Dissection of Two QTL for Grain Size Linked on the Long Arm of Chromosome 5 in Rice

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Abstract

Background: Most agronomical traits of crops are complex traits controlled by several major quantitative trait locus (QTL) and many minor QTL. Grain size determines grain weight and influences rice appearance quality. Identification of minor QTL is important for understanding the genetic and molecular network regulating grain size in rice. Following previous identification of QTL for grain weight and size using populations derived from the Teqing/IRBB52 indica rice cross, one QTL, qTGW5/qGL5 having significant effects on grain weight and length, was targeted for validation, dissection and fine-mapping.

Result: Firstly, the effect of qTGW5/qGL5 was validated using two near isogenic line (NIL) F2 populations. Then, qTGW5/qGL5 was dissected into two closely linked QTL for grain size using four sets of NILs with sequential segregating regions. One of them, qTGW5 with the IRBB52 alleles increased grain weight, length and width with the same allelic direction, was located within an 1896.4-kb region flanked by RM18865 and Fi25273. The other one, qGL5 controlling grain length, was further delimited into a 68.8-kb region using seven NIL-F2 populations. Six annotated genes were found in the qGL5 region, of which five showed nucleotide polymorphisms between the two parental lines. In three of the six annotated genes, significant expression differences were detected between qGL5-NILs.

Conclusions: Two closely-linked QTL having small effects for grain size in rice were separated using NIL-derived populations. One of them, qGL5 was fine-mapped into a 68.8-kb region containing six annotated genes. Our work lays a foundation for cloning minor QTL for grain size and offers potential targets for marker-assisted breeding in rice.

Background

Rice (Oryza sativa L.) is one of the most important cereal crops worldwide and a model monocot species for studying the molecular genetic mechanism of complex traits. Grain yield in rice is a complex trait determined by three component traits, i.e., number of panicles per plant, number of grains per panicle, and grain weight. All of them are typical quantitative traits controlled by multigene known as quantitative trait loci (QTL) (Xing & Zhang, 2010). Grain size is the major determinant of grain weight, playing an important role in grain quality that is related to preferences of consumers in various areas around the world (Calingacion et al., 2014). Therefore, rice researchers have paid a great attention to this trait (Li et al., 2019).

Over the past two decades, 20 QTL with major effects on grain size and weight in rice have been cloned. Twelve of them mainly control grain length and weight, including GS2/GL2/OsGRF4 on chromosome 2, GS3, SG3, OsLG3, OsLG3b/qLG3, GL3.1/qGL3, qTGW3 and GSA1 on chromosome 3, TGW6, GW6a and GL6 on chromosome 6, and GLW7 on chromosome 7. Six of them mainly affect grain width and weight, including GW2 and TGW2 on chromosome 2, GS5 and GW5/GSE5 on chromosome 5, GW6 on chromosome 6, and GW8 on chromosome 6. The remaining two QTL, GL7/GW7 and GS9, affect grain size but hardly influence grain weight (Li et al., 2019; Li et al., 2020; Dong et al., 2020; Wang et al., 2019; Ruan et al., 2020; Shi et al., 2020). These QTL are involved in multiple signaling pathways, including G protein signaling, ubiquitination-mediated proteasomal degradation and phytohormone signaling (Li et al., 2019). However, these results do not fully elucidate the molecular mechanisms of grain size and weight. More QTL Genes controlling grain size and weight should be identified to fill up the gaps in the genetic regulatory networks for grain size.

Most important agronomical traits are controlled by several major QTL and many minor QTL (Yamamoto et al., 2009), but previous studies mainly focused on major QTL. Cloning of minor QTL has been a great challenge, because the small genotypic effects are sensitive to the genetic background and greatly influenced by measurement bias. So far, minor QTL cloned in rice only include several ones for heading date (Matsubara et al., 2012; Wu et al., 2013; Shibaya et al., 2016; Chen et al., 2018; Cai et al., 2021) and one for grain weight (Chan et al., 2021).
In a previous study, QTL analysis for grain weight and size was performed using populations derived an *indica* rice cross between Teqing (TQ) and IRBB52 (Zhu et al., 2019a). One QTL region, *qTGW5/qGL5* flanked by markers RM18927 and RM3321 on chromosome 5 was selected for validation, dissection and fine-mapping in the present study. Two tightly-linked QTL were separated, of which *qTGW5* affecting grain size and grain weight was located in an 1896.4-kb region flanked by RM18865 and Fi25273, and *qGL5* controlling grain length was delimited into a 68.8-kb region flanked by Fi27369 and Fi27438. Our studies laid a foundation for cloning minor QTL for grain size.

**Methods**

**Plant materials**

A total of 13 populations were used in this study. They were derived from one F\(_9\) plant of the *indica* rice cross TQ/IRBB52 as described below and illustrated in Fig.1. This plant was only heterozygous in three regions, including Fi24431–Fi27642 on chromosome 5, RM20731 on chromosome 6, and pTA248–RM5926 on chromosome 11. Its selfing progenies were assayed with DNA markers in the three segregating regions. Two F\(_{11}\) plants carrying a single heterozygous segment, Fi24431–Fi27642 on chromosome 5, were selected and selfed. Two NIL-F\(_2\) populations were produced and named XJ1 and XJ2.

For further mapping of *qTGW5*, four plants with overlapped heterozygous segments within the Fi24431–Fi27642 region were selected from the XJ1 population. In each of the resultant NIL-F\(_2\) populations, homozygous non-recombinants were selected and selfed. Four sets of NILs were constructed, namely N1, N2, N3 and N4, each consisting of 35 TQ homozygous lines and 35 IRBB52 homozygous lines. QTL analysis using these populations resulted in dissection of two linked QTL, of which *qGL5* was selected for fine-mapping. Three plants that carried heterozygous segments covering the entire or partial region of *qGL5* were identified from the XJ2 population. They were selfed to produce three NIL-F\(_2\) populations in F\(_{14}\), namely J1, J2 and J3. By using these populations, the *qGL5* region was narrowed down to a region flanked by markers Fi27293–Fi27438. For further fine-mapping of *qGL5*, three STS markers, Fi27390, Fi27542 and Fi27600, located in the heterozygous region Fi27369-Fi27642 were designed. Then, four plants carrying heterozygous segments overlapped in the whole *qGL5* region were selected from the J1 population. They were selfed to produce four NIL-F\(_2\) populations, namely, K1, K2, K3 and K4, of which each consisted of 240 plants.

**Field experiments and phenotyping**

The rice populations were planted in the experimental stations of the China National Rice Research Institute located at Hangzhou (30°04′ N, 119°54′ E) in Zhejiang Province or Lingshui (18°31′ N, 110°00′ E) in Hainan Province (Table 1). For four sets of NILs (N1, N2, N3 and N4), the experiments followed a randomized complete block design with two replications. For each replication, 12 plants per line were planted in one row. The plant density was 16.7 cm × 26.7 cm in all trials. Field management followed local agricultural practice. At maturity, five middle plants in each row were harvested in bulk and sun-dried. Nine traits were measured for NIL populations, including heading date (HD), 1000-grain weight (TGW), grain length (GL), grain width (GW), number of panicles per plant (NP), number of grains per panicle (NGP), number of spikelets per panicle (NSP), spikelet fertility (SF) and grain yield per plant (GY). Fully filled grain were selected and evaluated for grain size and weight following the procedure reported by Zhang et al. (2016). Mean values of two replications were used for data analysis.

For the nine NIL-F\(_2\) populations, plants were harvested individually and sun-dried. Approximately 200 fully filled grains of each plant were randomly selected for the measurement of TGW, GL and GW.

**DNA extraction and genotyping**
The DNA was extracted from young leaves using a conventional method (Zheng et al., 1995). PCR amplification was performed according to the method of Chen et al. (1997). The products were visualized on 6% or 8% non-denaturing polyacrylamide gels using silver staining. A total of 16 polymorphic DNA markers were used, including five simple sequence repeats (SSR) and 11 InDel markers (Additional file 1: Table S1). SSR markers were selected from Gramene database (https://www.gramene.com) and InDel markers were developed according to the differences between TQ and IRBB52 detected with whole genome re-sequencing.

Sequence analysis

Sequence analysis was performed for six annotated genes located in the qGL5 region. Genomic DNA was extracted from young leaves using the DNeasy Plant Mini Kit (TIANGEN, China). The primers were designed according to the sequence of Nipponbare in MSU (http://rice.plantbiology.msu.edu/) (Additional file 1: Table S2). The reaction mixture (50 μL) consisted of 4 μL of sample DNA, 25 μL 2×PCR buffer of KOD, 10 μL 2mM dNTP, 1μL each of 10 mM forward and reverse primers, 1μL KOD enzyme and 13 μL of ddH2O. PCR thermal cycling program was performed according to the protocol of KOD-FX DNA polymerase (TOYOBO CO., LTD. Life Science Department OSAKA Japan). The PCR products were visualized on 1% agarose gels using Gelred staining. Positive clones were sequenced by the TsingKe Company (Hangzhou, China). The nucleotide and predicted amino acid sequences of TQ and IRBB52 were compared.

RNA extraction and real-time PCR analysis

Total RNA was isolated from 8 cm-long young panicles of NIL-TQ and NIL-IRBB52 using RNeasy Pius Mini Kit (QIAGEN, German). First-stand cDNA obtained via reverse transcription of total RNA using ReverTra AceR Kit (TOYOBO, Japan). Real-time PCR was conducted on Applied Biosystems 7500 using SYBR qPCR Mix Kit (TOYOBO, Japan) following the manufacturer's instructions. qRT-PCR reaction was carried out with a total volume of 20 μL, consisting 2 μL cDNA, 10 μL SYBR qPCR Mix, 0.4 μL of each gene-specific primer (Additional file 1: Table S3) and ddH2O. The rice Actin1 gene was used as the internal control. Three biological replicates and three technical replicates were used.

Data analysis

For the nine NIL-F2 populations, genetic maps of each population were constructed using Mapmaker/Exp 3.0 (Lander et al., 1987). QTL were determined using Windows QTL Cartographer 2.5 (North Carolina State University, Raleigh, NC, USA) (Wang et al., 2012). A threshold of LOD were calculated with 1,000 permutation test (P < 0.05) and used for claiming a putative QTL.

For the four NIL populations, two-way analysis of variance (ANOVA) was performed to test the phenotypic differences between the two genotypic groups in each population. The analysis was performed using the SAS procedure GLM (Dai et al., 2008). Given the detection of significant difference (P < 0.05), the QTL effect was estimated, including additive effect and the proportion of phenotypic variance explained (R2).

Results

Validation of qTGW5 using two NIL-F2 populations in F12

The two NIL-F2 populations tested in the first experiment, XJ1 and XJ2, was segregated in the same region covering qTGW5/ qGL5 (Fig. 2a). As shown in Table 2, significant genotypic effects were detected in both populations for all the three traits analyzed, TGW, GL and GW. In all cases, the enhancing alleles were derived from IRBB52. In XJ1, the additive effects were 0.33 g for TGW, 0.073 mm for GL and 0.012 mm for GW, explaining 22.0, 36.5 and 11.7% of the phenotypic
variance, respectively. In XJ2, the additive effects were 0.30 g for TGW, 0.094 mm for GL and 0.011 mm for GW, contributing 18.2, 49.9 and 16.9 % to the phenotypic variance, respectively. In both populations, the phenotypic variance explained were much larger for GL than for GW, indicating that this QTL affected grain weight mainly through grain length. This is in accordance with the detection of $qTGW5/qGL5$ in the previous study (Zhu et al. 2019a).

Dissection of $qTGW5$ into two QTL using four NIL populations

The four NIL populations tested in the second experiment, N1, N2, N3 and N4, were segregated within the whole $qTGW5/qGL5$ region (Fig 2 b). Results of QTL analysis for these populations are shown in Table 3. In N1 and N2, significant effects were detected for the three grain size traits. In N3 and N4, significant effects were detected for TGW and GL but not for GW. In all cases, the enhancing alleles were derived from IRBB52. As shown in Fig. 2b, the segregating region in N1 was different from those in N3 and N4. Meanwhile, the segregating region in N3 covered that in N4. These results suggest that at least two QTL were responsible for the grain size variations in these populations. One was located in the segregating region of N1, and the other in the common segregating region of N3 and N4.

In the N1 population, the QTL effect was stronger on TGW and GL than on GW, having $R^2$ of 23.1, 22.6, and 12.5%, respectively. In the N3 and N4 populations, the QTL effect was much stronger on GL than on TGW. The $R^2$ values were 41.6 % for GL and 25.7 % for TGW in N3, and 26.0 % for GL and 8.1 % for TGW in N4. Following the previous nomenclature of $qTGW5/qGL5$ (Zhu et al. 2019a), the QTL segregated in N1 was named $qTGW5$ and the QTL segregated in N3 and N4 was named $qGL5$. As shown in Fig. 2b, $qTGW5$ was located in the interval RM18865–Fi25273 and $qGL5$ in Fi27293–Fi27682. According to the physical positions in Nipponbare genome, the physical distances of RM18865–Fi25273 was 1896.4 kb and that of Fi27293–Fi27682 was 388.9 kb.

In addition, heading date and other yield traits, including NP, NGP, NSP, SF and GY, were analyzed in the four NIL populations. Non-significant effects were detected on HD and SF in all NIL populations. Significant effects ($P < 0.05$) were detected on NGP and NSP in N1, N2 and N4, and the enhancing alleles were all derived from TQ (Table 3). For NGP, the additive effects were 3.0, 5.2 and 2.9, with $R^2$ values of 5.0, 8.8 and 5.5%. For NSP, the additive effects were 3.5, 7.0 and 3.9, with $R^2$ values of 5.4, 12.5 and 7.1%. These results suggest that both QTL had pleiotropic effects on grain number and grain size with opposite allelic directions.

Fine-mapping of $qGL5$ using NIL-F$_2$ populations in F$_{14}$ and F$_{15}$

The $qGL5$ was further fine-mapped using seven NIL-F$_2$ populations. Among the three NIL-F$_2$ populations in F$_{14}$, significant genotypic effects were only detected for GL in J1 and J2 (Table 4), with the enhancing alleles all derived from IRBB52. In J1, the additive effects were 0.023 mm, with $R^2$ values of 6.6%. In J2, the additive effects were 0.028 mm for GL, with $R^2$ values of 14.0%. These results indicate that $qGL5$ was located within the segregating region of J1 and J2 but outside the segregating region of J3. Thus, the QTL was delimited into a 145-kb region flanked by InDel markers Fi27293 and Fi27438 (Fig. 2c).

Among the four NIL-F$_2$ populations in F$_{15}$, significant genotypic effects were only detected for GL in K2, K3 and K4 (Table 4). The enhancing alleles were all derived from IRBB52. The additive effects detected in the three populations were similar, ranging from 0.024 to 0.030 mm. Obviously, $qGL5$ was located within the common segregating region of K2, K3 and K4. As shown in Fig. 2d, this interval was flanked by InDel markers Fi27369 and Fi27438, corresponding to a 68.8-kb region in the Nipponbare genome.

Candidate genes of $qGL5$

According to Rice Genome Annotation Project (http://rice.plantbiology.msu.edu), there are six annotated genes in the 68.8-kb region for $qGL5$ (Additional file 1: Table S4). Two of them encode proteins with known functional domains.
\textit{LOC\_Os05g47780} encodes a RING-type E3 ubiquitin ligase that regulates plant iron responses and accumulation (Kobayashi et al., 2013) and participates in the control of stress response and seed development (Cooper et al., 2003). \textit{LOC\_Os05g47840} encodes an adenosine phosphate isopentenyl transferase which is a potential target protein of phytohormones such as gibberellins and cytokinins (Sakamoto et al., 2006). The remaining four annotated genes are \textit{LOC\_Os05g47790}, \textit{LOC\_Os05g47810}, \textit{LOC\_Os05g47820} and \textit{LOC\_Os05g47830} that encode expressed proteins.

Sequence comparisons of the six annotated genes were performed between full-length genomic fragments of TQ and IRBB52 (Additional file 1: Table S5). Differences were detected in all the genes except \textit{LOC\_Os05g47830}. For \textit{LOC\_Os05g47780}, three single nucleotide polymorphisms (SNPs) were detected, of which one occurred in intron and the others were synonymous. For \textit{LOC\_Os05g47840}, three SNPs and one 3-bp insertion/deletion were detected in the coding region. All these mutations lead to amino acid changes. The substitutions A220C, G871T and G895A lead to amino acid changes from Asparagine to Aspartic, Alanine to Serine and Alanine to Threonine, respectively. The 3-bp deletion at position 1024 in TQ resulted in the deletion of one Proline acid. A total of 11 SNPs were detected in the three other annotated genes, including five for \textit{LOC\_Os05g47790}, one for \textit{LOC\_Os05g47810}, and five for \textit{LOC\_Os05g47820}.

Transcript level comparisons of the six annotated genes were performed between the young panicles of NIL\textsuperscript{TQ} and NIL\textsuperscript{IRBB52} (Additional file 3: Fig S1). Significant expression difference was identified in three genes. As compared with NIL\textsuperscript{TQ}, the expression levels of NIL\textsuperscript{IRBB52} were 2.6 times higher in \textit{LOC\_Os05g47820}, 0.3 times higher in \textit{LOC\_Os05g47830} and 1.9 times higher in \textit{LOC\_Os05g47840}.

**Discussion**

Over the past two decades, great progress has been made in the cloning of major QTL in rice, but the cloning of minor QTL has been difficult especially for yield related traits. In the present study, two minor QTL associated with grain size and weight in rice were separated in a 4.5-Mb region on the long arm of chromosome 5. One of them, \textit{qTGW5} was located in an 1896.4-kb interval flanked by DNA markers RM18865 and Fi27253, affecting grain weight, grain length and grain width with the same allelic direction. The other one, \textit{qGL5} controlling grain length, was mapped within a 68.8-kb region flanked by Fi27369 and Fi27438. The two QTL were located in regions where no QTL associated with grain size and weight have been cloned, providing new candidates for gene cloning.

In rice, QTL controlling the same trait are often closely linked, forming QTL clustering (Yamamoto et al., 2014). For instance, two closely linked QTL for grain number were mapped on the short arm of chromosome 1 (Ashikari et al., 2005), two tightly linked loci, \textit{Hd3a} and \textit{Hd3b}, were dissected in the \textit{Hd3} region controlling heading date (Monna et al., 2002), and \textit{TGW6}, \textit{GW6a} and \textit{GL6} were closely linked in the 25.0-27.5 Mb region of the long arm of chromosome 6 (Ishimaru et al., 2013; Song et al., 2015; Wang et al., 2019). However, isolation of closely-linked QTLs is often very difficult. Generally, a large population is needed to create sufficient recombination sites and recombinants for dissection of closely linked QTL. Our studies offer a good approach to solve this problem. In our previous studies, six minor QTL for grain size and weight were dissected in a 7.1-Mb region on the long arm of chromosome 1 (Wang et al., 2015; Zhang et al., 2016; Dong et al., 2018). One of them, \textit{qTGW1.2b} was cloned using CRISPR/Cas9-targeted mutagenesis (Chan et al., 2021). Moreover, three closely-linked QTL for grain size on the long arm of chromosome 10 were dissected using NIL populations with sequential segregating regions (Zhu et al., 2019b). In this study, two tightly linked QTL for grain size were dissected within a 4.5-Mb region flanked by RM18865 and Fi27682 on the long arm of chromosome 5. One of them, \textit{qGL5} was delimited into a 68.8-kb region containing six annotated genes.

Ubiquitination-mediated proteasomal degradation and phytohormone signaling pathways play a critical role in regulating grain size (Li et al., 2019). Two annotated genes identified in the \textit{qGL5} region, encoding proteins with known functional domains, were involved in two of these pathways. \textit{LOC\_Os05g47780} encodes the putative RING-type E3 ubiquitin ligase that participate in the control of stress response and seed development (Cooper et al., 2003). There were two SNPs
differences with synonymous mutations in exons between two parents. LOC_Os05g47840 encodes adenosine phosphate isopentenyl transferase that is involved in the phytohormone signaling pathway (Taya et al., 1978; Sakamoto et al., 2006). Sequencing analysis indicated that there were three SNPs and a 3-bp insert/deletion between the two parents in coding region, and these differences resulted in non-synonymous mutations. Non-synonymous mutations were also detected in other three annotated genes located in the qGL5 region, including LOC_Os05g47790, LOC_Os05g47810 and LOC_Os05g47820. More evidence is needed to determine which genes is the most likely candidate for the causal gene underlying qGL5.

In cereal crops, grain size and number are the main components of yield, but there is a negative correlation between these two traits (Sadras, 2007). Previous studies on the trade-off between grain size and grain number have made by using mutant screens or identified genes/QTL controlling these two traits (Cloé et., 2009; Fletcher et al., 2015; Sukumaran et al., 2018). Some cloned QTL for grain size and weight showed pleiotropic effects for grain number, such as GW2, GL3.1, GL6 and GSA1 (Song et al., 2007; Qi et al., 2012; Wang et al., 2019; Dong et al., 2020). More recently, researchers found that GSN1 can coordinate the trade-off between grain number and grain size through conserved MAPK cascade (Guo et al., 2018; Xu et al., 2018). Furthermore, Guo et al (2020) found that OsER1 acts upstream of the OsMKKK10-OsMKK4-OsMPK6 kinase cascade to control the grain number. In this study, two minor QTL for grain size and weight were newly identified and showed pleiotropic effects on grain number. Opposite allelic directions were observed, with the IRBB52 allele enhancing grain size but reducing grain number. These two regions are good targets to further investigate the genetic and molecular mechanisms underlying the trade-off between grain number and grain size in rice.

**Conclusion**

Two closely linked minor QTL for grain size and weight in rice were separated in a 4.5-Mb region on the long arm of chromosome 5. The qTGW5 was located within an 1896.4-kb region, which controlled grain weight, length and width with same allelic direction, offering a new gene resource for enhancing grain yield. The qGL5 was situated within a 68.8-kb region containing six annotated genes. This QTL mainly affected grain size by controlling grain length, providing a potential gene resource for modifying grain appearance quality. Our work lays a foundation for cloning of minor QTL for grain size and provides potential targets for marker-assisted breeding in rice.

**Abbreviations**

ANOVA: Analysis of Variance; HD: Heading date; GL: Grain Length; GW: Grain Width; GY: Grain Yield per Plant; NGP: Number of Grains per Panicle; NSP: Number of of spikelets per panicle; NIL: Near Isogenic Line; NP: Panicle per Plant; QTL: Quantitative Trait Locus; R^2: Proportion of Phenotypic Variance Explained; RH: Residual heterozygote; SF: Spikelet fertility; SNP: Single Nucleotide Polymorphism; SSR: Simple Sequence Repeat; TGW: 1000-Grain Weight; TQ: Teqing

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

All authors are consent for publication.

**Availability of data and material**

The datasets supporting the conclusions of this article are included with in the article (and its additional files).
Competing interests

The authors declare that they have no conflict of interest.

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Author's contributions

JYZ, SBY and SQT designed the experiments. XJN and YJZ constructed the populations. XJN, YJZ, ZHZ and YYF performed the marker assay. XJN and YJZ conducted the field trials. XJN, ANC and JYZ analyzed the data. XJN and YJZ wrote the manuscript. All authors read and approved the final manuscript.

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Table 1 Rice populations and field experiments

| Generation | Name | Segregation region | Samples | Location and growing season |
|------------|------|---------------------|---------|----------------------------|
| F<sub>13</sub> | XJ1  | Fi24431–Fi27642     | 300 plants | HZ: May-Sep. 2018         |
| F<sub>13</sub> | XJ2  | Fi24431–Fi27642     | 300 plants | HZ: May-Sep. 2018         |
| F<sub>14:15</sub> | N1   | Fi24431–RM18927    | TQ: 35 lines; IRBB52: 35 lines | HZ: May-Sep. 2019         |
| F<sub>14:15</sub> | N2   | Fi25273–Fi27642    | TQ: 35 lines; IRBB52: 35 lines | HZ: May-Sep. 2019         |
| F<sub>14:15</sub> | N3   | RM274–Fi27642      | TQ: 35 lines; IRBB52: 35 lines | HZ: May-Sep. 2019         |
| F<sub>14:15</sub> | N4   | Fi27369–Fi27642    | TQ: 35 lines; IRBB52: 35 lines | HZ: May-Sep. 2019         |
| F<sub>14</sub> | J1   | Fi27369–Fi27642    | 240 plants | LS: Dec.2018-Apr.2019     |
| F<sub>14</sub> | J2   | Fi27369–Fi27642    | 240 plants | LS: Dec.2018-Apr.2019     |
| F<sub>14</sub> | J3   | Fi27438–Fi27642    | 240 plants | LS: Dec.2018-Apr.2019     |
| F<sub>15</sub> | K1   | Fi27369            | 240 plants | HZ: May-Sep. 2020         |
| F<sub>15</sub> | K2   | Fi27369–Fi27390    | 240 plants | HZ: May-Sep. 2020         |
| F<sub>15</sub> | K3   | Fi27369–Fi27438    | 240 plants | HZ: May-Sep. 2020         |
| F<sub>15</sub> | K4   | Fi27390–Fi27642    | 240 plants | HZ: May-Sep. 2020         |

a TQ, Teqing homozygote; IRBB52, IRBB52 homozygote

Table 2 QTL analysis for 1000-grain weight (TGW), grain length (GL) and grain width (GW) using two NIL-F<sub>2</sub> populations in F<sub>12</sub>

| Population | Trait | Phenotype | Mean±SD | LOD | A<sup>a</sup> | D<sup>0</sup> | R<sup>2</sup>%<sup>c</sup> |
|------------|------|-----------|---------|-----|-------------|-----------|----------------|
| Teqing    | IRBB52 | heterozygote |         |     |             |           |                |
| XJ1 TGW   | 23.60±0.51 | 24.35±0.55 | 23.77±0.55 | 13.9 | 0.33 | -0.23 | 22.0          |
| XJ1 GL    | 8.047±0.075 | 8.209±0.081 | 8.105±0.088 | 26.6 | 0.073 | -0.023 | 36.5          |
| XJ1 GW    | 2.861±0.028 | 2.888±0.025 | 2.871±0.027 | 7.1  | 0.012 | -0.006 | 11.7          |
| XJ2 TGW   | 23.12±0.45 | 23.74±0.60 | 23.56±0.40 | 13.01 | 0.30 | 0.11 | 18.2          |
| XJ2 GL    | 7.859±0.076 | 8.053±0.084 | 7.958±0.066 | 42.81 | 0.094 | 0.017 | 49.9          |
| XJ2 GW    | 2.796±0.021 | 2.817±0.022 | 2.812±0.019 | 11.16 | 0.011 | 0.008 | 16.9          |

<sup>a</sup> Additive effect of replacing a Teqing allele by a IRBB52 allele
Dominance effect

Proportion of phenotypic variance explained by the QTL effect

Table 3 Dissection of qTGW5 into two QTL using four sets of near isogenic lines in F13:14
| Population | Segregation region | Trait | Phenotype Mean ± SD | $P$   | $a^a$ | $R^2$(%)$^b$ |
|------------|-------------------|------|---------------------|-------|-------|-------------|
| N1         | Fi24431-RM18927   | TGW  | 22.46±0.24          | 22.80±0.24 | <0.0001 | 0.17        | 23.1        |
|            |                   | GL   | 7.723±0.043         | 7.777±0.038 | <0.0001 | 0.027       | 22.6        |
|            |                   | GW   | 2.719±0.011         | 2.731±0.013 | <0.0001 | 0.006       | 12.5        |
|            |                   | NP   | 9.9±0.9             | 9.9±0.9    | 0.7994  |             |             |
|            |                   | NGP  | 170.2±11.4          | 164.2±8.3  | 0.0058  | -3.0        | 5.0         |
|            |                   | NSP  | 190.4±11.7          | 183.4±10.3 | 0.0044  | -3.5        | 5.4         |
|            |                   | GY   | 36.51±3.90          | 35.71±3.52 | 0.3737  |             |             |
|            |                   | SF   | 89.2±1.6            | 89.6±1.6   | 0.2464  |             |             |
|            |                   | HD   | 86.9±1.0            | 87.5±0.8   | 0.0361  |             |             |
| N2         | Fi25273-Fi27642   | TGW  | 22.65±0.29          | 23.13±0.29 | 0.0004  | 0.24        | 29.2        |
|            |                   | GL   | 7.598±0.040         | 7.760±0.053 | <0.0001 | 0.081       | 68.9        |
|            |                   | GW   | 2.746±0.011         | 2.754±0.011 | 0.0016  | 0.004       | 6.8         |
|            |                   | NP   | 10.0±1.1            | 9.7±1.1    | 0.1886  |             |             |
|            |                   | NGP  | 179.4±12.3          | 168.9±11.6 | 0.0006  | -5.2        | 8.8         |
|            |                   | NSP  | 203.3±13.2          | 189.3±12.5 | <0.0001 | -7.0        | 12.5        |
|            |                   | GY   | 38.47±4.09          | 35.67±3.60 | 0.0017  | -1.40       | 6.8         |
|            |                   | SF   | 88.3±2.0            | 89.3±1.8   | 0.0161  |             |             |
|            |                   | HD   | 87.5±0.9            | 87.4±1.1   | 0.7972  |             |             |
| N3         | RM274-Fi27642     | TGW  | 21.75±0.21          | 22.06±0.16 | <0.0001 | 0.15        | 25.7        |
|            |                   | GL   | 7.588±0.029         | 7.654±0.027 | <0.0001 | 0.033       | 41.6        |
|            |                   | GW   | 2.705±0.011         | 2.707±0.012 | 0.2827  |             |             |
|            |                   | NP   | 10.0±1.0            | 10.0±1.0   | 0.8906  |             |             |
|            |                   | NGP  | 170.0±9.0           | 170.0±10.6 | 0.9966  |             |             |
|            |                   | NSP  | 197.6±10.6          | 195.1±10.6 | 0.3372  |             |             |
|            |                   | GY   | 35.86±3.50          | 36.35±4.31 | 0.5123  |             |             |
|            |                   | SF   | 86.1±1.4            | 87.2±3.0   | 0.2464  |             |             |
|            |                   | HD   | 87.6±1.3            | 87.8±1.3   | 0.4516  |             |             |
| N4         | Fi27369-Fi27642   | TGW  | 22.27±0.23          | 22.48±0.30 | 0.0005  | 0.10        | 8.1         |
|            |                   | GL   | 7.583±0.031         | 7.640±0.041 | <0.0001 | 0.029       | 26.0        |
|            |                   | GW   | 2.715±0.015         | 2.711±0.014 | 0.2089  |             |             |
|            |                   | NP   | 9.4±0.9             | 9.8±0.9    | 0.0338  | 0.19        | 2.7         |
|            |                   | NGP  | 178.3±9.7           | 172.5±8.7  | 0.0035  | -2.9        | 5.5         |
| Generation | Population | Trait | Phenotype | Mean±SD | LOD | A | B | R²(%) |
|------------|------------|-------|-----------|---------|-----|---|---|-------|
| F₁₄        | J1         | TGW   | 25.59±0.49| 25.69±0.50| 25.62±0.51| 0.21| 3.51 | 0.023 | -0.003 | 6.6  |
|            |            | GL    | 7.914±0.060| 7.961±0.061| 7.935±0.070| 3.51 | 7.1  |
|            |            | GW    | 3.030±0.029| 3.027±0.025| 3.026±0.026| 0.50 | 7.1  |
|            | J2         | TGW   | 25.34±0.37| 25.42±0.41| 25.43±0.45| 0.49| 7.1  |
|            |            | GL    | 7.982±0.039| 8.032±0.053| 8.015±0.050| 7.57 | 0.028| 0.009 | 14.0 |
|            |            | GW    | 3.013±0.024| 3.010±0.029| 3.014±0.029| 0.26 | 7.1  |
|            | J3         | TGW   | 25.56±0.44| 25.65±0.40| 25.60±0.43| 0.29| 7.1  |
|            |            | GL    | 7.905±0.044| 7.910±0.041| 7.898±0.044| 0.72 | 7.1  |
|            |            | GW    | 3.042±0.029| 3.045±0.030| 3.043±0.030| 0.05 | 7.1  |
| F₁₅        | K1         | TGW   | 22.94±0.47| 22.84±0.39| 22.87±0.45| 0.37| 7.1  |
|            |            | GL    | 7.796±0.066| 7.800±0.061| 7.791±0.059| 0.15 | 7.1  |
|            |            | GW    | 2.783±0.029| 2.780±0.027| 2.779±0.028| 0.10 | 7.1  |
|            | K2         | TGW   | 23.38±0.55| 23.50±0.53| 23.35±0.54| 0.64| 7.1  |
|            |            | GL    | 7.876±0.072| 7.937±0.087| 7.906±0.066| 4.25 | 7.1  |
|            |            | GW    | 2.810±0.035| 2.802±0.034| 2.800±0.032| 0.74 | 7.1  |
|            | K3         | TGW   | 23.32±0.55| 23.32±0.46| 23.37±0.46| 0.19| 7.1  |
|            |            | GL    | 7.809±0.066| 7.854±0.071| 7.837±0.071| 2.82 | 7.1  |
|            |            | GW    | 2.822±0.034| 2.804±0.028| 2.814±0.030| 2.12 | 7.1  |
|            | K4         | TGW   | 23.11±0.45| 23.21±0.45| 23.11±0.37| 0.70| 7.1  |
|            |            | GL    | 7.856±0.075| 7.905±0.071| 7.865±0.062| 3.88 | 7.1  |
|            |            | GW    | 2.807±0.033| 2.799±0.029| 2.803±0.025| 0.32 | 7.1  |

* Additive effect of replacing a Teqing allele by a IRBB52 allele

* Proportion of phenotypic variance explained by the QTL effect

Table 4 Fine mapping of qGL5 using seven sets of NIL-F₂ populations in F₁₄ and F₁₅
a Additive effect of replacing a Teqing allele by a IRBB52 allele

b Dominance effect

c Proportion of phenotypic variance explained by the QTL effect

Figures

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**Figure 1**

Development of the rice populations used in this study.
Figure 2

Segregating regions in the rice populations. a Two NIL-F2 populations in F11:12. b Four sets of NILs in F13:14. c Three NIL-F2 populations in F14. d Four NIL-F2 populations in F15.

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