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Identification of A Major Quantitative Trait Locus for Grain Weight In Rice Using Microsatellite Marker

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Abstract

Rice is one of the major staple foods in the world, especially in Asia. Improving yield potential of superior cultivars is important to meeting the demand for rice production, which is increasing due to human population increase, climate change, and degradation of agricultural resources, such as land and water. In this study, a BC_3F_2 population developed from an intraspecific cross between Ciherang and a new plant type line (B11143D) was used in a quantitative trait locus (QTL) analysis. Ciherang is a high yielding rice cultivar with good grain quality which has been planted in 37% of the irrigated rice area in Indonesia. The objective of this study was to identify QTL(s) for yield components on chromosome 12, which can be used to improve the elite cultivar Ciherang or other popular cultivars through marker-assisted breeding. A total of two hundred BC_3F_2 lines were evaluated in the greenhouse during this study. The population was observed for eight agronomic traits including days to heading (dth), plant height (ph), flag leaf length (fll), panicles per plant (ppl), panicle length (pl), grains per panicle (gpp), 1000-grain weight (gw), and yield (yld). Four simple sequence repeats (SSR) markers (RM3472, RM28048, RM28195, and RM1986) were used for targeted mapping on chromosome 12. Linkage analysis identified a QTL for 1000-grain weight located on chromosome 12 at position 53.5 cM–73 cM.

Abstrak

Identifikasi QTL Mayor untuk Sifat Berat Gabah pada Padi Menggunakan Marka Mikrosatelit. Padi merupakan salah satu komoditas pangan terpenting di dunia, terutama di benua Asia. Perbaikan potensi hasil kultivar unggul penting dilakukan untuk memenuhi permintaan produksi padi karena meningkatnya jumlah penduduk, perubahan iklim dan degradasi sumber daya pertanian seperti sumber daya lahan dan air. Pada penelitian ini, populasi BC_3F_2 yang dikembangkan dari persilangan antara Ciherang dan galur Padi Tipe Baru (B11143D) digunakan dalam analisis QTL. Ciherang merupakan kultivar padi yang memiliki potensi hasil tinggi dengan kualitas beras dan nasi yang bagus dan sekitar 37% dari sawah irigasi di Indonesia ditanami oleh varietas ini. Tujuan dari penelitian ini adalah mengidentifikasi QTL komponen hasil pada kromosom 12 yang bisa dimanfaatkan untuk memperbaiki kultivar Ciherang atau kultivar-kultivar populer yang lain melalui pemuliaan berbasis penanda molekuler. Dua ratus galur BC_3F_2 dievaluasi di rumah kaca selama penelitian. Populasi diamati 8 sifat agronomisnya yaitu umur berbunga, tinggi tanaman, panjang daun bendera, jumlah malai per tanaman, panjang malai, jumlah gabah isi per malai, bobot 1000 butir, dan hasil per tanaman. Empat penanda SSR (RM3472, RM28048, RM28195, dan RM1986) digunakan dalam pemetaan terarah pada kromosom 12. Analisis keterpautan mengidentifikasi QTL untuk bobot 1000 butir yang terletak pada kromosom 12 pada posisi 53,5 cM–73 cM.

Key words: SSR markers, new plant type, QTL analysis, 1000-grain weight

Introduction

Ciherang is an irrigated rice inbred cultivar released in the year 2000. Seventeen years after its release, Ciherang is still the most popular rice variety, cultivated in 37% of the irrigated rice area in Indonesia [1], partly because its grain quality characteristics are preferred by both traders and consumers. B11143D is a New Plant Type line superior to Ciherang in some agronomic characteristics. The grains per panicle for B11143D and

Ciherang are 207 and 133, respectively [2]. The 1000-grain weight of B11143D and Ciherang are 26.7 g and 21.1 g, respectively [2]. However, the panicle numbers per plant for B11143D and Ciherang are 12 and 29, respectively [2]. Introgression of genetic locus controlling for the superior traits of B11143D through backcrossing may improve the yield potential of Ciherang.

Grain yield is a quantitative trait that is affected by the three component traits including the number of panicles, number of grains per panicle, and grain weight or grain size, all of which are controlled by various genes [3]. The first stage in the identification of the genes that affect a quantitative trait is the mapping of the quantitative trait locus (QTL) that affects the trait. QTL is defined as a chromosomal region that affects a quantitative trait. At least 18 QTLs were identified in 9 out of 12 chromosomes for the panicle number of rice [4]. Four major QTLs for the number of grains per panicle have been reported and shown as distributed on chromosomes 1, 4, 6, and 7 [5-7]. A major QTL (R^2 > 10%, [8,9]) for grain weight was identified around the centromeric region of chromosome 3 [10,11]. Gene cloning showed that a gene encoding the putative transmembrane protein is responsible for this QTL [12]. Other major QTLs for grain weight were identified in chromosome 2, 5, and 8 [2,13,14].

During a previous experiment that used a BC_1F_1 population derived from a cross between Ciherang and B11143D, a QTL for yield component was identified on chromosome 12, where B11143D contributed the favorable allele [15]. The objective of the current study was to delimitate the QTL for yield component on chromosome 12 into a region of 20 cM.

To develop a mapping population, a set of 63 SSR markers distributed on 12 rice chromosomes were tested against the BC_3F_1 population. A BC_3F_1 line 2-3-6 carrying both parental alleles for the chromosome 12, but carrying only Ciherang's allele for majority of the regions on chromosomes 1-11, was selected [16]. This selected line was self-pollinated and the progenies were used for targeted mapping using SSR markers on chromosome 12. A QTL for 1000-grain weight was located between markers RM28048 and RM1986, with B11143D contributing the favorable allele. This is the first major QTL (R^2 = 26%) for grain weight identified on chromosome 12. The two markers flanking the QTL can be used for marker-assisted backcrossing to improve grain weight trait in Ciherang. A BC_3F_2 line heterozy gous for the region flanked by these two markers will be selected, and its progenies will be used for fine mapping of this trait as a step toward cloning of the gene responsible for the trait.

Material and Methods

Population development. A BC_3F_2 (Ciherang x B1114 3D) population was used as the mapping population. Backcrossing was performed with Ciherang as the recurrent parent and B11143D as the donor parent in the greenhouse of ICABIOGRAD, Bogor, Indonesia [2] [15]. A BC_3F_1 line carrying both Ciherang and B11143D alleles for the chromosome 12, but carrying only Ciherang's allele for about 85% of the regions on chromosomes 1-11, was selected (Figure 1) [16]. A total of 200 BC_3F_2 lines were produced by self-pollination of the selected BC_3F_1 line. The 200 BC_3F_2 lines, along with the parental lines, were grown in pots filled with paddy soil. Compost and NPK 16:16:16 compound fertilizer were supplied before planting. NPK fertilizer was supplied again at 21 and 45 days after planting.

Phenotypic evaluation. The BC_3F_2 and parental lines were grown in pots in the greenhouse of ICABIOGRAD, Bogor, Indonesia from June to October 2015. Morpholo gical observation was conducted on dth (days), ph (cm), fll (cm), ppl, pl (cm), gpp, gw (g), and gy (g).

SSR genotyping. The genomic DNA of the 200 BC_3F_2 lines and two parents were isolated from the leaves of 4-week-old seedlings using a modified Cetyl trimethylammonium bromide (CTAB) method [17]. The quality and quantity of DNA were evaluated by gel electrophoresis with λ DNA as a control. PCR amplification was performed in a 96-well plate. The total volume of reaction in each well was 10 µL, containing 2 µL of 50 ng genomic DNA as a template, 5.68 µL of ddH_2O, 0.12 µL of DreamTaq DNA Polymerase (5 U/µL), 1 µL of 5 mM SSR primer (mixed forward and reverse primers), 0.2 µL of 10 mM dNTPs (dATP, dCTP, dGTP, and dTTP), and 2µL of 10× Buffer PCR (Thermo Scientific). Four polymorphic SSR markers (RM3472, RM28048, RM28195 and RM1986) located on chromosome 12 were used for genotyping [2]. PCR amplification was performed by applying pre-denaturation at 94°C for 5 minutes, 30 cycles of denaturation, annealing, and extension at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 2 minutes [2]. Amplification products were separated on an 8% poliacrylamid gel electrophoresis, stained with ethidium bromide solution (20 mg/L) for 10 minutes, and visualized under trans-UV light using a Chemidoc Gel System (Bio-Rad). A BC_3F_2 line was scored as A if it showed the same band as Ciherang, B if it showed the same band as B11143D, and H (heterozygous) if it showed bands from both parents.

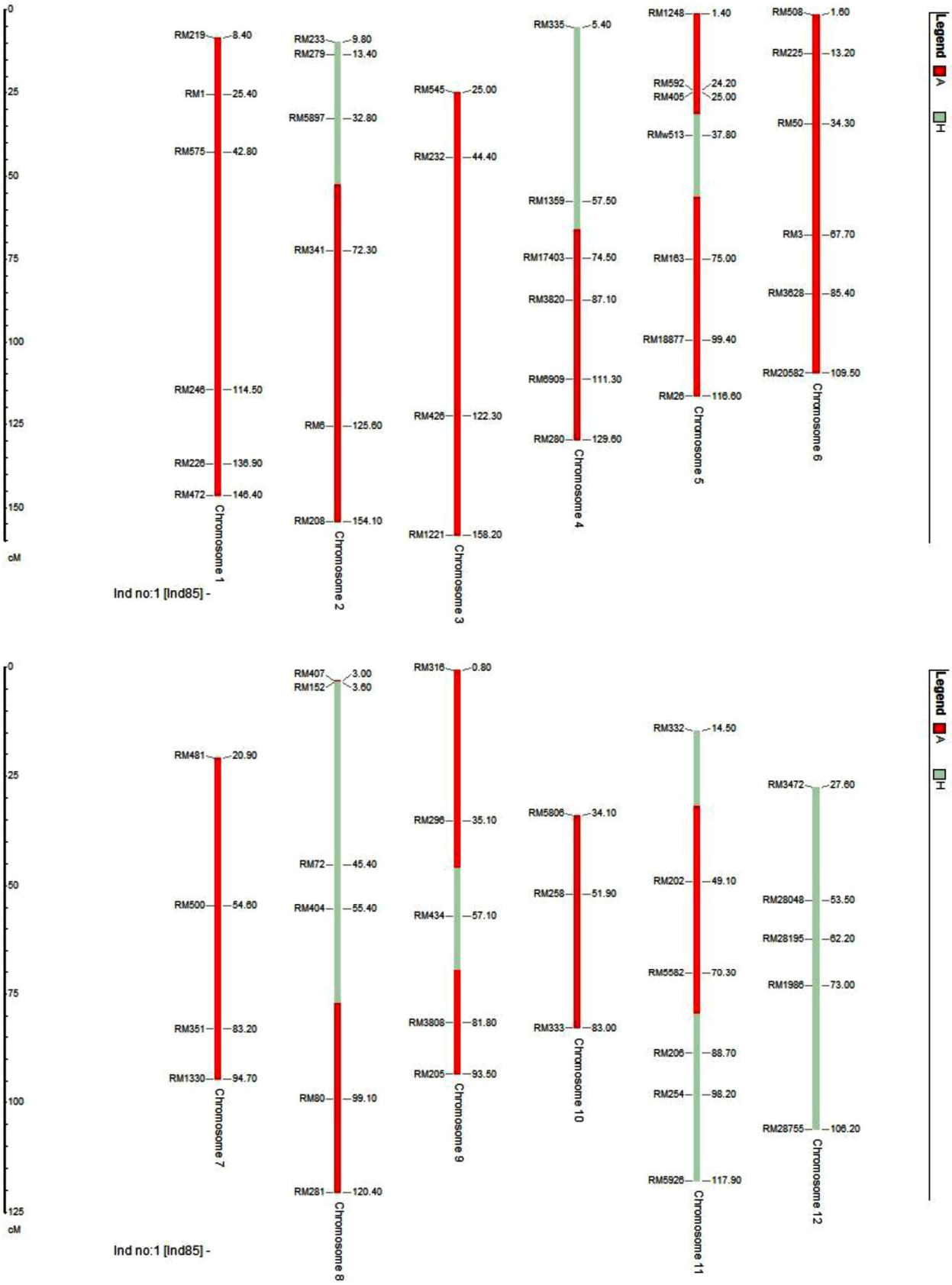

Figure 1. Graphical Genotypes for Chromosome 1 to 12 of the Selected BC_3F_1 Line 2-3-6 [16]

Table 1. Phenotype Performance of Parental Lines and a BC_3F_2 Population

Traits	Parental lines			BC_3F_2 population (n = 200)	
	Ciherang mean	B11143D mean	Ciherang vs B11143D[a]	Mean	Range
Days to heading (*dth*)	73.33	63.83	**	71.01	65 - 77
Plant height (*ph*)	96.92	126.17	**	98.96	75 - 188
Panicle per plant (*ppl*)	9.76	4.33	**	9.66	5.00 - 18.00
Flag leaf length (*fll*)	33.38	49.19	**	32.89	19.23 - 44.40
Panicle length (*pl*)	25.23	26.44	ns	25.03	18.33 - 27.67
Grains per panicle (*gpp*)	140.94	220.61	**	162.40	42.67 - 210.33
Percent seed set (*pss*)	94.12	92.52	ns	93.55	60.38 - 97.67
1000-grain weight (*gw*)	22.2	26.8	**	23.85	18.9 - 25.80
Grain yield per plant (*gy*)	25.13	23.42	ns	28.00	15.91 - 47.95

ns not significant
** Significant at $p < 0.01$
[a] Difference between two parents with t-test.

Data analysis. Descriptive statistics, mean comparison, and correlation among the traits were calculated using the Statistical Tool for Agricultural Research (STAR) software. QTL mapping was performed using Single Marker Regression implemented in QGene Ver. 4.3.8 [18]. Specific parameters were set for the mapping: the population structure was BC_3F_2, the type of cross (mating string) was "bbbs," and the genotype symbols were ABHxx-. Permutations of 10.000 iterations were used to determine the threshold of the QTLs in QGene. Subsequently, LOD values at $p < 0.05$ were used as the threshold to determine the significance of the QTLs. The visualization of marker genotypes along the chromosome was performed using Graphical GenoTypes Ver. 2.0 (GGT).

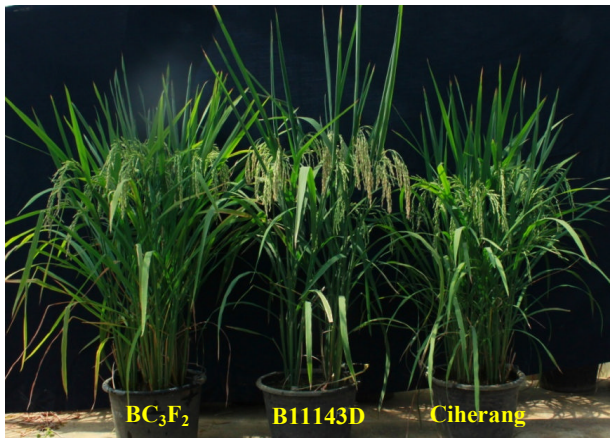

Figure 2. Plant Performance of Parental Lines and a Randomly Selected BC_3F_2 Line (84 Days After Planting)

Results and Discussion

Phenotypic evaluation. Consistent with a previous experiment [2], B11143D showed a higher grains number, higher grain weight, and lower panicle number compared to Ciherang (Table 1). B11143D flowered earlier than Ciherang (Table 1, Figure 2). The grain yield of B11143D was similar to that of Ciherang with means of 25.13 g and 23.42 g per plant, respectively (Table 1). In the BC_3F_2 population, grain yield showed transgressive segregation in both directions, as seen in how the progenies had a grain yield that fell outside of the range of either parent (the grain yield range in progenies was from 15.91 to 47.95 g). Plant height, panicle per plant, panicle length, and percent seed set also showed transgressive segregation in both directions (Table 1), indicating that the two parental lines contribute favorable alleles for these traits. Flag leaf length, grains per panicle, and 1000-grain weight showed transgressive segregation in a negative direction (Table 1, Figure 3), which indicates that B11143D, although containing a superior phenotype for these traits, possesses hidden negative alleles [19]. These alleles show up in the BC_3F_2 population in which 85% of the genome has been fixed to other parental line, i.e., Ciherang.

Days to heading was the only trait that showed transgressive segregation in a positive direction (there were some BC_3F_2 individuals with days to heading longer than two parents), indicating that, although it flowered earlier than Ciherang, B11143D contributes alleles for longer flowering time in the BC_3F_2 population. These findings on transgressive segregation, regardless of direction, show that the phenotype of a plant is only a modest predictor of the number of superior alleles it can contribute to the phenotype of interest, and that the breeding paradigm needs to shift from selecting plants on the basis of phenotype to evaluating them for the presence of the chromosomal segments associated with the desired traits [19].

Table 2. Pearson's Correlation Coefficients for Yield and Yield Components

Trait	*dth*	*ph*	*ppl*	*fll*	*pl*	*gpp*	*pss*	*gw*
ph	-0.160[a]							
ppl	0.497**	-0.101						
fll	-0.139	0.243**	-0.086					
pl	-0.069	0.309**	0.089	0.510**				
gpp	-0.048	0.198*	0.100	0.351**	0.653**			
pss	-0.099	0.124	-0.008	0.084	0.213*	0.507**		
gw	-0.123	0.118	-0.115	0.415**	0.470**	0.366**	0.338**	
gy	0.288**	0.018	0.657**	0.163[a]	0.413**	0.592**	0.171[a]	0.204*

* Significant at P<0.01
** Significant at P<0.001
[a] Significant at P<0.05
[b]*dth* days to heading, *ph* plant height, *ppl* panicle per plant, *fll* flag leaf length (cm), *pl* panicle length (cm), *gpp* grains per panicle, *pss* percent seed set (%), *gw* 1000-grain weight (g), *gy* grain yield per plant (g).

Figure 3. Seed Performances of Parental Lines and 3 Representative BC_3F_2 Lines

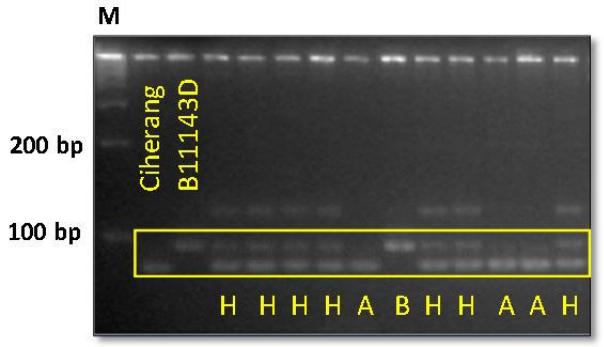

Figure 4. Scoring of BC_3F_2 Lines for the Genotype of RM28048 Marker. M: 100bp DNA Ladder, A: Homozygous Ciherang, H: Heterozygous, B: Homozygous B11143D

Grain yield showed a strong positive correlation with panicle number (r=0.657), moderate positive correlations with grain number (r=0.592) and panicle length (r=0.413), and weak positive correlations with grain weight (r=0.204) and days to heading (r=0.288), following the grouping of correlation value suggested by previous research [20]. There was no correlation identified between grain yield and plant height (Table 2).

These findings show that grain yield is associated more with yield components than with phenology (days to heading) or plant-type traits (plant height). Since grain yield is a complex trait with low heritability, indirect selection using DNA markers associated with yield components may contribute a significant genetic gain.

Determination of marker genotype. During analysis, the Ciherang allele was scored as A and the B11143D allele was scored as B. For RM28048, Ciherang showed a single band with size of 82 bp, whereas B11143D showed a single band with size of 94 bp (Figure 4). A BC_3F_2 line was scored as A if it showed only the 82 bp band, B if it showed only the 94 bp band, and H (heterozygous) if it showed both bands (Figure 4). The same scoring method was applied for the other 3 markers.

QTL analysis. A population of 200 BC_3F_2 plants developed from a selected BC_3F_1 plant was used for QTL mapping. The selected BC_3F_1 plant was hetero-zygous for the whole chromosome 12 and approximately 85% of the area on chromosomes 1-11 had the same genotype as Ciherang (Figure 1). Backcrossing was performed three times to recover the chromosomal regions from the recipient parent outside the chromosome 12 [21]. Recovery of the recipient parent's chromosomal regions was performed to minimize the effects of QTLs from the donor parent on chromosomes 1-11 so that the QTL

Table 3. Single Marker Analysis of Yield Components on a BC_3F_2 Population Derived from Ciherang x B11143D

Marker	Genotype	Yield Components						
		ppl[1]	fll	pl	gpp	pss	gw	gy
	A	9.58a[2]	32.78a	25.19a	160.22b	93.40a	23.60b	27.14a
RM3472	H	9.82a	32.66a	24.95a	160.44b	93.21a	23.80b	27.97a
	B	9.40a	33.56a	25.06a	169.39a	94.49a	24.20a	29.02a
LOD		0.35	0.78	0.38	1.00	0.35	3.00	0.30
R^2 (%)		0.80	1.70	0.90	2.40	0.79	5.50	0.70
Add effect		0.10	-0.60	0.002	-4.30	-0.405	-0.30	-0.70
	A	10.00a	31.95b	24.92a	157.12b	93.40a	23.30c	26.44b
RM28048	H	9.64a	33.08ab	25.05a	162.96ab	93.21a	23.90b	28.32ab
	B	9.29a	33.71a	25.14a	168.01a	94.49a	24.50a	29.31a
LOD		0.58	1.00	0.20	1.60	0.30	11.50	1.30
R^2 (%)		1.40	2.30	0.40	3.50	0.69	24.00	3.00
Add effect		0.36	-0.88	-0.12	-5.50	-0.42	-0.58	-1.44
	A	10.02a	32.26a	24.87a	156.88b	97.13a	23.30c	26.52b
RM28195	H	9.57a	32.84a	25.07a	163.70ab	93.13ab	23.80b	28.25ab
	B	9.42a	33.77a	25.17a	166.44a	89.28b	24.50a	29.27a
LOD		0.48	0.65	0.25	1.40	0.64	13.00	1.20
R^2 (%)		1.10	1.50	0.60	3.00	1.50	26.00	2.40
Add effect		0.30	-0.75	-0.16	-4.80	-0.41	-0.60	-1.36
	A	10.08a	31.62b	24.86a	154.85b	97.83a	23.20c	26.22b
RM1986	H	9.60a	32.87ab	25.02a	163.26a	93.08ab	23.80b	28.27ab
	B	9.42a	34.15a	25.22a	167.72a	89.76b	24.50a	29.10a
LOD		0.52	1.90	0.51	2.20	0.10	13.00	1.25
R^2 (%)		1.20	4.20	1.20	4.90	0.20	26.00	2.60
Add effect		0.34	-1.27	-0.19	-6.44	-0.20	-0.62	-1.45

[1]*ppl* panicle per plant, *fll* flag leaf length (cm), *pl* panicle length (cm), *gpp* grains per panicle, *pss* percent seed set (%), *gw* 1000-grain weight (g), *gy* grain yield per plant (g)
[2]Different letters on the same column indicate statistical significant according to the test of Duncan 5%.

on chromosome 12 could be handled as a single Mendelian factor [22], and so the progenies from the heterozygote for the QTL could be used for mapping by evaluating the four polymorphic markers on chromosome 12 (RM3472, RM28048, RM28195 and RM1986).

Depending on genome size and marker spacing, an LOD threshold between 2 and 3 is required to ensure an overall false positive rate of 5% [23], indicating a 5% risk of concluding that an association between a marker and a trait exists when there is no actual association. In this study, 10,000 permutations for each trait at an experiment-wise significance level of 0.05 provided an LOD threshold requirement of 3.00 to declare a significant association between a marker locus and a QTL. Based on the LOD score, a major QTL was identified as explaining 26% of the 1000-grain weight variation in the intervals between RM28048 (position 53.5 cM), RM28195 (position 62.2 cM), and RM1986 (73 cM), in which B11143D contributed the favorable allele (Table 3 and Figure 5). The association between grain weight and RFLP markers located on chromosome 12 was reported in a previous study that used a population derived from a cross between Zhenshan97 and Minghui63 [24], although it was categorized as a suggestive QTL, given that the LOD score was 2.6 and the R^2 value was 2.2% [25].

Implication for breeding and gene discovery. The major QTL for grain weight identified in this study will be useful for marker-assisted selection, since this QTL contributed 26% of the grain weight variation and is also detected in different genetic backgrounds [24]. Application of marker-assisted selection for backcross breeding has been successful in the improvement of traits controlled by one or a few QTLs with a large effect [21]. The consistency of the QTL region from this experiment and in another study [24] using different genetic backgrounds provides confidence that this QTL will be effective for improvement of grain weight on diverse rice varieties. In these primary mapping works,

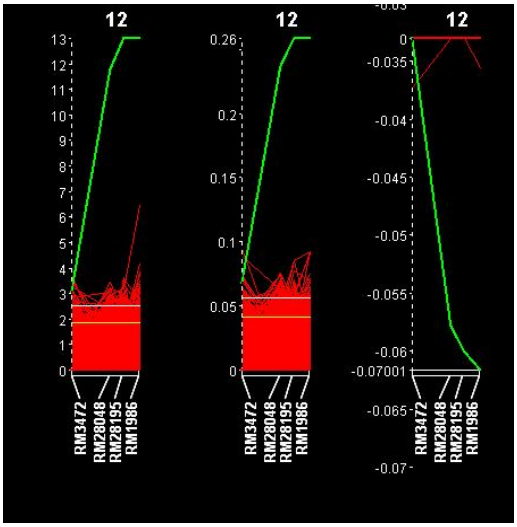

Figure 2. Single Marker Analysis for 1000-grain Weight (gw) of Chromosome 12 in a BC_3F_2 Population Using Four SSR Markers: RM3472 (LOD: 3.00; R^2: 5.50%; Add: -0.30), RM28048 (LOD: 11.50; R^2: 24%; Add: -0.58), RM28195 (LOD: 13; R^2: 26%; Add: -0.60), and RM1986 (LOD: 13; R^2: 26%; Add: -0.62)

(equal to 4.9 Mb in rice [26]). Currently, a large population (> 1000 individuals) of BC_3F_3 derived from a heterozygous BC_3F_2 line for the region flanked by RM28048 and RM1986 is being developed to perform the first fine mapping. Additional markers, including SSR and SNP, within the region flanked by RM28048 and RM1986 will need to be genotyped on the large BC_3F_3 population to allow for identification of rare recombinants. These recombinants will facilitate delimitation of the QTL to a smaller region of around 5-10 cM. The second fine mapping will then be performed to delimitate the QTL into a region of 1 cM (equal to 244 kb in rice [26]). With a gene density of one gene per 9.9 kb in rice [27], 244 kb may contain approximately 25 genes. This relatively small number of genes will allow for selection of the candidate genes based on their predicted functions, and the ability to proceed with validation of gene cloning using reverse genetics approaches, such as targeted mutation, silencing, or overexpression.

Conclusions

A major QTL for grain weight was identified on chromosome 12 at position 53.5 cM-73.0 cM in a population derived from Ciherang and B11143D. This major QTL is potentially useful for marker-assisted selection to improve the yield potential of modern rice varieties, especially for the 1000-grain weight trait, and it provides a primary target for fine mapping and identification of candidate genes responsible for the trait.

Acknowledgments

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