

Theor Appl Genet (2011) 122:327–340
DOI 10.1007/s00122-010-1449-8

ORIGINAL PAPER

Mapping 49 quantitative trait loci at high resolution through sequencing-based genotyping of rice recombinant inbred lines

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Received: 22 November 2009 / Accepted: 8 September 2010 / Published online: 28 September 2010
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Abstract Mapping chromosome regions responsible for quantitative phenotypic variation in recombinant populations provides an effective means to characterize the genetic basis of complex traits. We conducted a quantitative trait loci (QTL) analysis of 150 rice recombinant inbred lines (RILs) derived from a cross between two cultivars, *Oryza sativa* ssp. *indica* cv. 93-11 and *Oryza*

sativa ssp. *japonica* cv. Nipponbare. The RILs were genotyped through next-generation sequencing, which accurately determined the recombination breakpoints and provided a new type of genetic markers, recombination bins, for QTL analysis. We detected 49 QTL with phenotypic effect ranging from 3.2 to 46.0% for 14 agronomics traits. Five QTL of relatively large effect (14.6–46.0%) were located on small genomic regions, where strong candidate genes were found. The analysis using sequencing-based genotyping thus offers a powerful solution to map QTL with high resolution. Moreover, the RILs developed in this study serve as an excellent system for mapping and studying genetic basis of agricultural and biological traits of rice.

Communicated by T. Sasaki.

L. Wang and A. Wang contributed equally to this work.

Electronic supplementary material The online version of this article (doi:[10.1007/s00122-010-1449-8](https://doi.org/10.1007/s00122-010-1449-8)) contains supplementary material, which is available to authorized users.

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Introduction

Genetic variation of a complex trait is usually controlled by multiple loci. When studied in a recombinant population, the trait typically varies in a continuous manner. The use of molecular genetic markers decades ago enabled the detection of chromosomal regions harboring quantitative trait loci (QTL). Since then, the number of studies to map QTL has increased rapidly, fueled primarily by interests to identify the genetic control of agriculturally, medically, and ecologically important traits (Tanksley 1993; Lander and Schork 1994; Mackay 2001; Mauricio 2001). Advances in molecular biology and genomic techniques have then made it possible to narrow down a QTL to a few or even a single candidate gene (Doebley et al. 1997; Frary et al. 2000; Yano et al. 2000; Grisart et al. 2002). The cloning of QTL and identification of causative mutations have opened an avenue to unlock the genetic basis of complex phenotypic variation.

However, cloning QTL remains technically challenging. It either requires the development of near-isogenic lines (NILs) through repeatedly backcrossing with one of the mapping parents (Ashikari et al. 2005; Konishi et al. 2006; Song et al. 2007; Jin et al. 2008; Shomura et al. 2008; Xue et al. 2008; Li et al. 2006) or additional samples of natural variants for association of phenotype and candidate genes (Grisart et al. 2002; Van Laere et al. 2003; Sutter et al. 2007; Harjes et al. 2008). Positional cloning using NILs is time-consuming and labor-intensive because it takes a few generations of backcrossing to make NILs and thousands of recombinants to fine map the candidate genes. It could be prohibitively tedious and prolonged for organisms with relatively long life cycles or relatively few offspring from crosses. With regard to the other cloning strategy involving association analysis, the difficulty arises as it often relies on the presence of candidate genes with known function near the QTL.

The whole-genome sequencing approach takes advantage of a recently developed genotyping method that uses single nucleotide polymorphisms (SNPs) detected from whole-genome sequencing of a mapping population (Huang et al. 2009). This approach could substantially reduce the amount of time and effort required for QTL mapping. The SNPs were evaluated in sliding windows to generate recombination maps for the individuals. The maps were then aligned and used to define recombination bins for the entire population. Recombination bins can serve as a new and effective type of genetic markers for QTL analysis. It is different from conventional molecular markers, such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLPs), insertion–deletion markers (In/Del), and simple sequence repeat (SSR), which often have uneven distribution and low density on whole genome and need more time on genotyping. The bins, which presumably capture all recombination events in the population, provide available abundant markers based on dense SNPs for detailed genome-wide trait analysis.

In this study, we reported high-resolution QTL mapping through sequencing-based genotyping of 150 rice recombinant inbred lines (RILs). The population was developed from a cross between two rice cultivars with genome sequences, *Oryza sativa* ssp. *japonica* cv. Nipponbare and *Oryza sativa* ssp. *indica* cv. 93-11 (Goff et al. 2002; Yu et al. 2002; International Rice Genome Sequencing Project 2005). We identified 49 QTL within relatively small genomic regions for 14 agronomic traits. With a relatively high mapping resolution, we were able to identify the candidate genes for some QTL of large or moderate effect. The new genotyping method thus greatly improved the resolution and precision of QTL mapping for complex traits.

Materials and methods

Mapping population

The rice mapping population of 150 RILs was derived by single-seed descents from a cross between *Oryza sativa* ssp. *indica* cv. 93-11 and *Oryza sativa* ssp. *japonica* cv. Nipponbare. The population was developed in the experimental fields at China National Rice Research Institute in Hangzhou, Zhejiang Province, and Sanya, Hainan Province. After ten generations of self-fertilization following the initial cross, DNAs of the F₁₁ RILs were isolated for genotyping. Phenotyping was conducted in the Hangzhou field (N 30.32°, E 120.12°) from May to October, 2008, and in the laboratory following harvest.

Phenotyping

Of 18 individuals of each RIL and parent grown in the field, 5 plants were randomly chosen for phenotyping. A total of 14 traits were evaluated. Traits measured directly in the field include heading date, culm diameter, plant height, flag leaf length and flag leaf width, tiller angle, tiller number, panicle length, and awn length. Traits measured in the laboratory following harvest include grain length, grain width, grain thickness, grain weight, and spikelet number per panicle (Table S1).

Heading date was recorded as days from sowing to time when inflorescences had emerged above the flag leaf sheath for more than half of the individuals of line. Culm diameter, plant height, flag leaf length and width, and tiller angle were evaluated when panicles fully emerged. Culm diameter was measured at the thickest location of the third tiller node from the root; tiller angle was scored on a 1–6 scale (1, <10° between tiller and vertical; 6, >45°). Plant height was measured from the soil surface to the apex of the tallest panicle. On the main tiller, flag leaf length was measured from leaf blade and sheath boundary to the leaf apex; flag leaf width was measured at the widest location of the leaf. Tiller number, panicle length, and awn length were evaluated when grains fully matured. All flowered tillers of an individual were counted, the longest panicle was measured in length, and five grains located on the top of this panicle were chosen for measuring awn length. The total number of spikelets produced on the main tiller was counted.

The grain related traits were measured in the laboratory after grains were detached from panicles and awns were removed from the grains. For the sampled panicles of an individual, grains were mixed and 10 grains were randomly sampled for phenotyping. Grain length, width, and thickness were recorded at the maximal values for each grain using an electronic digital caliper. Grain weight was

initially obtained by weighing a total of 200 grains, which was then converted to 1,000-grain weight, a scale commonly used for yield evaluation.

Genotyping, linkage map, and QTL analysis

A high-throughput genotyping method was previously developed and tested using these 150 rice RILs (Huang et al. 2009). The RILs were genotyped based on SNPs generated from the whole-genome resequencing. A recombination map was constructed for each RIL. The recombination maps were aligned to determine recombination bins across the entire population with the minimal bin length of 100 kb adopted. Resulting bins were then treated as a genetic marker for linkage map construction using MAPMAKER/EXP version 3.0b (Lander et al. 1987).

Using this linkage map and phenotypic values, QTL analysis was conducted with the composite interval mapping (CIM) implemented in software Windows QTL Cartographer V2.5 (Wang et al. 2007) (<http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>). The CIM analysis was run using Model 6 with forward and backward stepwise regression, a window size of 10 cM, and a step size of 2 cM. Experiment-wide significance ($P < 0.05$) thresholds for QTL detection were determined with 1,000 permutations. The location of a QTL was described according to its LOD peak location and the surrounding region with 95% confidence interval calculated using WinQTLCart. The epistasis between QTL was estimated using R/qtl in the R package (<http://www.rqtl.org>) (Broman et al. 2003).

Simulation schemes

To evaluate the effect of marker density, two sets of markers with different density were simulated for QTL analysis. For the set with low marker density, 238 locations evenly distributed in the rice genome were designated with the density of 1 marker per 1.6 Mb based on physical position. Then each location was treated as one simulated marker, and the genotype of the marker was deduced from genotype of the recombination bin where the marker was located. In this way, genotypes of 150 individuals with a total of 238 simulated markers were obtained (Table S2). The set with high-marker density was simulated in the same way. The density was 1 marker per 164 kb, which generated a total of 2,330 markers.

To evaluate the effect of population size, 50 and 100 lines were randomly sampled from 150 RILs five times for QTL analysis, respectively. Moreover, genotypes and phenotypes were simulated five times for each population size (from 50 to 500 individuals) for QTL analysis using the simulation module in the software WinQTLCart. In the

simulation, chromosome number and marker position were imported according to 2,334 bins, and the QTL information was imported based on the 49 QTL mapped using the 150 RILs.

The way to construct genetic map and QTL analysis using the simulated markers and populations was the same as that for the 150 RILs.

Results

Phenotypic variation

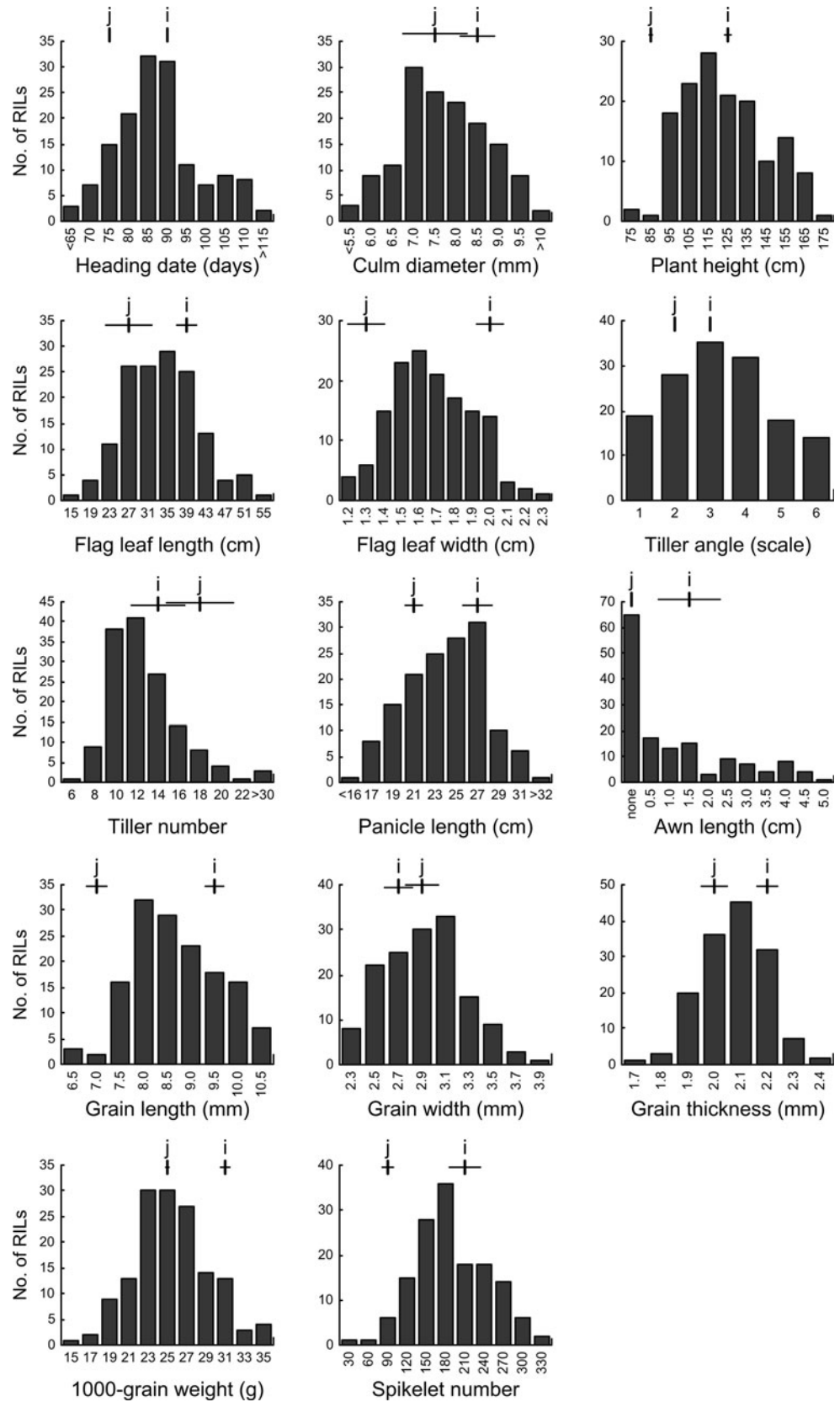
Phenotypic variation of the rice RILs and parents is illustrated in Fig. 1 and supplemental Fig. S1. Of the 14 traits evaluated, 10 showed significant differences between the *indica* and *japonica* mapping parental lines and 2 (culm diameter and tiller number) were not significantly different between the parental lines, while the significance level was not determined for heading date or tiller angle (Table S1). All traits showed transgressive segregation in the RIL population (Fig. 1).

The correlation of trait variation is illustrated in Fig. 2. Significantly positive correlation is found among nine traits (green shading), awn length, grain length, culm diameter, spikelet number, plant height, panicle length, flag leaf length, flag leaf width, and heading date. This group of nine traits shows significantly negative correlation with other four traits (yellow shading), tiller angle, tiller number, grain width, and grain thickness. Specifically, grain thickness is negatively correlated with spikelet number, panicle length, flag leaf length, and heading date; grain width is negatively correlated with awn length, grain length, culm diameter, spikelet number, and panicle length; tiller number is negatively correlated with culm diameter, spikelet number, panicle length, plant height, flag leaf length, and flag leaf width; tiller angle is negatively correlated with flag leaf length and width. Between these four traits, grain thickness and grain width are significantly positive correlation. The remaining trait (purple shading), 1,000-grain weight, was positively correlated with grain thickness and grain width, and shows both positive and negative correlations with the first group of nine traits. It is positively correlated with awn length, grain length, culm diameter, panicle length, and plant height, and negatively correlated with spikelet number and heading date.

Linkage map of recombination bins

A linkage map was constructed using 2,334 recombination bins which was obtained from the whole-genome resequencing of the 150 RILs (Huang et al. 2009), which resulted in a total genetic distance of 1,539.5 cM with an average interval of 0.66 cM between adjacent bins.

Fig. 1 Variation of phenotypic traits in RILs. Mean and standard deviation of the parents are indicated at the top of each histogram, with *i* and *j* representing *O. sativa* ssp. *indica* cv. 93-11 and *O. sativa* ssp. *japonica* cv. Nipponbare, respectively



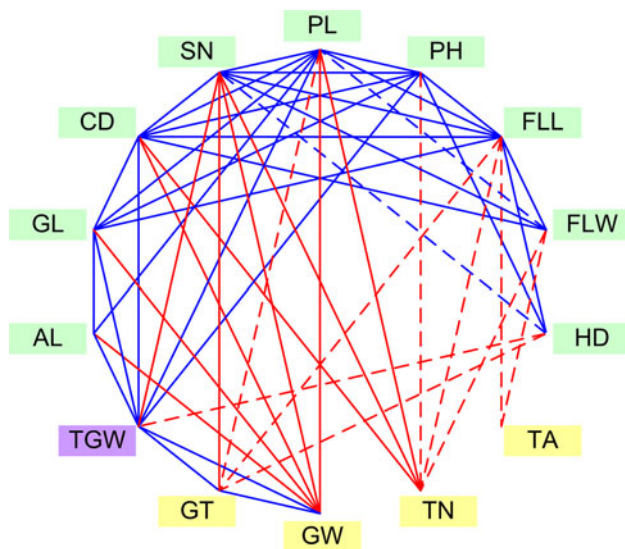


Fig. 2 The correlation of trait variation. *Blue and red lines* indicated positive and negative correlations, respectively. *Solid lines* $P < 0.01$; *dotted lines* $0.01 < P < 0.05$. *AL* awn length, *GL* grain length, *CD* culm diameter, *SN* spikelet number, *PL* panicle length, *PH* plant height, *FLL* flag leaf length, *FLW* flag leaf width, *HD* heading date, *TA* tiller angle, *TN* tiller number, *GW* grain width, *GT* grain thickness, *TGW* 1,000-grain weight

For each chromosome, the average genetic distance between adjacent bins ranging 0.66–0.82 cM, with the maximal distance between 2.1 and 8.3 cM (Table S3).

The linkage map constructed from the bins is compared with a map generated from an F_2 population of 186 individuals derived from a cross between *japonica* cv. Nipponbare and *indica* cv. Kasalath (Harushima et al. 1998) (Table S3). This represents the rice linkage map covered with the largest number of conventional molecular markers reported to date, where we found a total of 3,235 genetic markers including RFLP, RAPD, and STS from the most updated version (http://www.gramene.org/db/cmap/map_set_info?map_set_acc=jrgp-rfp-2000). The total genetic distance of the 12 chromosomes of these two maps is very close. The average genetic distance between adjacent bins with greater than zero distance is 0.72 cM on our map, smaller than the average of 1.03 cM for the conventional markers. The maximal genetic distance between adjacent markers is 8.3 and 15.6 cM on the bin and conventional maps, respectively. Furthermore, on the map with conventional markers, more than half (53.7%) of the adjacent markers have genetic distance of 0, whereas only about 7.9% of adjacent bins had zero genetic distance as calculated by MAPMAKER. In addition, 76.6% of adjacent bins had genetic distance between 0.1–1 cM, whereas 31.6% of adjacent markers have this level of resolution on the conventional map (Fig. 3; Table S4). Therefore, the map with recombination bins has well-distributed linkage distance and higher resolution than the conventional map.

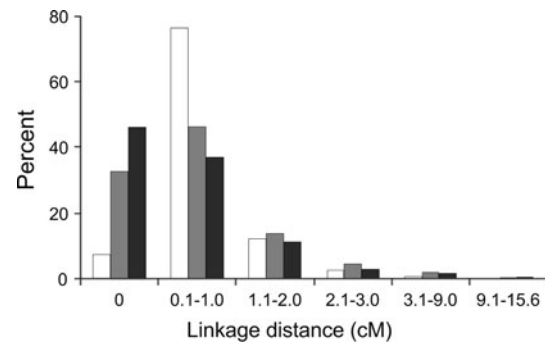


Fig. 3 Comparison of chromosomal coverage between bins, high-density simulated markers, and conventional molecular markers. *Bars* indicate the frequency of genetic distance between adjacent markers on the linkage maps. *White bars* bin markers from this study; *gray bars*, simulated markers from this study; *black bars* conventional molecular markers from a previously studied rice F_2 population

QTL analysis

The LOD thresholds for QTL calling were estimated from the permutation test and ranged from 2.85 for tiller number to 3.48 for flag leaf width. Based on these thresholds, a total of 40 QTL were called for the 14 traits, with phenotypic effect (R^2) of the QTL ranging 4.3–46.0% (Table 1). Considering the power of QTL detection with 150 RILs, we also reported QTL with LOD value higher than 3.0. This gives nine additional QTL, with phenotypic effect ranging 3.2–7.0% (Table 1). Thus a total of 49 QTL are detected on 12 rice chromosomes, with 1–5 QTL detected for each trait (Fig. 4). The region of each QTL identified in this study was based on the 95% confidence interval (CI) calculated using WinQTLCart (Wang et al. 2007). Of them, QTL that explained more than 10% of phenotypic effects were defined as major-effect QTL here. We totally identified 10 major-effect QTL, including qTA-9, qPH-1, qFLW-4, qGL-3, qGW-5, qAL-1, qAL-3, qPH-2, qHD-3, and qCD-2.

We searched the literatures and database for previously identified QTL from mapping populations also derived from crosses between *indica* and *japonica* cultivars. QTL detected in this study were compared with those previously identified by physically locations that could be clearly determined. In this condition, 18 of our QTL for 11 traits fell into the chromosomal regions containing the QTL identified in the previous studies (Table 2), including the top 5 large-effects QTL and another major-effect QTL (qHD-3). The remaining 31 were not found. These new QTL include two of the three QTL for culm diameter, two of the four QTL for plant height, two of the three QTL for flag leaf length, three of the four QTL for flag leaf width, two of the three QTL for tiller angle, one QTL for tiller number, four of the five QTL for panicle length, three of the four QTL for grain length, two of the five QTL for

Table 1 QTL identified from the analysis of the rice recombinant inbred lines

Trait	LOD threshold ^a	QTL	Chr.	LOD	LOD peak position (cM)	R ² (%) ^b	Additive effect ^c	95% CI (cM) ^d	QTL region (Mb) ^e												
									Recombination bins	Simulation of 2,330 markers	Simulation of 238 markers										
Heading date	3.33	qHD-3	3	5.40	3.7	11.0	4.03	3.1–4.7	0.6–1.3	0.572–1.392	0.8–8.8										
												qCD-2	2	5.71	135.5	10.4	0.38	135.2–137.1	33.4–34.2	33.372–34.356	31.2–34.4
Culm diameter	3.37	qCD-4	4	4.13	94.9	7.3	0.30	93.6–96.6	30.1–31.3	29.764–31.404	28.0–32.8										
												qCD-12	12	5.47	74.9	9.9	0.36	73.9–75.9	18.6–20.0	18.448–20.088	18.4–23.2
Plant height	3.46	qPH-1	1	17.18	191	30.0	–13.10	190.0–193.2	40.0–40.6	40.096–40.752	39.2–40.8										
												qPH-2	2	8.00	136.4	11.9	7.90	136.1–137.7	33.9–34.6	33.700–34.356	31.2–36.0
												qPH-3	3	5.27	138.5	7.6	6.21	137.6–139.7	32.6–33.3	32.224–33.536	
Flag leaf length	3.43	qPH-12	12	4.61	88.4	6.5	5.89	86.2–91.2	22.4–22.9	22.056–23.204	21.6–23.2										
												qFL-2	2	3.32	109.9	7.0	2.68	107.6–112.2	25.7–27.3	25.664–27.304	24.8–29.6
Flag leaf width	3.48	qFL-5	5	3.07	115.8	6.4	–1.96	112.2–118.3	27.5–29.0	27.468–29.272	4.0–8.8										
												qFL-12	12	3.73	35.4	7.8	2.21	34.7–36.5	6.2–7.0	6.148–7.132	
Tiller angle	3.37	qFLW-1	1	3.48	45.3	6.0	0.66	43.5–47.7	4.9–5.4	4.836–5.656	4.0–8.8										
												qFLW-4	4	8.68	100.7	16.4	–1.01	99.0–101.2	31.3–31.8	31.240–31.896	31.2–34.4
Panicle length	3.37	qFLW-7	7	4.34	80.4	7.4	0.67	74.4–82.2	24.4–25.6	0.080–0.736	26.4–31.2										
												qFLW-8	8	3.89	1.0	6.8	0.66	0.0–2.0	0.0–0.3	27.468–30.256	4.0–7.2
Tiller number	2.85	qTA-2	2	3.72	118.7	4.7	–0.34	114.1–121.3	27.8–30.0	21.236–21.892	20.0–23.2										
												qTA-6	6	3.22	32.2	4.1	–0.38	31.3–33.5	5.3–5.8		
Grain length	3.35	qTA-9	9	24.57	81.8	46.0	1.03	81.1–82.9	21.3–21.7	33.864–35.012	32.8–36.0										
												qTN-5	5	4.01	115.5	8.8	1.92	114.5–116.8	27.9–28.7		
Awn length	3.30	qPL-2	2	3.27	137.4	5.1	0.96	135.9–139.6	33.9–34.9	0.900–1.556	26.4–29.6										
												qPL-6a	6	4.13	3.9	6.5	–0.99	2.3–5.6	0.8–1.5		
Grain length	3.35	qPL-6b	6	4.09	62.7	6.4	1.07	62.3–64.1	13.1–17.6	13.036–17.628	16.8–18.4										
												qPL-12a	12	4.39	99.0	7.1	1.09	97.2–99.7	23.6–24.2	23.532–24.352	4.0–7.2
Grain length	3.35	qPL-12b	12	3.14	32.2	4.9	0.94	30.3–33.5	5.4–5.8	26.648–30.420	28.0–31.2										
												qAL-1	1	7.40	152.6	13.7	5.71	150.7–154.9	29.1–30.8	16.152–17.464	15.2–18.4
Grain length	3.35	qAL-3	3	6.98	77.8	12.9	5.62	77.6–80.2	16.7–17.4	27.960–28.616	26.4–29.6										
												qAL-4a	4	4.75	86.3	8.4	–4.57	86.3–87.6	28.1–28.4		
Grain length	3.35	qAL-4b	4	3.25	53.0	5.6	–4.02	48.9–55.9	19.6–20.7	19.596–20.744	26.4–29.6										
												qGL-1	1	5.81	149.7	8.1	0.29	147.6–150.4	27.7–29.1	26.976–29.764	26.4–29.6
Grain length	3.35	qGL-3	3	10.51	78.8	16.1	0.41	78.5–81.3	16.8–17.5	16.644–17.464	16.8–18.4										
												qGL-6	6	4.12	32.2	5.7	0.28	30.8–34.5	5.2–5.9	5.164–5.984	
Grain length	3.35	qGL-10	10	4.45	59.1	6.1	0.23	54.2–65.1	19.0–20.9	18.776–21.236											

Table 1 continued

Trait	LOD threshold ^a	QTL	Chr.	LOD	LOD peak position (cM)	R ² (%) ^b	Additive effect ^c	95% CI (cM) ^d	QTL region (Mb) ^e		
									Recombination bins	Simulation of 2,330 markers	Simulation of 238 markers
Grain width	3.26	qGW-2a	2	4.18	41.4	4.3	-0.07	41.1–44.3	9.0–9.8	8.936–9.920	
		qGW-2b	2	3.21	112.1	3.2	-0.06	111.5–113.4	27.1–27.5		
		qGW-3	3	7.02	82.2	7.3	-0.09	78.5–83.7	16.8–17.9	16.644–18.120	15.2–18.4
		qGW-5	5	12.61	25.1	14.6	-0.15	25.1–26.4	5.1–5.5	4.672–5.656	4.0–5.6
		qGW-6	6	5.32	60.4	5.5	-0.09	59.7–62.3	11.5–13.1	11.396–13.200	10.4–18.4
		qGT-5	5	4.77	23.0	9.2	-0.04	22.6–25.5	4.7–5.3	4.672–5.328	
Grain thickness	3.35	qGT-6	6	3.91	61.4	7.4	-0.04	60.1–62.5	11.6–16.9	11.560–16.972	10.4–20.0
		qGT-10	10	4.73	78.7	9.2	-0.04	77.2–82.2	22.4–23.2	22.384–23.204	
		qGT-11	11	3.73	45.9	7.1	0.03	45.6–47.3	10.6–11.0	10.248–11.068	
		qTGW-5a	5	3.29	9.1	6.7	-1.08	7.9–9.7	1.3–2.2		
		qTGW-5b	5	3.14	110.4	5.8	-0.96	107.8–111.5	27.0–27.4	26.812–27.468	
		qTGW-8	8	4.27	106.7	8.8	1.23	104.9–108.1	25.9–26.4	26.320–27.304	24.8–28.0
Spikelet number	3.43	qTGW-10	10	4.26	21.9	9.7	-1.28	21.2–23.1	11.7–11.9	10.740–12.052	2.4–12.0
		qSN-1a	1	4.63	37.3	8.8	18.98	35.0–40.9	3.5–4.4	3.360–3.852	36.0–40.8
		qSN-1b	1	5.08	181.2	9.8	-18.78	179.8–182.1	37.1–37.5	36.980–37.636	
		qSN-6	6	5.09	60.4	10.0	19.81	58.8–62.4	11.2–16.9	11.068–16.972	10.4–21.6
		qSN-8	8	4.36	18.3	8.3	16.99	16.7–20.0	2.3–3.0	2.376–3.196	0.8–4.0

^a Thresholds determined by 1,000 permutation

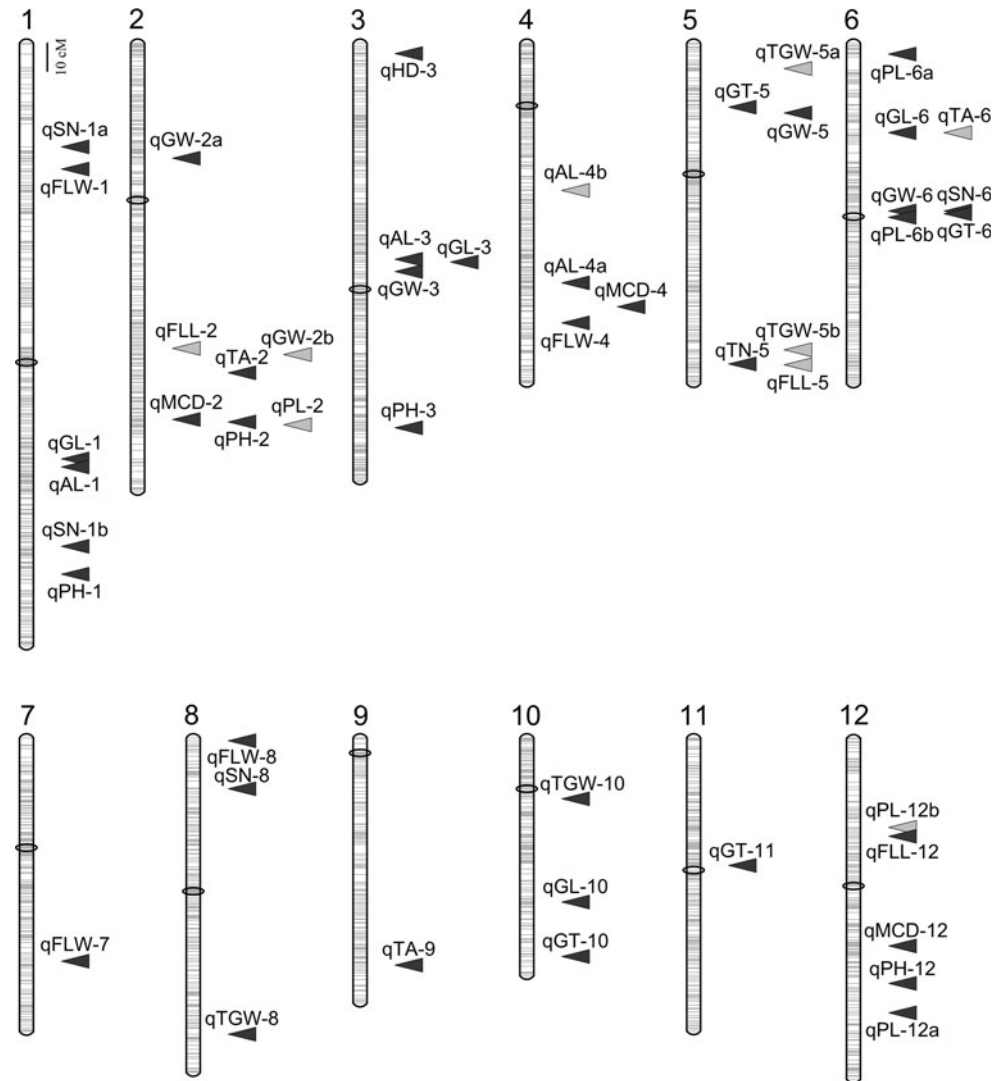
^b Percentage of phenotypic variation explained

^c Positive and negative values indicated additive effect contributed by the alleles of 93-11 and Nipponbare, respectively

^d Chromosome regions corresponding to the 95% confidence interval for the detected QTL

^e Regions between flanking bins or simulated markers of identified QTLs using high-throughput genotyping method (re-sequencing), 2,330 simulated markers, and 238 simulated markers by their physical locations, respectively. Blank spaces represent no overlapped QTL

Fig. 4 Chromosomal locations of detected QTL. Twelve rice chromosomes are numbered, with centromeres indicated by *open circles*. Positions of 2,334 bins used as genetic markers are indicated by *horizontal lines* spaced proportionally according to their genetic distance on the linkage map. *Arrow heads* indicate LOD peak locations of QTL. *Black arrow heads* QTL with LOD values above the threshold determined by permutation tests; *gray arrow heads* QTL with LOD values above 3.0 but below the permutation threshold. For QTL abbreviations, see Table 1



grain width, one of the four QTL for grain weight, and one of the four QTL for spikelet number. Almost all traits in this study have novel QTL except heading date. In addition, among 31 newly detected QTL, 4 QTL above mentioned with large effect have not been reported to date which is involved with awn length, plant height, and culm diameter.

We found that the LOD peak of a QTL always overlapped with the genetic position of a bin marker. This location of the QTL was then pinpointed on the chromosome (Fig. 4). The 95% confidence interval of the QTL location was determined (Table 1). If 95% confidence intervals of the two QTL were overlapped, these QTL were defined to be co-localized. A total of seven groups of co-localized QTL are identified, including (1) qFLL-2 and qGW-2b on chromosome 2; (2) qCD-2, qPH-2, and qPL-2 on chromosome 2; (3) qAL-3, qGL-3 and qGW-3 on chromosome 3; (4) qGT-5 and qGW-5 on chromosome 5; (5) qTN-5 and qFLL-5 on chromosome 5; (6) qTA-6 and

qGL-6 on chromosome 6; and (7) qGW-6, qGT-6, and qSN-6 on chromosome 6.

Epistasis analysis detected significant interaction between nine pairs of QTL for six traits (Table 3). These include one pair each for flag leaf length, flag leaf width, and awn length, and two pairs each for grain length, 1,000-grain weight, and spikelet number. Because only additive effect of QTL can be estimated from the RIL population, all interactions are additive-additive epistasis with effect sizes ranging 1.09–4.87%.

Candidate genes

With a relatively high mapping resolution, the small genomic regions of top five large-effects QTL are overlapped with the locations of five genes reported in previous studies (Fig. 5). For the QTL of the largest effect on tiller angle, qTA-9 ($R^2 = 46.0\%$) was located in the region which ranges from 21.3 to 21.7 Mb on chromosome 9. A strong candidate

Table 2 Comparison of QTL identified from this and previous studies of *indica* and *japonica* cultivars

Traits	QTL of this study		Previous studies					Reference	
	QTL	Physical position (Mb) ^a	QTL ^b	Flanking markers	Physical position (Mb)	Population type	Population size		Mapping parents ^c
Heading date	qHD-3	0.6–1.3	Hd9	R1468B-S12021	0.97–1.24	BC ₄ F ₃	198	Nipponbare(J), Kasalath(I)	Lin et al. (2002)
			qHDD3-1	RG104-RG348	0.46–1.42	DH	125	Azucena(J), IR64(I)	Hittalmani et al. (2003)
			QHd3a	C515-RG348a	0.69–1.42	RIL	254	Lemont(J), Teqing(I)	Mei et al. (2005)
Culm diameter	qCD-12	18.6–20	Unnamed	G2140-R3375	5.60–18.86	BIL	98	Nipponbare(J), Kasalath(I)	Kashiwagi and Ishimaru (2004)
Plant height	qPH-1	40–40.6	Unnamed	R494-C742	34.97–41.24	BC ₁ F ₇	98	Nipponbare(J), Kasalath(I)	Ishimaru et al. (2001)
			QTLph1	C86-C742	39.47–42.71	BIL	98	Nipponbare(J), Kasalath(I)	Ishimaru et al. (2001, 2004)
			Unnamed	R2414-C742	39.89–41.24	BIL	98	Nipponbare(J), Kasalath(I)	Kashiwagi and Ishimaru (2004)
Flag leaf length	qPH-12	22.4–22.9	QPh12	G402-RG20q	21.25–25.39	RIL	254	Lemont(J), Teqing(I)	Mei et al. (2005)
Flag leaf width	qFLL-5	27.75–28.6	QFl5	RM480-RM334	27.45–28.62	RIL	180	IRAT109(J), Zhenshan 97(I)	Yue et al. (2006)
Tiller angle	qFLW-4	31.3–31.8	QFlw4	RM255-RM349	31.35–33.08	RIL	180	IRAT109(J), Zhenshan 97(I)	Yue et al. (2006)
	qTA-9	21.3–21.7	Spk(t)	C1263-C506	20.37–21.85	BIL	98	Nipponbare(J), Kasalath(I)	Yamamoto et al. (1997)
			qTA-9	C609-C506	19.24–21.85	CSSL	65	Asominori(J), IR24(I)	Yu et al. (2005)
Panicle length	qPL-6a	0.8–1.5	QPl6	RZ762-C76	0–1.82	RIL	254	Lemont(J), Teqing(I)	Mei et al. (2005)
Grain length	qGL-3	16.8–17.5	qGL-3a	C80-C1677	16.19–18.36	RIL	71	Asominori(J), IR24(I)	Wan et al. (2006)
Grain width	qGW-2a	9–9.8	GW2	RM5897-RM2634	6.76–10.99	BC ₂ F ₂	190	WY3(J), Fenggaizhan-1(I)	Song et al. (2007)
	qGW-3	16.8–17.9	qGW-3	RM257-RM175	3.84–28.83	BIL	142	Nantehao(J), Baliila(I)	Yan et al. (2003)
			qGW-3	G332-C80	17.68–21.5	BIL	98	Nipponbare(J), Kasalath(I)	Li et al. (2003)
	qGW-5	5.1–5.5	qGW-5b	C249-R566	1.83–7.74	BIL	98	Nipponbare(J), Kasalath(I)	Li et al. (2003)
1,000-grain weight	qTGW-5a	1.3–2.2	Unnamed	R830-R3166	1.09–1.83	BIL	98	Nipponbare(J), Kasalath(I)	Ishimaru K (2003)
	qTGW-5b	27–27.4	Unnamed	RM87-RM334	27.16–28.62	DH	190	WYJ2(J), Zhenshan 97(I)	Jiang et al. (2004)
	qTGW-10	11.7–11.9	Unnamed	R2194-R2447	7.59–14.69	BIL	98	Nipponbare(J), Kasalath(I)	Ishimaru K. (2003)
Spikelet number	qSN-1a	3.5–4.4	Unnamed	MRG167-RM84	3.94–4.23	DH	190	WYJ2(J), Zhenshan 97(I)	Jiang et al. (2004)
	qSN-1b	37.1–37.5	qSNP-1b	OSR3-RM104	35.78–41.92	IL-LT	217	Lemont(J), Teqing(I)	Mei et al. (2006)
	qSN-6	10.9–13.1	QSn6	C235a-G294d	9.28–17.93	RIL	254	Lemont(J), Teqing(I)	Mei et al. (2005)

^a The physical position of the bin corresponding to the 95% confidence interval for the detected QTL

^b QTL identified in the previous studies of mapping population derived from crosses between *indica* and *japonica* cultivars. Those with flanking markers with known physical locations overlapping with the 95% confidence intervals of QTL found in this study are listed

^c The mapping parents of *indica* and *japonica* cultivars indicated by I and J, respectively

Table 3 Epistasis detected between QTL

QTL _i ^a	Bin _i ^b	QTL _j ^a	Bin _j ^b	LOD	R ² (%) ^c	F value
qFLL-5	Bin 1134	qFLL-12	Bin 2192	1.93	4.13	8.71**
qFLW-4	Bin 940	qFLW-7	Bin 1462	1.15	1.95	4.97*
qAL-1	Bin 180	qAL-4	Bin 921	1.73	3.11	7.82**
qGL-1	Bin 174	qGL-10	Bin 1936	1.24	1.69	5.39*
qGL-6	Bin 1185	qGL-10	Bin 1936	0.81	1.09	3.49*
qTGW-5a	Bin 983	qTGW-8	Bin 1673	1.59	3.10	6.92**
qTGW-5a	Bin 983	qTGW-10	Bin 1879	2.46	4.87	10.88**
qSN-1a	Bin 24	qSN-1b	Bin 229	1.09	1.92	4.71*
qSN-1b	Bin 229	qSN-8	Bin 1523	1.27	2.25	5.51*

*, ** Epistasis at the significance level of $P = 0.05$ and 0.01 , respectively

^a A pair of QTL with detected epistasis. See Table 2 for QTL abbreviations

^b Bins overlapping with the LOD peak location of QTL_i and QTL_j, respectively

^c Epistasis effect

gene, *TAC1*, that controls tiller angle was previously cloned (Yu et al. 2007) and is located in this region. For plant height, the major QTL, qPH-1 ($R^2 = 30.0\%$) has its LOD peak in the region of 600 kb on chromosome 1. On the same location, *SD1*, a gene responsible for rice semi-dwarfness and known as the rice green revolution gene (Sasaki et al. 2002), is located. The major QTL for flag leaf width, qFLW-4 ($R^2 = 16.4\%$), is mapped to the region which ranges from 31.3 to 31.8 Mb on chromosome 4, while a gene controlling leaf width, *Nall1*, was found in it (Qi et al. 2008). The QTL of largest effect on grain length, qGL-3 ($R^2 = 16.1\%$), has its LOD peak in the region of 700 kb on chromosome 3, which contains *GS3*, a gene controlling grain shape, was cloned (Fan et al. 2006). Finally, the major QTL for grain width, qGW-5 ($R^2 = 14.6\%$), is located in the region of 400 kb on chromosome 5, which is overlapped with the location where *qSW5* that controls grain width was previously cloned (Shomura et al. 2008).

Effects of genotype resolution and population size on QTL mapping

In order to evaluate the effect of high-throughput genotyping method on QTL mapping resolution, we designed a set of low density (238) and a set of high density (2,330) markers evenly distributed in the rice genome (Table S2) for QTL analysis. On the linkage map constructed by high-density simulated markers, which is comparably dense to bins, 755 pairs of adjacent markers (32.6%) have genetic distance of zero, which was more than the linkage map constructed by bins (Fig. 3; Table S4). It was likely because that these simulated markers were set to specific locations, such as regions around the centromeres and regions with low-recombination rates. Then 29 and 43 QTL were mapped using these low- and high-density simulated markers,

respectively, which overlapped with QTL identified using sequencing-based genotyping dataset (Table S5). The 95% confidence intervals (CI) of the latter set of QTL were significantly smaller than those of two former sets of QTL (paired-sampled t test, both $P < 0.001$; and independent t test, $P < 0.001$ and $P = 0.037$, respectively; Table S5). It suggested that QTL mapping resolution using the high-throughput genotyping method was increased significantly.

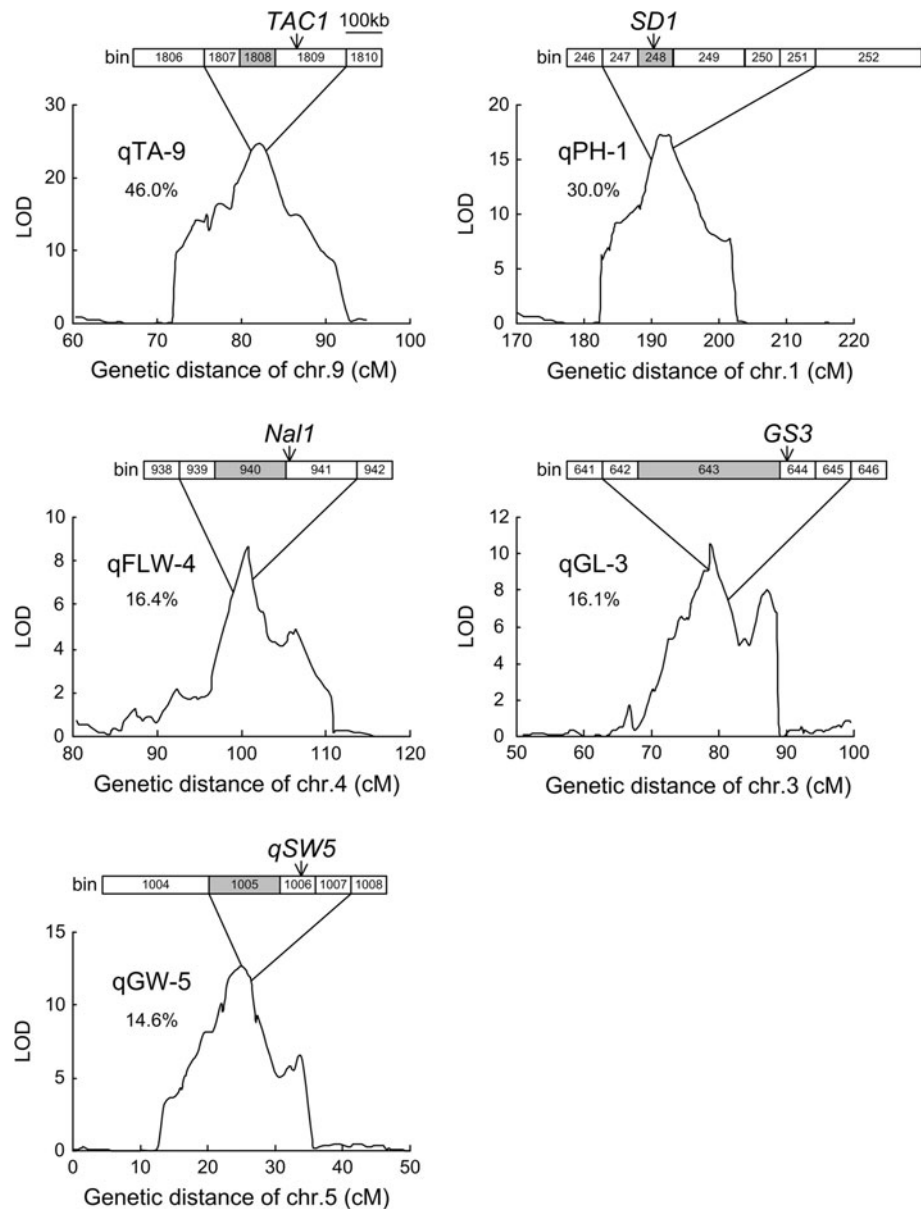
Moreover, in order to evaluate the effect of population size on QTL mapping resolution using the high-throughput genotyping method, two schemes were employed. In the scheme 1, 50 and 100 RILs were randomly sampled from original 150 RILs five times, respectively. We observed that the possibility with which five major QTL can be detected dropped to 76% (19 of 25) when population size decreased to be 100, and the possibility dropped to 44% (11 of 25) when the size got to be 50 (Table S6). It suggested that the smaller population sizes affected the sensitivity of QTL mapping dramatically. In the scheme 2, various sizes of population size (from 50 to 500 individuals) were simulated by Win-QTLcart. On average, the 95% confidence interval of five major QTL was changed from 6.7 to 1.4 cM (the mean value of five parallel simulation) when population size was transformed from 50 to 500, and their standard deviation was changed from 3.2 to 0.7 (Table S7, Fig. S2). It suggested that larger population size coupled with high-resolution genotype information could further increase QTL mapping resolution.

Discussion

Relationship of mapped QTL and previously identified genes

We found that there were strong candidate genes for the top five largest-effect QTL. For example, the known *TAC1*

Fig. 5 Precise locations of five QTL and candidate genes. Curves indicate chromosomal locations and LOD values of detected QTL. Names and phenotypic effect (R^2) of the QTL are indicated. Recombination bins near the LOD peaks of the QTL are illustrated as horizontal bars with their numbers labeled inside. Shaded bin overlaps the LOD peak of a QTL. The portion of a curve and corresponding bins defining the 95% confidence interval of a QTL are bracketed by two lines. The relative physical position of a candidate gene in the bin is indicated by an arrow head



gene (Yu et al. 2007) was found to be located in the region of qTA-9 on chromosome 9. Comparative genome sequencing analysis revealed that Nipponbare shared the same mutation with all *japonica* cultivars which cause small tiller angles than *indica* rice. This strongly supports the candidacy of *TAC1* for qTA-9. The same situation happened to other four QTL, qPH-1, qFLW-4, qGL-3, and qGW-5, which contains *SD1*, *Nal1*, *GS3*, and *qSW5*, respectively (Sasaki et al. 2002; Qi et al. 2008; Fan et al. 2006; Shomura et al. 2008). The detailed information for the strong candidate genes involved in five major QTL is shown in Fig. S3. Of the candidate genes for these QTL, four were cloned from QTL identified from other rice

cultivars. They are likely the genes with widespread large effect on natural variation among rice cultivars. Besides these genes, few others have been cloned for the natural variation of complex traits in rice.

There was no such strong evidence for the remaining QTL. However, we found that some candidate genes might support some of them, such as qHD-3. *OsSOC1* that activate flowering in rice was found to be located in the region of qHD-3 for heading date in this study (Tadege et al. 2003). *OsSOC1*, which encodes a MADS domain protein, is the ortholog of the Arabidopsis gene *AtSOC1*. *AtSOC1* and *OsSOC1* show a 97% amino acid similarity in their MADS domain (Tadege et al. 2003).

Co-localization of mapped QTL

A total of seven co-localized QTL groups were identified among 49 QTL. We found that five groups of them underlie significant correlation between ten pairs of traits. Among them, five pairs of traits have significantly positive correlation, including CD and PH, CD and PL, PH and PL, AL and GL, and GW and GT; the other five pairs have significantly negative correlation, including AL and GW, GL and GW, TN and FLL, GW and SN, and GT and SN. These co-localizations suggested that pleiotropic effect or linkage of the genes is likely to be responsible for the trait correlation. With the data in the present form, however, we could not distinguish these two alternatives. Nevertheless, not all correlations can be explained by QTL co-localization. This could be due to the effect of undetected QTL or reasons other than pleiotropy or linkage.

Genotype resolution and population size affect QTL mapping resolution

QTL mapping resolution, determined by the size of confidence interval of QTL, depends on the population size and marker density (Visscher et al. 1996; Da et al. 2000). In general, a gain of information, provided by a greater population size and a more markers density, resulted in smaller intervals (Bennewitz et al. 2002). The former determines the number of recombination events, while the latter determines the resolution of recombination breakpoints.

Development and exploitation of additional markers is one of the strategies to increase QTL mapping resolution (Gautier et al. 2006; Liu et al. 2008). In this study, QTL mapping resolution using the sequencing-based genotyping method (Huang et al. 2009) is significantly increased by comparing with those using simulated markers. Based on the comparison among linkage maps generated from bins, simulated markers, and conventional molecular markers, bins detect a larger number of recombination events with high accuracy. Evidently, recombination bins are superior to conventional molecular markers in genome coverage. Unlike conventional molecular markers, recombination bins defined by SNPs detected from whole-genome sequencing allow the entire genome to be evenly evaluated and accurately represent recombination events across the RILs, almost each one of which is available in QTL analysis. Although conventional markers are based on distribution of nucleotide polymorphism on the whole genome, a significant proportion of them is unavailable when their linkage distance are zero. This is also supported by the fact that physical sizes and regions surrounding centromeres have the largest bins and lowest recombination rates (Fig. S4). It suggested that the conceptual and technical advances in genetic markers have improved the accuracy of QTL mapping.

There was some difference in detected QTLs between genotyped 150 RILs and simulations. The possible basis is the specificity of composite interval mapping, which uses other markers to control genetic background when testing for the putative QTL (Kao et al. 1999).

Furthermore, population size plays a key role in QTL mapping resolution. In our study, QTL analysis using simulated populations showed that QTL mapping resolution increased with increase in population size, which is consistent with the simulation study by Visscher et al. (1996). Especially, increasing population size was more efficient than increasing marker density in QTL analysis when the individual number of population was less than 150 in this study (Table S6).

For this study, it is the advantage of the sequencing-based genotyping method that allows mapping population to be expanded and more deeply genotyped easily. Additional RILs derived from the same cross can be genotyped to increase the mapping population size. The RILs can be sequenced again at higher genome coverage to add more SNPs and detect additional recombination events. These two efforts together can cut the recombination bins into smaller sizes, which should help resolve QTL in a finer scale. The RIL population between rice cultivars, Nipponbare and 93-11, provides an excellent system for studying genetic basis of complex traits. Especially, individuals in RILs as permanent segregating populations are homozygous at all loci throughout the genome, and individuals within lines are highly homogeneous and thus can be used for repeated trials both over times and locations (Mei et al. 2005). Thus, the genotyped RIL population will serve as an effective study system for characterizing a variety of agriculturally important traits, such as yield, grain quality and nutrition, stress tolerance, and water and nutrient use efficiency (Zhang 2007).

Acknowledgments We thank Zhou TY, Zong G, Gu BG, Shi JP, Hu WH, and Hu J for assistance with field management, phenotypic evaluation, and sample preparation. This work was supported by the Ministry of Science and Technology of China (the China Rice Functional Genomics Programs, Grant # 2006AA10A102), the Chinese Academy of Sciences (Grant # KSCX2-YW-N-024), and the National Natural Science Foundation of China (Grant # 30821004) to BH.

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