Mol Breeding (2008) 22:517–526
DOI 10.1007/s11032-008-9195-1

Detection of Fusarium head blight resistance QTLs using five populations of top-cross progeny derived from two-row × two-row crosses in barley

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Received: 21 August 2007 / Accepted: 15 May 2008 / Published online: 27 May 2008

Abstract Fusarium head blight (FHB) resistance was evaluated in five recombinant inbred (RI) populations. The RI populations consisted of top-cross progeny derived from a diallel set of crosses. Each of five two-row barley lines differing in response to FHB were crossed with 'Harbin 2-row'. FHB severity was scored on an 11-point scale, where resistant = 0 and susceptible = 10, based on the 'cut-spike test'. Disease data were obtained for each population for 2 or 3 years. Linkage maps comprised of expressed sequence tag (EST) markers were developed for each population and used for quantitative trait locus (QTL) detection. Thirty two QTLs were detected using all data sets (individual populations and years). Thirteen QTLs were detected using averages across years; 10 of these were consistent across the individual year and average data sets. These QTLs clustered at 14 regions, with clusters on all chromosomes. At 11 of these clusters, Harbin 2-row contributed FHB resistance alleles. No QTLs were detected near the row type (*vrs1*) locus in any of the five RI populations, suggesting that the FHB resistance QTL in this region reported in two-row × six-row crosses may be pleiotropic effect of *vrs1*. QTL were coincident with the flowering type locus (*cly1*/*Cly2*) on chromosome 2H in every population. Some QTL × QTL interactions were significant, but these were smaller than QTL main effects. Considering the pleiotropic effect of spike morphology on FHB resistance, future FHB resistance mapping efforts in barley should focus on cross combinations in which alleles at *vrs1* are not segregating.

Keywords Barley · Fusarium head blight · *Hordeum vulgare* L. · Scab · Quantitative trait loci

Abbreviations

FHB	Fusarium head blight
RI	Recombinant inbred
EST	Expressed sequence tag
QTL	Quantitative trait locus
SNP	Single nucleotide polymorphism
AFLP	Amplified fragment length polymorphism
SSR	Simple sequence repeat
STS	Sequence tagged site
CAPS	Cleaved amplified polymorphic sequence

Introduction

Fusarium head blight (FHB) has long been a serious disease of barley (*Hordeum vulgare* L.) in Japan, which

Electronic supplementary material The online version of this article (doi:10.1007/s11032-008-9195-1) contains supplementary material, which is available to authorized users.

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has a high humidity climate due to the Asian monsoon. This disease has become increasingly important throughout North and South America and Europe, even in areas once thought to be climatically unsuitable for FHB epidemic development (Steffenson 2003). Due to the contamination of grain with mycotoxins, serious economic losses can be caused by FHB (Bai and Shaner 1994). Genetic resistance is the safest and most cost-effective method of disease control (Mesfin et al. 2003). Therefore, the development of FHB-resistant barley cultivars is a goal of breeding programs in many major barley production areas.

Because the fungus infects flowers, flowering time is of key concern when rating disease on germplasm or segregating populations. In order to synchronize environmental conditions for each artificial inoculation, Takeda and Heta (1989) developed the 'cut-spike test'. This method involves collecting inflorescences (spikes) from field-grown plants and inoculation of the spikes under growth chamber conditions at the exact time of flowering. Extensive comparative data support the use of the 'cut-spike test' as a tool for FHB resistance research and breeding. For example, Takeda et al. (1986) repeatedly evaluated 73 barley accessions over a 3-year period to compare FHB infection under field conditions with the 'cut-spike test'. There was good agreement between the two phenotyping procedures, especially for resistant accessions. Subsequently, Takeda and Heta (1989) used the 'cut-spike test' to evaluate 4,957 barley accessions from around the world and identified 23 highly resistant accessions. Takeda (1990) made crosses between accessions with different levels of resistance and estimated heritabilities of 0.25 (F_2–F_3) to 0.32 (F_3–F_4) based on response to selection and heritabilities of 0.46 to 0.51 (F_3–F_4) based on parent-offspring correlation coefficient. These results demonstrate the quantitative inheritance of FHB resistance and the confounding effects of genotype-by-environment interaction. Further evidence for quantitative inheritance of FHB resistance was provided by the analysis of a diallel set of crosses among barley accessions with different levels of resistance (Takeda and Wu 1996). These analyses included a 6 × 6 diallel of two-row × two-row crosses and an 8 × 8 diallel of involving four two-row and four six-row accessions. In both sets of crosses, resistance to FHB was determined by additive genetic variance.

Numerous reports address the possible associations between FHB resistance and inflorescence morphology, especially row type (Takeda and Heta 1989; Zhou et al. 1991; Mesfin et al. 2003; Dahleen et al. 2003; Steffenson 2003). Takeda (1990) analyzed data from an F_4 population segregating for two-row versus six-row inflorescence type (a phenotype determined by alleles at the *vrs1* locus) and found that two-row plants were significantly more resistant than six-row plants. Takeda and Wu (1996) found greater genetic variance in two-row × six-row crosses than in two-row × two-row crosses, suggesting that more resistance factors (including *vrs1*), were segregating in crosses between the two germplasm groups. Hori et al. (2005) reported FHB resistance quantitative trait locus (QTL) near *vrs1* in two-row × six-row cross, providing further evidence for pleiotropic effects of row type on FHB resistance. However, the coincidence of morphology and resistance could also be due to tight linkage. In either case, the issue of row type is of tremendous importance. For example, in North America, certain brewers specify six-row and it would be difficult to replace this specification with a two-row variety, even if it were more resistant.

Therefore, it is appropriate to remove the confounding effects of *vrs1* on FHB resistance by focusing on genetic analyses of crosses within a row type group, e.g. two-row × two-row. To this end, we used a 6 × 6 mating design involving only two-row accessions with different level of FHB severity. Progenies were derived from top-crosses using the most resistant line as the maternal and common parent. Multiple population analysis based on this top-cross design is expected to provide more information regarding FHB resistance QTL than that reported to date from single crosses (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000; Mesfin et al. 2003; Dahleen et al. 2003; Hori et al. 2005, 2006; Horsley et al. 2006). We used data from the multiple populations to compare QTL main effects, stability, and interactions.

Materials and methods

Plant materials

From a set of 6 × 6 diallel crosses (Takeda and Wu 1996), a subset of five top-cross populations was selected that involved one maternal parent and five

pollen parents. Five recombinant inbred (RI) populations were developed by single spike selection from the F_2 to the F_{12} generation. The maternal parent was Harbin 2-row and the pollen parents were Khanaqin 7 (RI1), Turkey 6 (RI2), Turkey 45 (RI3), Katana 1 (RI4) and Khanaqin 1 (RI5). The six parental lines all have two-row inflorescences and they differ in their levels of resistance to FHB (Takeda and Wu 1996). Harbin 2-row shows cleistflory (closed-flowering) and other five accessions show chasmoflory (open-flowering). Harbin 2-row is of European origin and was cultivated in Manchuria, China. Of the 4,957 barley accessions evaluated by Takeda and Heta (1989), it was one of the most resistant to FHB. The FHB severity scores from five seasons (2001–2005), and other passport data, for the six parental accessions are presented in Table 1. Ninety four RI lines were chosen at random from each of the five populations for QTL mapping.

Evaluation of FHB severity

The RI lines and their parents were grown in field experiment at the facilities of Okayama University, Kurashiki, Japan (34°35′ N and 133°46′ E). Experiments were fall-sown in 2000, 2002, 2003 and 2004, and FHB severity was scored in the spring of 2001, 2003, 2004 and 2005. Twenty plants of each line were grown in a single row. Within-row spacing was 4 cm and between-row spacing was 90 cm apart. Flowering type was recorded as "open flowering" if anthers extruded from the lemma or "closed flowering" when they did not. These phenotype data were used as biallelic scores for mapping *cly1*/*Cly2*.

The 'cut-spike test' is described in detail by Takeda and Heta (1989). Briefly, spikes were collected from the field at the time of flowering and spray-inoculated with a freshly prepared conidial suspension of 50 ml (concentration of 2×10^5 per ml) per inoculation tray (ca. 240–300 spikes). *Fusarium graminearum* strain OUZ78 was used as a source of inoculum. OUZ78 was collected from an infected barley spike in the field at Okayama University in 1983 (Kanatani and Takeda 1991). This isolate has moderate virulence and high conidia-formation ability. Inoculated spikes were incubated in a growth chamber at 25°C and 100% RH for 2 days, then at 18°C with ≥90% RH for 6 days. Photoperiod was maintained at 14 h light/24 h with a light intensity of 320 umol σ^{-1} m^{-2}. Disease symptoms on each spike were scored on the eighth day after inoculation using a scale of 0–10, where 0 (resistant) = 0% infected florets per spike and 10 (susceptible) = ≥60% infected florets per spike. Scores from three inoculated spikes per line per replicate were averaged and used for subsequent analyses. There were three replications per experiment. Thus a total of nine spikes were evaluated for each line per season. FHB severity was scored for RI2 in 2001; for RI3, RI4 and RI5 in 2003; and for all populations in 2004 and 2005.

Genotyping and linkage map construction

In order to align linkage maps—and QTL detected with these maps—for the five populations, the same 384 barley expressed sequence tag (EST) markers were assayed in each population. The markers provide complete genome coverage on the Haruna Nijo × H602 map (Sato et al. 2004). Data from markers showing size difference polymorphisms between parental PCR amplicons were used directly

Table 1 Origin, spike row type, flowering type, and Fusarium head blight (FHB) disease scores for six barley accessions used as parents of mapping populations

Accession	Accession no.	Origin	Row type	Flowering type	FHB severity (average and s.d.)			
					2001	2003	2004	2005
Harbin 2-row	OUC649	China	Two	Closed	2.3 ± 0.6	3.0 ± 0.7	3.3 ± 1.4	3.4 ± 1.3
Khanaqin 7	OUI767	Iraq	Two	Open	–	–	6.8 ± 1.7	7.0 ± 1.1
Turkey 6	OUT602	Turkey	Two	Open	7.7 ± 0.6	–	6.6 ± 1.6	6.9 ± 0.4
Turkey 45	OUT615	Turkey	Two	Open	–	7.3 ± 1.2	7.2 ± 1.5	7.3 ± 0.9
Katana 1	OUI626	Syria	Two	Open	–	7.1 ± 1.4	7.5 ± 0.8	7.3 ± 1.4
Khanaqin 1	OUI765	Iraq	Two	Open	–	8.3 ± 1.3	8.3 ± 1.1	7.7 ± 0.4

Data are from 2001 to 2005. Data for Harbin 2-row and Turkey 6 in 2001 and 2004 are after Hori et al. (2006)

for mapping. If there were no size-differences, PCR amplicons of genomic DNA from each of the six parental lines were sequenced using an ABI 3100 (Applied Biosystems Co.). Sequences were aligned by ClustalX (Thompson et al. 1997) to find single nucleotide polymorphisms (SNPs). Polymorphisms based on SNPs providing restriction sites were assayed as cleaved amplified polymorphic sequence (CAPS) markers using agarose gel electrophoresis. If no restriction site was present, or the restriction enzyme was too costly, SNPs were genotyped directly by fluorescence polarization (Greene et al. 2002). For each of the EST framework markers, locus name, chromosome location, map position, enzyme (if used for CAPS) and primer sequences are shown in Supplemental Table 1. An RFLP-sequence tagged site (STS) marker (cMWG699) was used to map *vrs1* on chromosome 2H (Tanno et al. 2002). The 383 marker polymorphisms revealed by simple sequence repeat (SSRs) and amplified fragment length polymorphism (AFLPs) in RI2 (Hori et al. 2006) were integrated with the EST marker information to develop a higher density map. The RFLP-STS and SSR (from Ramsay et al. 2000) were assayed as described by Hori et al. (2005).

Linkage maps were constructed using MAPMAKER/EXP ver. 3.0 (Lander et al. 1987) with a LOD threshold of 3.0. SSR markers were used as anchors on the seven barley linkage groups. The Kosambi mapping function (Kosambi 1944) was used to estimate marker distances.

QTL detection

QTL for each individual year, and for the average across years, were identified by composite interval mapping as implemented in QTL Cartographer ver. 2.5 (Wang et al. 2005). LOD thresholds were determined by 1,000 times permutations. Additive × additive interactions between QTLs for each year were calculated using the option available with QTL Cartographer ver. 2.5.

Results

Evaluation of FHB severity

Harbin 2-row was the most resistant accession, with FHB scores of 2.3–3.4 (Table 1). Scores for the five pollen parents ranged from moderate (6.6, 2004, Turkey 6) to susceptible (8.3, 2003 and 2004, Khanaqin 1). Frequency distributions for average FHB severity scores in the five RI populations, and their parents, are shown in Fig. 1. The range of severity varied from 3.3–10.0 (RI1) to 3.5–8.7 (RI4). Correlation coefficients for FHB scores between the two years

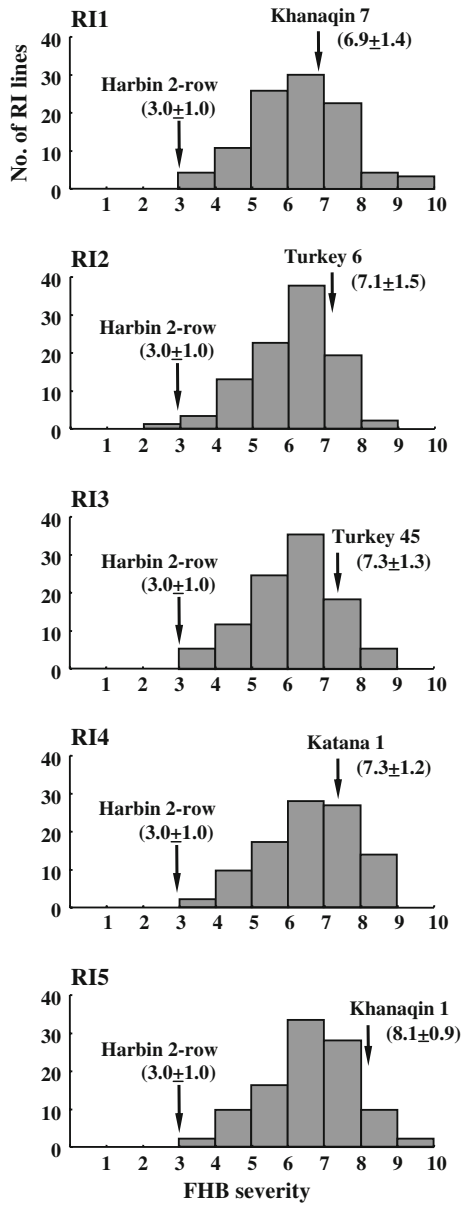

Fig. 1 Frequency distributions of FHB severity scores in five RI populations of barley averaged over years. The average FHB severity scores, and standard deviations, of the parental accessions are shown in parentheses and indicated by *arrows*

Table 2 Analyses of variance and broad sense heritabilities (estimated from variance components) for Fusarium head blight (FHB) disease scores in five recombinant inbred (RI) populations of barley

Population (cross combination)	Years	Variance			$H^{2,a}$
		Year	RI line	Error	
RI1 (Harbin 2-row × Khanaqin 7)	2004–2005	0.828	2.093	1.504	0.281
RI2 (Harbin 2-row × Turkey 6)[b]	2001, 2004–2005	0.501	3.101*	1.877	0.395
RI3 (Harbin 2-row × Turkey 42)	2003–2005	21.615*	3.752*	1.331	0.645
RI4 (Harbin 2-row × Katana 1)	2003–2005	12.000*	4.197*	1.484	0.646
RI5 (Harbin 2-row × Khanaqin 1)	2003–2005	4.373*	4.145*	1.227	0.704

* Significant at the 5% level

[a] Heritability estimate A/(A + E/N), calculated from number of years (N), variance components of RI line (A, variance of RI line/N) and error (E, error variance)

[b] FHB severity scores on RI2 in 2001 and 2004 are after Hori et al. (2006)

of data for each of the five populations were significant, positive, and moderate (0.32–0.66). Analyses of variance (Table 2) revealed significant differences between lines within populations, except for RI1 where there were many missing data points for 2004. The "year" effect accounted for a large and significant proportion of total variance whereas "error" (RI line × year) terms were much smaller. Across-year heritabilities were modest in RI1 (0.28) and RI2 (0.40), but moderate in other three populations (0.65–0.70).

Detection of FHB severity QTLs

Each of the five RI population linkage maps consists of 82–110 loci. Total map lengths range from 1078.3 to 1217.0 cM. Map positions for each of the barley EST markers are shown in Supplemental Table 1. The map of the RI2 population includes an additional 383 markers from Hori et al. (2006).

Figure 2 and Table 3 show QTL positions and parameters. A total of 32 QTLs were distributed on all

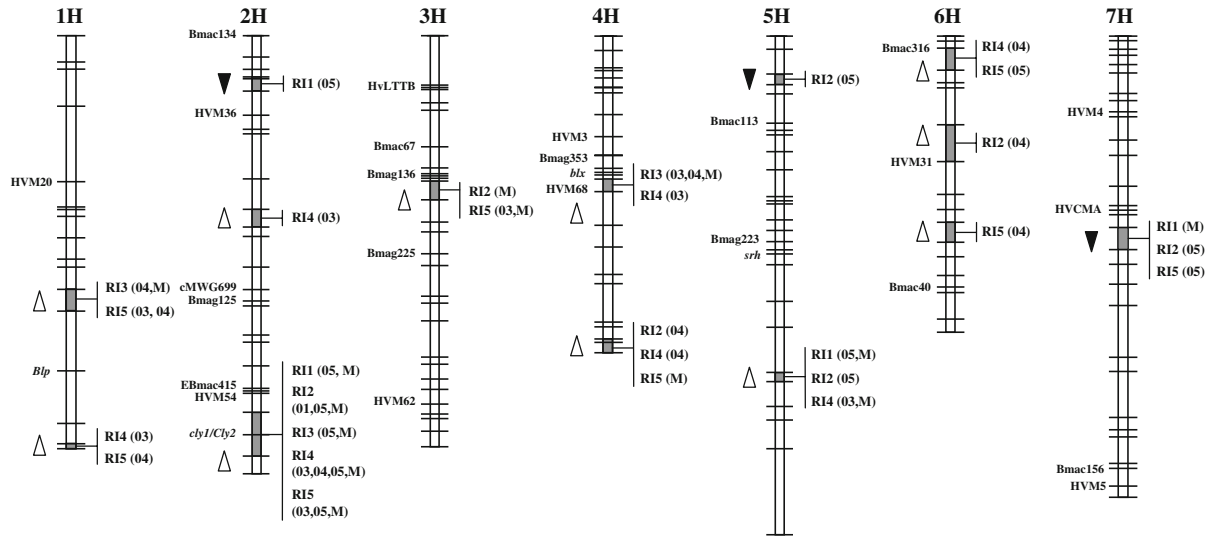

Fig. 2 A consensus linkage map of five RI populations of barley showing positions of FHB resistance QTLs. The year (number) and year means (*M*) for each QTL are shown in parentheses after the name(s) of each RI population(s) in which the QTL were detected. Linkage groups are shown with short arms at the top. Anchor marker names are shown on the left side of each chromosome cartoon. Harbin 2-row resistance and susceptibility alleles are indicated by *white* and *black triangles*, respectively. Marker intervals for QTL LOD peak positions are indicated by gray boxes. Data for RI2 in 2001 and 2004 are after Hori et al. (2006)

chromosomes in this germplasm array. Thirteen QTLs were detected for the five populations when averages across years were used. Ten of these QTLs overlapped with those detected in the individual year data sets and may therefore be the most robust. The other three QTLs did not coincide with any of the individual year QTLs for the same populations, but they did coincide with QTLs detected in other population/year combinations (Fig. 2). Therefore, these QTLs may be of biological importance. Considering all data, a total of 14 QTL regions were identified based on the within-year and across-year data sets (Fig. 2).

Harbin 2-row contributed FHB resistance alleles at 11 of the 14 QTL regions. It contributed susceptibility alleles on chromosome 2H in RI1, chromosome 5H in RI2 and chromosome 7H in RI2 and RI5. No QTL were detected in the vicinity of *vrs1* (as estimated by the position of cMWG699 on chromosome 2H) in any cross combination. A QTL located near *cly1*/*Cly2* on chromosome 2H was found in every RI population (Table 3), although the QTL peak position varied slightly in different populations. The magnitude of effect, and percentage of phenotypic variance accounted, for by this QTL were large (Table 3).

Interaction and additivity of FHB severity QTLs

Multiple interval mapping revealed significant interactions between QTLs on chromosomes 2H and 5H in RI1 (2005), 1H and 2H in RI4 (2003) and 1H and 6H in RI5 (2004) (Table 4). However, the percentage of variance accounted for (3.3–8.6%) and estimated additive effects (−0.4 to −0.3) were small compared to QTL main effects (Table 3). To estimate the effects of pyramiding multiple resistance QTL alleles, we classified the RI lines based on the number of resistance QTL alleles identified in each year and across years (Table 5). In every RI population, there was a trend toward greater resistance (lower FHB score) as RI lines had increasing numbers of resistance alleles.

Discussion

Association between FHB severity and morphological traits

One of our primary objectives was to investigate the relationship of degree of FHB resistance with allelic

Table 3 Position, LOD score, percentage of variance accounted for, and additive effect for Fusarium head blight (FHB) resistance QTLs detected in five recombinant inbred (RI) populations of barley

Population	Year	Chromosome	Marker interval (cM)	Position[a] (cM)	LOD[b]	Var.[c] (%)	Additive effect[d]
RI1	2004	–	–	–	–	–	–
	2005	2H	k02122–k07090	0.0	3.9	11.9	0.5
		2H	k08168–k04213	6.0	7.0	29.0	−0.8
		5H	k03846–k04431	12.0	2.8	12.2	−0.5
	Mean	2H	k04017–k08168	3.5	5.3	20.3	−0.6
		5H	k03846–k04431	13.5	3.2	13.8	−0.5
		7H	k06311–k07517	1.0	2.9	10.4	0.5[e]
RI2	2001	2H	FXLRRfor_XLRRrev119-STS_FEgtaMacg677	0.5	2.6	10.1	−0.7
	2004	4H	FMacgEcgt288-HVM67	2.5	1.9	9.1	−0.4
		6H	FMataEagc408-HVM11	7.0	1.9	8.7	−0.5
	2005	2H	FXLRRfor_XLRRrev119-STS_FEgtaMacg677	0.5	3.6	9.4	−0.6
		5H	MMacgEcgt269-k08607	22.0	3.1	10.5	0.5
		5H	MMaccEgcc354-FMacgEcca91	6.6	2.2	6.3	−0.4
		7H	k06732-FMactEggg533	0.0	2.1	5.2	0.4
	Mean	2H	K08168-FXLRRfor_XLRRrev119	0.0	5.1	15.4	−0.4
		3H	HVM27-FMacgEgtg1313	0.5	5.0	15.0	−0.4[e]

Table 3 continued

Population	Year	Chromosome	Marker interval (cM)	Position[a] (cM)	LOD[b]	Var.[c] (%)	Additive effect[d]
RI3	2003	1H	k00304–k03340	2.5	5.7	20.2	−1.0
		4H	*blx*-k08286	6.5	5.6	20.0	−0.8
	2004	4H	k08286–k05053	3.0	2.1	11.4	−0.5
	2005	2H	*cly1*/*Cly2*-k03299	2.5	4.3	20.0	−0.7
	Mean	1H	k00304–k03340	0.0	3.1	10.6	−0.4
		2H	*cly1*/*Cly2*-k03299	0.0	2.8	9.3	−0.4
		4H	k08286–k05053	0.0	4.7	16.5	−0.5
RI4	2003	1H	k06992–k09230	0.5	3.2	8.3	−0.5
		2H	k03662–k08164	6.0	2.6	8.0	−0.5
		2H	*cly1*/*Cly2*-k03299	0.0	10.0	31.7	−1.0
		4H	k08238–k06766	0.5	3.9	10.3	−0.6
		5H	k03993–k09350	4.0	2.9	10.8	−0.6
	2004	2H	*cly1*/*Cly2*-k03299	12.0	3.3	18.6	−0.7
		4H	k00136–k10866	0.0	2.2	7.6	−0.4
		6H	k04740–k08009	0.5	2.9	10.4	−0.3
	2005	2H	*cly1*/*Cly2*-k03299	8.0	2.0	20.9	−0.7
	Mean	2H	*cly1*/*Cly2*-k03299	0.0	7.9	25.9	−0.6
		5H	k03993–k09350	0.0	4.3	12.9	−0.4
RI5	2003	1H	k00680–k10026	9.0	3.1	13.9	−0.7
		2H	*cly1*/*Cly2*-HVM54	0.0	5.2	16.4	−0.8
		3H	k03264-HVM13	0.5	2.9	8.5	−0.5
	2004	1H	k00680–k10026	2.5	2.1	8.0	−0.4
		1H	k08497–k09230	4.5	2.7	12.6	−0.5
		6H	k07387–k00885	6.5	2.3	9.5	−0.4
	2005	2H	*cly1*/*Cly2*-HVM54	4.0	2.7	19.0	−0.6
		6H	k08009–k05295	28.0	2.1	15.2	−0.5
		7H	Bmac222-HVCMA	32.0	2.8	20.4	0.5
	Mean	2H	*cly1*/*Cly2*-HVM54	0.5	3.7	13.9	−0.4
		3H	k03264-HVM13	0.0	2.9	8.6	−0.4
		4H	EBmac701-k07229	0.0	2.7	8.7	−0.4[e]

The years each population was evaluated are indicated. Data for RI2 in 2001 and 2004 are after Hori et al. (2006)

[a] Distance of peak LOD score position from the left side marker

[b] Peak LOD score

[c] Explained variance

[d] Estimated additive effect of Harbin 2-row alleles

[e] QTL only detected by year mean

variation at the *vrs1* locus. We hypothesized that if no resistance QTL were detected in any of five two-row × two-row mapping populations, this would be good evidence that the FHB resistance QTL detected in two-row × six-row populations were pleiotropic effects of alleles at *vrs1*. No FHB resistance QTL were coincident with *vrs1* in any of five populations, each of which involved a male parent from a distinct geographic location and showing a different level of FHB resistance (Fig. 2 and Table 3). Takeda and Heta (1989) and Steffenson (2003) reported that two-rowed accessions show higher resistance to FHB than six-rowed accessions. Takeda (1990) also reported significant differences in level of resistance between

Table 4 Significant QTL × QTL interaction for Fusarium head blight (FHB) resistance detected in three of five recombinant inbred (RI) populations of barley

Population	Year	QTL1		QTL2		Var.[a] (%)	Weight[b]
		Chr.	Marker interval	Chr.	Marker interval		
RI1	2005	2H	k08168–k04213	5H	k03846–k04431	8.6	−0.4
RI4	2003	1H	k06992–k09230	2H	*cly1*/*Cly2*-k03299	3.3	−0.3
RI5	2004	6H	k07387–k00885	1H	k08497–k09230	4.6	−0.3

[a] Explained variance

[b] Estimated interaction effect

two- and six-row forms in an F_4 segregating population and observed that *Vrs1vrs1* heterozygotes (which are two-rows with larger lateral spikelets) showed levels of FHB resistance between two-row homozygotes (lowest severity) and six-row homozygotes (highest severity). Cumulatively, these data suggest that spike morphology (number of spikelets per rachis node and/or size of lateral spikelets) has a direct effect on the level of FHB resistance. Since *vrs1* was recently cloned (Komatsuda et al. 2007), it will now be possible to more directly determine if differences in FHB resistance are observed when spike morphology is altered.

A flowering morphology-related FHB resistance QTL was found in every RI population (Fig. 2 and Table 3). FHB resistance QTLs at this chromosome location were reported by other investigators in other germplasm (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000; Mesfin et al. 2003; Dahleen et al. 2003; Hori et al. 2005, 2006; Horsley et al. 2006). Turuspekov et al. (2004) mapped a flowering type locus—*cly1*/*Cly2*—to the long arm of chromosome 2H coincident with the position of this flowering type QTL. An association between FHB resistance and flowering type using isogenic lines was reported by Yoshida et al. (2005), who concluded that the flowering type largely controlled the level of FHB resistance. Thus, it is likely that this morphological trait locus also has a pleiotropic effect on FHB resistance. The mechanism may be avoidance of infection due to the physical barrier of a closed flower. Since there are no apparent negative of cleistogamy, this may be a useful approach to achieving higher levels of FHB resistance. Because *Fusarium graminearum* is a saprophytic parasite, true genetic resistance may be a challenge to identify and breed for. Alternatively, accumulation of alleles conferring morphological characteristics such as two-row spike, cleistogamy, and perhaps even husk thickness and rigidity may lead to acceptable levels of FHB disease control.

A strategy for introgression of FHB resistance QTL

The continuous phenotypic frequency distributions for FHB scores in all RI populations suggests that FHB resistance is quantitatively inherited (Fig. 1), a conclusion supported by the low to modest heritabilities (Table 2). These results agree with the results of selection experiments by Takeda (1990). The basis of this quantitative inheritance is a large number of FHB resistance QTL distributed throughout the genome, the most notable being that associated with cleistogamy. Those QTL showing large and consistent main effects are the most suitable targets for resistance breeding. We detected a total of 14 QTL clusters (Fig. 2 and Table 3). The highly resistant maternal parent Harbin 2-row contributed resistance alleles at 11 of these clusters. One approach to breeding for higher levels of FHB resistance would be to remedy the three "QTL allele defects" in Harbin 2-row. However, the level of improvement might be modest, considering the limited number of lines showing transgressive segregation for resistance (Fig. 1).

QTL interactions were significant in several cases (Table 4), but were always less than QTL main effects (Table 3). The additivity of FHB resistance is supported by the inverse relationship between number of FHB resistance alleles and FHB score (Table 5). As Mesfin et al. (2003) suggested, FHB resistance may be controlled by many QTLs with minor effects and several major QTLs associated with morphological and agronomic traits.

Table 5 Fusarium head blight (FHB) scores versus number of resistance QTL alleles in each of five recombinant inbred (RI) populations of barley

Population	No. of QTL alleles	No. of RI lines	FHB severity[a]
RI1	3	13	4.8 ± 1.1
	2	38	6.5 ± 1.4
	1	29	6.8 ± 1.1
	0	13	7.7 ± 1.2
RI2	6	1	5.7
	5	3	4.9 ± 2.0
	4	21	5.7 ± 1.3
	3	21	6.3 ± 1.4
	2	17	6.3 ± 1.5
	1	8	6.6 ± 0.8
RI3	3	8	3.7 ± 1.8
	2	31	6.7 ± 1.4
	1	37	7.0 ± 1.2
	0	11	7.9 ± 0.9
RI4	7	1	5.0
	6	7	4.7 ± 1.8
	5	5	4.3 ± 0.9
	4	18	6.8 ± 1.5
	3	30	7.6 ± 1.3
	2	14	8.1 ± 0.9
	1	3	7.8 ± 0.7
	0	1	9.0
RI5	6	4	5.5 ± 1.8
	5	13	6.5 ± 1.2
	4	26	5.8 ± 1.0
	3	24	6.5 ± 1.3
	2	9	6.8 ± 0.9
	1	3	6.8 ± 0.5
	0	3	7.9 ± 0.5

[a] Average and s.d. of FHB severity for 2 years in RI1 and 3 years in RI2, RI3, RI4 and RI5

In this research, we used parental accessions with varying levels of FHB resistance. However, most of the major resistance QTL alleles were already present in the most resistant accession, Harbin 2-row. This key finding would likely have been overshadowed if we used cross combinations segregating for *Vrs*/*vrs1*. We therefore believe that it is most appropriate to conduct genetic analyses and breeding efforts in cross combinations in which relevant resistance factors will come to light, free of the overshadowing pleiotropic effects of inflorescence and floral morphology.

Acknowledgements We would like to thank Dr. Patrick M. Hayes for his critical reading of the manuscript, Ms. N. Nankaku and Y. Motoi, Res. Inst. Biores., Okayama Univ., for their technical assistance.

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