Quantitative Trait Locus Analysis for Grain Size Related Traits of Rice

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Molecular Plant Breeding, 2016, Vol.7, No.12 doi: 10.5376/mpb.2016.07.0012

Received: 15 Mar., 2016
Accepted: 30 Mar., 2016
Published: 06 Apr., 2016

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Preferred citation for this article:
Zeng W., Cai Z.Q., Wang Y.L., Jiang Q.G., Wang J., Li S.R., Peng L., Qin B.X., Chen B.S., Li R.B., and Luo J.J., 2016, Quantitative Trait Locus Analysis for Grain Size Related Traits of Rice, Molecular Plant Breeding, 7(12): 1-19 (doi: 10.5376/mpb.2016.07.0012)

Abstract Grain shape and size, which are characterized by grain length, grain width, grain thickness, and length to width ratio, are the key determinants of grain weight. In this study, a 188-individual F2 population, which derived from a cross between large grain Indica cultivar Nangyangzhan and medium grain Indica cultivar Ce253, were used to analyze grain size related QTLs with 110 molecular markers. Inclusive Composite Interval Mapping (ICIM), was applied to genome-wide detection of QTLs underlying grain size related traits. For grain length, 4 QTLs were detected on chromosome 1, 3, 4, and 9, respectively. qGL-3, a major effect QTL, explained 27.60% phenotypic variation of grain length. Four QTLs for grain width were detected on chromosome 2, 3, and 5. Among them, qGW-5a was major QTL for grain width, which explained 21.30% of phenotypic variation. Five QTLs for length to width ratio, which were distributed on chromosome 2, 3, 5, and 12, were identified. The range of their phenotypic contribution varied from 8.60% to 16.86%. For TGW QTLs, only two were identified on chromosome 5 and 6, respectively, with which 13.26% and 9.04% phenotypic contribution. In our results, qGL-4 and qGL-9 for grain length were novel loci. They have not been reported in literature previously. Our results shed new light on further fine mapping and understanding the molecular genetic mechanism of novel grain size QTLs.

Keywords Digenic epistasis; Genetic linkage map; Grain size traits; QTL; Rice (Oryza sativa)

Introduction Rice (Oryza sativa L.), one of the most widely grown food crop in the world, feeds more than half of the world population. The increasing rice grain yield is of great strategic significance for world food security. Rice grain yield is determined by grain weight, number of grains per panicle, and number of panicles per plant (Fan et al. 2006). Wherein, grain weight is determined by grain size, which defined as the combination of several grain associated characters, including grain length (GL), grain width (GW), grain thickness (GT), and length to width ratio (LWR) (Lou et al., 2009). In breeding application, rice grain size is significantly correlated with grain weight, and therefore, usually being used to evaluate grain weight, as defined by 1000-grain weight (TGW) (Zuo and Li, 2014). Thus, the dissection of molecular genetic mechanism of rice high yield related characters has gradually been a hotspot in recent. Most grain size related traits are known to be regulated by multiple gene loci, referred to as quantitative trait loci (QTLs) (Tan et al., 2000). The genetic basis of grain size traits is complex and easily affected by the environment. Therefore, understanding the molecular genetic basis of grain size traits is of great significance for rice grain yield improvement.

In the last two decades, the development of molecular marker technology and the QTL mapping strategy facilitates QTL identification and cloning. Many grain size related QTLs have been identified and some major effect loci have been cloned and functional investigated (Tan et al., 2000). To date, more than 400 QTLs for rice grain size and grain weight have been identified from different genetic backgrounds and different mapping populations, in which 103 QTLs have been reported to be associated with grain length, 95 QTLs reported to be
associated with grain width, and 167 QTLs reported to be associated with grain weight. The QTLs for grain thickness, however, was relatively less reported (Huang et al. 2013).

Among the above-mentioned QTLs, 13 genes associated with grain size and shape, including several major QTLs, such as GS3 (Fan et al., 2006, 2009; Mao et al., 2010), GW2 (Song et al., 2007), GW5 (Shomura et al., 2008), GS3 (Li et al., 2011), and GW8 (Wang et al., 2012) have been isolated by map-based cloning strategy. Some of them have been functionally characterized at genetic, biochemical, and cell biological levels. A major QTL for grain length and weight, GS3, was first fine mapped and isolated using a population derived from crossing between MH63 and Chuan 7, and further was functionally characterized as negative regulator for grain length (Fan et al., 2006, 2009; Mao et al., 2010). A mutation in MH63 GS3 allele caused a 178 aa-truncation in the C-terminus of its predicted protein results in the formation of longer grain rice (Fan et al. 2006, 2009; Mao et al., 2010).

GW2, a major QTL for grain width initially identified in cross between a large grain cultivar, WY3, and a small-grain cultivar, FENGAIZHAN-1, encodes a RING-type E3 ligase of proteasomes, and involves in targeting its substrates to proteasome-regulated proteolysis. A 1-bp deletion in the allele of GW2 in WY3 resulting in a premature stop in its exon 4 in protein translation, causes large and width grain in WY3. In cell biological level, GW2 negatively regulating cell division. Loss function of GW2 accelerates cell division and increases cell number in spikelet hull of grains (Song et al., 2007). GW5, another gene for grain width, encodes a 144-aa nuclear protein with an arginine-rich domain. It has been shown that GW5 physically interacts with polyubiquitin and it is likely to act as a component of proteasome pathway to regulate the cell division of outer glume of rice spikelets (Shomura et al., 2008). The deletion in this gene may have been the target for selection of large grain rice during rice domestication (Weng et al., 2008; Shomura et al., 2008; Wan et al., 2008). GS5, a major QTL for grain width, grain filling, and grain weight, encodes a serine carboxypeptidase and acts as a positive regulator of grain size (Li et al., 2011). GW8, third major QTL for grain width and grain yield, was isolated from a cross between HJX74 and Basmati385, and being characterized as SQUAMOSA promoter-binding protein-like 16, referred to as OsSPL16, which belongs to the family of SPB-domain transcription factor. A mutation in the promoter of Basmati385 GW8 allele results in the slender grain shape of Basmati385 (Wang et al., 2012).

The molecular genetic studies of the rice grain size in the literature show that rice grain size traits are controlled by complicated genetic networks. Many different biochemical and physiological pathways involve in the formation of the diverse grain sizes and shapes (Heang and Sassa, 2012). Although more than 10 genes have been isolated and functionally characterized in the past two decades, many genes associated with rice grain size still poorly understood. Also it is expected that many QTLs still need to be identified. Thus, in this study, a F₂ population derived from the cross between a large grain indica rice variety, Nanyangzhan (hereafter, referred to as NYZ) and a medium grain indica rice variety, Ce253 was used to identify QTLs for grain length, width, thickness, length to width ratio, and grain weight of rice. The objective of this study was to identify novel QTL loci for grain size in the NYZ and Ce253 genetic background to facilitate the understanding of complex genetic regulatory network of grain size comprehensively.

1 Results
1.2 Phenotypic performance of the parents and F₂ population
In phenotypic evaluation, twenty randomly chosen fully filled grains from each rice plant were used for measuring grain length, grain width, grain thickness, and calculating length to width ratio (hereafter, referred to as GL, GW, GT, LWR, respectively). One hundred grains from each genotype was weighted and converted to 1000-grain weight, and hereafter referred to as TGW. The performances of grain size related traits showed significant differences between two parents respectively (Figure 1). Although the grains of two parents are exhibited long shape, the length, width, thickness, and TGW of grains of NYZ were distinct from those of Ce253 (Figure 1; Table 1). There was no significant difference observed in LWR between NYZ (3.68) and Ce253 (3.97) in student T-test.
In $F_2$ population, all five grain size related traits were shown continuous variation with normal or nearly normal distribution, exhibiting the characteristics of quantitative inheritance (Figure 2). One or both directions of transgressive segregation presented in all traits except TGW (Figure 2; Table 1).

**Table 1** Performance of grain size related traits of the parents and their $F_2$ population

| Traits   | Parents | $F_2$ population |
|----------|---------|------------------|
|          | Ce253   | NYZ              |
| GL (mm)  | 10.96   | 13.65            |
| GW (mm)  | 2.76    | 3.71             |
| GT (mm)  | 1.90    | 2.43             |
| LWR      | 3.97    | 3.68             |
| TGW (g)  | 24.91   | 50.16            |
|          | Range   | Mean  | SD   | Kurtosis | Skewness |
| GL (mm)  | 10.40–13.49 | 11.93 | 0.58 | -0.04 | 0.14 |
| GW (mm)  | 2.64–3.76   | 3.17 | 0.25 | -0.36 | 0.22 |
| GT (mm)  | 1.7–2.38    | 2.13 | 0.09 | 1.56  | -0.43 |
| LWR      | 2.96–4.78   | 3.78 | 0.35 | -0.11 | 0.16 |
| TGW (g)  | 25.66–48.78 | 34.39 | 4.28 | 0.73  | 0.35 |

**1.2 Correlations among five rice grain size related traits**

To study the pair-wise correlation relationships between rice grain size related traits, we performed correlation coefficient analysis using the data obtained from phenotypic evaluation. On the whole, GL and GW showed significant correlations with LWR. GL was positive correlated with LWR ($R^2=0.507$, $P<0.01$), whereas, GW was negative correlated with LWR ($R^2=-0.843$, $P<0.01$). GL, GW, and GT also showed significant positive correlations with TGW as expected (Table 2). The results indicated that GL, GW, and GT are important for the formation of TGW.

**Table 2** Coefficients of pairwise correlation among the grain size related traits in Ce253/NYZ $F_2$ population

|        | GL   | GW   | GT     | LWR  | TGW  |
|--------|------|------|--------|------|------|
| GL     | 1    |      |        |      |      |
| GW     | 0.026| 1    |        |      |      |
| GT     | -0.022| -0.034| 1      |      |      |
| LWR    | 0.507**| -0.843**| 0.020 | 1    |      |
| TGW    | 0.414**| 0.414**| 0.216**| -0.131| 1    |
Note: ** Significance at $P \leq 0.01$ levels

Figure 2 Phenotypic distribution of rice grain size related traits, (A) grain length, (B) grain width, (C) grain thickness, (D) length to width ratio, (E) 1000-grain weight in the Ce253/NYZ F$_2$ population. Open arrow and closed arrow indicate the means of Ce253 and NYZ, respectively. Red curve designates GaussAmp function fitting distribution of frequency.

1.3 Genotyping using DNA molecular markers

The total of 820 SSR and IN/DEL markers were used to detect and screen genetic polymorphism between the two parents NYZ and Ce253. One hundred and ten polymorphic markers (13.4%), which well-distributed on the whole genome of rice, were obtained (Table 3; Supplement Table 1). The individuals of F$_2$ population were genotyped with these polymorphic markers by PCR. The molecular markers’ genotypes of the population were utilized for the construction of genetic linkage map. The obtained linkage map spanned 2185.27 cM in length with an average...
distance of 19.87 cM between adjacent markers (Table 3).

### Table 3 Molecular marker polymorphism and their linkage analysis

| Chromosome | Total marker tested | Polymorphic percentage (%) | Polymorphic markers | Chromosome length\(a\) |
|------------|---------------------|-----------------------------|---------------------|-----------------------|
| 1          | 90                  | 14.4%                       | 13                  | 276.13                |
| 2          | 72                  | 13.9%                       | 10                  | 224.16                |
| 3          | 82                  | 12.2%                       | 10                  | 246.68                |
| 4          | 102                 | 9.8%                        | 10                  | 129.22                |
| 5          | 60                  | 15%                         | 9                   | 232.15                |
| 6          | 90                  | 11.1%                       | 10                  | 194.44                |
| 7          | 45                  | 15.5%                       | 7                   | 201.52                |
| 8          | 58                  | 17.2%                       | 9                   | 183.15                |
| 9          | 50                  | 14%                         | 7                   | 138.45                |
| 10         | 55                  | 14.5%                       | 8                   | 104.24                |
| 11         | 62                  | 11.3%                       | 7                   | 136.28                |
| 12         | 54                  | 14.8%                       | 8                   | 118.85                |
| Total      | 820                 | 13.4%                       | 110                 | 2185.27               |

Note: \(^a\) Unit: cM

### 1.4 QTL mapping for grain size related traits

Fifteen QTLs for 5 grain size related traits with phenotypic variation ranged from 4.81 to 27.60% were identified, respectively (Figure 3, 4; Table 4). LOD scores of identified QTLs varied from 2.50 to 9.40.

Four QTLs were detected for GL on chromosome 1, 3, 4 and 9, respectively. qGL-3, a major QTL for GL, which was mapped in the interval flanking by markers 03M36.3 and 03M33.1, accounted for 27.60% of phenotypic variation. All the QTLs have positive additive effect on phenotypic variation (Table 4), indicating the alleles derived from NYZ at those QTL loci have increasing effect on long grain phenotype.

Four QTLs for GW were mapped on chromosome 2, 3, and 5, with phenotypic contributions varying from 8.16% to 21.33%. Among them, qGW-5a and qGW-5b were located at the intervals on the chromosome 5, flanked by two pairs of markers, M06502-M040 and M13153-M06526.62, respectively (Figure 3, 4; Table 4). The major QTL qGW-5a, with the largest LOD score (9.40), explained 21.33% of phenotypic variation. All the QTLs have positive additive effect on phenotypic variation except qGW-3 (Table 4). The results suggest that wider grain phenotype was mainly controlled by the alleles derived from NYZ.

Although no statistically significant difference of LWR between two parents (Supplement Figure 1), five QTLs underlying LWR were detected on chromosome 2, 3, 5, and 12, with the range of phenotypic contributions from 8.60% to 16.86% (Figure 3, 4; Table 4). Of which, both qLWR-3a and qLWR-3b were mapped on chromosome 3, and flanked by 03M24.29-RM5488 and RM14391-M023, respectively. All QTLs have negative additive effect to LWR except qLWR-3b, indicating the QTL alleles from Ce253 mainly contributed to the higher values of LWR.

Two identified TGW QTLs qTGW-5 and qTGW-6, were mapped on chromosome 5 and 6, respectively. Both of them have positive additive effect on TGW. This indicated that QTL alleles from NYZ increase TGW (Table 4).

We identified any QTL for GT in this study (Supplement Figure 2).

It is worth noting that there were three chromosome regions clustered with QTLs for grain size related traits in our study. The first region, which was delimited by markers M018 and RM1211 on chromosome 2, was clustered with qGW-2 and qLWR-2. The second region was mapped in the interval flanking by markers RM14391 and M023 on chromosome 3, with which qGW-3 and qLWR-3b clustered. The third region, flanked by M06502 and M09668 on chromosome 5, was clustered with qTGW-5, qGW-5a, and qLWR-5. These results suggest that these regions might...
exist a single QTL with pleiotropic effect for grain size related traits (Figure 4; Supplement Figure 3).

**Figure 3 Genome-wide scanning of grain size related QTLs for (A) grain length, (B) grain width, (C) length to width ratio, (D) 1000-grain weight in F$_2$ population using QTL IciMapping 4.0**

**1.5 Digenic epistasis analysis**

The total of 37 locus pairs showed significant epistatic effect on all the grain size related traits in this study (Supplement Table 2). These loci were distributed on all the rice chromosomes. The contribution of epistatic effect of detected locus pairs to phenotypic variation ranged from 12.42 to 64.09%. Among them, 24 pairs had a higher effect than that of $qGL-3$, a QTL with the highest phenotypic contribution in this study (Table 4; Supplement Table 2). These indicated that the effect of digenic interaction also plays a key role in the variation of grain size related phenotypes, and accounts for a large proportion of genetic variation. Among the identified QTLs, five were involved in digenic epistasis, therein, three for GL ($qGL-1$, $qGL-3$, $qGL-4$), one for LWR ($qLWR-12$), and one for TGW ($qTGW-5$), respectively. $qLWR-12$ involved in five-pair digenic interactions (Supplement Table 2), indicating that $qLWR-12$ also involves in controlling GL and GW through digenic interactions.

**2 Discussion**

Rice grain weight, one of the key determinants for rice grain yield, is determined by grain length, width, thickness, and length to width ratio, which is referred to as grain size related traits. Grain size related traits have been received attention increasingly since 1990s. A great number of QTLs have been identified for rice grain size and shape by using different mapping populations (Bai et al., 2010, 2012; Huang et al., 2013; Lou et al., 2009). However, most mapping populations used in those studies derived from crossing between medium grain size parents (Bai et al., 2010). In this study, a large grain size parent, NYZ, and a relatively small grain size parent, Ce253, were used to generate mapping population for grain size QTL analysis. A total of 15 QTLs were identified for rice GL, GW, LWR, TGW except GT (Figure 4; Table 4). Among these, two QTLs $qGL-4$ and $qGL-9$, with minor effect for grain length, were reported for the first time here.
Figure 4 Genetic linkage map showing putative grain size QTLs detected in Ce253/NYZ population
Note: The numbers on left of chromosome designate position (Unit: cM) of the markers labeled on right of chromosome

Table 4 Chromosome location of QTLs for grain size related traits in F2 population

| Traits | Chromosomes | QTLs | Intervals | LOD | PVE(%) | Add |
|--------|-------------|------|-----------|-----|--------|-----|
| GL     | 1           | qGL-1| M008-RM11258 | 2.64| 17.64  | 0.1713 |
|        | 3           | qGL-3| 03M36.3-03M33.1 | 7.77| 27.60  | 0.422 |
|        | 4           | qGL-4| RM16943-M09496 | 3.11| 8.36   | 0.2493 |
Among four grain length QTLs, qGL-3, flanked by markers 03M36.3 and 03M33.1, was mapped at the end of the short arm of chromosome 3 with 27.60% phenotypic contribution (Table 4). It should be mapped at the same locus as qGL3c, another QTL for grain length, which was identified in a mapping population also using NYZ as favorable grain size parent (Bai et al., 2010). It has been reported that QTL for grain size and shape traits mainly controlled by cloned loci, such as GS3, G5, G3, GW, and G8, with major effect on grain length, grain width, and/or grain weight (Fan et al., 2006, 2009; Mao et al., 2010; Song et al., 2007; Shomura et al., 2008; Li et al., 2011; Wang et al., 2012). In our results, two major QTLs qGW-5a and qLWR-5, were detected clustered at the interval M06502-M09668 on chromosome 5, which was also reported in previous reports (Huang et al., 2009; Lou et al., 2009). These suggest that the hotspot identified in different research reports might be harbored a pleiotropic QTL for grain length, width and grain weight (Figure 4; Table 4; Supplement Figure 3). However, in this study, no QTL was identified syntenic with GS5, GW2, and G8 loci or located at their vicinity (Song et al., 2007; Wang et al., 2012). Such phenomenon also observed in the other two study cases (Lou et al., 2009; Bai et al., 2010). This indicates that different genetic background, different mapping population, and different methods for QTL analysis may result in such discrepancy observed among different reports. In addition, no QTL for GT was identified in this study. The reasonable interpretation might be the complex genetic basis of GT. It has been suggested that GT is influenced by several distinct factors, such as, polygene controls with additive effects, maternal effect, and, to some certain extent, affected by environmental conditions (Yang et al., 2001; Huang et al., 2013).

Epistasis plays an important role in the genetic basis of rice flowering time and heterosis (Yamamoto et al., 2000). In this study, all grain size related traits were influenced by epistatic interaction. The contributions of digenic interactions to phenotypic variation were higher than those of their single QTLs. The effect of digenic interactions to phenotypic variation of GL ranged from 12.42% to 46.17%. The effect to GW ranged from 16.41% to 41.88%, and to GT ranged from 18.73% to 33.84%. The effect of digenic interactions for LWR ranged from 18.73% to 33.84%. Moreover, the digenic interactions to phenotypic variation of TGW had the largest effect (25.18% to 64.09%) by comparison with those of GL, GW, and GT (Supplement Table 2). The observation of the large number of digenic interactions and a large proportion of phenotypic contribution involving in regulating the variation of grain related traits also have been previously reported in rice (Lou et al., 2009) and in other crop plants, such as maize (Ma et al., 2007), wheat (Wu et al., 2012). These results showed that digenic interaction

| Traits | Chromosomes | QTLs | Intervals | LOD | PVE(%) | Adda |
|--------|-------------|------|----------|-----|--------|------|
| GW     | 9           | qGL-9| RM566-9M10.51 | 2.59 | 5.57  | 0.1167 |
|        | 2           | qGW-2| M018-RM1211 | 2.50 | 4.81  | 0.0809 |
|        | 3           | qGW-3| RM14391-M023 | 3.35 | 8.16  | -0.0908 |
|        | 5           | qGW-5a| M06502-M040 | 9.40 | 21.33 | 0.1634 |
|        | 5           | qGW-5b| M13515-0SM26.62 | 3.84 | 8.65  | 0.0917 |
| LWR    | 2           | qLWR-2| M018-RM1211 | 5.08 | 11.06 | -0.1602 |
|        | 3           | qLWR-3a| 03M24.29-RM5488 | 4.43 | 8.60  | -0.1524 |
|        | 3           | qLWR-3b| RM14391-M023 | 3.91 | 10.91 | 0.1673 |
|        | 5           | qLWR-5| M06502-M040 | 7.51 | 16.86 | -0.2005 |
|        | 12          | qLWR-12| M110-12M27.47 | 3.14 | 14.34 | -0.1995 |
| TGW    | 5           | qTGW-5| M040-M09668 | 4.04 | 13.26 | 2.042 |
|        | 6           | qTGW-6| 06M17.5-6M13.5 | 3.57 | 9.04  | 1.4532 |

Note: aThe percentage of phenotypic variation of QTL explained; bThe additive effects of QTL. Positive Add indicates the alleles derived from NYZ increasing the effect on that trait, and, whereas, negative Add indicates the alleles derived from NYZ decreasing the effect on that trait.
plays important role in the variation of grain size related phenotypes. And also suggested that the highly complicated genetic regulatory network involves in the development of grain characters of crop plants. Thus, elucidating regulatory QTL networks underlying rice grain development, and understanding the mechanism of those QTLs are of great significance for QTL pyramiding in grain yield breeding application. In breeding program, the major effect QTLs are, of course, considered in priority for grain size modification. However, epistatic QTLs, even if their LOD score low to undetectable, also be another choice for grain size modification in molecular breeding design. All the epistatic QTLs and their combinations in breeding practice can produce diverse size of rice grain to meet the different preferences of different regions of the world people.

3 Materials and Methods

3.1 Population development and field experiments
A set of 188-individual F$_2$ population was developed from a cross between large/long grain indica variety, NYZ (1000-grain weight is 50.16 g), and medium/long grain variety, Ce253 (1000-grain weight is 24.91 g). In Spring of 2014, F$_2$ population and its parents were grown in Experimental Field of Guangxi University, Nanning, China. Each seedling was transplanted to 1-row plot with 6 plants in each row in a 15 cm (between rows) × 10 cm (within rows) pattern. The leaves of each individual and their parents were collected at seedling stage and used for molecular marker genotypic analysis (Luo et al., 2008). At crop maturity, each individual and parents were harvested and naturally dried for grain size phenotypic evaluation.

3.2 Evaluation of grain size related traits
For the measurement of grain length and grain width, the 20 seeds of each plant were lined up in two rows and taken the images by digital camera. The digital images were used to analyze and measure grain length and width by using a Bio-image analyzer, Digimizer (Ver. 4.2.6.0). Grain thickness of each genotype was determined by the average of the thickness values of 20 grains from each rice plant. The thickness of each grain was measured using a Vernier caliper with three replications. One hundred grains from each genotype was weighted and converted to 1000-grain weight.

3.3 DNA preparation and DNA marker genotyping
The genomic DNA for individual plant was extracted from young leaf tissues using the CTAB method as described by Murray and Thompson (1980). DNA was then dissolved in ddH$_2$O and stored for subsequent analysis. PCR reaction mixture (total volume 10 uL) contained 5 uL 2×Es Taq MasterMix (CWBO, Cat# CW0690D), 50 ng template DNA, and 5uM primers. PCR reaction cycles was performed with an initial 2-min period at 94℃, followed by 30 cycles of denaturing at 94℃ (30 s), annealing at 58℃ (30 s), extension at 72℃ (1 min), and a final 2-min extension step at 72℃. PCR products were analyzed by 2.5~3% agarose electrophoresis and stained with GelRed (Biotium, Cat# 41 003) for visualization.

SSR markers were obtained from the Gramene database (http://www.gramene.org/). The IN/DEL markers were developed using genomic DNA sequences of Nipponbare and 9 311 as references (Supplement Table 1). The primers were synthesized by Sunbiotech (Beijing, China) and SBS Genetech (Shanghai, China), respectively. All the primers were used for identifying the polymorphic DNA markers between NYZ and Ce253 by PCR.

3.4 Linkage map construction and QTL analysis
QTL IciMapping 4.0 (Wang et al., 2014) was used to construct genetic linkage map and QTL analysis. The total of 110 polymorphic DNA markers (41 SSR and 69 IN/DEL), which well-distributed on 12 chromosomes, were genotyped for genetic linkage map construction. According to the manual of QTL IciMapping 4.0, the genotypes of DNA markers were defined as follows, the band of male parent (NYZ) was coded as 2, the band of female parent (Ce253) was coded as 0, and heterozygote was coded as 1, missing genotypes were coded as -1. In QTL analysis, the statistical method, Inclusive Composite Interval Mapping (ICIM-ADD, ICIM-EPI) was used for genome-wide identification of grain size associated QTLs by combining phenotypic data and genetic linkage map. A threshold of LOD≥2.5 was used to indicate the significant main effect QTL ($P\leq0.001$), and a threshold of
LOD≥5.0 used to declare significant digenic epistatic interactions (P≤0.001). Additive effects, dominant effects, digenic epistatic effects, and phenotypic contributions were obtained in the computational results of QTL IciMapping 4.0. QTL nomenclature was done as described by McCouch (2008).

3.5 Statistical analysis
Phenotypic correlation analysis was performed with Pearson correlation type using SPSS (Ver.19). Phenotypic distribution frequency analysis and graphic plotting of F2 population was performed using Origin 9, and fitted frequent distribution with GuassAmp function shown as follow.

\[ y = y_0 + Ae^{-\frac{(x - x_c)^2}{2w^2}} \]

Authors’ contributions
JL, RL, and BC conceived and designed the experiments. WZ and ZC performed the experiments. YW, QJ, JW, SL, LP, and BQ did the field trials and involved in grain size related traits’ investigation, and further data processing. JL drafted the manuscript. RL and BC revised the manuscript. All authors approved the final version of manuscript.

Acknowledgments
The work was financially supported by the Natural Science Foundation of Guangxi Province (No. 2014GXNSFAA118075), and the Scientific Research Foundation for the Returned Overseas Chinese Scholars ([2013] No.1792, State Education Ministry of China). We thank reviewers for the careful reading of the manuscript and their constructive comments.

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Supplementary Figures

Figure 1

![Figure 1](image1)

Note: LOD • Markers’ Position — Threshold: 2.5

Figure 2

![Figure 2](image2)

Figure 3

![Figure 3](image3)
## Table 1 Sequences of molecular markers

| Marker name | Chr. | Position (M)* | Forward (5'-3') | Reverse (5'-3') | Product size (bp) |
|-------------|------|---------------|-----------------|-----------------|-------------------|
| 01M0.45     | 1    | 0.45          | TGCATGCACATTCATTTTATT | GCACAGAGCTAGGGTGCAAT | 200               |
| M001        | 1    | 3.3           | TCACCCGATATCCTCCATCAT | TAAAGACGGATGGTGCAAAACG | 191               |
| M004        | 1    | 7.4           | GAGCTATGGTTGTATGTGCTGT | TAGTCACTTATGTGCTGATCG | 178               |
| M005        | 1    | 9.4           | CCTCAGAAATGAGTCTTCTTG | CCGAAAGAAGTTACAGGAGT | 131               |
| RM1081      | 3    | 12.9          | AACCCTCTAACAGATTCAAA | TTGGAAATGTGCTCAACTGA | 389               |
| M008        | 1    | 18.7          | CGCACATCTGAAATATTGCC | TGTCACTCATATCAGGAC | 194               |
| RM1125      | 8    | 22.9          | GCTCCACCATTCTACCATACA | CAATATCAGCCTAGCACAA | 167               |
| RM488       | 1    | 24.8          | CAGCTAGGTGTGTGGAGCTG | TACGAACAAACGCGATATCG | 110               |
| M012        | 1    | 28            | CCACTGAGTATGCTACTATG | AGCCGGTTTAATGATATGC | 164               |
| M013        | 1    | 32.1          | CAATGACCACTGCAAGCATAA | TCCATCATTTTCAGCCTTTC | 146               |
| RM226       | 1    | 34            | GAAGCTAAAGTCTGGGAGAA | AATGGCCTTAACCAAGTAGG | 289               |
| RM1186      | 5    | 36.3          | CAACTCATCGCCTCTTCTT | GCAATTCATGAGTTGGAAT | 395               |
| RM1202      | 1    | 39.1          | TAAATTTTGGCAGCTACGC | GTAGCGATGCAACTACAGT | 464               |
| M015        | 2    | 1.8           | GGGTGTTGTTATATCACCT | AATCTAGTTTGTGGCTGC | 185               |
| M01763      | 2    | 3.94          | TGCACCCAAGAAAAATATAA | GCCAAGCATATTTCGATTC | 171               |
| RM1267      | 8    | 5.8           | AGGAGAGCAGCCCATCCTC | TCTGAGAGGCCTACCCGAT | 559               |
| M05076      | 2    | 7.68          | CTTTCATCATATCGGCTGG | AGCTTGTCTTTCCGCTG | 147               |
| 02M11.16    | 6    | 11.16         | AAGCGTAACCTTTTCGTTT | ATACTGTCACCGTGTCCTCAA | 149               |
| M018        | 2    | 15.2          | GTATTTGTTATGCACTATGC | CGAAGACGATAGCAGCGCAG | 157               |
| RM1211      | 2    | 18.55         | GACACGGGTTTCAGTAGATGCTAG C | GCCACATCAGCATGTTTAGTAGAAACG | 154               |
| RM1342      | 9    | 21.2          | ACTCAGACCTATGCTGTCCTTG | AAGGAGTGCTCTAGAATACG | 296               |
| M020        | 2    | 28            | ATGTCAAGTTGAGAACGTGA | AGCATGAAACATTTCGCCAG | 158               |
| RM250       | 2    | 32.79         | GTTTCAAAACAGCTGATCA | GATGAAGGCCCTCCCACGAG | 219               |
| RM1412      | 4    | 34.2          | CTCAGTCAGCAACTCGACGTA | ATCCTCTTCTGCTCAATTAG | 191               |
| M023        | 3    | 0.33          | TTCAATCCCTTCCGAACC | AGCTTGGCTGAATGATC | 113               |
| RM1439      | 1    | 2.7           | GGGAGGAATTTTGATAGGTA | ATGTGACTGCTTGCTGGAATTA | 293              |
| Accession | Position | Sequence 1 | Sequence 2 | Length |
|-----------|----------|------------|------------|--------|
| 03M6.79   | 3        | GGGAGTGCAAGAAAAATAA | ACAAACCTAAGTGGCAGC | 142    |
| 03M10.7   | 5        | CTTAAAATTGTGAGGGACCTCG | GCTCTGATTAAGAAGACG | 139    |
| RM6929    | 3        | TTTTCTCGAGGGTGAGGAGAGAGG | CTAGCTAGCCAGATGCTG | 185    |
| RM1524    | 7        | GACACTCACGAGGAAGAGTAG | ATCTGAACGGCTGAGATTTGT | 312    |
| RM5488    | 3        | GGTCTACGCTGACACATGAAACC | TCTTACGCTCCTCCTCTCCT | 168    |
| 03M24.2   | 9        | ATCCAAATGACTGAAAGATGC | TTTTAAGGAAAACCACTG | 164    |
| M033      | 3        | TCAGTGACCAGCCACATGA | CTTTCCGGTCTCTGAGCA | 154    |
| 03M33.1   | 3        | TCAATTCTCTATCTGTGCAA | AGCTCCGATCCATTTCAAA | 155    |
| 03M36.3   | 3        | ATTTAGCTCCCTCCTGTTC | CATTCTAGTAGTGGACGA | 173    |
| RM1635    | 4        | ACAAGTGATCACAATGGCTC | CTATGCTCTCATGGTAGT | 258    |
| M037      | 4        | CACATGATCTGGCTTTCATT | ATGCATACGTCGAAATCT | 179    |
| M04658    | 4        | CAATTCACCTCCCACCTCTGT | CCAATAGGAAAATCAATGC | 143    |
| S12020    | 4        | TGGTTGATTTGCTAGGACC | ACCCTGTCAATATACTC | 1270   |
| RM1664    | 9        | CTCCCTTCATGCGTAAGCTCT | GCAACACAGGATCCCTCAACAA | 342    |
| M09496    | 4        | TTGACCTGTTCAAGAAAACA | TCAAGCCAATATGGAAGAC | 159    |
| RM1694    | 3        | CTCCATGCGGTGACCATTGTAG | GATGAGCAGGAGCAGAGAG | 387    |
| 04M23.6   | 1        | CCACATTTCCTCAATACCTTA | GTCCTAAAGCAATGAAGC | 152    |
| M038      | 4        | GGCAATGTGCTGTCCTGATT | AGGACGAGCACAACATACCC | 181    |
| M039      | 4        | GTGTCCTCTGTAAGGATT | TCAAGTGCTCTTTCTTATG | 152    |
| RM1783    | 6        | TTGCTCGCCCTCAAATGTCTAAT | ATATCGTGCTTTCCCTGAC | 238    |
| 05M4.28   | 5        | ACGTAACACGACAGTGCC | TTGTTACTTCCCAATTTA | 130    |
| M040      | 5        | TGCTGAGATTTCCATGAG | GTGCAATTCAGCTTGATTC | 142    |
| 05M10.2   | 4        | TTAACTGCTCTGTGCTG | ACCACTCAATCATCTAGC | 152    |
| M06502    | 5        | GTCATACTGCAAGACACG | AGACTTGTGAGATTGCAG | 161    |
| M09668    | 5        | GATGGACTCGAGGGAGAG | TATCTTACCTTTTTACG | 161    |
| M13153    | 5        | TCAAGGAGGACACATAACCA | TAAAGGCTGATGTATTC | 182    |
| 05M26.6   | 2        | CTGGAGAGAAACAAATGGAGGAG | CTTTTAGGATGGTGCACC | 135    |
| M16113    | 5        | GTCACAAATATGAGCCTGAGAT | TGTCCTTCTCTTCTTCTT | 185    |
| RM1923    | 4        | AACTCCCTGTCTCTAGAGTT | GTTGGAGTCTAACCCTCA | 299    |
| Accession | Length | Sequence 1                     | Sequence 2                     | Length |
|-----------|--------|--------------------------------|--------------------------------|--------|
| 6M4.57    | 6      | TATAAGGGAATGAACTGCTG           | TTTGACTCAATGGAGTGTGA110TTT    | 130    |
| M051      | 6      | GGTGTTGTTTJAGGCTGTCA           | GTCCTCATCACTGAGCAGACT         | 132    |
| M056      | 6      | CAAGAGATTGTGACTGTGTTGG         | CGTCTCAATTGGAGAGACAGA          | 136    |
| 6M13.5     | 6      | GCCAGTTCAACTGTA                 | CACTCCAGICTCTCTCTCC           | 129    |
| 06M17.5   | 6      | AGACGAGACCATAAAGCAGCA          | GGGGCTAAGCTGATATT             | 143    |
| 06M20.9    | 6      | CAGCCATAAGCTACTGACTA           | CTGGGAATTCCTGTCAAATGA          | 160    |
| M14082    | 6      | TACCTCAATTGCTAGTAG             | GTCAGACCAGCAAGAGGAGG           | 168    |
| M060      | 6      | TGACCGGACACGCACGTC             | GACAGGGTTAATCACACAGG           | 148    |
| 06M30.3    | 6      | GCCACGAGACTTAAAGCACA          | TAGTTATGGGCCGTACTAGC           | 175    |
| M063      | 7      | ACTACCTACATCCTCTCATTG          | CTGACATGCTTTTCTCACTG          | 103    |
| M070      | 7      | GCATGTGTTTGGTGTTGCA            | CCACAGGAGATTCTAGCAGA          | 198    |
| RM2130.9   | 7      | GAGTGGATTTACTGACAAGC          | GATTTCTTGGGACCCATAC           | 531    |
| RM2139.2   | 7      | GCAAGTGAGAGTTAACAAGT          | GACAGGTGACAATACACATC          | 189    |
| 07M15.3    | 7      | TGATATAAATGGAGCTCCGC          | TGGTATTAGGTGGTTTCACTTGG       | 164    |
| 07M20.1.1  | 7      | ACCTTGACAGTCTCTGTGCTC        | CCAGGATATGACTGACTGCTCT        | 162    |
| 07M24.3    | 7      | CATAGGATCGAATTTTCTCTG         | CAAACCCCTATCTTATAGC           | 138    |
| 08M0.01    | 8      | GATGTCCAAGAGCTACTG           | ATGTCGACTACAATTGGCC            | 173    |
| RM2241.6   | 8      | CCCAGCTCTCTAAGCATCTC         | TAACCTTCCGCCTCTCACACA         | 336    |
| M077      | 8      | GGTGTTGCTTGATTTCCCCTTG       | GAAACAGATCAGCCTCCTCACTG       | 165    |
| RM8271     | 8      | AGCACTCCGAGTTGTTTAGG          | AATGGGCTGCTTTGCTGACTT         | 360    |
| RM72       | 8      | CGGCGGCTAAAACAAATGAG          | GCATCGGCTCTACTAAGGG           | 166    |
| 08M18.4.1  | 8      | CGTGATTATAGCTGCTG            | AAGATGAGTGGTCAACATT           | 143    |
| 08M21.0.3  | 8      | ACAGTCTAAATCAACCCTG          | ATTAGTCTGCTTTCCGTCACAA        | 162    |
| 08M25.5.7  | 8      | ACCTGATCTCATCCTAGG           | AAGAGATTGTGTAAGTGT            | 137    |
| RM2364.9   | 8      | GAGCGTGCTGCTGTTGCTG          | AAGAGAGTGTTTGGGAAGCT          | 385    |
| RM2377.8   | 9      | AACACAGCCTAAGGTTCTGC         | GCTTCCGCCCCATAGTCTTC          | 328    |
| 9M7        | 9      | ATTCTTTGAGAGAGCAGGGG         | GAGAGGCCTGTTAATCATGTC         | 113    |
| 9M10.51    | 9      | GGTGAGAGATTCTTGAGTTG         | TATCAAAATTAACAAACGGG          | 189    |
| RM566      | 9      | AATATGGTGGCCTCCTACACTCC      | TGAATCAGGCCAACAAACACTG        | 143    |
| Marker | Position | Product Size | Primer Sequences | Note: Physical position of the markers on the chromosomes referred to Nipponbare genomic DNA sequences. |
|--------|----------|--------------|------------------|--------------------------------------------------|
| M087   | 9        | 16.9         | TGGCCACAGCATGTTAAAAA CCCACATGTCAGTATGCGTA | 90 |
| RM201  | 9        | 19.87        | GTACTCTCGCGCTTACACACTCC TTAGTGACCGGGGACGACAGC | 395 |
| 09M22.8 | 9       | 22.81       | AGTTCGGTTAAACCTCTTTC AACCAATACACAAAGCAGA | 134 |
| M093   | 10       | 0.06         | CAGCCACTGCGACCTTAGTC GTCCCATTTTGGCCTTTTC | 159 |
| M094   | 10       | 4.9          | TATGGATGAAGGGCCACAG ACGGAGTCTCTTCCTGTC | 138 |
| RM2514 | 6        | 7.1          | GAGAGAGAGAGACGACGAC TATCCAGGCAATACACACC | 223 |
| M095   | 10       | 9.9          | GCCGATCCATGCTATCCTCAA GACAAGGGTTTGGCACAAGA | 156 |
| 10M12.6 | 6     | 12.66        | ACTACGCAGTTGTTTTACGA TTTTAGAGAGGAAAGAGC | 141 |
| RM2542 | 5        | 14.4         | CAGGCGGACACGCTCTTGC GGGCACGCGTTTCTTCTTGT | 155 |
| RM2553 | 9        | 16.7         | AAGCCCATATCATTTGTCCTTC CACCACTTTGCACATTACAG | 283 |
| M098   | 10       | 21           | TAGCCCGCTAGGCACACTCAACT CGGGTGCTGCGAGATAT | 128 |
| 11M2.51 | 11     | 2.51         | AATTACACAGAAGACATCAT GATGAATGCTCTTGGCTCT | 130 |
| M04308 | 11      | 6.25         | ATTCTAAGGGAGGGACGATAG CTATGTTTCTTCCGTT | 192 |
| 11M11.1 | 6     | 11.16        | GCCATGTCTATTTCTCTGC GTATGAGAGGAGGCGCATAC | 142 |
| M101   | 11       | 13.3         | GTTGCGCGCTTAAATATGTGG GCTTTAAACACAAATCATCA | 166 |
| 11M20.7 | 7     | 20.77        | AATGGTGAAAGTCTATCAGGAGAA ACTGTTAATTAACACTGAACA | 135 |
| 11M24.4 | 2     | 24.42        | GGGCTCTTTGGATAAATCTCT AAGGAAAAATGCTAAACAAA | 143 |
| M104   | 11       | 28.1         | CATTTTCACTCTTTGGCTAG GGAGCTCTATGAGA ACTTATG | 152 |
| M105   | 12       | 0.12         | CACATTTGCACATCATAATCTT GGAATCCCGGTTGAGCTG | 130 |
| L1251  | 12       | 4.6          | GTAGCTAAAGGCAAATGGGCA CATGGAATACCTCCTCAAAAT | 313 |
| M04442 | 12      | 7.19         | TGGCTAGAAATAGTGGGAA CCAGTTATCCACAGTGAAAT | 179 |
| 12M13.0 | 5     | 13.05        | ATTAGAAAGCAGGGTGAAGC AAGGATGGCTGGAATAAGA | 140 |
| M09555 | 12      | 16.7         | CTGACGAGGATGTATGAGAA ACACACATGCAAACCTTGA | 169 |
| M109   | 12       | 20.6         | TAGCAAGCAGCATGCTCTTCT AATCGCTCAGCGGATCTT | 137 |
| M110   | 12       | 22.8         | CTGTCAAAAATGCGTTCGAT AATGCCAATCCCTGGAATT | 184 |
| 12M27.4 | 7     | 27.47        | TAGCCCAATCATTTGCTTTC TACCTGCAACAGGTGATCAA | 139 |
Table 2: Digenic epistasis identified for rice grain size related traits in Ce253/NYZ F2 population

| Trait ID | Trait Name | Chromosome 1 | Position 1 | Marker interval | QT L | Chromosome 2 | Position 2 | Marker interval | QT L | PVE (%) | A1 | A2 | D1 | D2 | AA | AD | DA |
|----------|------------|--------------|------------|----------------|------|--------------|------------|----------------|------|----------|----|----|----|----|-----|----|----|
| 1        | GL         | 4            | 35         | M038-04M23     | 10   | 85           | RM25539-M  | 5.17           | 30.06 | -0.3    | 0.093 | 0.101 | 0.12 | 0.164 | 0.78 | -0.245 |
| 1        | GL         | 10           | 45         | 10M12.66-R     | 11   | 95           | 11M11.16-11|M20.77         | 5.84           | 30.99 | -0.0    | -0.074 | 0.396 | 0.31 | 0.261 | -0.07 | 0.593 |
| 1        | GL         | 6            | 0          | RM19234-6      | 12   | 35           | M04422-12M | 5.36           | 12.42 | 0.24       | 0.044 | -0.31 | -0.13 | 0.037 | -0.41 | -0.308 |
| 1        | GL         | 8            | 75         | RM8271-RM      | 12   | 85           | M110-12M27 | 6.04           | 34.17 | -0.0      | -0.477 | 0.315 | 0.08 | 0.026 | 0.17 | 1.059 |
| 2        | GW         | 1            | 135        | M008-RM11      | 2    | 155          | 02M11.16-M | 5.58           | 24.61 | -3E-04    | -0.133 | -0.02 | -0.11 | 0.182 | -0.04 | 0.229 |
| 2        | GW         | 7            | 0          | RM21309-07     | 8    | 85           | M20.11     | 6.38           | 17.53 | -0.0      | 0.011 | -0.04 | -0.13 | 0.15 | -0.008 |
| 2        | GW         | 1            | 30         | M001-M004      | 8    | 160          | 08M21.03-08 | 5.08           | 41.88 | 0.10      | 0.013 | -0.33 | -0.48 | 0.045 | -0.22 | 0.003 |
| 2        | GW         | 9            | 10         | M022-81-R      | 11   | 40           | M04308-M10 | 5.86           | 16.41 | 0.01      | -0.115 | 0.088 | -0.00 | 0.128 | -0.02 | 0.177 |
| 2        | GW         | 6            | 55         | M64.57-M05     | 11   | 50           | M101-11M11 | 5.28           | 23.23 | 0.07      | -0.188 | 0.125 | 0.10 | -0.10 | -0.03 | 0.253 |
| 2        | GW         | 2            | 240        | M20-RM250      | 12   | 110          | M110-12M27 | 5.87           | 27.85 | -0.0      | -0.073 | 0.085 | 0.07 | 0.007 | 0.23 | 0.264 |
| 3        | GT         | 1            | 25         | M001-M004      | 2    | 185          | RM1211-RM  | 5.70           | 21.28 | -0.0      | -0.110 | 0.105 | 0.03 | -0.13 | 0.07 | 0.149 |
| 3        | GT         | 1            | 155        | RM488-M01      | 3    | 140          | RM5488-RM  | 6.22           | 21.92 | 0.07      | -0.065 | 0.057 | 0.05 | 0.036 | -0.11 | 0.052 |
| 3        | GT         | 3            | 205        | 03M10.75-03    | 4    | 80           | RM16943-M  | 6.61           | 21.82 | -0.1      | -0.056 | 0.066 | 0.08 | -0.07 | 0.15 | 0.059 |
| 3        | GT         | 3            | 150        | RM15247-R      | 5    | 150          | M040-M0966 | 5.01           | 30.27 | -0.0      | 0.051 | -0.03 | -0.09 | -0.04 | -0.04 | -0.069 |
| LWR | GT | 03M10.75-03M6.79 | 07M15.3-M070 | 3.12 | 5.85 | 33.84 |
|-----|----|------------------|--------------|------|------|-------|
|     |    | RM1211-RM13429   | 09M22.81-RM201 | 0.035 | 6.24 | 26.86 |
|     | 2  | RM25146-M095      | 10M12.66-RM25425 | 0.13 | 8.5 | 18.73 |
|     | 10 | RM1211-RM13429    | RM25425-RM25539 | 0.1 | 5.04 | 28.53 |
|     | 2  | M008-RM11258hM28 | 02M11.16-M018  | 0.0 | 5.14 | 20.72 |
|     | 1  | LWR-12           | RM6929-03m1075 | 0.0-1 | 8.44 | 37.16 |
|     | 2  | RM1211-RM13429   | RM8271-RM23469 | 0.0 | 5.14 | 37.00 |
|     | 5  | 05M10.24-RM17836 | 08M18.41-08M21.03 | 0.0 | 6.66 | 59.41 |
|     | 3  | 03M33.1-M033     | 08M21.03-08m2557 | 0.0 | 5.11 | 33.88 |
|     | 7  | LWR-260          | 08M21.03-08m2557 | 0.0 | 5.11 | 33.88 |
|     | 1  | M12021           | M21.03-08m2557 | 0.0 | 5.29 | 38.01 |
|     | 4  | RM12678-M05076    | 11M11.16-11m20.77 | 0.0 | 6.56 | 31.69 |
|     | 2  | M21.03-08m2557   | 12M13.05-M09955 | 0.0 | 5.8 | 32.87 |
|     | 3  | 03M33.1-M033     | M109-M110      | 0.0 | 5.65 | 38.91 |
|     | 6  | LWR-5            | M109-M110      | 0.0 | 6.13 | 19.69 |
|     | 8  | LWR-145          | M110-12M27.47h | 0.0 | 6.42 | 29.92 |
|     | 11 | LWR-55           | M110-12M27.47h | 0.0 | 5.26 | 36.58 |

Note: The table represents data from Molecular Plant Breeding 2016, Vol.7, No.12, 1-19.
| Year | TGW | M33.1 | gGL | M36.3-03 | RM14391 | M6.79 | RM6.79-RM14391 | M6.79 | RM21309-07 | M05076 | M101-11M11 | M105-076-02M | M20.77 | Phenotypic Variance (%) | QTL | QTL | QTL | QTL | QTL | QTL |
|------|-----|-------|-----|---------|---------|------|----------------|------|--------------|------|-------------|-------------|--------|-------------------------|-----|-----|-----|-----|-----|-----|
| 2016 | 3   | 35    | -3  | 03M36.3-03 | 5.46   | 3     | 255            | 03M6.79-RM14391 | 5.03  | 49.55       | 98    | 3.03        | 7          | 3       | -2.8                     | 51  | 2.223 | -0.64 | 0.71 | -4.59 | 3.40 |
|      | 235 |       |     | 03M10.75-03 | 5.03   | 40    | 05M10.24-RM17836 | 6.65 | 64.09       | 18    | 1.33        | 5          | 1       | 3.7                       | 86  | 2.446 | -3.79 | -4.02 | 4.03 | 3.96 |
|      | 250 |       |     | 03M6.79-RM14391 | 5.03  | 40    | M095-10M12       | 6.65 | 64.09       | 18    | 1.33        | 5          | 1       | 3.7                       | 86  | 2.446 | -3.79 | -4.02 | 4.03 | 3.96 |
|      | 55  |       |     | RM12678-M05076 | 5.03  | 40    | M095-10M12       | 6.65 | 64.09       | 18    | 1.33        | 5          | 1       | 3.7                       | 86  | 2.446 | -3.79 | -4.02 | 4.03 | 3.96 |
|      | 100 |       |     | M05076-02M11.16 | 5.03  | 40    | M095-10M12       | 6.65 | 64.09       | 18    | 1.33        | 5          | 1       | 3.7                       | 86  | 2.446 | -3.79 | -4.02 | 4.03 | 3.96 |
|      | 235 |       |     | 03M10.75-03 | 5.03  | 40    | M095-10M12       | 6.65 | 64.09       | 18    | 1.33        | 5          | 1       | 3.7                       | 86  | 2.446 | -3.79 | -4.02 | 4.03 | 3.96 |

Note: a Main effect QTLs, identified at the threshold of LOD≥2.5 in this study, involve in degenic interaction for the variation of rice grain size related traits; b The percentage of phenotypic variation of epistatic QTL explained; c The additive effects of epistatic QTLs at position 1 and 2, respectively; d The dominant effects of epistatic QTLs at position 1 and 2, respectively; e The epistatic effects of two QTL positions in additive×additive model; f The epistatic effects of two QTL positions in additive × dominant model; g The epistatic effects of two QTL positions in dominant×additive model; h The epistatic effects of two QTL positions in dominant × dominant model.