	LFS, S/I, 2R/6R
	Bmag0125	2	10	Frederickson/Stander, M92-299/M81	DON, KD
QTL3	ABC252	2	13	Frederickson/Stander	DON
	Ksuf15	2	13	Chevron/M69	
	EBmac0415	2	13	Frederickson/Stander	DON
QTL4	Bmac0067	3	6	Frederickson/Stander	
	ABC261B	3	7	Gobernadora/CMB643	
QTL5	ABG452	5	7	Chevron/M69, Chevron/Stander	DON
	HVM020	5	7	Frederickson/Stander	
QTL6	Bmag0173	6	6	Chevron/M69	KD, HD
	MWG2227a	6	6	Frederickson/Stander	
	HVM065	6	6	Stander/MNS93	KD
	Bmag0807	6	6	M92-299/M81	KD, HD

[a]Location on barley 'Steptoe' × 'Morex' BIN map (http://barleygenomics.wsu.edu/)
[b]*LFS* Lateral floret size, *S/I* seeds per inflorescence, *DON* deoxynivalenol concentration, *HD* heading date, *KD* kernel discoloration, *2R/6R* two-rowed or six-rowed spike morphology

$$t = \frac{P_r - P_s}{\sqrt{P(1-P)/2n_r + P(1-P)/2n_s}},$$

where n_r is the number of individuals in the resistant tail and n_s is the number of individuals in the susceptible tail. We used a significance level of $P<0.10$ for detection of variation associated with a QTL region.

Results and discussion

Genetic diversity of FHB-resistant sources

Genetic diversity analysis, based on SSR markers distributed across the genome, revealed low similarity (maximum 41%) between resistant and susceptible lines (Fig. 1). However, susceptible cultivars and elite breeding lines developed at the University of Minnesota exhibited more than 78% similarity. Overall, the genotypes clustered into two groups that were 19% similar to each other. Group 1 consisted of seven resistant genotypes: 'Atahualpa', 'Kitchin', 'Frederickson', 'Zhedar#1', 'AC Oxbow', 'Chevron', and 'Gobernadora'. Within this group, 'Frederickson' and 'Zhedar#1' were the most alike (93% similar), while other FHB resistance sources were less than 57% similar. 'Atahualpa' and 'Kitchin' were the most diverse sources within group 1, showing 22% similarity to the rest of the cluster. The second group consisted of the 11 susceptible cultivars or elite breeding lines, and three resistant sources ('CIho4196', 'PFC88209', and 'HOR211'). Resistant sources within group 2 were only 33% similar to susceptible lines. 'CIho4196' and 'PFC88209' were 51% similar to each other; 'HOR211' was the most unique source within group 2, showing 25% similarity to the rest of the cluster. This suggests that (with the exception of 'Frederickson' and 'Zheddar#1') the FHB-resistant sources investigated in this study were relatively diverse and could carry different genes for FHB resistance. A recent study found that many of the FHB QTL identified in a population with 'Zheddar#2', a cultivar related to 'Zheddar#1', were coincident with those found in a mapping study with 'Frederickson' (Dahleen et al. 2003; Mesfin et al. 2003).

Evaluation of selective genotyping on previously mapped population

Using single-marker QTL analysis, we detected seven of the ten QTL regions for FHB resistance identified in the original study using CIM, with r^2 values ranging from 6% to 29% (Table 4). Subsequently, selective genotyping identified three of these seven QTL regions for FHB resistance. This indicates that selective genotyping can be used to detect major QTL regions. Ayoub and Mather (2002) conducted a similar analysis with grain and malting quality traits in three barley populations developed by the North American Barley Genome Project and also found they were able to detect most major QTLs. They also detected many spurious QTLs and concluded that it was necessary to validate QTL identified using selective genotyping.

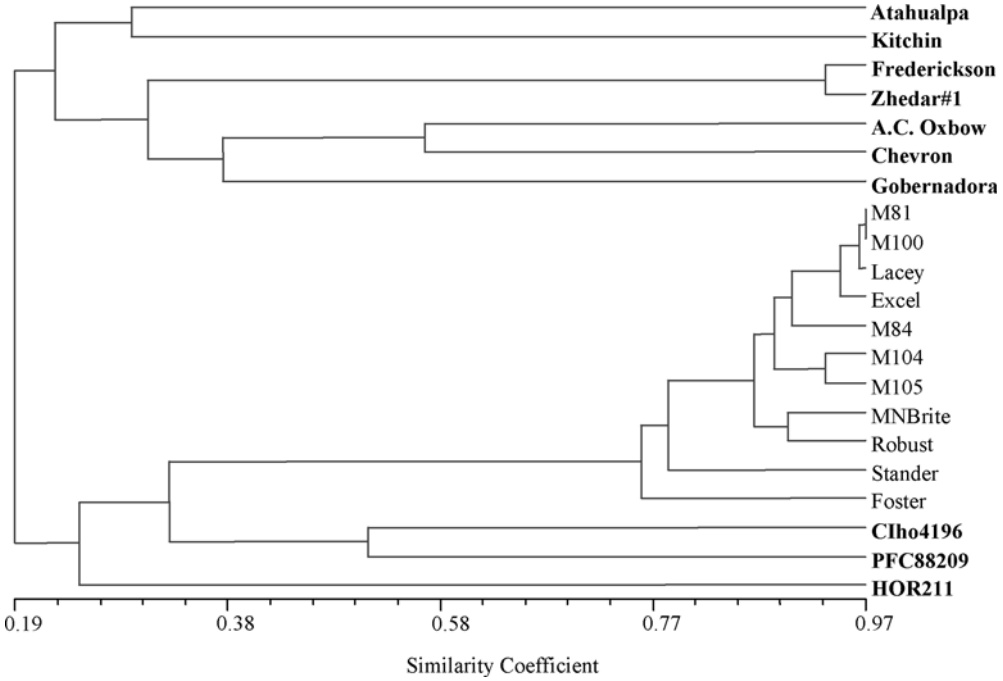

Fig. 1 Sequential, agglomerative, hierarchical, nested cluster analysis showing genetic diversity among the ten Fusarium head blight-resistant lines (*boldface*) and 11 susceptible cultivars and elite breeding lines

Table 4 Comparison of composite interval mapping (*CIM*), marker-by-marker regression (*MMR*) and selective genotyping (*SG*) for detecting QTL for FHB resistance, using the 'Chevron'/'M69' population

Chromosome no.	Interval[b]	Environment	CIM[a]		MMR		SG
			LOD	r^2 [c]	*P*	r^2	*t*
1	MWG530	HA-1997	8.13	10.0	0.375	NA[d]	0.31
	MWG564				0.0167	6.1	0.86
1	MWG836	StP-1997	4.33	10.0	<0.001	13.0	−2.27*
	ABG476				0.0770	NA	−0.50
1	ABG476	CR-1997	3.97	0.6	0.738	NA	0.50
	BCD98b				0.291	NA	−0.80
2	ABG459	CR-1997	4.63	7.2	NA	NA	NA
	MWG520a				<0.001	26.5	3.50*
2	MWG887	CR-1997	5.08	13.5	<0.001	23.4	3.15*
	ABC306				<0.001	29.4	3.00*
2	KSUF15	CR-1997	3.52	16.0	0.004	8.6	−1.88
	ABG497a				NA	NA	NA
3	ABC171	HA-1997	5.96	8.4	0.368	NA	0.31
	CDO395				0.212	NA	1.25
4	ABG705b	HA-1997	3.62	4.4	NA	NA	NA
	ABC303				0.053	NA	1.00
5	ABG452	HA-1997	5.26	7.1	0.258	NA	0.75
	ABG74				0.0186	5.8	1.00
7	CDO400	HA-1997	6.86	8.6	0.0805	NA	0.50
	CDO59b				0.0209	5.5	1.97

[a]CIM analysis presented by de la Peña et al. (1999)
[b]Markers flanking the peak of the LOD scan
[c]Percentage phenotypic variance explained by QTL
[d]*NA* Not applicable (dominant marker)
Significance level: *$P<0.05$

Selective genotyping in advanced breeding populations

Significant line differences ($P<0.05$) were observed for FHB severity in eight of the nine advanced breeding populations (Table 5). Pop 8, which was not significant ($P=0.13$) was included in the selective genotyping analysis, but should be interpreted cautiously. Line-by-environment interactions were significant for FHB severity in four populations. Even in those populations where there was significant line-by-environment interaction, the lines selected at the extremes for selective genotyping were consistent across environments. In general, late heading was not associated with disease resistance. While there was significant variation for HD in all the populations (Table 5), the resistant and susceptible tails differed for HD in only four of the populations (Table 6). In these four populations, the resistant tail was slightly earlier than the susceptible tail in two populations. This is contrary to what has been observed in other studies where resistance is linked to late heading and thought to be due to the host escaping infection by the pathogen, which can occur only when the head has emerged from the boot. Two populations (7 and 9) showed the familiar association between lower disease and late heading (Table 6). All of the sources of resistance included in this study were late heading (data not shown) and the reduced variance for HD in the advanced breeding population reflects the selection imposed against late heading in the breeding program.

Selective genotyping analysis indicated that SSR markers linked to the six previously identified FHB QTL regions explained some variation for resistance in six of the nine advanced breeding populations (Table 7). However, none of the six FHB QTL regions were associated with FHB severity in populations derived from 'PFC88209' or 'Hor211', suggesting that these sources contain novel genes for FHB resistance. For the other

Table 5 Mean square and heritability (*h*) values for nine breeding populations evaluated for FHB severity and heading date (*HD*)

Population	FHB					HD				
	Mean square				*h*	Mean square				*h*
	Env (*E*)	Reps	Lines (*L*)	*L*×*E*		Env (*E*)	Reps	Lines (*L*)	*L*×*E*	
1	27,009**	153*	145**	85**	0.59	3,917**	3.2*	536**	71**	0.99
2	16,570**	4	109**	77	0.35	1,718**	0.2	73**	17	0.99
3	21,033**	175	244**	179*	0.42	3.3*	1.3	2.2**	0.5	0.57
4	29,606**	241*	84**	34	0.30	2,672	4.0*	3.0**	0.4	0.76
5	–	57*	12*	–	0.17	–	0.1	2.6**	–	0.58
6	1251**	64	45*	25	0.24	7666**	2.7	3.7**	1.3	0.64
7	660**	133**	25**	21**	0.51	8941**	3.7*	17.8**	2.0**	0.89
8	42,359**	1,336**	90	80	0.11	3,175**	2.0**	2.4**	0.6**	0.78
9	3,454**	89**	43**	34**	0.41	2,063	3.0**	3.6**	0.5*	0.85

Significance levels: *$P<0.05$, **$P<0.01$

Table 6 FHB severity means, HD means, and *t*-test results for HD from the resistant and susceptible tails of the seven advanced breeding populations used in selective genotyping

Resistant source	Population name	Mean of FHB severity (%)			Mean of HD (number of days)			P^a
		Population (mean ±SD)	Resistant tail	Susceptible tail	Population (mean ±SD)	Resistant tail	Susceptible tail	
Frederickson	Pop 1	15.1±6.1	7.60	26.3	24.5±1.2	24.2	24.9	NS
Atahualpa	Pop 2	17.4±5.2	11.4	24.5	22.6±0.7	22.5	22.4	NS
	Pop 3	23.2±7.8	14.4	34.1	26.6±0.7	26.2	27.0	0.010
AC Oxbow	Pop 4	21.9±4.6	15.4	29.6	22.5±0.9	23.0	22.4	NS
HOR211	Pop 5	3.95±2.7	1.25	NA[b]	21.3±1.2	20.2	NA	NA
	Pop 6	10.2±5.0	4.3	20.5	21.9±1.2	23.8	25.6	NS
PFC88209	Pop 7	7.2±2.7	3.6	10.6	27.9±3.4	30.8	26.5	0.013
Zhedar#1	Pop 8	16.8±4.8	7.6	26.3	24.7±0.8	24.0	25.1	0.019
Atahualpa and Zhedar#1	Pop 9	8.67±3.3	4.41	14.0	23.8±1.0	24.7	22.8	0.002

[a] *P*-value for *t*-test comparing HD for the resistant and susceptible tails
[b] *NA* Not applicable, *NS* not significant
Significance level for one-tailed *t*-test: **P*<0.05

Table 7 Association of SSR markers linked to six previously identified FHB QTL with FHB severity, based on *t*-tests of the frequency of the resistant-parent allele in the resistant and susceptible tails of nine breeding populations

QTL (cM)[a]	Marker	Resistant sources								
		Frederickson	Atahualpa		AC Oxbow	HOR211		PFC88209	Zheddar#1	Atahualpa and Zhedar#1
		Pop 1	Pop 2	Pop 3	Pop 4	Pop 5	Pop 6	Pop 7	Pop 8	Pop 9
QTL1										
44	Bmag0140	F[b]	NS[d]	F	F	NS	F	F	NS	NS
50	Bmac0093	F	F	F	F	NS	F	F	F	NP[c]
52	EBmac0521	F	F	NS	F	NS	NS	F	NS	NS
QTL2										
63	Bmag0125	F	F	F	NS	NS	F	NS	F	NP
QTL3										
103	HVM54	NS	F	F	3.18***	NS	F	NS	2.12*	NS
105	EBmac0415	NP	F	F	NS	NP	F	NS	2.12*	NS
QTL4										
55	Bmac0209	NP	−2.12*	NP	F	NS	F	F	F	−3.18***
55	Bmag0905	NS	−2.48**	NS	F	NS	F	F	F	−3.18***
60	Bmag0138	NS	−2.48**	NS	F	NS	F	F	F	−3.18***
QTL5										
62	Bmag0770	F	F	F	−1.77*	NS	NS	NS	F	NP
66	Bmag0345	F	F	F	−1.77*	NS	NS	F	F	NP
70	Bmag0347	F	F	F	NP	NS	NS	NS	F	NP
QTL6										
84	Bmag0807	2.83**	2.48**	NS	F	NS	NS	NS	F	F
109	EBmac0602	2.83**	NS	2.48**	F	NS	NS	NS	F	NS

[a] CentiMorgan position as reported in Ramsay et al. (2000)
[b] Marker locus is fixed for a susceptible parent allele
[c] Not polymorphic between resistant source and susceptible parent
[d] *NS* No significant association (p>0.10)
Significance levels: **P*<0.10, ***P*<0.05, ****P*<0.01

sources of resistance, at least one of the QTL regions was associated with resistance.

At some loci, the association of the QTL and resistance could not be tested because the marker(s) was/were not polymorphic between the resistant source and susceptible parents. In other cases, the selection imposed during breeding of advanced resistant lines resulted in the fixation of loci for the susceptible parent allele. In the former case,

it is not possible to test whether segregation at that QTL is affecting disease resistance. In the latter, fixation of the susceptible allele permits us to postulate that segregation at that locus is not responsible for the phenotypic variation in disease resistance observed in the population.

Fixation of susceptible parent alleles was more common for markers at QTL1 and QTL2 than at the other loci, and neither of these regions was associated with FHB severity in the breeding populations (Table 7). The QTL1 region is associated with HD (*eam6*) and QTL2 is associated with spike morphology (*vrs1*) (Table 2). Breeding for six-rowed malting barley imposes selection at both of these loci for early heading (relative to the late heading-resistant sources) and six-rowed spike morphology. The QTL3 region was associated with variation for resistance in populations derived from 'AC Oxbow' and 'Zheddar#1'. For both QTL4 and QTL5, the frequency of the resistant-parent allele was actually lower in the resistant tail for several populations, indicating that the susceptible parent contributed the allele conditioning higher levels of resistance. In the case of Pop 9, both parents were resistant and the negative *t*-value resulted from the association of the 'Zheddar#1' allele with resistance. In all the other cases, the resistant-parent allele was associated with lower FHB severity. The QTL6 region was associated with FHB severity in populations derived from 'Frederickson' and 'Atahualpa'. Pop 1 was derived from a previously mapped FHB-resistant source (Frederickson). The QTL6 region is coincident with a minor QTL for FHB that was detected in the 'Frederickson'/'Stander' population (Mesfin et al. 2003).

Variation for resistance in the population utilizing the sources of resistance 'Atahualpa' and 'Zhedar#1' (Pop 9) may be due to an association with HD. Significant differences were observed for HD in *t*-tests of the resistant and susceptible tails (Table 6). The three markers in the QTL4 region were associated with HD in this population, based on *t*-tests (data not shown). Given the potential that late heading can result in disease escape, we cannot be certain that FHB resistance at this locus is not the result of pleiotropy.

Plant breeding programs should effectively use the wealth of information derived from QTL mapping studies to develop new cultivars. To date, QTL information has been used primarily in marker-assisted introgression of one or more desirable alleles into an elite background or through marker-based recurrent selection. We have shown that it is possible to use previously generated QTL information for FHB resistance to investigate the genetics of resistance in breeding populations derived from resistant sources that have not been studied. This should lead to more efficient exploration of genetic diversity and avoid situations, like that in wheat, where the same major QTL for FHB on wheat chromosome 3BS has been identified as the primary determinant of resistance in at least eight separate mapping populations (Bai et al. 1999; Waldron et al. 1999; Anderson et al. 2001; Gupta et al. 2001; McGowan et al. 2001; Bowen 2002; Buerstmayr et al. 2002; Shen et al. 2003).

Selective genotyping can be easily incorporated into standard breeding schemes. It requires relatively little genotyping. To determine if nine populations were segregating for known FHB resistance genes, only 14 markers were screened on 136 individuals. Because the phenotypic data were collected as a part of routine screening protocol, we were able to select populations in which we had good quality data before investing in genotyping. Often in standard mapping studies the genetic maps are constructed first, and QTL mapping is conducted after sufficient phenotypic data have been collected. In the case of diseases like FHB, entire disease nurseries can be lost due to weather conditions that lead to either too much or too little disease. Selective genotyping conducted in advanced breeding populations also reduces the probability of identifying major disease resistance QTLs that are coincident with QTLs for undesirable or confounding agronomic and morphological traits. This feature is particularly important for FHB resistance in barley, which has been associated with late heading and numerous other traits (Steffenson 2002).

We have identified variation for FHB resistance in our breeding populations that is not likely due to loci that have been previously identified in QTL mapping studies. The obvious next step is to map the locations of these new resistance QTLs. Recent studies have suggested that mapping QTLs in breeding populations, similar to those used in this study, should be possible using association genetics (Jannick and Walsh 2002). Many of the same advantages for using breeding populations in selective genotyping are relevant to association genetics studies: relevant germplasm, taking advantage of phenotypic data that is routinely collected in breeding, and the potential of large population sizes if the study is extended to many breeding lines over a period of years. Leveraging the wealth of phenotypic data from breeding programs to investigate QTL has tremendous potential.

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