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Identification of QTLs associated with Fusarium head blight resistance in Zhedar 2 barley

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Abstract Fusarium head blight (FHB) in barley and wheat, caused by *Fusarium graminearum*, is a continual problem worldwide. Primarily, FHB reduces yield and quality, and results in the production of the toxin deoxynivalenol (DON), which can affect food safety. Identification of QTLs for FHB severity, DON level and related traits heading-date (HD) and plant-height (HT) with consistent effects across a set of environments, would provide the basis for marker-assisted selection (MAS) and potentially increase the efficiency of selection for resistance. A segregating population of 75 double-haploid lines, developed from the three-way cross Zhedar 2/ND9712//Foster, was used for genome mapping and FHB severity evaluation. A linkage map of 214 RFLP, SSR and AFLP markers was constructed. Phenotypic data were collected in replicated field trials from five environments in two growing seasons. The data were analyzed using MQTL software to detect quantitative trait locus (QTL) × environment (E) interactions. Because of the presence of QTL × E, the MQM procedure in MAPQTL was applied to identify QTLs in single environments. We identified nine QTLs for FHB severity and five for low DON. Many of the disease-related QTLs identified were coincident with FHB QTLs identified in previous studies. Only two of the QTLs identified in this study were consistent across all five environments, and both were Zhedar 2 specific. Five of the FHB QTLs were associated with HD, and two were associated with HT. Regions that appear to be promising candidates for MAS and further genetic analysis include the two FHB QTLs on chromosome 2H and one on 6H, which were also associated with low DON and later heading-date in multiple environments. This study provides a starting point for manipulating Zhedar 2-derived resistance by MAS in barley to develop cultivars that will show effective resistance under disease pressure.

Keywords *Hordeum vulgare* · Disease resistance · Gene mapping · Molecular markers

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Introduction

Fusarium head blight (FHB) in barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) has been a continual problem worldwide and has caused devastating losses to the US production since the early 1990s (McMullen et al. 1997; Windels 2000). Primarily caused by *Fusarium graminearum* Schwabe, FHB reduces yield and quality, and results in the accumulation of the mycotoxin deoxynivalenol (DON), which can affect food safety (Steffenson 1998; Salas et al. 1999). DON is of particular concern in malting and brewing. Even though much of the toxin is washed out during the steeping stage, *Fusarium* re-growth during germination can result in unacceptable amounts of DON in the malt and in the final beer (Schwarz et al. 1995), leading the malting and brewing industry to purchase only barley without detectable DON contamination.

All current malting-barley cultivars grown in the US are susceptible to FHB and DON accumulation. Resistance sources have been identified in both two-rowed and six-rowed barley but these provide only partial resistance to FHB, and it has been difficult to transfer two-rowed resistance into six-rowed genotypes (Rudd et al. 2001). Under high-disease pressure, even accessions with low disease levels had unacceptable DON concentrations (Prom et al. 1997). Studies of genetic distances based on molecular-marker data show that many of the partially resistant accessions are closely related, indicating that many sources of resistance may carry the same resistance alleles (Urrea-Flórez 2000).

Several studies have identified QTLs for FHB resistance and low DON in US barley (reviewed by Kolb et al. 2001). Regions associated with disease and mycotoxin levels have been found in all seven barley chromosomes. Many QTLs also were associated with heading date, plant height and spike morphology traits (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000; Mesfin et al. 2003). Each resistance locus has a limited effect on FHB severity, so it is likely that breeders will need to combine genes from several sources to develop cultivars with adequate resistance. Knowledge of the locations and effects of each resistance locus in different accessions will increase efficiency in combining these genes.

The objectives of this study were to map QTLs associated with FHB severity and DON accumulation from the exotic Chinese resistance source Zhedar 2, determine associations of the FHB QTLs with heading date and plant height, and compare resistance QTLs with those found in other mapping studies. Zhedar 2 has a different origin than the other sources of FHB resistance previously mapped; thus it may contain QTLs not found in the other accessions.

Materials and methods

Germplasm

Three barley genotypes were used in this study: Foster, ND9712 and Zhedar 2. Foster and ND9712 are US six-rowed genotypes susceptible to FHB infection caused by *F. graminearum*. Zhedar 2 is a two-rowed accession from China that showed some resistance to FHB (Prom et al. 1997) and it has a different origin than the other sources of FHB resistance previously mapped. The initial crosses were made between ND9712 and Zhedar 2 in Fargo, N.D. The resulting F_1 plants were crossed to Foster to increase adaptation. The plants from the three-way cross were crossed with *H. bulbosum* (Chen and Hayes 1989) to develop a Zhedar 2/ND9712//Foster doubled-haploid (DH) population consisting of 75 lines.

Field evaluation

The DH lines, parents, and the resistant check cultivar 'Chevron' were evaluated in the US and China in five environments from 1996 to 1997. Traits measured in Hangzhou, China, in 1997 (Ha97) included FHB severity, DON level, heading date (HD) and plant height (HT). FHB, DON and HD were measured at Langdon and Fargo, North Dakota, US, in 1996 (La96, Fa96). FHB and DON were measured in Shanghai, China, in 1996 (Sh96). Only FHB was measured at Hangzhou, China, in 1996 (Ha96). At each location DH lines were replicated twice and the parents were sown every 25 plots, in an augmented block design.

Field inoculation was conducted by infesting autoclaved barley and maize (*Zea mays* L.) grain with *F. graminearum* and spreading the grain in the experimental plots. Overhead irrigation was used to augment infection in the nursery. FHB severity was scored by sampling ten randomly selected spikes from each DH line and parent row, during the mid to late dough-developmental stage. FHB severity was reported as the percentage of infected kernels from the ten spikes. Seed harvested from the disease nurseries were tested for DON using the method of Tacke and Casper (1996).

Marker analysis

DNA extraction, digestion and Southern hybridization were conducted as described by Dahleen (1997). For RFLP analysis, six restriction enzymes were used to digest genomic DNA (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I). DNA genomic clones from wheat and barley, and barley and oat cDNA libraries that identified polymorphisms between the parents, were tested on the progeny.

SSR loci were amplified in a 20-μl reaction mixture containing 50 ng of template DNA, 0.3 μM of each primer, 200 μM of each dNTP, 2 mM of $MgCl_2$, 1 unit of *Taq* DNA polymerase and 1 × *Taq* buffer supplied with the enzyme using the conditions described by Ramsay et al. (2000). Amplified products were separated on 4% SFR agarose (Amresco, Solon, Ohio, USA)—TAE gels and visualized by ethidium bromide staining.

AFLP loci were amplified with the kit and methods provided by Life Technologies (Invitrogen, Carlsbad, Calif.) using the *Eco*RI and *Mse*I primers recommended for barley. Resistance gene analog (RGA) loci were amplified using the primers and conditions described by Chen et al. (1998). For both marker types, primers were labeled with [γ-^{33}P]ATP, and amplified sequences were visualized by exposure of the acrylamide gels to X-ray film.

Two phenotypic markers were evaluated and used for mapping, *Rpg1*, on chromosome 7H that confers resistance to stem rust, and *vrs1* (six-row spike) on 2H that controls fertility of lateral spikelets and spike type.

Statistical analysis and QTL mapping

Pearson and Spearman rank correlations between traits were calculated using SAS (SAS Institute 1999). Single-locus associations between markers and phenotypic data for the DH lines were determined using one-way ANOVA (SAS Institute 1999). Linkage maps were developed using Mapmaker 3.0 (Lander et al. 1987). Markers were only considered polymorphic when the Zhedar 2 allele differed from both the ND9712 and Foster alleles. Foster and ND9712 were treated as identical genotypes for the analysis because they differed for less than 5% of the markers. This resulted in a population structure where the observed recombination in the DH lines, $R=(2r-r^2)/2$, with r equal to the gametic recombination frequency (N. Tinker, personal communication 1999). Using the Mapmaker data-type F_2 intercross with codominant (A/B) markers, providing us with the appropriate relationship between R and r. Log-odds ratio (LOD) scores of 3 to 6 were examined using the Kosambi map function (Kosambi 1944). A LOD score of 4.0 was set to develop the linkage map. The complete DNA marker data set of 703 markers was used to construct the linkage groups. A subset of 214 markers was used as a framework map for this analysis. These markers were chosen on the basis of LOD, maximal information content and gene order. MQTL (Tinker and Mather 1995a, b) software was used to detect both QTLs and QTL × environment interaction using simple interval mapping (SIM) and simplified composite interval mapping (sCIM). QTL analysis parameters included a chromosome walking pace of 1 cM and an experiment-wise Type-I error rate of 5%, with 5,000 permutations

Table 1 Means of the parents (Foster, ND9712 and Zhedar 2) and doubled-haploid (DH) progeny for Fusarium head blight severity (FHB % infected kernels), deoxynivalenol content (DON μg/g), heading date (HD days after planting), and plant height (HT cm) in Hangzhou (Ha96, Ha97) and Shanghai (Sh96), China, and Fargo (Fa96) and Langdon (La96), North Dakota, USA

Trait	Environment	Foster	ND9712	Zhedar 2	DH		
					Mean	Range	Std dev.
FHB	Ha97	21.5	23.9	7.0	20.6	1.0–48.2	10.9
	Fa96	11.8	9.1	1.2	10.8	2.5–27.4	4.9
	La96	44.0	47.2	3.6	42.2	11.0–66.9	14.3
	Sh96	39.6	24.4	1.2	16.8	0.2–43.3	12.0
	Ha96	2.5	2.8	0.2	3.1	0.4–12.2	1.9
DON	Ha97	6.3	6.7	2.7	10.7	1.3–69.9	9.6
	Fa96	27.1	25.0	6.4	34.6	8.3–71.1	15.3
	La96	50.5	112.1	13.6	89.3	25.4–160.0	34.7
	Sh96	31.6	18.0	6.8	16.7	0.9–52.4	12.2
HD	Ha97	141	145	155	148.8	137.0–155.0	4.9
	Fa96				63.5	56.7–69.0	2.6
	La96				55.8	51.0–61.5	2.3
HT	Ha97	103	104	119	104.6	88.0–122.0	8.7

run to calculate the significance threshold. MQTL also was used to detect QTL × QTL epistatic effects. MAPQTL (Van Ooijen et al. 2002) was used to identify QTLs in single environments. The markers with LOD values greater than 4.0 were used as co-factors for running a multiple-QTL method (MQM) on data from each environment for the four traits. sCIM (Tinker and Mather 1995a, b) and MQM (Jansen 1993; Jansen and Stam 1994) used background markers to account for other QTLs and thus gave better resolution of multiple linked QTLs. Because of the presence of the QTL × environment interactions, the results obtained by MQM for QTLs in single environments are presented in this report. A LOD threshold of 3.0 was used (Van Ooijen 1999) as a criterion for detecting QTL (Qi et al. 1999).

Results

Trait analysis

Means and ranges for measured traits for the DH population and parents are shown in Table 1. Disease levels ranged from very low in Hangzhou 1996 (Ha96) to very high in Langdon 1996 (La96). Susceptible parents Foster and ND9712 had at least a 3-fold higher FHB severity compared to the resistant parent Zhedar 2 in all environments. The DH progeny means generally were higher than the averages of the parent, although transgressive segregants with FHB severities less than the resistant parents were observed in several environments. The FHB values for the Zhedar 2-derived DH lines fit a normal distribution at Ha97 and La96, but were skewed towards susceptibility at Fa96 and were skewed towards resistance in Shanghai 1996 (Sh96) and Ha96 (data not shown).

DON concentrations also varied across environments; even the resistant parent Zhedar 2 showed unacceptably high levels of toxin, especially at La96. Transgressive segregants with low DON were observed at Ha97 and Sh96, but not at Fa96 and La96, where overall toxin levels were higher. DON levels for the DH lines were normally distributed at La96, but were skewed towards low DON at Fa96, Sh96 and Ha97.

The three-way cross using two adapted genotypes helped increase the number of progeny with acceptable values for HD and HT. Progeny means at each location were close to the Foster and ND9712 means, although earlier and shorter transgressive segregants were observed. HD at Fa96 and La96, plus HT at Ha97 were normally distributed, while HD at Ha97 was skewed towards earliness.

Table 2 Pearson correlation coefficients and significance between Fusarium head blight severity (FHB), deoxynivalenol content (DON), heading date (HD) and plant height (HT) evaluated at five test environments; Hangzhou, (Ha97) and Shanghai (Sh96), China, and Fargo (Fa96) and Langdon (La96), North Dakota, USA

Environment	Trait	DON	HD	HT
Ha97	FHB	0.257*	–0.338**	–0.471***
	DON	–	ns	–0.404***
	HD	–	–	ns
Fa96	FHB	0.594***	ns	NT
	DON	–	0.293*	NT
La96	FHB	0.626***	–0.700***	NT
	DON	–	–0.517***	NT
Sh96	FHB	0.547***	NT	NT

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability level, respectively; ns, not significant ($P>0.05$); NT, not tested

Pearson correlation coefficients are shown in Table 2. Spearman rank correlations between locations for each trait were not homogeneous, so coefficients are shown for each environment. Correlations between FHB and DON were positive but, although significant, were rather low, with only La96 showing a correlation above 0.60. Correlations between HD and FHB ranged from non-significant to significantly negative, and between HD and DON ranged from positive to non-significant to negative. This variation in correlation reflects the strong relationship between the environment, and disease and toxin levels. These association of resistance, low DON accumulation, taller plants and late heading are consistent with the possibility that the genes that control these traits are linked or have pleiotropic effects.

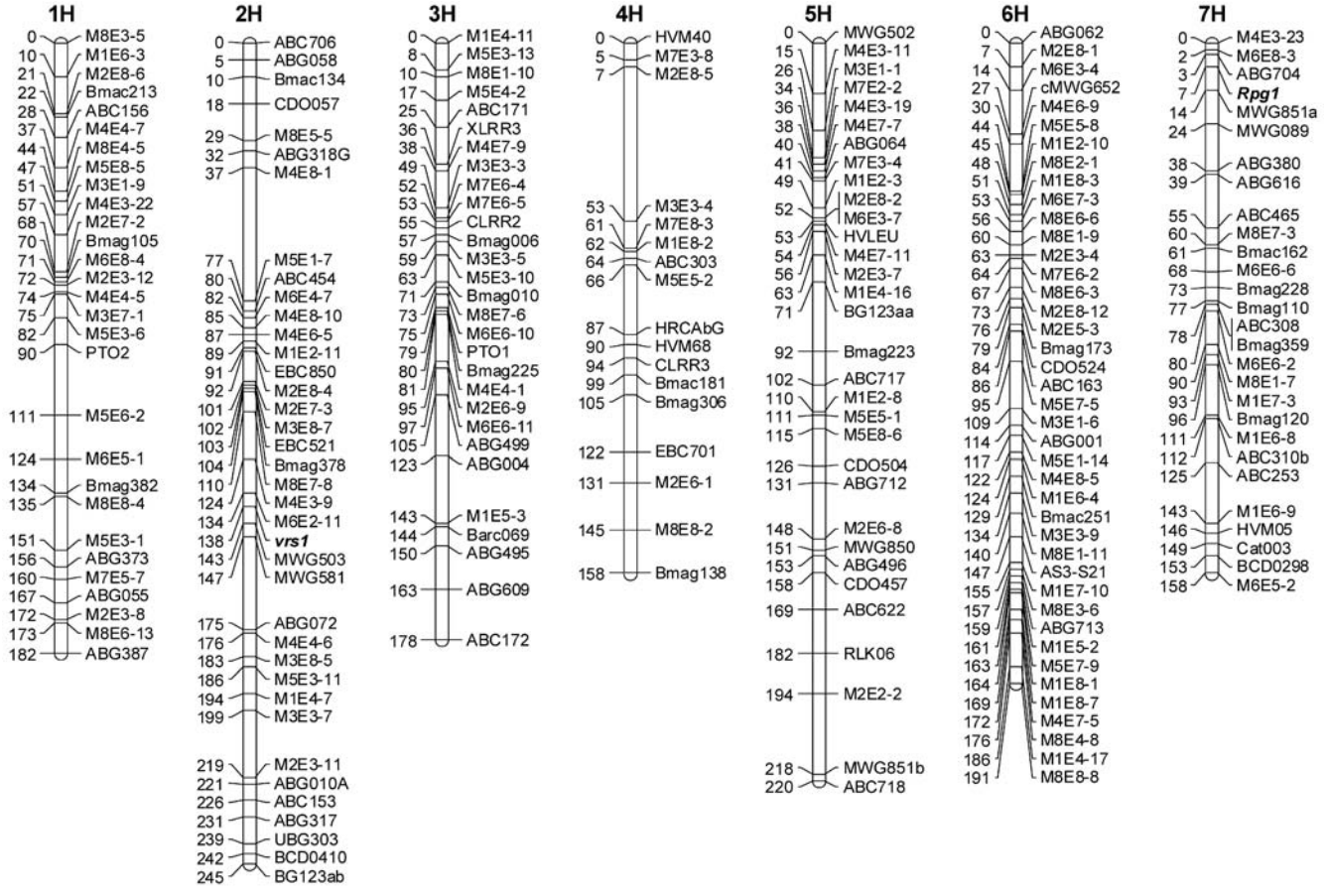

Fig. 1 Linkage map of the Zhedar 2/ND9712//Foster doubled-haploid population. Phenotypic markers *vrs1* and *Rpg1* are shown in *bold italics*. Cumulative distances between markers are given in centiMorgans

Linkage map construction

A linkage map was constructed to determine the positions and effects of QTLs and genes controlling these traits. Altogether 703 molecular markers, that identified polymorphism between Zhedar 2 and the two susceptible parents, were analyzed in the progeny. Many markers cosegregated in the seven chromosomes. Since cosegregating markers do not provide any additional information, only one marker with the fewest missing observations was retained at a position while dropping the redundant markers. In total, 214 markers were used to construct the linkage map that was used for QTL analysis (Fig. 1). This map included 123 AFLP, 53 RFLP, 29 SSR and 7 RGA markers plus the *Rpg1* locus for stem rust resistance and the *vrs1* locus for spike morphology. The total map distance was 1,330.8 cM, with an average two-locus interval of 6.2 cM and all seven chromosomes represented. RFLP and SSR marker order followed that of previously published maps. We were unable to find markers for some large gaps, particularly in the short arm of chromosome 4H, but most chromosome bins were covered by at least one marker (Fig. 1).

The three-way cross gives an expected segregation ratio of 3:1 (ND9712 or Foster:Zhedar 2). All markers were tested by chi-square for a fit to this ratio and more than half (115/214) showed skewing, probably because of the small population size. The distal short arm and the proximal end of the long arm of chromosome 2H (12 markers) and the proximal end of short arm of chromosome 4H (five markers) had an over-representation of alleles from the resistant parent. Portions of each chromosome were skewed towards the susceptible parents, especially a large region around the centromere of chromosome 3H and most of the long arm of chromosome 6H. The distortion that we have seen was probably due to gametophytic or sporophytic factors resulting in the preferential selection of particular alleles.

QTL identification

NQTL, the windows version of MQTL, was used to test the QTL × environment interaction (Fig. 2). Because the interaction was significant for FHB and DON in all chromosomes except 4H, and HD showed a significant interaction on 1H and 2H, individual environments were evaluated in the QTL analysis using MapQTL. For example at the ABC622 marker in the interval CDO457–RLK06 in 5H, the Zhedar 2 allele reduced

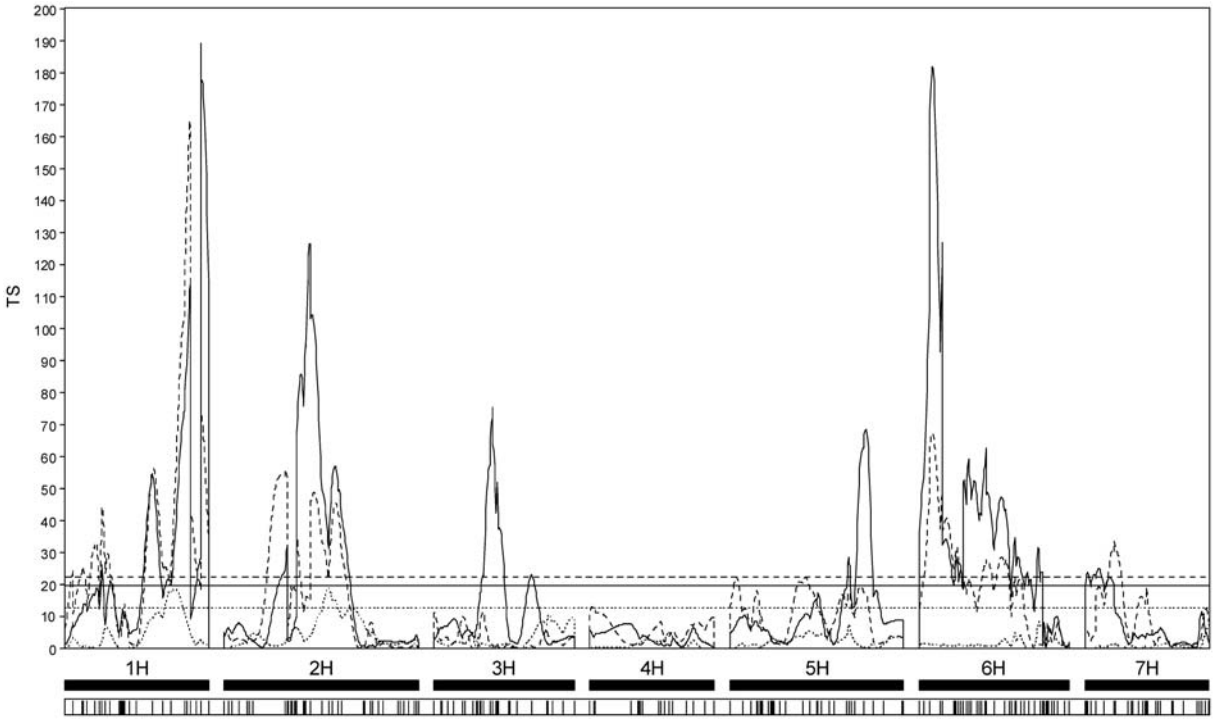

Fig. 2 Scan of test statistics (Y-axis) for QTL × environment interaction for the whole genome. Scans are shown for FHB (*solid lines*), DON (*broken lines*) and HD (*dotted lines*). *Horizontal lines* indicate thresholds for testing estimated from 5,000 permutations. Chromosomes 1H, 2H, 3H, 4H, 5H, 6H and 7H are shown along the X-axis, with the marker positions (*code bar*) as in Fig. 1

FHB severity by 6.77 in Ha97 and by 4.32 in Sh96, while the Foster/ND9712 allele decreased FHB by 2.63 in Fa96 (Table 3). For each trait in each environment, the cofactors were selected independently and varied from 12 to 15 selected markers.

QTLs were found for all traits in the different environments and distributed in all chromosomes except 4H and 7H (Table 3). Nine QTLs were identified for resistance to FHB in this population. The number and locations of QTLs detected varied between the five environments (Table 3). Two QTLs for FHB resistance were present in all environments on chromosome 2H in marker interval M5E1-7 to EBC850 and near *vrs1* in marker interval M4E3-9 to MWG581. At these two regions, the Zhedar 2 allele contributed to resistance (negative additive effects). The distance between the two regions was approximately 50 cM. In La96 and Ha96, a third QTL was identified on 2H located in the interval M5E3-11 to M3E3-7. A fourth QTL was identified on 1H for resistance in Ha96 and a fifth was detected approximately 45-cM apart in Fa96. An additional QTL was located on 1H in Fa96. A seventh QTL was located on 5H in three environments. The resistance was contributed by Zhedar 2 in Ha97 and Sh96, whereas, in Fa96, the additive effects were positive because an allele from the susceptible parent reduced FHB severity. Two QTLs were identified on 6H separated by 100 cM. The percentage of phenotypic variance explained (%exp) for FHB by a single QTL ranged from 5.3% in Sh96 to 17.1% in Fa96 (Table 3). The total amount of phenotypic variation (R^2) for FHB explained by all significant QTLs varied from a low of 28.4% in Sh96 to a high of 47.3% in Fa96 (Table 3).

Five significant QTLs were detected for DON. One QTL on 2H (M4E3-9 to MWG581) was consistent across the four environments and the second QTL on the same chromosome (interval M5E1-7 to M1E2-11) was present in three of four environments (Fig. 1, Table 3). In both QTLs, the resistant parent Zhedar 2 contributed to a low DON. Another three QTLs were identified on chromosome 6H. One of these three was identified only in La96 and the other two in three environments. The allele from Zhedar 2 contributed to low DON accumulation in one region on 6H (M6E3-4 and M4E6-9). The susceptible parent alleles contributed to a low DON at the other QTLs on 6H. These QTLs explained substantially less of the total phenotypic variance for DON than QTLs for FHB (Table 3). The marker interval of the two QTLs on 2H and in marker interval Bmac251–AS3-S21 on 6H overlapped with the QTLs found for FHB in the same regions (Fig. 1, Table 3).

Five QTLs associated with late heading date (HD) were located on chromosomes 2H, 3H and 6H that explained 4.9%–18.7% of the phenotypic variance in individual environments (Table 3). The alleles contributed by Zhedar 2 were associated with late HD for all QTLs in all environments, except on 3H in Ha97 and La96. The QTL in 6H flanked by marker interval M6E3-4 to M4E5-8 was identified in all three environments. This region also was associated with resistance to FHB in La96 and

Table 3 Identification of significant (LOD>3.0) QTLs for FHB, DON, HD and HT in 75 DH lines of Zhedar 2/ND9712//Foster, chromosome location, explained phenotypic variance (%exp), additive effect of the Zhedar 2 allele (add) and percent of phenotypic variance explained by QTLs (R^2) in five environments

Trait/ chrom	Marker interval		**Ha97** LOD	%Exp	Add	**La96** LOD	%Exp	Add	**Fa96** LOD	%Exp	Add	**Sh96** LOD	%Exp	Add	**Ha96** LOD	%Exp	Add
FHB																	
1H	M4E4-7	M5E8-5													4.09	12.3	2.42
1H	M5E3-6	PTO2							4.25	17.1	2.81						
1H	M7E5-7	ABG055							4.48	8.4	−1.52						
2H	M5E1-7	EBC850	3.82	7.2	−3.07	3.67	6.6	−2.86	3.27	7.2	−2.53	3.19	5.3	−3.79	4.35	9.6	−0.21
2H	M4E3-9	MWG581	4.19	16.9	−4.82	5.40	14.5	−11.59	5.08	9.0	−1.82	6.04	14.1	−7.39	5.42	12.1	−0.67
2H	M5E3-11	M3E3-7				4.04	10.7	−5.72							3.64	10.8	−0.72
5H	CDO457	RLK06	5.71	14.2	−6.77				4.15	13.8	2.63	5.44	9.3	−4.32			
6H	M6E3-4	M4E6-9	4.82	5.9	−3.91	5.04	9.6	−6.5				4.02	5.4	−3.98			
6H	M4E8-5	M3E3-9				3.92	6.0	6.7									
R^2				37.2			41.6			47.3			28.4			39.3	
DON																	
2H	M5E1-7	M1E2-11				4.72	6.9	−10.75	3.91	8.4	−4.57	3.19	5.3	−3.87			
2H	M4E3-9	MWG581	4.89	7.4	−2.68	4.05	9.3	−10.03	3.68	11.8	−4.95	4.88	8.7	−7.39			
6H	M6E3-4	M4E6-9	3.3	8.2	−3.48	4.52	5.5	−10.91				3.62	6.6	−2.36			
6H	M5E7-5	ABG001				3.84	12.9	14.9									
6H	Bmac251	AS3-S21	11.46	10.9	12.39	3.84	9.8	17.47				3.90	9.3	9.27			
R^2				22.5			38.8			17.7			23.8				
HD																	
2H	M2E7-3	M8E7-8	4.49	14.4	1.09	4.53	19.1	1.36									
2H	M4E3-9	*Vrs1*	5.15	12.2	1.92												
2H	M5E3-11	M3E3-7				5.55	18.7	1.39	3.44	14.8	1.31						
3H	M1E5-3	ABG495	3.27	9.2	−1.66	6.62	9.1	−1.10									
6H	M6E3-4	M4E5-8	3.8	4.9	0.15	3.92	12.3	1.11	4.69	11.4	0.54						
R^2				37.8			52.3			21.6							
HT																	
2H	M8E7-8	M6E2-11	9.6	16.2	3.80												
3H	M2E6-9	ABG499	12.94	17.3	3.51												
R^2				31.7													

Sh96. The three QTL regions on 2H were also associated with the resistance to FHB. Two QTLs on 2H, in marker intervals M2E7-3–M8E7-8 and M4E3–9–*vrs1*, also showed association with a low DON level. At La96, 52.3% of the total phenotypic variance in HD was explained by the QTLs (Table 3). Only two QTLs were identified for increased plant height (HT), on chromosomes 2H and 3H. HT was measured only in the 1997 experiment at Hangzhou, China. The QTL on 2H also was associated with FHB resistance, a low DON and later heading date. These two QTLs explained 31.7% of the total phenotypic variance for HT (Table 3).

Additive QTL × QTL epistasis between FHB loci was detected in all environments except Hangzhou 1996. Most of the effects were small, and were not consistent across locations. Epistatic interactions were observed more frequently at Fargo 1996 and Langdon 1996 than at Shanghai 1996 and Hangzhou 1997. No dominant QTL × QTL epistatic interactions were detected.

Discussion

A three-way cross, Zhedar 2/ND9712//Foster, DH population was used to map QTLs associated with FHB and related traits in this study. Three-way crosses have been applied successfully in rice to map and identify QTLs for wide-compatibility (Wang et al. 1998), to map centromeric regions (Wang et al. 2000), and to map R genes (Wang and Xiao 2002).

The 214 marker base map developed covers a total linkage distance of 1,330.8 cM. The orders and distances of RFLP and SSR markers are consistent with previous published maps (Kleinhofs et al. 1993; Kasha et al. 1995; Hayes et al. 1997; Zhu et al 1999; Ramsay et al 2000). Two large gaps were observed in our map, which consisted of a 40-cM segment on the short arm of chromosome 2H and a 46-cM segment on the short arm of 4H. Gaps often have been observed in barley genome maps. For example, gaps of more than 30 cM were present on chromosomes 3H, 4H, 5H and 7H in a map of Rolfi × CIho9819 (Manninen et al. 2000), and the Harrington × TR306 map had gaps greater than 50 cM on chromosome 1H, and greater than 25 cM on chromosomes 2H, 3H, 4H and 5H (Tinker et al. 1996). Also, gaps larger than 25 cM were presented in the QTL mapping for FHB resistance using a DH population derived from a cross between Chevron and Stander (Ma et al. 2000). Some of the gaps may occur because the parents originated from common ancestors.

Several genetic mapping studies have reported resistance to FHB in European, ICARDA/CIMMYT and Japanese barley lines (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000; Mesfin et al. 2003). These maps essentially identified the same QTLs. This is the first report of molecular markers used to identify QTLs for resistance to FHB using a barley line of Chinese origin, Zhedar 2. We hoped that Zhedar 2 would contain additional QTLs that could be used to breed resistant cultivars. Comparison of Zhedar 2 FHB QTL locations with those identified in Chevron (de la Pena et al. 1999; Ma et al. 2000), Gobernadora (Zhu et al. 1999) and Fredrickson (Mesfin et al. 2003) indicate that these four cultivars mostly carry the same resistance loci. The allele at the *vrs1* locus that is present in Zhedar 2 (*vrs1.d*) is not present in other crosses in which QTLs for FHB resistance were identified. The *vrs1.d* allele was identified in "Svanhals" (Woodward 1949; Franckowiak and Lundquist 1997) and is present in many early heading cultivars from East China (Franckowiak, unpublished). The *vrs1* alleles in FHB resistant parents in the other studies are *vrs1.a* in Chevron (Woodward 1949) and *vrs1.t* in CMB643 (Vivar 2001). Since Zhedar 2 is closely related to the Swedish cultivar Svanhals based on molecular markers (Urrea-Flórez 2000), the FHB QTL for resistance near *vrs1* may have originated in European landraces. The situation on chromosome 2H is very complex which makes it difficult to compare QTL regions from different maps. Intercrossing resistant lines with different genetic background sources will show whether these QTLs are truly allelic.

Only two of the nine QTLs were unique to Zhedar 2. The Zhedar 2 specific QTL identified on chromosome 6H (M6E3-4 to M4E6-9) explained 9.6%, 5.9% and 5.4% of the phenotypic variation in La96, Ha97 and Sh96, respectively. This QTL, significant in three environments out of five, might be a valuable QTL in breeding programs for FHB resistance. The QTL located on chromosome 2H (M5E3-11 to M3E3-7) was identified in two environments and explained about 11% of the variation in La96 and Ha96. The value of this QTL for breeding is questionable because it was significant in only two of the five environments tested. These two unique QTLs were associated with a later heading date. Four DON QTLs were in locations previously associated with a lower DON level (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000; Mesfin et al. 2003). Only one QTL was specific to Zhedar 2, flanked by markers M6E3-4 and M4E6-9 on chromosome 6H. This QTL is significant in three of four environments and explained the phenotypic variation that ranged between 5.5% and 8.2%. This region also was associated with FHB resistance and later heading, which confirmed the importance of this QTL for FHB resistance breeding.

The presence of the photoperiod sensitive gene *Eam6* (early heading 6) in the US Midwest six-rowed barley cultivars (Franckowiak and Konishi 2002) may explain the QTL (M2E7-3 to M8E7-8) for heading date on chromosome 2H. The *Eam6* gene responds to long-day conditions and is located near the centromere of chromosome 2H (Toho-oka et al. 2000). A second heading-date QTL (M4E3-9 to *vrs1*) may be associated with the qualitative gene *lin1* (lesser internode number 1) for the reduced number of fertile spikelets per spike, which is also present in the US Midwest six-rowed barley (Swenson and Wells 1944; Franckowiak 2002). The plant height QTL in the region M8E7-8 to M6E2-11 of chromosome 2H may correspond to the *hcm1* (short culm) locus

(Swenson and Wells 1944; Franckowiak 1997). Previously reported gene order for the *vrs1*, *lin1*, *hcm1* and *Eam6* loci fits with the orders of QTLs on chromosome 2H (Fig. 1).

Correlation among the four traits may be caused by linked or plieotropic QTLs. The correlation coefficients observed among FHB, DON, HD and HT suggested that Zhedar 2 alleles at chromosome regions associated with FHB resistance were associated with low DON accumulation, late HD and taller plants. Morphological traits also have been associated with FHB resistance in barley (Rudd et al. 2001). The *vrs1* gene that determines the two-rowed spike-type in the marker interval M4E3-9 to MWG581 on chromosome 2H was linked in coupling with FHB resistance, low DON and late HD alleles. A QTL for plant height identified on 2H at marker interval M8E7-8 to M6E2-11 (*hcm1* locus) was linked to the *vrs1* locus. Published reports indicate that two-rowed barley is more resistant to FHB than six-rowed barley (Gocho and Hirai 1987; Takeda and Heta 1989; Xihang et al. 1991; Rudd et al. 2001), most likely because of linkage between *vrs1* and a resistance gene.

The QTL × environment (E) interaction was significant for FHB, DON and HD. Some QTLs for FHB showed allelic effects that changed their effects depending on the environment. These QTLs are not likely to be useful for marker-assisted selection. Similar results were observed for barley yield QTLs (Tinker et al. 1996) and for oat β-glucan content QTLs (Kianian et al. 2000). The interaction observed across environments for the measured traits was most likely caused by environmental conditions at each location, such as differences in photoperiod, temperature, rainfall and humidity. Tinker et al. (1996) suggested that some QTL × E interactions are always present. The heterogeneity between all the test environments influenced the number and location of QTLs. Indeed, numerous QTLs were identified in single environments such as the QTL for FHB resistance on chromosome 1H in the interval M4E4-7 to M5E8-5 detected only in Ha96, and the QTL for low DON in interval region M5E7-5 to ABG001 on chromosome 6H in La96. However, four FHB QTLs were identified in three or more environments, including the chromosome-2H QTLs in the intervals M5E1-7 to EBC850 and M4E3-9 to MWG581, which were significantly associated with FHB resistance in all five environments. The use of markers associated with QTLs consistent across environments is more reliable and efficient for marker-assisted selection (MAS) because the QTLs are expressed regardless of the environmental conditions (Teulat et al. 2001).

The MQM analysis performed using the five environments supports the presence of QTLs for FHB resistance and correlated traits in the Zhedar 2. Studies also have been conducted to identify QTLs for resistance to FHB from the cultivars Chevron, Gobernadora, CMB643 and Fredrickson. Even though several QTLs were found to be specific to one location or genetic background, several consistent regions emerged from this work. The QTL identified in the chromosome 2H at marker interval M4E3-9 to MWG581, including *vrs1*, was significant in all environments for FHB and DON, and in one environment for HD. The same region was associated with type-I and type-II resistance, low DON, the number of seeds per spike, and lateral floret size (*Vrs1.t*) in the Gobernadora × CMB643 DH population (Zhu et al. 1999). The ICARDA/CIMMYT barley breeding program screened for FHB type-II resistance, and a QTL controlling this trait has been mapped to the same region of 2H (Vivar et al. 1999). One cross that included Chevron (Ma et al. 2000), and the cross to Fredrickson (Mesfin et al. 2003), also showed that the 2H region contains QTLs for FHB and DON. The presence of this large-effect QTL in diverse materials from China, Europe, Japan and Mexico was unexpected. However, it is possible that the resistance gene had a single origin and was transferred throughout the world by germplasm exchange. It was not determined if genes for these traits were linked or pleiotropic (Zhu et al. 1999; Rudd et al. 2001); however, association of the FHB QTL with three different alleles at the *vrs1* locus favors linkage. Projects are underway to fine-map critical QTLs and to develop near-isogenic lines with a single resistance QTL. The presence of environment-specific QTLs for FHB resistance emphasizes that improvement of resistance will probably come through a combination of stable QTLs involved in the expression of traits significantly correlated with resistance. The information suggests that critical genes for local adaptation (maturity, plant height and plant architecture) need to be fixed before enhanced FHB resistance can be achieved. Several QTLs for FHB resistance and their close linkage to adaptation-genes creates a complex breeding problem. Recombinants need to be identified, but selection for them is hampered by laborious screening methods and complex environment-genotype interactions observed in the assessment of FHB reactions. Regions that appear to be promising candidates for MAS and further genetic analysis including the two FHB QTLs on chromosome 2H, and one on 6H which were also associated with low DON and later heading date in multiple environments. This study provides a starting point for manipulating Zhedar 2-derived resistance by MAS in barley to develop cultivars that will show effective resistance under disease pressure.

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