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

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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 BC3F2 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 BC3F2 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 (pl), 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.

Identification 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 BC3F2 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-pupuler yang lain melalui pemuliaan berbasis penanda molekuler. Dua ratus galur BC3F2 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]. Introgenesis 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 (R² > 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 BC₁F₁ 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 BC₁F₁ population. A BC₁F₁ 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 (R² = 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 BC₂F₁ line heterozygous 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 BC₁F₁ (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 BC₁F₁ 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 BC₂F₂ lines were produced by self-pollination of the selected BC₁F₁ line. The 200 BC₂F₂ 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 BC₂F₂ and parental lines were grown in pots in the greenhouse of ICABIOGRAD, Bogor, Indonesia from June to October 2015. Morphological observation was conducted on dth (days), ph (cm), fl (cm), ppl, pl (cm), gpp, gw (g), and gy (g).

SSR genotyping. The genomic DNA of the 200 BC₂F₂ 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 ddH₂O, 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% polyacrylamid 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 BC₂F₂ 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$_3$F$_1$ Line 2-3-6 [16]
Table 1. Phenotype Performance of Parental Lines and a BC$_3$F$_2$ Population

| Traits                      | Parental lines | BC$_3$F$_2$ population (n = 200) |
|-----------------------------|----------------|----------------------------------|
|                             | Ciferang mean  | B11143D mean                     | Mean   | Range         |
| Days to heading (dih)       | 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 (flf)      | 33.38          | 49.19                            | 32.89  | 19.23 - 44.40 |
| Panicle length (pl)         | 25.23          | 26.44                            | 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                            | 93.55  | 60.38 - 97.67 |
| 1000-grain weight (gwi)     | 22.2           | 26.8                             | 23.85  | 18.9 - 25.80  |
| Grain yield per plant (gy)  | 25.13          | 23.42                            | 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$_3$F$_2$, 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).

**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$_3$F$_2$ 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$_3$F$_2$ 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$_3$F$_2$ 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$_3$F$_2$ 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].
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Table 2. Pearson’s Correlation Coefficients for Yield and Yield Components

| Trait   | dth  | ph   | ppl  | fll  | pl   | gpp  | pss  | gw   |
|---------|------|------|------|------|------|------|------|------|
| dth     |      |      |      |      |      |      |      |      |
| ph      | -0.160* |      |      |      |      |      |      |      |
| ppl     | 0.497** | -0.101 |      |      |      |      |      |      |
| fll     | -0.139 | 0.243** |      |      |      |      |      |      |
| pl      | -0.069 | 0.309** | 0.089 |      |      |      |      |      |
| 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* | 0.413** | 0.592** | 0.171* | 0.204* |

* Significant at P<0.01
** Significant at P<0.001
* Significant at P<0.05

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 BC3F2 Lines

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.

Figure 4. Scoring of BC3F2 Lines for the Genotype of RM28048 Marker. M: 100bp DNA Ladder, A: Homozygous Ciherang, H: Heterozygous, B: Homozygous B11143D

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 BC3F2 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 BC3F2 plants developed from a selected BC3F1 plant was used for QTL mapping. The selected BC3F1 plant was heterozygous 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$_1$F$_2$ Population Derived from Ciherring x B11143D

| Marker | Genotype | Yield Components |
|--------|----------|------------------|
|        |          | $ppl^1$  | $fl^1$  | $pl$   | $gpp$  | $pss$  | $gw$   | $gy$   |
| RM3472 | A        | 9.58a$^2$ | 32.78a | 25.19a | 160.22b| 93.40a | 23.60b | 27.14a |
|        | 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  |
| RM28048| A        | 10.00a    | 31.95b | 24.92a | 157.12b| 93.40a | 23.30c | 26.44b |
|        | 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  |
| RM28195| A        | 10.02a    | 32.26a | 24.87a | 156.88b| 97.13a | 23.30c | 26.52b |
|        | 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  |
| RM1986 | A        | 10.08a    | 31.62b | 24.86a | 154.85b| 97.83a | 23.20c | 26.22b |
|        | 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, $fl$ 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,
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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.

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