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Efficient construction of high-density linkage map and its application to QTL analysis in barley

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Abstract Using a High Efficiency Genome Scanning (HEGS) system and recombinant inbred (RI) lines derived from the cross of Russia 6 and H.E.S. 4, a high-density genetic map was constructed in barley. The resulting 1,595.7-cM map encompassed 1,172 loci distributed on the seven linkage groups comprising 1,134 AFLP, 34 SSR, three STS and *vrs1* (kernel row type) loci. Including PCR reactions, gel electrophoresis and data processing, 6 months of work by a single person was sufficient for the whole mapping procedure under a reasonable cost. To make an appraisal of the resolution of genetic analysis for the 95 RI lines based on the constructed linkage map, we measured three agronomic traits: plant height, spike exsertion length and 1,000-kernel weight, and the analyzed quantitative trait loci (QTLs) associated with these traits. The results were compared on the number of detected QTLs and their effects between a high-density map and a skeleton map constructed by selected AFLP and anchor markers. The composite interval mapping on the high-density map detected more QTLs than the other analyses. Closely linked markers with QTLs on the high-density map could be powerful tools for marker-assisted selection in barley breeding programs and further genetic analyses including an advanced backcross analysis or a map-based cloning of QTL.

Keywords Barley · High Efficiency Genome Scanning (HEGS) · Genetic map · QTL

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Introduction

Cultivated barley (*Hordeum vulgare* ssp. *vulgare*) is a diploid (2n =14) and self-fertilizing species with a large genome size of 4.9×10^9 bp/1C (Arumuganathan and Earle 1991). Barley is an important crop species in the world and has been subject to considerable genetic mapping and QTL studies. Barley is assumed to be a model genome system for Triticeae species, because its self-fertile diploid genetic system has advantages for phenotypic expression of gene and homozygous material development, and its chromosomes are homoeologous to cultivated wheats and rye, which are polyploids and outbreeding, respectively.

Construction of a linkage map with molecular markers is a key step in the linkage analysis of biologically or agronomically important traits. Several types of molecular markers, such as restriction fragment length polymorphism (RFLP; Tanksley 1993), random amplified polymorphic DNA (RAPD; Williams et al. 1990), amplified fragment length polymorphism (AFLP; Vos et al. 1995), simple sequence repeat (SSR; Litt and Luty 1989) and sequence-tagged site (STS; Olson et al. 1989), have been used in barley mapping and have advantages and disadvantages.

In particular, AFLP seemed to be more efficient than other DNA marker techniques because it can detect many polymorphic bands per assay unit. In addition, AFLP markers have been confirmed reproducible and reliable by a number of workers, and used for the barley map construction and trait mapping (Becker et al. 1995; Qi et al. 1998; Ramsay et al. 2000; Costa et al. 2001; Mano et al. 2001). However, AFLPs in previous studies still required laborious work; the uses of a large heavy gel, a long exposure time of the radioisotope or a combination

of fluorescent-labeled primers and DNA sequencer. Recently, Kawasaki et al. (unpublished) developed a simple and high-throughput electrophoresis method named the High Efficiency Genome Scanning (HEGS) system. The HEGS was proved to be extremely useful for the scanning of AFLP bands. The combination of the HEGS system and AFLP technique, which uses non-denatured acrylamide gel, discontinuous Tris-Glycine buffer and Vistra Green staining, allows a single person to construct a map in a short period under a reasonable cost. Using this system, high-density maps were constructed in rice (Murai et al. 2001; Shimizu et al., personal communication) and *Pyricularia oryzae*, a causal fungus of rice blast disease (Motomura et al., personal communication).

One of the important objectives to develop a high-resolution map is to identify the precise location of genes. If markers are close enough to the gene, they can be directly used for the marker-assisted selection. Also, fine mapping, and following map-based cloning of QTLs, was clearly demonstrated for rice heading-date using the high-density map of Yano et al. (2000).

Another important objective is the rapidity of map development. Many agronomic and quality traits may be controlled by a number of genetic factors. However, the segregating genes are restricted by the parental combination of the cross. Since the Mendelian genetic analysis including QTL analysis can identify genes of difference among parents, the rapid construction of the genetic map is necessary to collect genetic information among a diverse range of germplasm.

The objectives of the investigation reported here were: (1) to develop a high-density genetic linkage map in barley using the HEGS/AFLP system, and (2) to evaluate the resolution of the high-density map by detecting QTLs in comparison with a skeleton map.

Materials and methods

Plant materials and DNA isolation

One hundred and twenty five F_9 RI lines derived from the cross of Russia 6 and H.E.S. 4 have been developed by single-seed descent in the field at the Research Institute of Bioresources in Okayama University, Kurashiki, Japan. Russia 6 is a two-row spring barley from Russia. H.E.S. 4 is a six-row spring barley from Afghanistan. We used 95 RI lines to construct a linkage map and to detect QTLs associated with agronomic traits.

A small amount of DNA (1–5 μg) was isolated from 50 mg of fresh leaf tissue from each line and parents, according to the method of Langridge et al. (1997) without adding RNase in the final dissolution TE buffer.

SSR amplification and STS analysis

The primer sequences for SSR markers used in this study were obtained from Liu et al. (1996) and Ramsay et al. (2000). The PCR amplification procedure was described by Costa et al. (2001). After the reaction, polymorphic markers between the parents were used as anchors on the seven barley linkage groups.

Table 1 Sequence of adaptor, universal and selective primers. The sequence profiles are shown from the 5′-end to the 3′-end

Primer name	Sequence
*Eco*RI adaptors	CTCGTAGACTGCGTACC AATTGGTACGCAGTCTAC
*Eco*RI universal primer (E+0)	GACTGCGTACCAATTC
*Eco*RI selective primers (E+3)	GACTGCGTACCAATTCNNN[a)]
*Pst*I adaptors	CTCGTAGACTGCGTACC ACGTGGTACGCAGTCTAC
*Pst*I universal primer (P+0)	GACTGCGTACCACGTC
*Pst*I selective primers (P+3)	GACTGCGTACCACGTCNNN
*Mse*I adaptors	GACGATGAGTCCTGAG TACTCAGGACTCAT
*Mse*I universal primer (M+0)	GATGAGTCCTGAGTAA
*Mse*I selective primers (M+3)	GATGAGTCCTGAGTAANNN

[a] N means the single nucleotide either A, C, G or T

Three RFLP markers were selected to fill gaps of SSR markers and converted to STS markers (ABC155, ABC253 and cMWG699) in this study (http://wheat.pw.usda.gov/ggpages/probes/abc.html). PCR amplifications of ABC155 and ABC253 were performed as described by Blake et al. (1996). cMWG699 which was closely localized with the *vrs1* locus on chromosome 2H was amplified as described by Tanno et al. (2002).

DNA amplification for AFLP

AFLP was analyzed based on the description by Vos et al. (1995) with some modifications. Fifty nanograms of genomic DNA were completely digested with two restriction enzymes, either *Eco*RI (1.5 U)/*Mse*I (1.5 U) or *Pst*I (1.5 U)/*Mse*I (1.5 U), in a volume of 25 μl for 3 h at 37 °C. Both enzyme combinations digested the DNA samples under the same protocol. The fragments were ligated to 50 μM of *Mse*I adaptor and either 5 μM of *Eco*RI or *Pst*I adaptor (Table 1) by 25 U of T4 DNA Ligase (Takara, Tokyo, Japan) for 3 h at 37 °C. The digested and ligated DNA was then pre-amplified with universal primers (M+0 and either E+0 or P+0; Table 1). These primers had no additional selective nucleotides at the 3′ ends. After the preparation of the pre-amplified solution at 0.06 ng/μl concentration, the products were amplified using the selective primers (Table 1) that had three additional nucleotides (E+3/M+3 or P+3/M+3). Each 10-μl reaction in the selective amplification contained non-labeled selective primers either E+3 (7 ng)/M+3 (15 ng) or P+3 (7 ng)/M+3 (15 ng). In the pre-amplification and selective amplification, Ex *Taq* DNA polymerase (Takara, Tokyo, Japan) was used for PCR reactions. Universal and selective primers were synthesized by SAWADY tech., Tokyo, Japan, and Invitrogen, Tokyo, Japan. PCR plates with a 384-well format (ThermoFast 384; ABgene, Epsom, England) and a dual 384-well unit PCR thermal-cycler (GeneAmp PCR System; Applied Biosystems, Tokyo, Japan) were used in the selective amplifications.

Electrophoresis and detection of polymorphism

The electrophoresis used a conventional system for protein analysis with Tris-Glycine electrophoresis buffer. A polyacrylamide non-denatured gel was prepared with a flat tooth comb on a 24.5 cm (height) × 26.5 cm (width) glass plate. The running gel (1 mm thickness) consisted of two types of polyacrylamide gels, a 6% stacking gel containing 125 mM of Tris-HCl (pH 6.8) and a 13% separation gel containing 375 mM of Tris-HCl (pH 8.8). The 6% stacking gel was piled on the polymerized 13% separation gel. The acrylamide solution consisted of 37.5:1 for acrylamide (Daiichi Pure Chemicals, Tokyo, Japan) to bis-acrylamide (Wako Pure

Chemical Industries, Osaka, Japan). The running buffer consisted of 25 mM Tris and 192 mM of Glycine. PCR products (10 μl) were mixed with 2 μl of loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, 30% Glycerol) to apply on a gel, and 6 μl was loaded on the gel.

For the quick precise-sample application, either a eight-linked or a 16-linked syringe was used to load the PCR products. The samples were electrophoresed at 100 V for 1.5 h and then at 250 V for 7 h without temperature control. The gels were scanned by the fluorescent gel scanner (FluorImager 595; Amersham Biosciences, Uppsala, Sweden) after staining with Vistra Green (Amersham Biosciences, Uppsala, Sweden).

AFLP fragment scoring and map construction

The scoring of polymorphic bands could be done manually by checking the parental differences and their segregation in RI lines. To accelerate this procedure, we ordered a custom development of the software package (Tecs Co.; Itako, Japan). The software can recognize differential band positions between parents on the gel image (BMP file) and automatically score the segregation of polymorphic bands in the RI lines. Then the multiple segregation data are transferred to the Excel spreadsheet. The data in the Excel spreadsheet can easily translate to the MAPMAKER format. SSR, STS and morphological markers were used for the anchors of seven barley linkage groups, using their previous map locations. The segregation data of AFLP, SSR, STS and morphological (*vrs1*) markers were used for the linkage analyses performed by MAPMAKER/EXP ver. 3.0 (Lander et al. 1987) and MAPL98 (Ukai et al. 1995). At first, AFLPs were assigned to the anchor markers at the LOD threshold of 3.0. Marker orders in each linkage group were confirmed by both the ripple command of MAPMAKER/EXP ver. 3.0 and the metric MDS command of MAPL98. The Kosambi mapping function was used to calculate map distances (Kosambi 1944).

After the development of a high-density map, a skeleton map was constructed using anchor markers and selected AFLP loci with marker intervals of 10–20 cM.

Phenotype evaluation and QTL analysis

The RI lines were grown in the field of Kurashiki in 2001. Phenotypes were scored for row type, plant height, spike exsertion length and 1,000-kernel weight. Row type (*vrs1* locus) was used as a morphological marker for the map construction. Plant height (PH) was measured by the height from the ground level to the top of the tallest tiller for ten plants per RI line. Spike exsertion length (SEL) was measured by the length of stem from the flag leaf to the bottom of the spike in a main tiller for ten plants in each RI line. Thousand-kernel weight (TKW) was measured from a bulk sample of grains harvested in each RI line.

QTL analysis was carried out by the simple interval mapping (SIM) and by the composite interval mapping (CIM), using software packages MAPMAKER/QTL ver. 1.1b (Lander and Bostein 1989) and QTL Cartographer ver. 1.16 (Basten et al. 1994), respectively. The results of QTL analysis were compared between the skeleton map and the high-density map. We used a LOD threshold of 2.0 for declaring the presence of putative QTLs. The LOD peak of each significant QTL was considered as the QTL location on the linkage map.

Results

Detection of polymorphism between Russia 6 and H.E.S. 4

Parental screening revealed 33 polymorphic SSRs among 79 primer pairs. All the three STS markers showed polymorphism between parents. AFLP analyses detected 820 polymorphic primer combinations (518 in E+3/M+3 and 302 in P+3/M+3) between parents among 1,548 primer combinations. Only 33 gels from five series of electrophoresis completed parental screenings of 1,548 AFLP primer combinations. On average, 60 fragments

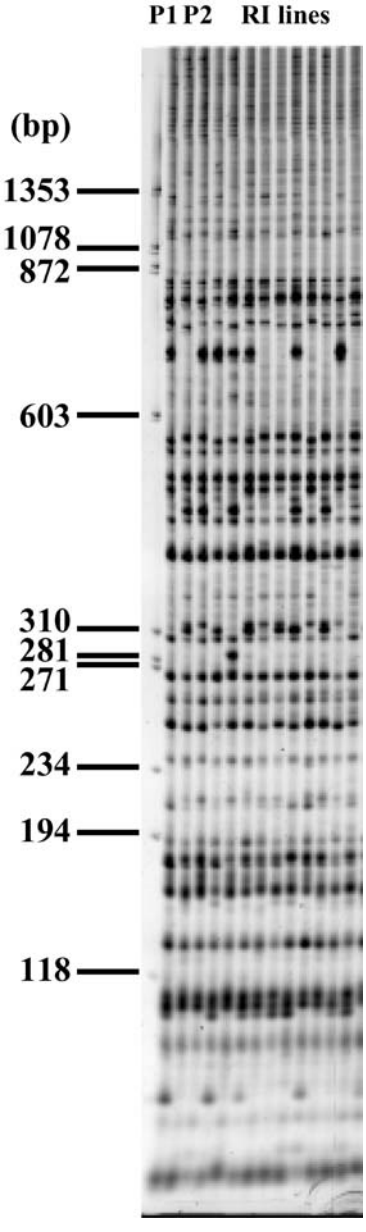

Fig. 1 An example of the HEGS/AFLP electrophoresis gel stained with Vistra Green. Molecular weight markers of the ϕX174/*Hae*III digest were indicated on the *left side*

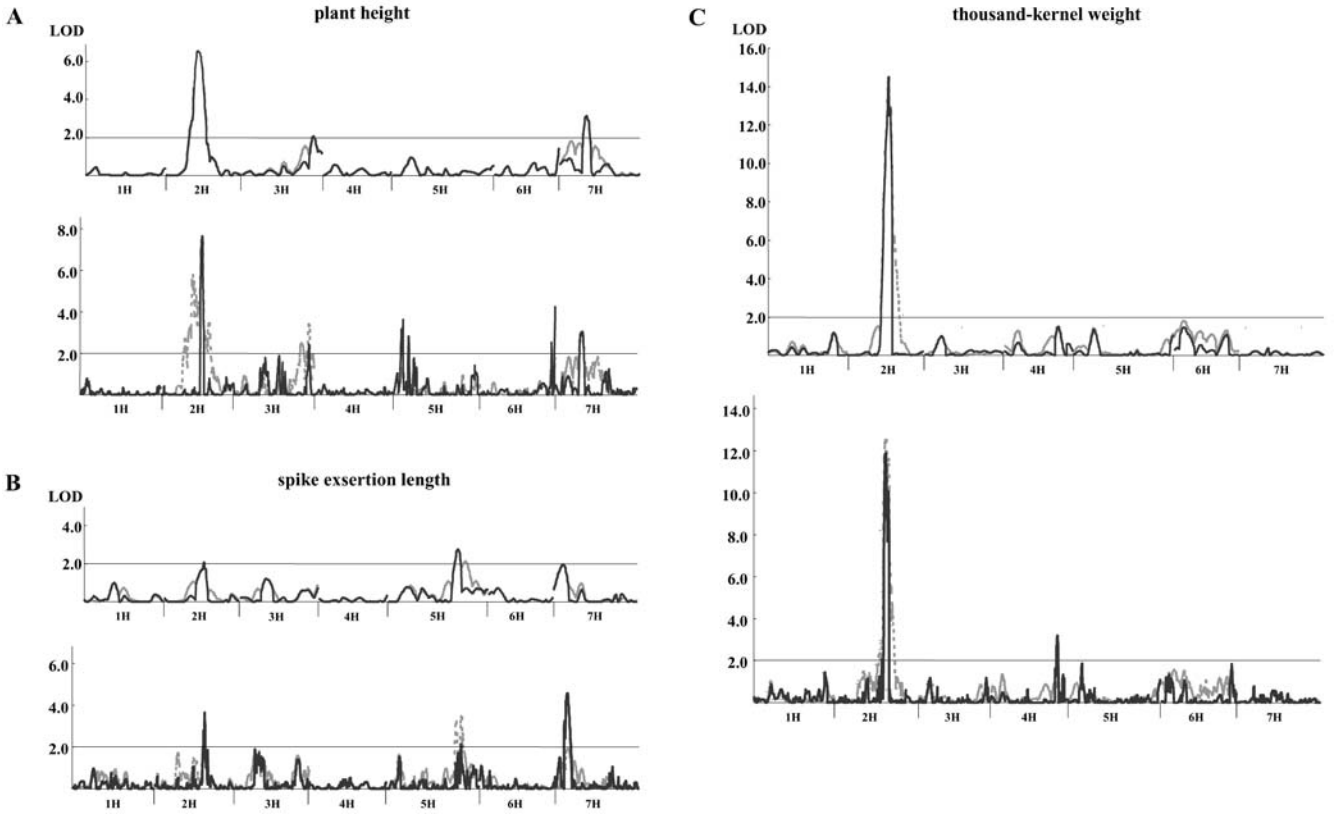

Fig. 2 Scans of QTL analysis for three agronomic traits (**A**: plant height, **B**: spike exsertion length and **C**: 1,000-kernel weight) using the skeleton map (above) and the high-density map (below) in the RI population derived from Russia 6 × H.E.S. 4. LOD-score profiles of chromosomes are shown in the order of 1H, 2H, 3H, 4H, 5H, 6H and 7H, oriented from short arms. The results of simple interval mapping (SIM) and composite interval mapping (CIM) analysis are shown by a *broken line* and a *solid line*, respectively

were generated and 6.6 polymorphic bands were obtained for each primer combination between Russia 6 and H.E.S. 4 by the HEGS system. The polymorphic ratio (polymorphic bands/total bands) between Russia 6 and H.E.S. 4 was estimated as 5.8%.

Map construction in the RI population

Ninty five RI lines were used for the linkage map construction. Figure 1 shows an example of the AFLP gel profile obtained from the parents and RI lines with the HEGS system. A total of 1,685 polymorphic bands were detected from 224 E+3/M+3 and 32 P+3/M+3 primer combinations. As one person can process eight primer combinations (100 lanes × 8 gels = 800 lanes) every day, 32 days were spent to analyze all the polymorphic bands among RI lines.

Including 1,685 AFLP, 34 SSR, three STS markers and one morphological (*vrs1*) marker, a total of 1,782 segregation data were used for the map construction. Only one SSR marker (HVM2) amplified two polymorphic bands, each located on chromosome 2H or 5H. The final linkage map generated by MAPMAKER/EXP ver. 3.0 and MAPL98 included 1,172 loci consisting of 1,113 E+3/M+3 AFLPs, 21 P+3/M+3 AFLPs, 34 SSRs, three STSs and *vrs1*.

Most of the location of anchor markers such as SSR, STS and *vrs1* were not different from the previous reports. ABC155 was localized on the chromosome 5H and ABC253 was localized on chromosome 7H. cMWG699 was closely localized with the *vrs1* locus on chromosome 2H. However, five SSR markers (EBmac684, HVM2, HVM4, HVM5 and HVM51) showed different locations from the ones in previous maps. For example, EBmac684 was located on the long arm of chromosome 5H by Ramsay et al. (2000) but was located on the centromeric region of chromosome 2H in this study. HVM51 was located on chromosome 5H by Liu et al. (1996) but was located on chromosome 7H in this study.

Of the 1,134 AFLP loci mapped in the present population, 103 loci (9.1%) were seemed to be codominant. The number of distorted markers was 51 at the 5% level and 19 at the 1% level in the total of 1,172 markers. The region of segregation distortion was observed on the long arm of chromosome 3H.

The total map distance was 1,595.7 cM. The average marker density in the whole genome was 1.4 cM/locus. The degree of marker density was different among the regions of the constructed map. Marker clusters were

Table 2 QTLs for three agronomic traits on the skeleton map and the high-density map detected by simple interval mapping (SIM) and composite interval mapping (CIM)

Trait	Algorithm	Chromosome	The skeleton map					The high-density map				
			Marker interval	Position[b] (cM)	LOD[c]	Var.[d] (%)	Weight[e]	Marker interval	Position (cM)	LOD	Var. (%)	Weight
Plant height (cm)	SIM	2H	EBmac684-*vrs1*	2.0	6.5	28.7	12.8	MEgggMatc799-FEctaMata590	2.0	7.6	33.2	13.8
		3H	FEggaMagg288-HVM62	18.0	2.1	19.4	10.5	MEggcMatc305-NEacaMcag36	0.0	3.5	15.6	9.5
		7H	FEctcMagc455-MEaagMatt269	18.0	3.1	17.6	10.0	MEaagMatt269-FEcggMacc79	6.0	3.1	18.4	10.2
	CIM	2H	EBmac684-*vrs1*	2.0	6.5	28.5	12.8	MEgggMatc799-FEctaMata590	2.0	7.6	32.8	13.8
		3H	FEggaMagg288-HVM62	18.0	2.1	19.7	10.7	MEggcMatc305-NEacaMcag36	0.0	2.4	8.7	7.4
		5H[a]	–	–	–	–	–	FEgggMacc754-NEaagMcgt41	2.0	3.6	21.0	15.3
		6H[a]	–	–	–	–	–	MEgcaMatt220-MEcccMact377	0.0	4.2	18.6	–13.9
		7H	FEctcMagc455-MEaagMatt269	18.0	3.1	17.5	10.1	MEaagMatt269-FEcggMacc79	4.0	3.0	16.0	9.6
Spike exsertion length (cm)	SIM	2H	*vrs1*-Bmag125	2.0	2.1	11.3	3.5	FEgagMaat824-MEgtaMata458	0.0	2.2	9.9	3.3
		5H	MEgcgMagc120-MEcccMatt1067	14.0	2.8	18.7	4.4	MEcgcMacg279-FEgcaMacc179	0.0	3.5	15.5	4.1
	CIM	2H	*vrs1*-Bmag125	2.0	2.1	11.1	3.4	FEgagMaat824-MEgtaMata458	0.0	3.7	14.6	6.8
		5H	MEgcgMagc120-MEcccMatt1067	16.0	2.8	18.6	4.5	MEcgcMacg279-FEgcaMacc179	0.0	2.1	6.9	3.8
		7H[a]	–	–	–	–	–	Bmag7-MEaccMata451	6.0	4.6	26.0	6.3
Thousand-kernel weight (g)	SIM	2H	*vrs1*-Bmag125	4.0	14.5	53.7	11.1	*vrs1*-cMWG699	0.0	12.6	46.7	10.3
	CIM	2H	*vrs1*-Bmag125	4.0	14.5	53.2	11.1	*vrs1*-cMWG699	0.0	11.9	37.5	13.2
		4H[a]	–	–	–	–	–	FEggaMtgg116-FEgggMcaa585	4.0	3.2	19.2	–9.4

[a] QTL detected only by CIM on the high-density map
[b] Distance of peak LOD score position from the left side marker
[c] Peak LOD score of significant marker interval
[d] Explained variance of peak LOD score
[e] Estimated additive effect of Russia 6 or H.E.S. 4 allele shown by *normal* or *minus*, respectively

detected on each linkage group. In contrast, marker poor-regions were observed both on the long arm of chromosome 4H and the long arm of chromosome 5H. (See electronic supplementary materials for map details).

QTL detection on agronomic traits

QTL analyses were carried out for three agronomic traits both on the skeleton map and high-density map using MAPMAKER/QTL ver. 1.1b (SIM) and QTL Cartographer ver. 1.16 (CIM) (Fig. 2). Table 2 showed the characteristics of the detected QTLs. The numbers of QTLs detected were three for PH, two for SEL and one for TKW in both SIM and CIM on the skeleton map, and SIM on the high-density map. CIM on the high-density map detected more QTLs (five for PH, three for SEL and two for TKW) than the other analyses (Table 2). Most of the marker intervals including QTLs existed in coincidence or a very close region, between the skeleton map and the high-density map (Fig. 2). The QTLs for SEL and TKW on chromosome 2H were coincident with the *vrs1* locus that determines the inflorescence row type (Table 2). The QTL associated with PH was also detected near the *vrs1* locus.

On the six QTLs common both in the skeleton map and the high-density map, the average distances between the QTLs and the flanking marker were 9.7 cM (SIM) and 10.0 cM (CIM) on the skeleton map, and 1.3 cM (SIM) and 1.0 cM (CIM) on the high-density map, respectively. The marker intervals were narrowed on the high-density map.

Discussion

Through-put of the HEGS system

Although a polymorphic ratio of 5.8% is not high compared with those in other reports (11.3%, Becker et al. 1995; 14.0%, Castiglioni et al. 1998; 22.1%, Mano et al. 2001), it is sufficient for the usual genetic analysis with the HEGS/AFLP system, because only 32 days were spent to detect all the polymorphic primer combinations in the present analysis. Accordingly, we succeeded to construct the high-density linkage map within 6 months under a reasonable cost. The handling of the HEGS system is more reasonable and easier than any other method, such as a system using the DNA sequencer with fluorescent primers, or one by radioisotope labeling of primers and denatured polyacrylamide gels.

The population size of this study was designed to be easy and applicable for the 96-well plate format because we used two parental lines and 94 RI lines in one AFLP trial. Because of the difference in amplification, we used one RI line different between the E+3/M+3 primer combinations and the P+3/M+3 primer combinations in a set of 94 RI lines, although the other 93 RI lines were the same in both primer combinations. Thus, 95 RI lines were used for map construction in this study. Even this population size is not large, it is reasonable to use this system for handling multiple samples in the map development from the viewpoint of cost and throughput.

Construction of the high-density linkage map

Five SSR markers showed different locations from the ones in previous maps. These discrepancies might be caused by the higher copy number of the target sequence in the barley genome or the amplification of different genomic region from the primer sequences used. We cannot assume genomic duplication between each original and the present marker region since the positional difference occurred in the individual SSR marker but not by a set of markers arranged on the genome. These possibilities could not be further confirmed because the genomic sequences including SSR repeats were not available.

The high-density barley linkage map we generated with 1,172 markers seemed to cover the whole genome, because the number of markers on the seven barley chromosomes was large, and there was no significant map-distance extension from our preliminary map consisting of 678 markers (1,594.5 cM, data not shown) to the final map (1,595.7 cM). This map length was longer than those of Ramsay et al. (2000; 1,173 cM) or Costa et al. (2001; 1,387 cM). The differences might be due to the higher recombination frequency in the Russia 6 × H.E.S. 4 cross. Moreover, it was reported that the use of AFLP markers and RI population structure increased the map length (Mano et al. 2001).

Some of the AFLP markers (103; 9.1%) showed co-dominance between parents in this study. Groh et al. (2001) and Menz et al. (2002) also reported the percentages of co-dominant AFLP markers in oat (33%) and sorghum (12%), respectively. Detected co-dominant markers are useful to identify heterozygotes in backcross and F_2 analyses. Even the rate of co-dominance is not quite high, the HEGS/AFLP system seemed to be effective to pick up the PCR-based co-dominant markers quickly.

The region of segregation distortion was observed in this study. The most-possible genetic cause of segregation distortion is the abortion of male or female gametes, followed by the selective fertilization of particular gametic combinations. There were no known distortion factors found for the parents in this study. Segregation distortion is rather frequent and sometimes observed in barley mapping populations (Costa et al. 2001).

Clusters of AFLP markers were observed on every chromosome. These clustered AFLPs which may be located in regions of suppressed recombination, appear to be mostly on the centromeric region as reported by Becker et al. (1995) and Qi et al. (1998) in barley, and Castiglioni et al. (1999) in maize.

The advantage of detailed QTL analysis using the high-density map

Some of the detected QTLs in the present study were reported on the similar position in other studies (TKW: Jui et al. 1997; PH: Marques-Cedillo et al. 2001; Teulat et al. 2001). Detected QTLs for SEL and TKW on chromosome 2H may be a result of the pleiotropic effect of the *vrs1* locus or expression of the tight-linked gene for *vrs1* (Marques-Cedillo et al. 2001).

QTLs for a range of other economically important traits are reported throughout the barley genome (Mather et al. 1997; Zhu et al. 1999; Marques-Cedillo et al. 2001; Teulat et al. 2001). These previous studies used maps with adequate marker intervals to raise the significance of QTL detection and to prevent errors of marker orders. However, very closely linked markers to QTLs are detected on the high-density map (1.0 cM; Table 2). These QTL flanking markers could be immediately available for marker-assisted selection in barley breeding.

Although some detected QTLs were coincident between the skeleton map and the high-density map, closely linked markers with a QTL on the high-density map have more promising uses than the ones on the skeleton map. If there are two QTLs closely linked within several cM on the repulsion phase, a marker between QTLs can separate two QTL effects. This separation function may be most effective in the combination of the high-density map and CIM, which can separate QTLs by a higher resolution than SIM. Since genes tend to cluster in gene-rich regions on the barley genome (Künzel et al. 2000), more repulsion QTLs can be separated with a higher marker density.

The coverage and density (1.4 cM/locus) of this map are satisfactory for QTL analysis, and some QTL positions were coincident with flanking-marker loci (Table 2). However, a mis-scoring of markers causes serious errors on the marker order and the following QTL estimation. The allelic difference of one line from 95 RI lines may correspond to the approximate distance of 1.0 cM on the present map, which is less than the average marker density (1.4 cM). From this viewpoint, the present map reached almost the highest resolution with the present population size.

The advanced backcross QTL analysis is an alternative strategy to identify and separate QTLs (Tanksley et al. 1995). Higher marker density helps to include a target QTL into a backcrossed line without carrying unnecessary factors. Restriction of the marker interval of QTLs was reported in rice based on the high-density map constructed using RFLP markers and F_2 progenies derived from backcross lines (Yamamoto et al. 1998). They carried out advanced backcross QTL analysis to detect tightly linked markers with QTLs.

In rice and tomato, map-based cloning was elegantly demonstrated with the sufficient resources and the appropriate genetic stock, and it suggests that a QTL can be converted to a Mendelian locus and clone based on the high-density linkage map (Frary et al. 2000; Yano et al. 2000). For the first step to achieve map-based cloning, construction of a high-resolution linkage map is required. Although STS conversion of closely linked AFLP markers with a QTL for an agronomic trait can make the procedure simpler (Meksem et al. 2001), it may be difficult because the proportion of the repeated elements may be higher in the barley genome than the ones in rice or tomato.

The AFLP technology is often used to build a BAC-based physical map in plant species integrated into the genetic map (Klein et al. 2000; Meksem et al. 2001). A BAC library is available in barley with an average insert size of 106 kb (Yu et al. 2000) and 115 kb (Saisho et al., personal communication). However, the average resolution on the physical distance scanned by 1,172 markers on the barley genome of 4.9×10^9 bp/1C (Arumuganathan and Earle 1991) is about 4,500 kb/marker in the present map. There is still a large gap between the physical distance and the resolution of the high-density map in barley. Accordingly, it may be reasonable to approach the target gene by the backcrossing strategy, and then raise the number of individuals segregating only on the restricted genome segment, which includes the target gene. The mapping strategy in this study might demonstrate a reasonable starting point for gene discovery within BAC contigs linked to QTLs.

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