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Quantitative trait loci associated with resistance to Fusarium head blight and kernel discoloration in barley

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Abstract Resistance to Fusarium head blight (FHB), deoxynivalenol (DON) accumulation, and kernel discoloration (KD) in barley are difficult traits to introgress into elite varieties because current screening methods are laborious and disease levels are strongly influenced by environment. To improve breeding strategies directed toward enhancing these traits, we identified genomic regions containing quantitative trait loci (QTLs) associated with resistance to FHB, DON accumulation, and KD in a breeding population of $F_{4:7}$ lines using restriction fragment length polymorphic (RFLP) markers. We evaluated 101 $F_{4:7}$ lines, derived from a cross between the cultivar Chevron and an elite breeding line, M69, for each of the traits in three or four environments. We used 94 previously mapped RFLP markers to create a linkage map. Using composite interval mapping, we identified 10, 11, and 4 QTLs associated with resistance to FHB, DON accumulation, and KD, respectively. Markers flanking these QTLs should be useful for introgressing resistance to FHB, DON accumulation, and KD into elite barley cultivars.

Key words Fusarium head blight · Scab · Kernel discoloration · Black point · Kernel blight · Deoxynivalenol (DON) · RFLP · Barley

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Introduction

Kernel diseases are major constraints in barley (*Hordeum vulgare* L.) production. Fusarium head blight (FHB), caused primarily by *Fusarium graminearum* Schwabe, is currently the most destructive disease of barley in the midwestern United States. Losses to midwest barley growers due to FHB during the period of 1993–1996 were estimated at 129 million bushels (McMullen et al. 1997). A related disease of lesser importance is kernel discoloration (KD), also known as black point or kernel blight. This disease results in a black to dark-brown discoloring of the palea and lemma and is caused by several organisms including *Bipolaris sorokiniana* (Sacc.) Shoemaker, *Alternaria* spp., and *F. graminearum* (Mathre 1997).

Losses due to FHB are mainly attributed to production of the mycotoxin, deoxynivalenol (DON), in infected grain. The malting and brewing industry rejects barley kernels containing detectable levels of DON to avoid a phenomenon known as beer "gushing" and to alleviate concerns about public perception of barley containing mycotoxin. In addition, the presence of DON in animal feed is associated with mycotoxicoses of swine and cattle (Mirocha et al. 1976). Both FHB and KD cause discoloration which can result in reduced market value and in severe cases, downgrading from malting to feed quality.

Six-row barley cultivars currently grown in the midwestern US are susceptible to FHB and KD. Chevron (CI 1111), a six-row barley variety introduced from Switzerland in 1914, exhibits resistance to FHB and KD in field tests (Immer and Christensen 1943; Banttari et al. 1975; Miles et al. 1987). However, it has poor agronomic characteristics that include weak straw, late heading, tall plants, thin kernels, and low yield. Chevron has been used in breeding programs as a source of resistance to FHB, DON accumulation, and KD. To date, selection for resistant genotypes has been difficult due, in part, to the lack of effective screening methods and large environmental effects on disease development. In addition, breeding efforts with Chevron indicate that resistance is quantitatively inherited.

Molecular markers are useful for estimating the number and location of genes involved in quantitative traits. They have been utilized successfully to identify chromosomal locations of genes for quantitative traits in many crop plants (Dudley 1993; Patterson 1995). There are several saturated linkage maps of restriction fragment length polymorphism (RFLP), isozyme, and morphological markers developed for barley (Heun et al. 1991; Graner et al. 1991; Kleinhofs et al. 1993; Becker et al. 1995; Langridge et al. 1995, Sherman et al. 1995). These maps have been used to identify quantitative trait loci (QTLs) for many agronomic and quality traits (see for examples Heun 1992; Hayes et al. 1993; Tinker et al. 1996) and provide a rich resource for studying the genetics of kernel diseases in barley.

Identifying molecular markers associated with resistance to FHB, DON accumulation, and KD for eventual use in marker-assisted selection (MAS) is especially compelling considering the influence of environment on the development of these diseases. In this paper, we describe our efforts to understand the genetic basis of resistance to FHB, DON accumulation, and KD.

Materials and methods

Parents and mapping population

The mapping population consisted of 101 $F_{4:7}$ families developed from a cross between the cultivar Chevron and elite breeding line M69. The lines in the mapping population traced to F_4 plants obtained by single-seed descent. After the F_4 generation, the lines were bulk-harvested and evaluated for heading date (HD), height (HT) and disease resistance in the $F_{4:7}$ and $F_{4:8}$ generations. 'Chevron' is a potential source for resistance to FHB and KD (Banttari et al. 1975; Rassmusson 1997) and presumably resistance to DON accumulation. M69 is susceptible to FHB and KD and is an advanced generation line from the University of Minnesota barley breeding program. Chevron is taller, later maturing, and more susceptible to lodging than M69.

The mapping population was evaluated at the University of Minnesota Agricultural Experiment Stations at Crookston, St. Paul, and Morris in 1995, 1996, and 1997; North Dakota State University Research Station at Fargo in 1997; and in Hangzhou, China in 1997. We obtained data for FHB, KD, DON, maturity, and height from some but not all the nurseries. Insufficient FHB in some cases and excessive FHB in others precluded the use of data from all nurseries, and extreme lodging made other nurseries unusable. We seeded the mapping population in one-row plots, 1.8–2.4 m long, spaced 30 cm apart in a randomized complete block design with two replications at each location unless otherwise noted.

Disease and mycotoxin assessments

We obtained disease data on FHB at Crookston in 1995 and 1997, St. Paul in 1997, and Hangzhou in 1997. The Crookston nursery in 1995 was naturally infected (non-inoculated), whereas the nursery in 1997 was artificially inoculated by spreading maize seed colonized with 15 different *F. graminearum* isolates collected in the Red River Valley of Minnesota in 1996. In the 1997 nurseries, we used a misting irrigation system 2 weeks before anthesis for a period of 1 month. On days without rain, we misted the plots for 15 min h^{-1} for a 10-h period at night. The Hangzhou nursery was inoculated by spreading maize and barley grain colonized by local isolates of *F. graminearum* (5 g/plot) at weekly intervals for 4 consecutive weeks while maintaining high humidity with mist irrigation. In all nurseries, we assessed FHB severity by visually estimating percentage of infected kernels in each of 20 heads per plot 18–21 days after anthesis and calculating the plot mean.

Deoxynivalenol (DON) concentration was determined on harvested grain from Crookston in 1995, Fargo in 1997, and Hangzhou in 1997 following the methodology described by Tacke and Casper (1996). In 1997 we determined DON levels from a single replicate.

KD was scored on grain samples obtained from the FHB nurseries at Crookston and Morris in 1995. In addition, we scored KD in 1995 and 1997 on grain samples from a St. Paul KD nursery that was inoculated each day from anthesis through physiological maturity with 33 ml per plot conidial suspension of *B. sorokiniana* (5×10^4 conidia/ml). These plots were sprinkler-irrigated for 15–30 min twice a day, except when it rained, from anthesis to harvest. We assessed KD severity using a 1–5 scale, where 1 was the brightest and 5 was the most discolored grain as described by Miles et al. (1987).

Agronomic trait evaluations

We quantified heading date (HD) at Crookston in 1995 and St. Paul in 1995, 1996, and 1997 as the number of days from planting to anthesis. We measured plant height (HT) at Crookston in 1995, St. Paul in 1996 and 1997, and the KD nursery at St. Paul in 1996 as the distance from the ground to the top of the spike, excluding awns.

DNA markers

Barley genomic DNA was isolated and DNA gel blot analysis performed as described in de la Pena et al. (1996). We screened more than 300 genomic and cDNA probes previously mapped in barley, wheat, rice, and oat (Gill et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993; Phillips and Vasil 1994) for RFLPs between 'Chevron' and M69 using the four restriction endonucleases *Eco*RI, *Eco*R V, *Hind*III, and *Dra*I.

Data analysis

Analysis of variance (ANOVA) was performed for a randomized complete block design with genotype as source of variation using Proc GLM (SAS Institute 1985). We calculated correlation coefficients between traits using Proc CORR.

Map construction and QTL analysis

We performed linkage analysis using MAPMAKER/EXP 3.0 (Lincoln et al. 1992), by evaluating the mapping population as an F_2 using two point analysis to identify linkage groups at a LOD score of 3.0. We used multipoint analysis to determine the order and interval distances for the markers in each linkage group and then scaled the interval distances between markers in the F_2 map to produce a recombinant inbred line (RIL) map using the equation $rf_{RIL}=rf_{F2}/(2-2rf_{F2})$, where rf_{F2} and rf_{RIL} are the recombination fractions for the F_2 generation and estimated recombination fractions for a recombinant inbred population, respectively. We assigned linkage groups to chromosomes based on published maps (Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993; Qi et al. 1996).

We employed composite interval mapping (CIM) to identify genomic regions associated with the traits evaluated using the software PLABQTL (Utz and Melchinger 1996). Markers to be used as cofactors were selected by a stepwise regression procedure with the default selection parameters. We used a threshold LOD value of 3.16 for detection of a QTL corresponding to experiment-wise and comparison-wise significant levels of P=0.05 and P=0.0007, respectively, as calculated by the chi-square approxi-

Table 1 Means and *P*-values for Fusarium head blight (FHB), kernel discoloration (KD), DON concentration (DN), heading date (HD), and plant height (HT) (*nd* not determined)

Trait	Environments	Chevron	M69	Population	Population range	*P*-value[a]
FHB[b]	Crookston 1995	17.7	40.7	21.6	4.0–58.0	<0.001
FHB	Hangzhou 1997	8.0	41.0	25.0	9.0–48.0	<0.001
FHB	Crookston 1997	11.0	38.0	17.0	3.0–59.0	<0.001
FHB	St. Paul 1997	2.2	6.5	4.2	1.1–10.3	0.003
KD[c]	Crookston 1995	1.1	4.2	2.1	1.0–4.0	<0.001
KD	Morris 1995	1.0	4.0	2.0	1.0–3.5	<0.001
KD	St. Paul 1995	1.1	4.0	2.2	1.0–4.5	<0.001
KD	St. Paul 1997	1.3	3.2	2.7	1.0–4.0	0.001
DN[d]	Crookston 1995	2.3	8.8	4.5	0.6–11.1	0.004
DN	Hangzhou 1997	2.0	7.2	5.3	0.4–17.9	nd
DN	Fargo 1997	1.8	18.6	8.6	0.2–26.5	nd
HD[e]	Crookston 1995	44.7	40.1	43.4	37.0–50.0	<0.001
HD	St. Paul 1995	56.3	52.7	55.5	50.0–63.0	<0.001
HD	St. Paul 1996	59.0	56.3	60.2	55.0–65.0	<0.001
HD	St. Paul 1997	59.0	50.0	51.0	45.0–66.0	<0.001
HT[f]	Crookston 1995	108	86	102	87–120	<0.001
HT	St. Paul 1996	107	95	104	92–119	<0.001
HT	St. Paul 1997	94	72	85	72–99	<0.001
HT	St. Paul KD 1997	100	85	96	86–110	<0.001

[a] Test for significant variation among the $F_{4:7}$ families
[b] FHB severity (% infected kernels)
[c] KD score on a 1–5 scale (1=no dislocation, 5=heavily discolored)
[d] ppm vomitoxin
[e] Days to anthesis
[f] Plant height in centimeters

mation suggested by Zeng (1994). We estimated the proportion of phenotypic variance explained by each QTL marker using the coefficient of determination (R^2), which is based on the partial correlation of a putative QTL with the trait adjusted for cofactors in the multi-locus model. For each QTL, we used the regression coefficient from the multi-locus model to estimate the additive effect of the 'Chevron' allele (alpha). Alpha values are relative to the mean phenotypic values presented in Table 1. For example, an alpha value of –5 for a FHB QTL indicates that a 'Chevron' allele at that QTL reduces the percentage of infected kernels by 5% in that environment.

Results

Inheritance of resistance

In this study, we observed less FHB and KD disease on 'Chevron' than M69 in the environments tested (Table 1). This is in agreement with previous research that identified 'Chevron' as having resistance to FHB and KD (Immer and Christensen 1943; Miles et al. 1987). 'Chevron' exhibited a range of resistance to FHB (2–18% diseased kernels) depending upon the environment, indicating that FHB resistance is environmentally influenced. In addition, 'Chevron' kernels accumulated lower levels of DON than M69 in all environments tested (Table 1). Taken together, these data suggest that 'Chevron' is a source of resistance to these kernel diseases.

The mean values of the mapping population for resistance to FHB, KD and DON accumulation were within the range of the parents with only one exception, although in general the means were closer to the 'Chevron' parent (Table 1). The range and distribution (data not shown) of phenotypes for the mapping population indicated that resistance to FHB, DON accumulation, and KD were inherited in a quantitative fashion. We observed significant differences ($P<0.01$) among the lines in the mapping population for all of the traits evaluated.

Correlation of traits

We observed significant correlations among environments for a particular trait and among different traits (Table 2). Resistance to FHB was positively correlated among environments in 2 out of 6 cases, indicating that there was genotype×environment (G×E) interaction. Resistance to DON accumulation was not correlated among the three environments, indicating that there was a significant G×E interaction for this trait. In contrast, resistance to kernel discoloration was significantly correlated among all four environments, indicating that there was little G×E interaction. Heading date and plant height were positively correlated among the four environments.

There were several interesting correlations among traits. FHB severity was positively correlated with KD severity in 7 of the 16 cases (Table 2). FHB and KD severity were both positively correlated to DON accumulation in Crookston 1995, but negatively correlated in Hangzhou 1997. In general, FHB and KD severity were negatively correlated to heading date and plant height, indicating that late heading and tall plants are associated with FHB and KD resistance. However, the relationships were more pronounced with heading date than with height. DON accumulation was negatively correlated to heading date and height in the Crookston 1995 environment and positively correlated to heading date in the Hangzhou 1997 environment. These correlations among resistance, taller plants and late heading are consistent with the possibility that the genes that control these traits are linked or have pleiotropic effects. To determine the position and effects of genes controlling these traits, we constructed a linkage map with RFLP markers that could be used to conduct a search of the genome for QTLs.

Table 2 Correlation coefficients among Fusarium head blight (FHB), kernel discoloration (KD), DON concentration (DN), heading date (HD), and plant height (HT)

	FHB-H97	FHB-C97	FHB-S97	KD-C95	KD-S95	KD-M95	KD-S97	DN-C95	DN-H97	DN-F97	HD-C95	HD-S95	HD-S96	HD-S97	HT-C95	HT-S96	HT-S97	HT-SKD97
FHB-C95[a]	ns	0.52**	ns	0.44**	0.23*	ns	0.41**	0.73**	–0.22*	ns	–0.52**	–0.51**	–0.51**	–0.52**	–0.46**	–0.23*	–0.22*	–0.27*
FHB-H97[b]		0.23*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	–0.22*	ns	ns	ns	ns
FHB-C97[c]			ns	0.46**	0.40**	ns	0.46**	0.53**	–0.20*	ns	–0.54**	–0.54**	–0.62**	–0.64**	ns	ns	ns	ns
FHB-S97[d]				ns	ns	0.22*	ns	ns	ns	ns	0.31**	0.24*	0.24*	0.26*	ns	0.30**	ns	ns
KD-C95					0.61**	0.54**	0.46**	0.62**	ns	0.24*	–0.47**	–0.51**	–0.44**	–0.52**	–0.27**	ns	–0.30**	–0.27*
KD-S95[e]						0.51**	0.43**	0.52**	–0.27**	0.27**	–0.35**	–0.42**	–0.38**	–0.38**	ns	ns	ns	ns
KD-M95[f]							0.24*	0.32**	ns	ns	ns	–0.21*	ns	–0.22*	ns	–0.23*	–0.21*	–0.31**
KD-S97								0.44**	ns	ns	–0.49**	–0.47**	–0.50**	–0.48**	–0.22*	ns	–0.24*	ns
DN-C95									ns	ns	–0.69**	–0.64**	–0.64**	–0.61**	–0.48**	–0.28*	–0.39**	–0.38**
DN-H97										ns	0.31**	0.28**	0.33**	0.26*	ns	ns	ns	ns
DN-F97[g]											ns	ns	ns	ns	ns	ns	ns	ns
HD-C95												0.90**	0.82**	0.79**	0.28**	0.20*	0.36**	ns
HD-S95													0.84**	0.83**	0.33**	ns	0.39**	ns
HD-S96[h]														0.83**	0.33**	ns	0.40**	0.23*
HD-S97															0.24*	ns	0.35**	0.20*
HT-C95																0.38**	0.58**	0.54**
HT-S97																	0.50**	0.64**
HT-SKD97[i]																		0.61**

**, * Significant at the 0.01 and 0.05 probability level, respectively; ns, not significant (P>0.05)
[a] Crookston 1995 [b] Hanzhou 1997 [c] Crookston 1997 [d] St. Paul 1997 [e] St. Paul 1995 [f] Morris 1995 [g] Fargo 1997 [h] St. Paul 1996 [i] St. Paul KD nursery 1997

Genetic markers and linkage map

Three hundred RFLP probes from barley, wheat, rice, and oat were screened for polymorphisms between 'Chevron' and M69. We identified 94 polymorpisms between the parents using 87 of these probes and used these to genotype the 101 $F_{4:7}$ lines. Thirty markers exhibited significant segregation distortion; the largest region was on chromosome 2 where the bias was toward 'Chevron'. The mean percentage 'Chevron' alleles for the entire population was 53%. The average percentage homozygosity was 81.5%, which is less than the expected 87.5% for an F_4 population.

We constructed maps with the marker data using the F_2 and RIL models in the MAPMAKER program. Since there was considerable heterozygosity in the marker data set, we decided to use the F_2 map and scale the recombination fractions to an RIL to approximate an F_4. We were able to group 94 RFLP markers to construct the genetic linkage map. Twenty-one linkage groups were identified and assigned to chromosomes based on published barley maps (Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993; Qi et al. 1996). Sixty six of the markers placed on this map (marked in bold-type in Fig. 1) are consistent with respect to placement and order on a chromosome with the consensus barley map (Qi et al. 1996) with three minor differences. On chromosome 1 the order of ABC253 and ABC305 is reversed; on chromosome 2, MWG950 and MWG557 are between MWG520 and MWG887, and on chromosome 5, the order of ABC152b and ABC164 are reversed. Of the remaining 28 markers, 18 have been previously placed in similar positions on other barley and wheat maps (GrainGenes search 6/98; A. Kleinhofs personal communication). Ten probes identified new loci (BCD98b, ABG497a, MWG2227a, ABG705b, BCD1087a, ABC706c, CDO542b, CDO59b, MWG503b, and ABG10b).

QTLs analysis

We identified QTLs associated with resistance to FHB, DON accumulation, and KD and heading date and height using CIM (Fig. 1). Ten QTLs were associated with FHB resistance. In 7 of the 10 cases, the allele from the resistant parent, Chevron, contributed FHB resistance (as indicated by the negative alpha values in Table 3). These

Fig 1. RFLP linkage map of the barley genome and LOD score scan for QTL associated with disease resistance, plant height, and heading date. The positions and names of RFLP markers are shown on the *horizontal axis*. Markers printed in *bold* are common to the integrated barley map (Qi et al. 1996). *Thick horizontal lines* represent linkage groups and *thin horizontal lines* chromosomes. Chromosomes are labeled *below* followed by the wheat chromosome designation in *parentheses*. The *horizontal dotted line* is the threshold for detection of a QTL (LOD=3.16). The scans for QTL for each environment are represented individually. Discontinuities in the LOD score scans correspond to *breaks* between linkage groups ▶

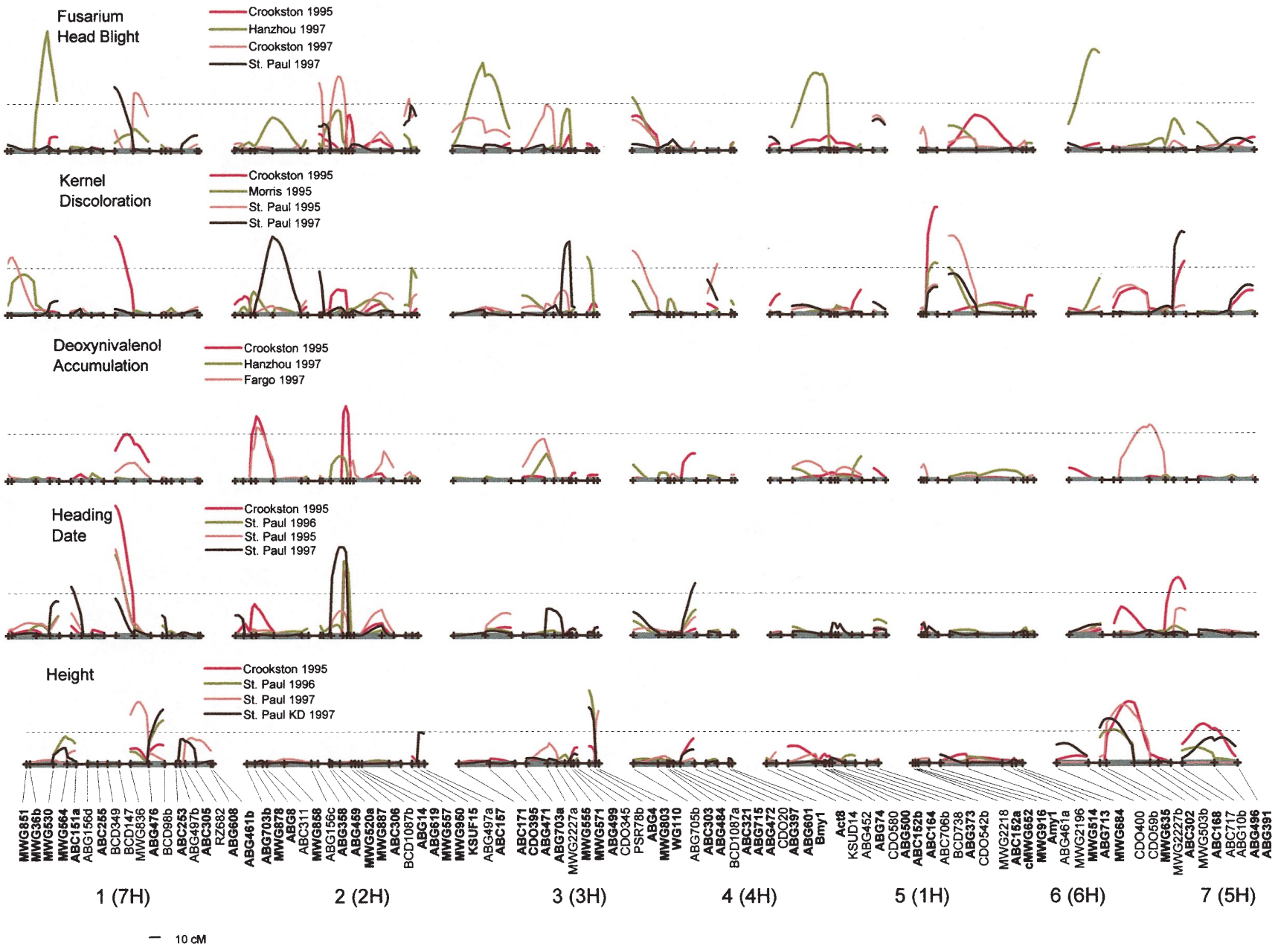
Fusarium Head Blight
Crookston 1995
Hanzhou 1997
Crookston 1997
St. Paul 1997
Kernel Discoloration
Crookston 1995
Morris 1995
St. Paul 1995
St. Paul 1997
Deoxynivalenol Accumulation
Crookston 1995
Hanzhou 1997
Fargo 1997
Heading Date
Crookston 1995
St. Paul 1996
St. Paul 1995
St. Paul 1997
Height
Crookston 1995
St. Paul 1996
St. Paul 1997
St. Paul KD 1997
MWG851 MWG36b MWG530 MWG664 ABC151a ABG156d ABC255 BCD349 BCD147 MWG836 ABG476 BCD98b ABC253 ABG497b ABC305 RZ682 ABG608 ABG461b ABG703b MWG878 ABG8 ABC311 MWG858 ABG156c ABG358 ABG469 MWG520a MWG887 ABC306 BCD1087b ABG14 ABG619 MWG557 MWG960 KSUF15 ABG497a ABC167 ABC171 CDO395 ABG471 ABG703a MWG2227a MWG555 MWG571 ABG499 CDO345 PSR78b ABG4 MWG803 WG110 ABG705b ABC303 ABG484 BCD1087a ABC321 ABG715 ABG472 CDO20 ABG397 ABG601 Bmy1 Act8 KSUD14 ABG452 ABG74 CDO580 ABG500 ABC152b ABC164 ABC706b BCD738 ABG373 CDO542b MWG2218 ABC152a cMWG652 MWG916 Amy1 ABG461a MWG2196 MWG514 ABG713 MWG684 CDO400 CDO59b MWG635 MWG2227b ABC302 MWG503b ABC168 ABC717 ABG10b ABG496 ABG391
1 (7H)
2 (2H)
3 (3H)
4 (4H)
5 (1H)
6 (6H)
7 (5H)
10 cM

Table 3 Quantitative trait loci associated with Fusarium head blight severity at three environments (*nd* not determined)

Chromosome/ linkage group	Marker interval[a]	Crookston 1997			St. Paul 1997			Hanzhou 1997		
		LOD	R-sqr[b]	Alpha[c]	LOD	R-sqr	Alpha	LOD	R-sqr	Alpha
1.1	MWG530-MWG564							8.13	10.0	–2.69
1.3	MWG836-ABG476				4.33	10.0	0.73			
1.3	ABG476-BCD98b	3.97	0.6	0.71						
2.2	ABG459-MWG520a	4.63	7.2	–2.98						
2.2	MWG887-ABC306	5.08	13.5	–5.63						
2.3	KSUF15-ABG497a	3.52	16.0	3.70						
3.1	ABC171-CD0395							5.96	8.4	–2.27
4.1	ABG705b-ABC303							3.62	4.4	–2.23
5.2	ABG452-ABG74							5.26	7.1	–2.49
7.1	CDO400-CDO59b							6.86	8.6	–2.61
	Multi-locus model	13.46	45.9		nd	nd		6.26	26.3	

[a] Markers flanking the peak of the LOD scan
[b] Percentage phenotypic variance explained by QTLs
[c] Effect of 'Chevron' allele on disease severity (percentage kernels infected) expressed as regression coefficient from multi-locus model

Table 4 Quantitative trait loci associated with kernel discoloration severity at four environments

Chromosome/ linkage group	Marker interval[a]	Crookston 1995			St. Paul 1995			Morris 1995			St. Paul 1997		
		LOD	R-sqr	Alpha	LOD	R-sqr	Alpha	LOD	R-sqr	Alpha	LOD	R-sqr	Alpha
1.1	MWG36b-MWG530				3.93	13.3	–0.36						
1.3	MWG836-ABG476	5.36	10.9	–0.26									
2.1	ABC311-MWG858										5.32	19.8	–0.31
2.3	ABG497a-ABC157							3.18	7.6	0.19			
3.2	ABG499-CDO345										4.96	6.2	0.18
3.3	ABG4-MWG803							3.91	7.7	–0.21			
4.1	ABG705b-ABC303				4.34	10.9	0.42						
4.2	CDO20-ABG397				3.45	5.5	–0.22						
6.1	cMWG652a-MWG916	7.29	23.0	–0.38				3.54	6.2	–0.19			
6.2	Amy1-ABG461a				5.36	25.3	–0.51	3.19	13.5	–0.26			
7.2	ABC168-ABC717	3.61	11.5	0.27							5.63	7.2	0.20
	Multi-locus model	9.99	36.6		9.84	36.2		9.34	34.7		7.62	29.3	

[a] Markers flanking the peak of the LOD scan
[b] Percentage phenotypic variance explained by QTLs
[c] Effect of 'Chevron' allele on disease severity (disease score 1–5) expressed as regression coefficient from multi-locus model

markers were located on chromosomes 1, 2, 3, 4, 5, and 7 (Fig. 1, Table 3). On chromosome 1, two adjacent marker intervals were identified in separate environments. In a region on chromosome 2, flanked by the markers MWG887 and ABG14, 1 QTL was detected in one of the environments and 2 additional QTLs were detected just below the LOD threshold at two other environments (LOD=2.78 and 2.46). The marker interval KSUF15-ABG497a was also detected at two environments, one below the LOD threshold (LOD=3.0). The other QTL were identified in a single environment.

Eleven QTLs for KD resistance were identified on all chromosomes except chromosome 5 (Fig. 1, Table 4). The allele contributed by Chevron was associated with KD resistance for 7 of the 11 QTLs identified. We identified 2 major QTLs for KD resistance on chromosome 6 that explained between 6% and 25% of the phenotypic variance; one linked to MWG916 was detected at two environments and one linked to Amy1 detected at two environments and at a third environment below the LOD threshold (LOD=2.7). A QTL located on chromosome 7, linked to ABC168, was also detected at two environments (Table 4).

We identified 4 QTLs for resistance to DON accumulation on chromosomes 1, 2, and 7 (Fig. 1, Table 5). The marker interval ABC311-MWG858 on chromosome 2 was associated with resistance to DON accumulation in two environments. For all 4 QTLs, 'Chevron' contributed the allele associated with resistance to DON accumulation.

We identified 7 QTLs each for heading date and 6 QTLs for height (Tables 6 and 7). 'Chevron' contributed alleles for late heading and tall plants for most of these loci. The heading date QTLs located on chromosome 1 (MWG836-ABG467) and the region flanked by MWG887 and ABG14 on chromosome 2 were detected in multiple environments, while the others were detected in a single environment. Three QTL for height on chromosomes 1, 3, and 7 were detected in multiple environments (Fig. 1).

Table 5 Quantitative trait loci associated with accumulation of deoxynivalenol in harvested grain at two environments

Chromosome/ linkage group	Marker interval[a]	Crookston 1995			Fargo 1997		
		LOD	R-sqr[b]	Alpha[c]	LOD	R-sqr	Alpha
1.3	MWG836-ABG476	3.17	17.0	–1.17			
2.1	ABC311-MWG858	4.36	23.8	–1.28	3.61	6.3	–1.52
2.2	ABC306-BCD1087b	5.02	25.8	–1.55			
7.2	ABC302-MWG503b				3.72	11.2	–1.93
	Multi-locus model	7.83	43.1		3.45	15.1	

[a] Markers flanking the peak of the LOD scan
[b] Percentage phenotypic variance explained by QTLs
[c] Effect of 'Chevron' allele on accumulation of DON in kernels (ppm) expressed as regression coefficient from multi-locus model

Table 6 Quantitative trait loci associated with heading date at four environments (*nd* not determined)

Chromosome/ linkage group	Marker interval[a]	Crookston 1995			St. Paul 1995			St. Paul 1996			St. Paul 1997		
		LOD	R-sqr[b]	Alpha[c]	LOD	R-sqr	Alpha	LOD	R-sqr	Alpha	LOD	R-sqr	Alpha
1.2	ABG156b-ABC255										3.64	5.5	–0.21
1.3	MWG836-ABG476	9.65	29.4	1.68	6.41	20.1	1.30	6.01	25.2	1.17			
2.2	MWG887-ABC306										6.56	22.6	1.55
2.2	ABC306-BCD1087b							5.53	22.7	1.35			
2.2	BCD1087b-ABG14	4.83	21.5	1.67									
4.1	ABG715-ABG472										3.85	15.9	–0.82
7.2	ABC168-ABG717	4.28	11.8	–0.96									
	Multi-locus model	12.33	43.0		nd	nd		10.57	38.2		7.98	30.5	

[a] Markers flanking the peak of the LOD scan
[b] Percentage phenotypic variance explained by QTLs
[c] Effect of 'Chevron' allele on heading date (d) expressed as regression coefficient from multi-locus model

Some of the markers on chromosome 2 were biased in the direction of Chevron and may have contributed to the inconsistencies in marker order between our map and the consensus map of Qi et al. (1996). In order to avoid problems in the QTL analysis that could be introduced by incorrect marker order, we analyzed the data for this chromosome using marker by marker regression. All of the QTLs identified by CIM interval mapping were also identified by regression (P<0.01) except the QTL for DON accumulation on chromosome 2 between ABC311-MWG858 (P=0.05).

Several regions of the genome were associated with more than one trait. On chromosome 1, the marker interval MWG836-ABG476 was associated with all five traits studied (Fig. 1). For this region, the 'Chevron' allele reduced DON accumulation and KD severity and increased heading date, plant height, and FHB severity. On chromosome 2, the Chevron allele for QTLs in the marker interval ABC311-MWG858 was associated with reduced KD severity, reduced DON accumulation, and reduced FHB severity (LOD=2.3). A second region on chromosome 2, ABG459-MWG520a, was also associated with Chevron-derived resistance to FHB and KD (LOD=2.96). Also, on chromosome 2 the Chevron allele for QTLs in the region spanning MWG887 to ABG619 was associated with reduced FHB severity, reduced DON accumulation, and late heading. QTLs for resistance to FHB and KD were associated with the marker interval ABG705b-ABC303 on chromosome 4. However, in this case the effect of the Chevron allele reduced FHB severity and increased KD severity. On chromosome 7, the QTL associated with KD severity was also associated with heading date.

Discussion

This is the first report of molecular markers used to identify QTLs for resistance to FHB and KD in barley. The results from this genetic study of Chevron-derived resistance to kernel diseases are in agreement with the practical experience of the Minnesota barley breeding program efforts to develop KD-resistant varieties. The cultivar MNBrite was derived from a cross involving Chevron followed by eight crossing cycles and phenotypic selection for KD resistance (Rasmusson et al. 1998). MNBrite has significant KD resistance and intermediate FHB resistance (Rasmusson unpublished results). Two regions on chromosome 2 (ABC311-MWG858 and ABG459-MWG520a) are associated with resistance to KD and FHB and confer resistance when the Chevron allele is present, suggesting that FHB resistance in MNBrite resulted from selection for KD resistance. Neither of these regions is associated with heading date or height, which is in agreement with the fact that MNBrite is similar to other Minnesota cultivars with respect to height and maturity (Rasmusson et al. 1999). We expect

Table 7 Quantitative trait loci associated with height at four environments

Chromosome/ linkage group	Marker interval[a]	Crookston 1995			St. Paul 1996			St. Paul 1997			St. Paul KD 1997		
		LOD	R-sqr[b]	Alpha[c]	LOD	R-sqr	Alpha	LOD	R-sqr	Alpha	LOD	R-sqr	Alpha
1.3	MWG836-ABG476							6.02	35.6	4.61			
1.3	ABG476-BCD98b				4.23	22.1	2.45				5.27	21.9	2.32
3.3	ABG4-MWG803				7.08	21.5	2.73				5.40	12.5	1.87
3.3	MWG803-WG110							5.08	12.9	2.06			
7.2	MWG2227b-ABC302	6.00	12.1	–2.48	3.23	8.5	–1.67	5.67	24.5	–3.55	4.31	7.5	–1.33
7.3	ABG10b-ABG496	3.81	13.6	3.11									
	Multi-locus model	4.95	20.6		11.68	41.3		12.9	44.5		9.67	35.7	

[a] Markers flanking the peak of the LOD scan
[b] Percentage phenotypic variance explained by QTLs
[c] Effect of 'Chevron' allele on plant height (cm) expressed as regression coefficient from multi-locus model

that since *F. graminearum* is a causal organism for both KD and FHB that resistance to these diseases is conditioned by at least some of the same QTLs. It is also interesting to note that while MNBrite is resistant to KD itis not as resistant as Chevron, suggesting that some, but not all of the Chevron-derived alleles conferring resistance have been introgressed into MNBrite. We are currently attempting to verify if the Chevron alleles on chromosome 2 were introgressed into MNBrite and if MAS could be useful to introgress additional Chevron-derived alleles for resistance to KD or FHB into MNBrite.

There are several criteria to consider when deciding which QTL to emphasize in a MAS program. First, it is necessary to identify those regions with sufficient evidence for the presence of a QTL. This is accomplished by setting appropriate thresholds for the detection of QTLs and by detecting QTLs in the same region in repeated environments or mapping populations. In this study, several QTLs exceeded the LOD threshold of 3.16 and were detected in more than one environment: KD resistance on chromosomes 6 and 7, and resistance to DON accumulation on chromosome 2. In addition, a region on chromosome 2 was associated with FHB in one environment above the 3.16 LOD threshold and in two other environments slightly below the threshold. An independent mapping study involving the cultivars Chevron and Stander identified QTLs for FHB resistance with some of the same markers used in this study (N. Lapitan personal communication). Consistent with our results, in the Chevron/Stander population FHB was associated with BCD1087b and CDO395 on chromosomes 2 and 3, respectively. Identification of the above QTLs in multiple environments and, in some instances, independent mapping populations suggests that they are robust and, therefore, promising regions to employ MAS.

A second consideration in the selection of QTLs for MAS is the detection of QTLs for other traits in the same region as the target QTL. We observed significant phenotypic correlations among resistance to FHB, DON accumulation, KD, and heading date. These phenotypic correlations appear to be due, in part, to coincidence of QTLs for these traits. On chromosome 2, the 19-cM region spanning markers MWG887 to ABG619 is associated with FHB severity, DON accumulation, and heading date. In this case, late heading is associated with resistance to FHB and DON accumulation. Since it seems likely that late heading plants have lower levels of FHB due to reduced exposure to inoculum, one should proceed with MAS in this region with caution. Additional fine mapping in this region may permit us to determine if these traits are controlled by different loci or are the result of pleiotropy. Late heading and taller plants are undesirable traits in barley and their linkage to resistance would need to be separated to be useful in barley improvement. In contrast to these examples of negative linkages, QTL for KD severity, FHB severity, and DON accumulation were identified in the same region on chromosome 2 (ABC311–MWG858). In this case, the 'Chevron' allele was associated with reduced disease severity and DON accumulation and was not associated with late heading.

One last criterion to consider is the magnitude of the effect of a QTL as measured by R^2 or alpha. The QTL identified for the disease-related traits, FHB, DON accumulation, and KD, explain from 1% to 26% of the phenotypic variance. For each of these traits, we identified at least one QTL with an R^2 greater than 15%. However, one should use caution in placing too much emphasis on these values since they can be significantly influenced by population size and the number of markers used in the multi-locus model (Beavis 1998). In simulation studies, QTLs analyses using population sizes of 100 identified only a subset of the total number of simulated QTLs and often over-estimated their effects.

Given these criteria, several regions appear to be promising candidates for MAS and further genetic analysis including: the region on chromosome 2 associated with FHB resistance at multiple locations; the region on chromosome 3 associated with FHB resistance (CDO395); two regions on chromosome six associated with KD resistance; and the region on chromosome 2 associated with resistance to DON accumulation, KD, and FHB. We have initiated both traditional phenotypic selection and MAS in the Chevron/M69 population for the development of resistant barley cultivars. The MAS program will also permit further analysis of regions as-

sociated with multiple traits to determine if the effects are due to multiple loci or a single locus. This study provides a starting point for manipulating Chevron-derived resistance by MAS in barley to develop varieties that will be useful under continued disease pressure.

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