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ORIGINAL PAPER

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QTL analysis of Fusarium head blight resistance using a high-density linkage map in barley

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Abstract Fusarium head blight (FHB) resistance was evaluated in a set of recombinant inbred (RI) lines from a cross between Russia 6 (resistant) and H.E.S. 4 (susceptible), which had one of the widest differences of FHB resistance reactions among ca. 5,000 barley germplasm accessions in Okayama University. Field-grown spikes were sampled and inoculated by the 'cut-spike test'. Resistance reactions on the parents and RI lines were scored by eleven grades, from resistant (0) to susceptible (10). Quantitative trait loci (QTL) analysis detected three QTL: two located on the long arm of chromosome 2H, and another on the short arm of chromosome 5H. A QTL located on chromosome 2H was coincident with the *vrs1* locus, which governs inflorescence row type. The other QTL on chromosome 2H was positioned in the vicinity of cleistogamy locus (*cly1* or *Cly2*) that determines inflorescence opening/closing. Resistant gene analog (RGA) and expressed sequence tag (EST) markers with homology for disease resistance genes were integrated into the high-density linkage map. Most of these markers were not localized near the identified resistance QTL, except for one RGA marker (FXLRRfor_XLRRrev170) localized in the vicinity of the *cly1*/*Cly2* locus. Five AFLP markers localized in the vicinity of the identified QTL were sequenced to convert them into sequence tagged site (STS) markers. Genotyping of each RI line using two AFLP–STS markers and the *vrs1* locus indicated that the RI lines with three Russia 6 QTL alleles exhibited the same level of high FHB resistance reactions as Russia 6. In contrast, RI lines with three susceptible alleles showed reactions close to H.E.S. 4. Therefore, the markers closely linked to the QTL can be efficiently used for the selection of resistance.

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Introduction

Fusarium head blight (FHB) or scab is one of the most serious diseases of barley and causes significant loss in both yield and quality (Atanasoff 1920; Ayoub and Mather 2002; Steffenson 2003). FHB has been serious in East Asia where the climate is moist during the growing period (Takeda and Heta 1989; Zhu et al. 1999). Many aspects of the Barley–Fusarium pathosystem have been studied on pathogen identification, population biology, epidemiology, biological and chemical controls, and forecasting in Japan (Higashi and Kato 1954; Nakagawa and Hori 1986). The genetic basis for FHB resistance in barley has been investigated because the cultivation of resistant plants is the most cost-effective method of controlling the disease (Mesfin et al. 2003).

Reliable and accurate genotyping of FHB resistance is difficult in barley. In particular, it is difficult to evaluate barleys with different maturities under the same inoculation condition. To overcome this problem, Takeda and Heta (1989) developed the 'cut-spike test', which entails the collection of spikes from field-grown plants and their inoculation under controlled conditions at the exact time of flowering. Using this technique, they evaluated 4,957 accessions for resistance to FHB and identified 23 accessions exhibiting a high level of resistance. Based on the resistance information, Takeda (1990) made five cross combinations between barley accessions with different resistant reactions and examined their genetic behaviors in the F_2, F_3, and F_4 generations. From the frequency distributions, he assessed the quantitative genetic factors controlling the resistance. He estimated relatively high heritability ($h^2 = 0.51$) from the parent-offspring correlation between F_3 and F_4 generations. Quantitative inheritance with high heritability in FHB resistance was also supported by the genetic analysis of a diallel cross among barleys with different levels of resistance (Takeda and Wu 1996).

After the serious FHB epidemics of the 1990 s in the Upper Midwest region of the United States, a number of

studies were completed on the genetics including mapping of FHB resistance in breeding. These studies identified many resistance quantitative trait loci (QTL) scattered across the genome, although with small effects, and strong associations between FHB resistance and certain morphological or physiological traits (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000; Mesfin et al. 2003; Dahleen et al. 2003). For example, two-rowed varieties showed higher FHB resistance than six-rowed varieties in several studies (Takeda and Heta 1989; Chen et al. 1991; Steffenson et al. 1996). Mesfin et al. (2003) and Dahleen et al. (2003) reported a QTL for FHB resistance near the *vrs1* locus (controlling row type) on chromosome 2H using populations derived from two-rowed × six-rowed crosses. However, they could not clarify whether FHB resistance was due to the pleiotropic effects of the *vrs1* locus or to linkage with the *vrs1* locus. Other than row type, several other traits such as heading date and plant height were coincident with FHB resistance in some studies (de la Pena et al. 1999; Ma et al. 2000). Late heading lines of spring barley tend to have lower severity of FHB while early heading lines tend to have higher severity under field conditions, apparently because late heading lines have less chance of pathogen attack in certain conditions (Steffenson 2003).

Knowing the genetic basis of resistance is important to achieve a higher level of resistance through the additive effects of resistance loci with different functions. Some QTL analyses for disease resistance reported QTL coincident with resistant gene analog (RGA) markers (Pflieger et al. 1999), which were generated from conserved motifs of disease resistance genes, and localized on the linkage maps in cereals (Leister et al. 1998). Expressed sequence tags (EST) are also useful markers when homologous sequences are annotated on the nucleotide or protein databases, e.g., Genbank. Thus, QTL analysis with RGA or EST markers may help to estimate the genetic basis of FHB resistance.

To establish a simple and effective method of selecting for FHB resistance, it would be advantageous to develop sequence tagged site (STS) markers linked with QTL conferring FHB resistance. Amplified fragment length polymorphism (AFLP) markers are useful for saturation mapping of QTL because a large number of polymorphic fragments can be amplified (Hori et al. 2003). AFLP markers must then be converted into STS markers for easy use in breeding. This technique has been used successfully in several crops including barley (Meksem et al. 2001; Guo et al. 2003).

The objectives of this investigation are (1) to detect QTL for FHB resistance on a high-density linkage map using the widest range of resistance materials identified from ca. 5,000 barley accessions by Takeda and Heta (1989), (2) to characterize them by comparison with the related agronomic traits, RGA, or EST markers, and (3) to develop effective STS markers for the selection of FHB resistance in practical breeding.

Materials and methods

Plant materials

One hundred and twenty-five F_{10} recombinant inbred (RI) lines derived from a cross between Russia 6 and H.E.S. 4 were developed by single-seed descent from F_2 individuals in the field at the Research Institute for Bioresources, Okayama University, Kurashiki, Japan. Russia 6, which is a two-row spring barley introduced from Russia, was selected as one of the most highly resistant cultivars by Takeda and Heta (1989). H.E.S. 4, which is a six-row spring barley collected from Afghanistan, was one of the most susceptible cultivars identified by the same authors. Ninety-five RI lines were randomly selected and used to construct a linkage map and to identify the loci for FHB resistance and some agronomic traits.

Phenotype evaluation

The RI lines were grown in the field at Kurashiki during the winter barley growing seasons of 2000–2001 (2001 season) and 2002–2003 (2003 season). Twenty plants were grown in a single row 90 cm apart with plants spaced by 4 cm. The whole plots were replicated twice. FHB inoculation was carried out by the 'cut-spike test' developed by Takeda and Heta (1989). Spikes from each line were collected from the field at the time of flowering, cut at the second internode from the top, and placed on a test-tube stand, which was set on a stainless-steel tray flooded with running tap water. *F. graminearum* strain OUZ78, which possesses moderate virulence and is maintained at the Research Institute for Bioresources, Okayama University, was used as a source of inoculum. The conidial suspension was grown in modified Bailey's medium (Joffe 1963) on a shaker at 25°C under continuous fluorescent light and adjusted to a concentration of 2×10^5 per ml with sterilized, deionized water. Before the inoculation, 0.05% Tween-20 was added to the suspension to increase adhesion of conidia on the spikes. Spikes were spray inoculated with a freshly prepared conidial suspension of 50 ml per tray (ca. 240–300 spikes). The inoculated spikes were then placed in a plant growth chamber maintained at 25°C and 100% humidity by a centrifugal humidifier with 15 min on-and-off intervals for 2 days under a 14 h photoperiod condition with light intensity 176.4 μmol^{-1} m^{-2}. After the infection period, the spikes were moved to another growth chamber maintained at 18°C with more than 90% relative humidity for 6 days under a 14 h photoperiod condition with light intensity of 176.4 μmol^{-1} m^{-2}. Disease symptoms were scored on the 8 days after inoculation using a 0–10 scale, where 0 indicated 0% infected florets per spike (resistant), and 10 indicated over 60% infected florets per spike (susceptible). One replication consisted of three spikes and the inoculations had five replications

in the 2001 season and three replications in the 2003 season. The mean score of replications for each RI line was used for QTL analysis.

The phenotypes were also scored on row type, fertility of lateral florets, flowering type, culm length, ear length, spike-exsertion length, awn length, number of spikelets, rachis-internode length (spike density), glume length, thousand-kernel weight, and heading date. Fertility of lateral florets was scored as inflated lateral florets or small lateral florets on two-rowed RI lines. Flowering type was scored as to whether anthers were exserted (opening) or not (closing) from the lemma at anthesis. Spike-exsertion length was measured by the length from the flag leaf to the bottom of the spike in a main tiller. Scoring of other traits followed the standard methods of the Barley Germplasm Center, Research Institute of Bioresources, Okayama University, described at the barley germplasm database (http://shigen.lab.nig.ac.jp/barley/). Row type, fertility of lateral florets, flowering type, and heading date of the RI lines were scored in both the 2001 and 2003 seasons. Culm length, ear length, spike-exsertion length, awn length, number of spikelets, rachis-internode length, glume length, and thousand-kernel weight were scored only in the 2001 season, because these traits showed high heritabilities (data not shown).

DNA marker analysis

A high-density linkage map (1,172 loci) comprised mostly of AFLP and SSR markers was developed by Hori et al. (2003). Resistance gene analog (RGA) markers amplified with degenerate primers were also positioned on the linkage map. Primer sequences and amplification condition of the RGA markers were according to Chen et al. (1998).

Thirty-eight EST sequences that showed high similarity for wheat R gene '*Cre3*' (Lagudah et al. 1997) and barley R genes 'b4', 'b5', 'b6' (Leister et al. 1998) were collected from NCBI nucleotide database (nt) by TBLASTX research (Altschul et al. 1990) with threshold e-value $1e^{-20}$. Thirty-one core EST markers with known map positions were also selected from the linkage map of Haruna Nijo × H602 (Sato et al. unpublished data). Sequence-specific primers were designed by the software package Primer3 (Rozen and Skaletsky 2000) with parameter settings of product size 200–500 bp, optimum primer size 20 mer, and optimum primer Tm 60°C. The primers were used to amplify DNA fragments from genomic DNA samples of Russia 6 and H.E.S. 4. Amplifications by EST primer sets were conducted by the following cycling conditions: 94°C for 2 min, five cycles of 94°C for 30 s, 65°C for 30 s, 72°C for 2 min decreasing by 1°C per cycle, 35 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 2 min, and 72°C for 7 min. PCR products were cycle sequenced by ABI 3100 DNA sequencer (Applied Biosystems, Foster, USA). CAPS (Konieczny and Ausubel 1993) or dCAPS markers (Neff et al. 1998) were designed when the parental sequences included single nucleotide polymorphisms (SNP). PCR products from genomic DNA samples of the RI lines were then digested with the respective recognizing restriction enzyme (1 unit) with the appropriate buffer. Electrophoresis was performed using the High Efficiency Genome Scanning (HEGS) system (Hori et al. 2003).

STS marker conversion

The AFLP procedure used was according to Hori et al. (2003). Ten AFLP markers located in the vicinity of the identified FHB resistance QTL were selected for conversion into STS markers. AFLP fragments on the acrylamide gels were removed with sterilized toothpicks and dissolved in 50 µl sterilized water. After the fragments were re-amplified with the same AFLP primer combinations, PCR products were cloned using pGEM-T Easy Vector Systems kit (Promega, Madison, USA). The isolated plasmids were used for sequencing analysis with the M13 primer set on the ABI 3100 DNA sequencer. After the sequencing analysis, CAPS markers were designed when the sequences of PCR products included SNP between Russia 6 and H.E.S. 4. TAIL-PCR (Liu et al. 1995) was used to detect polymorphisms when the parental sequences did not show the differences. We followed the methods of Liu et al. (1995) for arbitrary primer sequences and their accompanying PCR conditions. The corresponding three-nested primers were designed based on the target AFLP fragment sequences using Primer3 with the parameters set as above. Sequences homologous with each AFLP fragment were searched for on the NCBI nucleotide database (nt) by the BLASTN program (Altschul et al. 1990). Segregation data of successful AFLP-STS markers were subsequently used to map and validate their locations on the linkage map. All PCR products were electrophoresed on a 3% agarose gel at a constant power of 100 V.

Data analysis

The segregation data of morphological, RGA, EST, and AFLP-STS markers were integrated into the barley high-density linkage map using MAPMAKER/EXP Version 3.0 (Lander et al. 1987) and MAPL98 (Ukai et al. 1995). The Kosambi mapping function was used to calculate map distances (Kosambi 1944).

The QTL analysis was carried out by simple interval mapping (SIM) and multiple QTL model (MQM) using software packages MAPMAKER/QTL Version 1.1b (Lander and Bostein 1989) and by composite interval mapping (CIM) using QTL Cartographer Version 1.16 (Basten et al. 1994). LOD threshold 2.0, which was estimated by the permutation test using QTL Cartographer, was used to declare the presence of putative QTL. The LOD peaks of each significant QTL were considered as the QTL locations on the linkage map.

Results

Phenotypes for FHB resistance and agronomic traits

Figure 1 shows the scores of the parents and RI lines for FHB resistance in the 2001 and 2003 seasons. The means and standard errors of FHB resistance scores of Russia 6 and H.E.S. 4 were 2.4 ± 0.2 and 9.6 ± 0.2 in the 2001 season, and 4.4 ± 0.2 and 9.4 ± 0.3 in the 2003 season. Russia 6 showed markedly lower FHB scores than those for H.E.S. 4 in both the seasons. The RI lines differed markedly in their FHB resistance scores, ranging from 2.3 (resistant) to 10.0 (susceptible) in the 2001 season, and from 3.0 to 10.0 in the 2003 season, respectively. The means and standard deviations of total RI lines were 6.6 ± 1.9 in the 2001 season and 7.7 ± 1.7 in the 2003 season. Broad-sense heritabilities of the RI lines estimated from analysis of variance (Falconer 1989) were 0.79 in the 2001 season and 0.52 in the 2003 season. The correlation coefficient for resistance scores of the RI lines between the 2001 and 2003 seasons was significant at the 1% level ($r = 0.53$).

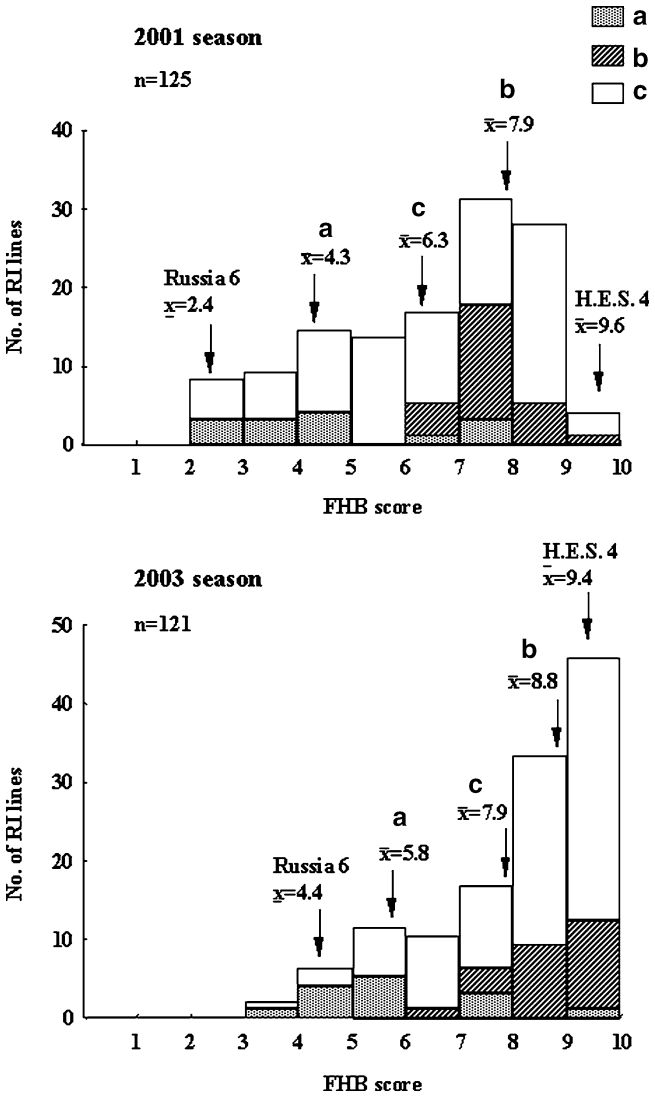

Fig. 1 Frequency distributions of FHB scores and marker genotypes at QTL regions in the RI population derived from a cross between Russia 6 and H.E.S. 4 in the 2001 and 2003 seasons. Genotypes of RI lines are classified into **a** RI lines possessing three Russia 6 alleles at the three QTL regions for FHB resistance, **b** RI lines possessing three H.E.S 4 alleles, and **c** lines other than **(a)** and **(b)**. The scores of Russia 6, H.E.S. 4, and averages of **a**, **b**, and **c** are indicated by *arrows*. FHB score 0 indicates 0% infected florets per spike (resistant), and FHB score 10 indicates over 60% infected florets per spike (susceptible)

Correlation coefficients were calculated between the resistance score and nine agronomic traits in the 2001 and 2003 seasons (Table 1). Four of the nine agronomic traits were significantly correlated with the resistance score, but at low *r*-values (< 0.37). Rachis-internode length ($r = 0.28$) showed a significant positive correlation with the resistance score, indicating higher susceptibility in the RI lines with lax spikes. Spike-exsertion length ($r = -0.25$) and thousand-kernel weight ($r = -0.37$) showed significant negative correlations with the resistance, indicating higher resistance in the RI lines with longer spike exsertion or larger grain sizes. Heading date was significantly correlated ($r = 0.22$) with the resistance score in the 2001 season, but not in the 2003 season ($r = -0.06$).

Genotyping by morphological and molecular markers

Row type (*vrs1*), flowering type (*cly1*/*Cly2*), and fertility of lateral florets (*int-c*) were mapped as morphological markers on the linkage map constructed previously (Hori et al. 2003). Each trait segregated into the expected ratio of 1:1 in the RI population. Row type (two-rowed or six-rowed) conferred by *vrs1* was scored in all RI lines and mapped to the long arm of chromosome 2H, as reported by Mesfin et al. (2003) and Dahleen et al. (2003). Flowering type (opening or closing) conferred by *cly1*/*Cly2* was located on the long arm of chromosome 2H, as reported by Turuspekov et al. (2004). The fertility of lateral florets was scored only in two-rowed RI lines and mapped on the short arm of

Table 1 Correlation coefficients (*r*) between FHB resistance and agronomic traits in the RI population derived from the cross of Russia 6×H.E.S. 4

Trait[a]	r[b]
Culm length	−0.17
Ear length	0.18
Awn length	0.04
Spike exsertion	−0.25**
Glume length	−0.06
Number of spikelets	−0.21
Rachis-internode length	0.28**
Thousand-kernel weight	−0.37**
Heading date (2001)	0.22*
Heading date (2003)	−0.06

[a]Heading dates were scored both in the 2001 and 2003 seasons, other traits were scored only in the 2001 season
[b]*,** significant at the 5% and 1% levels, respectively

chromosome 4H, which is most likely the *int-c* locus previously reported (Lundqvist and Franckowiak 1997; Fig. 2).

Using 16 degenerated primer pairs, 43 amplified fragments showed polymorphisms between Russia 6 and H.E.S. 4, and segregated in the RI population. Forty polymorphic bands were localized on the linkage map with four to nine RGA markers on each chromosome (Fig. 2). Sequence comparisons of PCR products from 22 EST primer sets, which were selected by homology search with R genes, indicated some SNP sites between Russia 6 and H.E.S. 4. Based on the recognition sequences of restriction endonucleases, five and one primer sets were designed as CAPS (BAH36F09, HU03k01r, BAAK29F13, BAH21C03, and rBAAL2G09) and dCAPS (BAH29F22) markers, respectively. They were localized on chromosomes 5H (one), 6H (two), and 7H (three) (Fig. 2). Thirty-one of the position-anchored EST markers were also mapped in positions similar to these on the reference linkage map of a doubled haploid population derived from the cross between Haruna Nijo and H602 (Sato et al. unpublished data; Fig. 2).

QTL detection of FHB resistance and agronomic traits

Figure 3 shows LOD score profiles by CIM analysis for FHB resistance and nine agronomic traits on chromosomes 2H and 5H. QTL detected in this study existed in coincident or very close regions with flanking markers on the high-density map (Tables 2, 3). Three QTL for FHB resistance were detected, two on chromosome 2H and one on chromosome 5H (Table 2). All the QTL alleles originated from the resistant parent Russia 6 contributed higher FHB resistance. Peak LOD positions were coincident or very close in both the 2001 and 2003 seasons; however, the variances explained by each QTL were different between the two seasons. The QTL on chromosome 2H coincident with the *vrs1* locus was not detected in the 2003 season. The *cly1*/*Cly2* locus and one

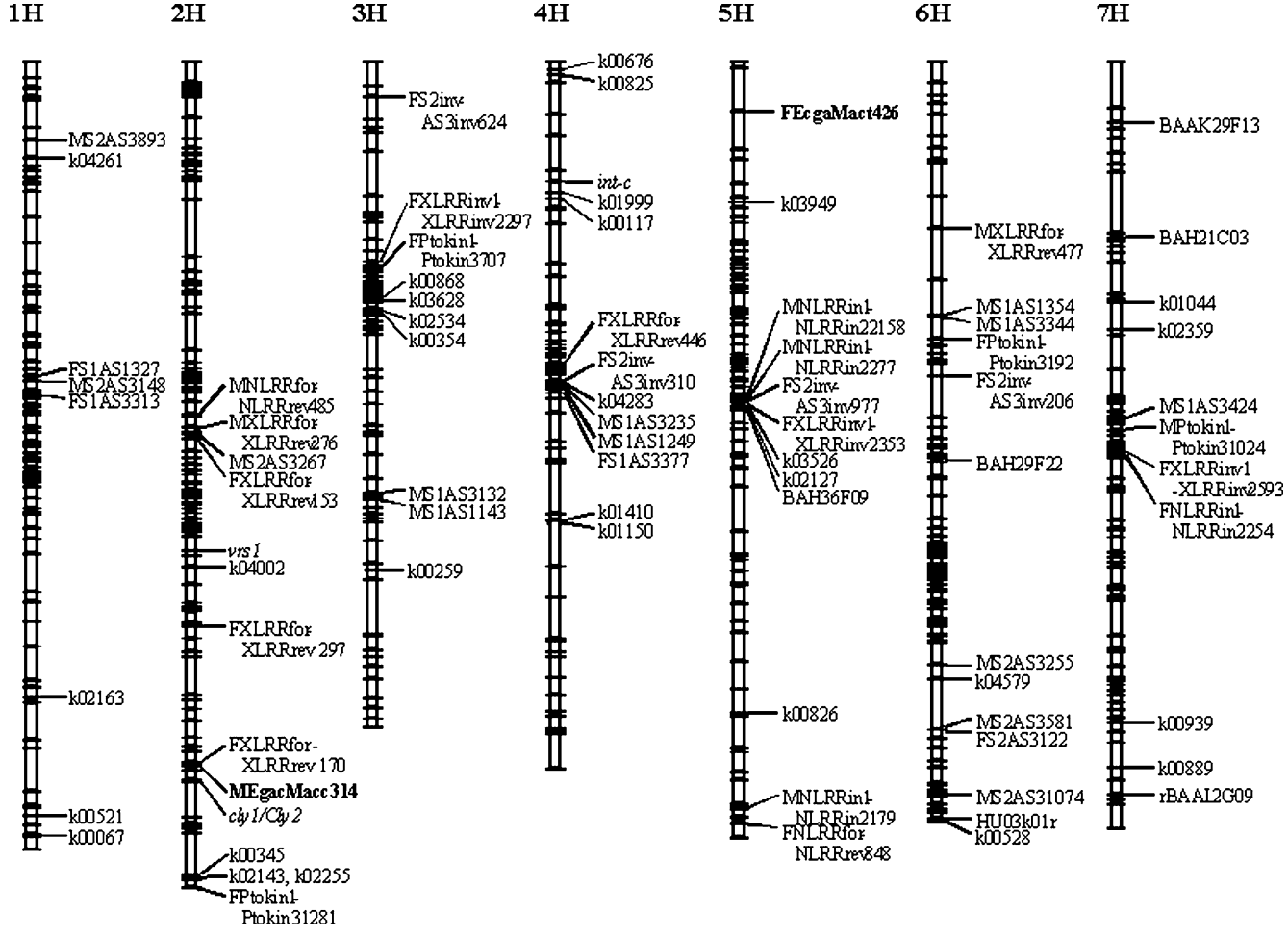

Fig. 2 Distribution of morphological (*vrs1*, *cly1*/*Cly2* and *int-c*), RGA, EST, and AFLP–STS (MegacMacc314 and FEcgaMact426) markers on the high-density linkage map in the RI population derived from Russia 6×H.E.S. 4. Morphological and AFLP–STS markers are indicated in *bold*. Linkage groups are orientated with the short arms on the *top*. *Crossbars* are shown as loci mapped previously (Hori et al. 2003). Marker names are shown on the *right side* of each chromosome. EST markers starting with 'k' (e.g., k04261) are selected based on the linkage map of the different segregating population (Haruna Nijo × H602; Sato et al. unpublished data)

Table 2 QTL for FHB resistance detected by simple interval mapping (*SIM*), composite interval mapping (*CIM*), and multiple QTL model (*MQM*)

Year	Algorithm	Chromosome	Marker interval	Position[a] (cM)	LOD[b]	Var.[c] (%)	Weight[d]
2001	SIM	2H(a)	*vrs1*-cMWG699	0.0	2.8	13.0	-1.3
		2H(b)	MEgacMacc314-FEgtaMacg677	0.0	3.5	16.7	-1.5
		5H(c)	NEaacMcat28-MEccgMacg1057	2.6	3.6	18.7	-1.6
	MQM	(a + b)			5.7	25.9	
		(a + c)			5.4	28.6	
		(b + c)			5.0	25.7	
		(a + b + c)			8.0	37.3	
	CIM	2H	*vrs1*-cMWG699	0.0	2.5	10.1	-1.2
		2H	MEgacMacc314-FEgtaMacg677	0.0	3.2	13.5	-1.4
		5H	NEaacMcat28-MEccgMacg1057	2.6	3.4	15.5	-1.5
2003	SIM	2H(b)	MEgacMacc314–FEgtaMacg677	0.0	6.0	26.1	-1.8
		5H(c)	NEaacMcat28–MEccgMacg1057	2.6	2.3	11.2	-1.2
	MQM	(b + c)			6.8	35.8	
	CIM	2H	MEgacMacc314–FEgtaMacg677	0.0	6.0	25.9	-1.8
		5H	NEaacMcat28–MEccgMacg1057	2.6	3.4	15.9	-1.4

[a]Distance of peak LOD score position from the left side marker
[b]Peak LOD score
[c]Explained variance
[d]Estimated additive effect of Russia 6 allele

RGA marker (FXLRRfor_XLRRrev170) were localized in the vicinity of the QTL with the largest effect on chromosome 2H (Fig. 3). Total LOD scores with explained variances of MQM are shown in Table 2. The MQM effects were smaller than the sum of additive effects of each QTL, indicating some degree of interaction among resistance QTL. In particular, combination of the QTL located on the vicinity of the *cly1*/*Cly2* locus and the QTL located on chromosome 5H showed lower explained variance (25.7%) than the simple sum of additive variances (35.4%) of the two QTL.

Several QTL were also detected by both SIM and CIM analyses on nine agronomic traits (culm length, ear length, spike-exsertion length, awn length, number of spikelets, rachis-internode length, glume length, thousand-kernel weight, and heading date). SIM and CIM analyses found totals of nine and 17 QTL for these agronomic traits, respectively (Table 3). One (SIM) and three (CIM) QTL for heading date were detected in both the 2001 and 2003 seasons. QTL analysis of culm length, ear length, spike-exsertion, and thousand-kernel weight was already reported in Hori et al. (2003). Fewer QTL were detected by SIM than CIM in all traits except glume length (Hori et al. 2003; Table 3). Figure 3 shows the locations of QTL for the agronomic traits on chromosomes 2H and 5H, on which FHB QTL were localized. QTL for all nine traits were localized on chromosome 2H, and QTL for plant height, spike-exertion length, glume length, and number of spikelets were detected on chromosome 5H.

STS marker development

Ten AFLP markers located closely on each side of the three resistance QTL were converted into STS markers. All AFLP markers showed dominant segregation in the RI population. Seven of the ten AFLP fragments re-amplified from the fragment samples on the acrylamide gel were sequenced. Sequences of MEggaMcct188 and FEgacMatt73 were too short to design a primer set. A CAPS marker was developed for FEcgaMact426 and mapped to its original map position. In contrast, four AFLP fragments (FEggcMgga439, MEgacMacc314, FEgtaMacg677, and MEccgMacg1057) did not show any polymorphisms (Table 4). TAIL-PCR revealed that MEgacMacc314 included a 57-base insertion-deletion polymorphism between Russia 6 and H.E.S. 4. A pair of primers was then designed to include the polymorphic sequences in order to map them to the original position. The positions of two AFLP-STS markers (MEgacMacc314 and FEcgaMact426) are shown in Fig. 2, and their segregation patterns are shown in Fig. 4.

Using the *vrs1* locus and the two AFLP-STS markers closely linked to the identified QTL, the RI lines were genotyped to show the effect of combination among the FHB resistance loci in the 2001 and 2003 seasons (Fig. 1). Distances from the QTL were 0.0 cM for the *vrs1* locus on chromosome 2H, 0.0 cM for MEgacMacc314 on chromosome 2H, and 9.0 cM for FEcgaMact426 on chromosome 5H. Based on phenotypes of the *vrs1* locus and genotypes of the two markers, we classified the RI population into three subgroups: (a) 15 RI lines with three alleles from Russia 6, (b) 24 RI lines with three alleles from H.E.S. 4, and (c) other RI lines. The means and standard errors of FHB resistance scores of the RI lines with three Russia 6 alleles and the RI lines with three H.E.S. 4 alleles were 4.3 ± 0.5 and 7.9 ± 0.2 in the 2001 season and 5.8 ± 0.4 and 8.8 ± 0.3 in the 2003 season, and the difference was significant at the 1% level with

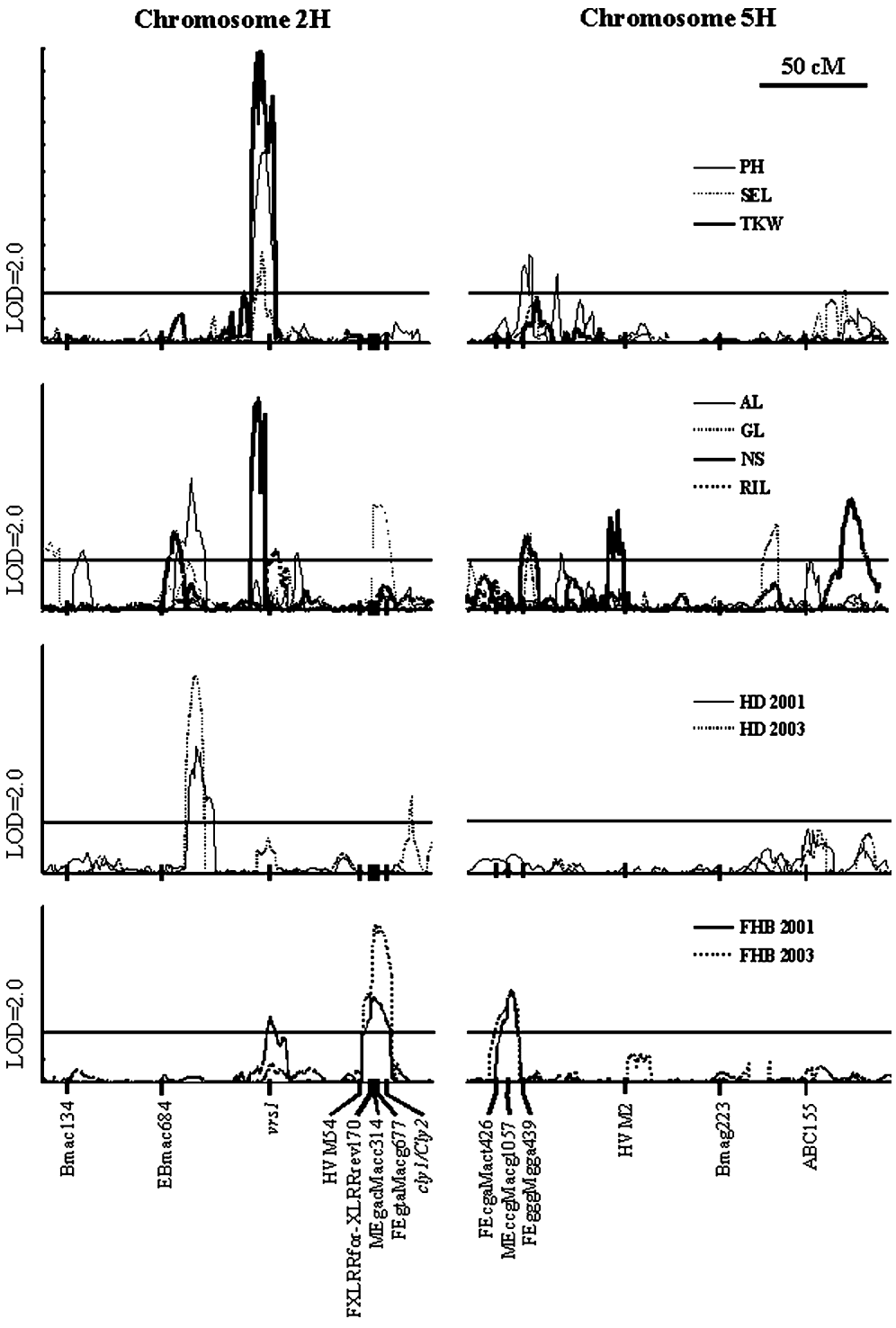

Fig. 3 LOD scans from QTL analysis (composite interval mapping) of FHB resistance and agronomic traits on chromosomes 2H and 5H of the high-density map in the RI population derived from Russia 6×H.E.S. 4. LOD score profiles of chromosomes are shown from the *left* oriented by the short arm. Locations of morphological, SSR, RGA, and AFLP-STS markers are indicated by the *vertical lines* under the LOD *curves*. Each trait is indicated as abbreviation letters in *bold*; FHB 2001 and FHB 2003 are resistance scores for FHB in the 2001 and 2003 seasons, HD 2001 and HD 2003 are heading dates in the 2001 and 2003 seasons. The other abbreviations of traits are awn length (*AL*), glume length (*GL*), number of spikelets (*NS*), rachis-internode length (*RIL*), plant height (*PH*) including culm length and ear length, spike-exsertion length (*SEL*), and thousand-kernel weight (*TKW*). The results for PH, SEL, and TKW are quoted from Hori et al. (2003)

t value = 7.37 in the 2001 season and 3.27 in the 2003 season. However, four RI lines possessing three Russia 6 alleles showed susceptible reactions (average disease scores of the two seasons were 7.5, 7.5, 7.9, and 9.5). These RI lines showed the same degree of susceptibility in both the 2001 and 2003 seasons, which might have come from recombination between the AFLP-STS markers and the QTL for FHB resistance.

Discussion

Evaluation of FHB resistance

The present analysis gives clear insight into the elements of FHB resistance in barley. Even though disease severity (mean disease score) for the RI lines in the 2003

Table 3 QTL associated with agronomic traits on the high-density map detected by simple interval mapping (*SIM*) and composite interval mapping (*CIM*)

Trait[a]	Algorithm	Chromosome	Marker interval	Position[b] (cM)	LOD[c]	Var.[d] (%)	Weight[e]
Awn length (mm)	SIM	2H	Bmac93–EBmac684	0.2	4.9	21.2	20.9
	CIM	2H	Bmac93–EBmac684	0.2	7.9	23.4	22.3
		3H	FEccgMacc84–FEgcgMatt264	1.2	2.8	7.3	12.2
		6H	FPaaaMctc377–NEaacMcga25	2.0	2.9	8.0	−12.8
Glume length (mm)	SIM	2H	MEggcMatt194–FEgtgMacg86	2.3	3.1	14.6	1.6
		3H	FEgacMacc687–FEctaMact284	1.6	2.5	11.2	1.4
		5H	MEggaMtgg228–FEggcMatc197	1.2	2.5	11.3	1.4
	CIM	1H	FEacgMatt982–MEgtcMacg210	1.9	2.6	6.7	−1.1
		2H	FEgtgMacg86–k00345	2.3	5.6	15.6	1.6
		3H	FEgacMacc687–FEctaMact284	1.6	4.0	10.6	1.3
Number of spikelets	SIM	2H	FEgacMacg102–MEggaMccc1164	1.9	11.7	45.3	4.4
		5H	MEcccMatt547–MEcctMacg403	0.4	3.1	13.9	2.4
	CIM	1H	MEtcaMatt558–MEggcMatt360	0.0	3.5	5.8	1.6
		2H	FEgacMacg102–MEggaMccc1164	1.9	13.2	28.2	4.1
		5H	FEtcgMact182–MEcccMagc78	0.5	3.1	4.4	1.4
Rachis-internode length (mm)	SIM	7H	MEgacMagg504–FEataMaat666	3.3	15.2	53.1	8.5
	CIM	2H	k02143–k02255	4.8	9.9	16.8	-5.0
		7H	MEgacMatt212–FNLRRin1NLRRin2254	2.1	21.9	57.8	9.1
Heading date (2001) (days)	SIM	2H	FEgcaMagc453–MEgagMagt172	3.5	4.5	21.7	5.5
	CIM	2H	FEgcaMagc453–MEgagMagt172	1.9	8.8	26.0	6.2
		6H	MEggaMact180–FEtggMatc643	1.5	3.4	8.7	3.6
		7H	FEgggMatc372–FEctcMagc455	0.7	3.5	9.0	3.6
Heading date (2003) (days)	SIM	2H	FEgcaMagc453-MEgagMagt172	3.7	10.4	39.6	3.8
	CIM	2H	FEgcaMagc453-MEgagMagt172	1.9	14.3	43.4	4.1
		6H	FEtggMatc643-MEacgMagt790	1.5	5.7	10.6	2.0
		7H	MEgatMatc58-MEctaMata894	0.9	3.8	9.7	1.9

[a]Heading dates were scored both in the 2001 and 2003 seasons. Other traits were scored in the 2001 season only
[b]Distance of peak LOD score position from the left side marker
[c]Peak LOD score
[d]Explained variance
[e]Estimated additive effect of Russia 6 allele

season was significantly higher than that in the 2001 season (t value = 7.10, $P < 0.01$), the correlation coefficient between the two seasons was significant at the 1% level ($r = 0.53$). Other reference materials also showed higher scores in the 2003 season than in the 2001 season (data not shown). This difference might be caused by the higher precipitation during the flowering period in the 2003 season (125 mm) than in the 2001 season (29 mm). Broad-sense heritabilities were relatively high for disease severity ($h^2 = 0.79$ in the 2001 season and $h^2 = 0.52$ in the 2003 season), indicating the reliability of experiments or the large effect of the QTL. However, the large environmental variances in the 2003 season might be the reason for the decrease in the power of QTL detection.

The QTL analysis detected two QTL located on the long arm of chromosome 2H and another on the short arm of chromosome 5H. Epistatic interactions might be expected between the QTL located on the vicinity of the *cly1*/*Cly2* locus and the QTL located on chromosome 5H by the comparisons between total explained variances in MQM analysis and those of individual QTL in SIM analysis (Table 2). Interactions among QTL for FHB resistance are very important to express resistant reaction when multiple QTL are introgressed into the same genetic background.

One of the major objectives of this paper was to find the maximum number of QTL between the parents, which had the most different FHB reactions from evaluation of ca. 5,000 barley accessions using the 'cut-spike test' by Takeda and Heta (1989). The present study was not designed to detect QTL by environment interactions by planting the population in various conditions, but to confirm resistance factors, which were observed by the previous germplasm evaluation under the same method. The advantage of the 'cut-spike test' is the precise control of inoculation condition. It is important to detect QTL by reducing experimental errors. In this study, low significant correlations between FHB resistance score and the agronomic traits were observed (Table 1), and positions of the QTL for FHB resistance were coincident or very close in both the two seasons (Table 2). Thus, we might detect most of FHB resistance QTL between the parents using the 'cut-spike test'.

Comparison with other QTL studies for FHB resistance

The resistant parent Russia 6 in this study was one of the 23 elite resistant varieties showing a very high degree of FHB resistance among 4,957 barley accessions tested by

Table 4 Characters of AFLP–STS primers, which are located in the vicinity of QTL associated with FHB resistance in the recombinant inbred lines derived from a cross between Russia 6×H.E.S. 4

Marker name	Chromosome	Position[a] (cM)	Primer sequence[b]		Product size (bp)			BLASTn hit information		
			Forward	Reverse	Russia 6	H.E.S. 4	Difference	Sequence name	Score	*E*-value
MEgacMacc314	2H	0.0	AGAGATCCCTGCTCAGCTTG	TCGTATTAAGGCCGCATAGG	581	524	57	*Triticum aestivum* cDNA clone: whyf5d18, 3′ end	105	4E-20
FEgtaMacg677	2H	0.6	GCACGTAGCGTTCAACATCA	AACTTTTCCCAACCCTTTCC	208	208	0	*Oryza sativa* chromosome 9 clone PAC0645D04	84	2E-13
FEcgaMact426	5H	9.0	CCGTGTGTCGTCTAGGTCAA	CAACTTTGGTGGGACGTAGG	335	335	0	*Triticum aestivum* cDNA clone AZO4100A18	44	0.051
MEccgMacg1057	5H	2.2	CATGAACATGCAGCCAAGTC	TGAGACTGAAAAGCGGGTATG	180	180	0	*Triticum aestivum* (mercia) clone D10_j122_plate_2 embryo 14 days post anthesis	44	0.096
FEgggMgga439	5H	8.0	AACGATTTCCCTTTGGAAGC	GTTGCAAGAAGCCTGGGATA	254	254	0	HB04P19r BC *Hordeum vulgare* subsp. *vulgare* cDNA clone HB04P19 5-PRIME	343	4E-92

[a]Distance from peak LOD score position on the linkage map
[b]Primer sequences are shown in order from 5'-end to 3'-end

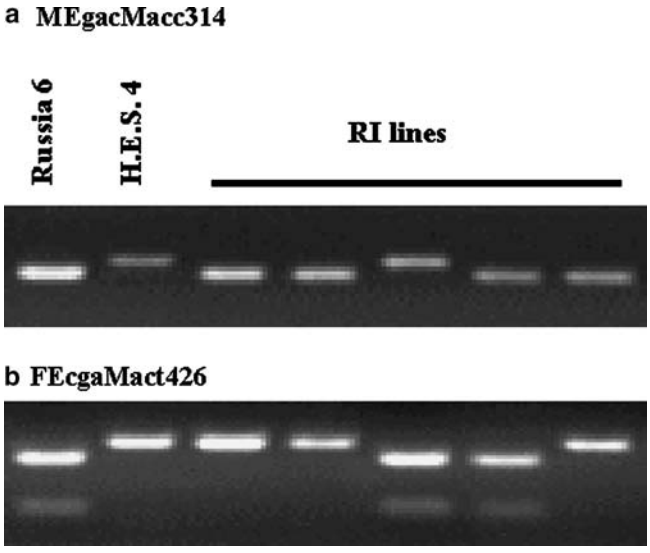

Fig. 4 Electrophoresis images of marker genotyping in MEgacMacc314 (**a**) and FEcgaMact426 (**b**) located in the vicinity of QTL for FHB resistance on chromosomes 2H and 5H, respectively

Takeda and Heta (1989). Several previous studies also reported QTL for FHB resistance, which were scattered across the genome with small effects (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000; Mesfin et al. 2003; Dahleen et al. 2003). As a source of germplasms for studying the genetics of FHB resistance, Mesfin et al. (2003) used Frederickson, which showed the highest degree of resistance described by Takeda and Heta (1989). De la Pena et al. (1999) and Ma et al. (2000) used Chevron, which is considered one of the most resistant six-rowed barleys (Steffenson 2003). Zhu et al. (1999) used Gobernadora and CMB643, which show moderate resistance to FHB in China. Dahleen et al. (2003) used Zhender 2, which also shows moderate resistance to FHB. All these resistance accessions might be descended from different sources and origins.

To assess the diversity for FHB resistance in the barley gene pool, integration of multiple mapping results is of particular importance. In this study, two QTL were detected on the long arm of chromosome 2H and another on the short arm of chromosome 5H. de la Pena et al. (1999), Zhu et al. (1999), Mesfin et al. (2003), and Dahleen et al. (2003) detected a QTL in the vicinity of the *vrs1* locus on chromosome 2H. Ma et al. (2000), Mesfin et al. (2003), and Dahleen et al. (2003) reported a QTL within a marker interval ksuD22-ABC153 on chromosome 2H, which is likely coincident with the *cly1*/*Cly2* locus. de la Pena et al. (1999) and Ma et al. (2000) reported a QTL within CDO400-CDO59B and MWG502-BG739 on the short arm of chromosome 5H. These QTL might be located on the same chromosomal regions as the identified QTL in this study.

The AFLP analysis can easily construct a high-density linkage map and obtain a number of markers linked more closely with QTL than other methods such as RFLP or SSR. In this study, most of the molecular markers were generated by AFLP analysis (Tables 2, 3), although previous QTL studies for FHB resistance used RFLP. Thus, we could not determine the precise locations of the identified QTL in the present study compared to the previous studies. Since RFLP technique is robust but not practical for marker-assisted selection and RFLP-STS markers sometimes fail to localize on the original map positions, it might be necessary to integrate the position-anchored PCR markers (AFLP-STS, SSR, and EST markers) used in this study into the linkage maps of other studies. We are currently trying to introduce EST markers from a high-density barley transcript map (Sato et al. unpublished data) into multiple mapping populations. This procedure might be much easier and more informative to coordinate QTL among the multiple populations.

Association between FHB resistance and agronomic traits

A LOD peak was coincident with the *vrs1* locus in this study (Table 2, Fig. 3), and two-rowed RI lines also showed significantly higher resistance than six-rowed lines in the 2001 season. Several massive germplasm screening studies have shown that two-rowed barleys are more resistant to FHB than six-rowed barleys (Takeda and Heta 1989; Chen et al. 1991). However, the *vrs1* locus might not be a primary factor for FHB resistance in the present parental combination, since the difference between row types, which might be nullified by the higher disease level, was not significant in the 2003 season.

Zhu et al. (1999), Mesfin et al (2003) and Dahleen et al. (2003) also reported a QTL in the vicinity of the *vrs1* locus. Dahleen et al. (2003) suggested that this was most likely due to a linkage between the *vrs1* locus and a resistant locus. They considered that if the resistance gene had a single origin and was transferred to other accessions by crosses between germplasms, resistant accessions would possess the same alleles at the *vrs1* locus and the resistance locus. However, it is inconsistent with the fact that several FHB-resistant two-rowed varieties used by the previous studies possess the different *vrs1* alleles. It is especially important to resolve the question in breeding six-rowed barley for FHB resistance. In this study, the *vrs1* locus might be one of the FHB resistance factors because the QTL position was coincident with the *vrs1* locus. We are currently mapping the resistance on two-rowed × two-rowed barley populations to see whether the *vrs1* locus was associated with FHB resistance or not. If we can identify resistance QTL linked to the *vrs1* locus in the two-rowed × two-rowed populations, it should be possible to develop six-rowed barleys with elevated levels of FHB resistance.

Zhu et al (1999) and Steffenson (2003) summarized data showing a relationship between anther exsertion and FHB severity. In this study, closed flowering lines were

significantly more resistant than open flowering lines in both the 2001 and 2003 seasons. The means and standard errors of FHB resistance scores of closed flowering lines and open flowering lines were 5.7 ± 0.3 and 7.1 ± 0.2 in the 2001 season, and 6.8 ± 0.3 and 8.6 ± 0.2 in the 2003 season, respectively. The differences were significant at the 1% level with t value = 4.12 and 5.74 in the 2001 and 2003 seasons. In this study, one QTL for FHB resistance was detected within 1.7 cM from the *cly1*/*Cly2* locus on the long arm of chromosome 2H (Fig. 3). One RGA marker (FXLRRfor_XLRRrev170) was also localized in the vicinity (1.1 cM) of the QTL. It might be suggested that the *cly1*/*Cly2* locus had pleiotropic effect conferring the resistance, or was linked with resistance. More precise analysis will be necessary to reveal the relationship between resistance and the responsible genes in this region.

The QTL for plant height, glume length, and number of spikelets were localized near the resistance QTL located on the short arm of chromosome 5H. Moreover, although the high-density linkage map encompassed 40 RGA and 37 EST markers, which were annotated by BLAST homology searches, none of these markers were localized in the vicinity of this QTL (Fig. 2). This QTL must be investigated in detail in the future for its efficient utilization to obtain any critical information.

A significant positive correlation between heading date and FHB score ($r = 0.22$) was detected with late heading RI lines showing the highest susceptibility in the 2001 season (Table 1), although Takeda and Heta (1989) reported that FHB resistance was independent of heading date. QTL analyses detected an effect for heading date on chromosome 2H in both the 2001 and 2003 seasons (Fig. 3). Therefore, the low but significant correlation between heading date and FHB score in the 2001 season might come mainly from the linkage between QTL for heading date and FHB resistance on chromosome 2H. An association between late heading date and low FHB infection in the field inoculation test of barley was reported by Steffenson (2003) and other mapping studies.

Rachis-internode length (spike density) was also significantly correlated with FHB resistance ($r = 0.28$, $P < 0.05$). The positive correlation indicates higher resistance in lines with dense spikes. A possible explanation for the positive correlation might be the minor QTL for rachis-internode length, which was identified with linked position (5.9 cM) to the resistance QTL on chromosome 2H (Fig. 3). Zhu et al. (1999) and Ma et al. (2000) found coincident QTL for spike density and FHB reaction in two-rowed × two-rowed and six-rowed × six-rowed barley populations, respectively. On the other hand, Takeda and Heta (1989) identified a number of highly resistant lines with dense spikes as well as ones with lax spikes.

Correlation coefficients of spike-exsertion length ($r = -0.25$) or thousand-kernel weight (-0.37) with FHB resistance were significant. Since both QTL for spike-exertion length and thousand-kernel weight were detected in the vicinity of the resistance QTL, these correlations might be due to the pleiotropic effect of the *vrs1* locus. To estimate effects by these agronomic traits precisely, more number of measures of the agronomic traits were necessary.

Presence of mycotoxins including deoxynivalenol (DON) and nivalenol (NIV) cause the most serious economic damage from FHB infection (Tanaka et al. 1988). Several FHB resistance QTL are reported to coincide with DON concentration (de la Pena et al. 1999; Ma et al. 2000). However, we could not estimate toxin concentration due to the use of the 'cut-spike test', which does not allow materials to be grown until maturity. This is one of the disadvantages of the 'cut-spike test'.

Development of AFLP-STS markers

The AFLP markers located in the vicinity of the identified QTL were converted to STS markers. Since no tightly linked STS markers with QTL for FHB resistance have been reported in barley so far (de la Pena et al. 1999; Zhu et al. 1999; Ma et al. 2000; Mesfin et al. 2003; Dahleen et al. 2003), two resistance-linked AFLP-STS markers with sequence information could efficiently compare resistance QTL locations with other mapping populations.

Based on genotypes of the two AFLP-STS markers and phenotype of the *vrs1* locus, FHB resistance scores were compared between the two subgroups with either three Russia 6 alleles or H.E.S. 4 alleles (Fig. 1). These patterns suggested that the AFLP-STS markers developed in this study could be used in marker-assisted selection to achieve high level of FHB resistance. However, four RI lines possessing three Russia 6 alleles showed susceptible reactions. These RI lines might have been mis-scored or perhaps there was recombination between the STS marker and the resistance QTL, since FEcgaMact426 was 9.0 cM distant from the QTL on chromosome 5H.

It would be more important to estimate the effects of individual resistance alleles in common genetic backgrounds. To develop near isogenic lines with each resistance allele, we are backcrossing RI lines by marker-assisted selection using the AFLP-STS markers linked to the resistance QTL. Moreover, more rigorous validation of the resistance alleles might be obtained when the identified resistance QTL are transferred into other genetic backgrounds. The present study could lead to a Russia 6-derived breeding system for FHB resistance including identification of candidate genes associated with FHB resistance in barley.

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