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

xiaojun Niu

China National Rice Research Institute <https://orcid.org/0000-0002-6884-7369>

Zhenhua Zhang

China National Rice Research Institute

Aye Nyein Chan

China National Rice Research Institute

Yeyang Fan

China National Rice Research Institute

Shaoqing Tang

China National Rice Research Institute

Sibin Yu

Huazhong Agricultural University: Huazhong Agriculture University

Jieyun Zhuang

China National Rice Research Institute

Yujun Zhu (✉ zhuyujun@caas.cn)

State Key Laboratory of Rice Biology and Chinese National Center for Rice Improvement, China National Rice Research Institute, Hangzhou 310006, China <https://orcid.org/0000-0001-6409-1990>

Original article

Keywords: Quantitative trait locus, Close linkage, Grain size, Minor effect, Rice

Posted Date: April 1st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-367162/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

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 μ L) consisted of 4 μ L of sample DNA, 25 μ L 2 $\times$ PCR buffer of KOD, 10 μ L 2mM dNTP, 1 μ L each of 10 mM forward and reverse primers, 1 μ L KOD enzyme and 13 μ L 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 μ L, consisting 2 μ L cDNA, 10 μ L SYBR qPCR Mix, 0.4 μ L 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 (R^2).

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.

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). *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 *LOC_Os05g47790*, *LOC_Os05g47810*, *LOC_Os05g47820* and *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 *LOC_Os05g47830*. For *LOC_Os05g47780*, three single nucleotide polymorphisms (SNPs) were detected, of which one occurred in intron and the others were synonymous. For *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 *LOC_Os05g47790*, one for *LOC_Os05g47810*, and five for *LOC_Os05g47820*.

Transcript level comparisons of the six annotated genes were performed between the young panicles of NIL^{TQ} and NIL^{IRBB52} (Additional file 3: Fig S1). Significant expression difference was identified in three genes. As compared with NIL^{TQ}, the expression levels of NIL^{IRBB52} were 2.6 times higher in *LOC_Os05g47820*, 0.3 times higher in *LOC_Os05g47830* and 1.9 times higher in *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, *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, *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, *Hd3a* and *Hd3b*, were dissected in the *Hd3* region controlling heading date (Monna et al., 2002), and *TGW6*, *GW6a* and *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, *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, *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 *qGL5* region, encoding proteins with known functional domains, were involved in two of these pathways. *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 al., 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-OsMCK4-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; R²: 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.

Funding

This work was supported by Outstanding Talents for "Ten Thousand Talents Program" of Zhejiang Province (2020R51007), Central Public-interest Scientific Institution Basal Research Fund (CPSIBRF-CNRRI-202112) and a project of the State Key Laboratory of Rice Biology(2020ZZKT10105).

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.

Acknowledgements

Not applicable.

Author information

¹State Key Laboratory of Rice Biology and Chinese National Center for Rice Improvement, China National Rice Research Institute, Hangzhou 310006, China; ²State Key Laboratory of Crop Genetic Improvement and National Center of Plant Gene Research (Wuhan), Huazhong Agricultural University, Wuhan 430070, Hubei, China;

³Advanced Center for Agricultural Research and Education, Yezin Agricultural University, Naypyitaw 15013, Myanmar.

References

1. Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles E.R, Qian Q, Kitano K, Matsuoka M (2005) Cytokinin oxidase regulates rice grain production. *Science* 309: 741–745
2. Calingacion M, Laborte A, Nelson A, Resurreccion A, Concepcion JC, Daygon VD, Mumm R, Reinke R, Dipti S, Bassinello PZ, Manful J, Sophany S, Lara KC, Bao J, Xie L, Loaiza K, El-hissew A, Gayin J, Sharma N, Rajeswari S, Manonmani S, Rani NS, Kota S, Indrasari SD, Habibi F, Hosseini M, Tavasoli F, Suzuki K, Umemoto T, Boualaphanh C, Lee HH, Hung YP, Ramli A, Aung PP, Ahmad R, Wattoo JI, Bandonill E, Romero M, Brites CM, Hafeel R, Lur H-S, Cheaupun K, Jongdee S, Blanco P, Bryant R, Lang NT, Hall RD, Fitzgerald M (2014) Diversity of global rice markets and the science required for consumer-targeted rice breeding. *PLoS One* 9:e85106
3. Cai M, Zhu S, Wu M, Zheng X, Wang J, Zhou L, Zheng T, Cui S, Zhou S, Li C, Zhang H, Chai J, Zhang X, Jin X, Cheng Z, Zhang X, Lei C, Ren Y, Lin Q, Guo X, Zhao L, Wang J, Zhao Z, Jiang L, Wang H, Wang J (2021) DHD4, a CONSTANS-like family transcription factor, delays heading date by affecting the formation of the FAC complex in rice. *Mol Plant* 14:330-343
4. Chan A, Wang L, Zhu Y, Fan Y, Zhuang J, Zhang Z (2021) Identification through fine mapping and verification using CRISPR/Cas9-targeted mutagenesis for a minor QTL controlling grain weight in rice. *Theor Appl Genet* 134:327-337
5. Chen J-Y, Zhang H-W, Zhang H-L, Ying J-Z, Ma L-Y, Zhuang J-Y (2018) Natural variation at qHd1 affects heading date acceleration at high temperatures with pleiotropism for yield traits in rice. *BMC Plant Bio* 18:112
6. Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR (1997) Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor Appl Genet* 95:553–567

7. Cloé Paul-Victor, Lindsay A Turnbull (2009) The effect of growth conditions on the seed size/number trade-off. *PLoS One* 4:e6917
8. Cooper B, D Clarke J, Budworth P, Kreps J, Hutchison D, Park S, Guimil S, Dunn M, Luginbühl P, Ellero C, A Goff S, Glazebrook J (2003) A network of rice genes associated with stress response and seed development. *Proc Natl Acad Sci U S A* 100:4945-4950
9. Dai W, Zhang K, Wu J, Wang L, Duan B, Zheng K, Cai R, Zhuang J (2008) Validating a segment on the short arm of chromosome 6 responsible for genetic variation in the hull silicon content and yield traits of rice, *Euphytica* 160: 317–324
10. Dong N, Sun Y, Guo T, Shi C, Zhang Y, Kan Y, Xiang Y, Zhang H, Yang Y, Li Y, Zhao H, Yu H, Lu Z, Wang Y, Ye W, Shan J, Lin H (2020) UDP-glucosyltransferase regulates grain size and abiotic stress tolerance associated with metabolic flux redirection in rice. *Nat Commun* 11:2629
11. Dong Q, Zhang Z, Wang L, Zhu Y, Fan Y, Mou T, Ma L, Zhuang J (2018) Dissection and fine-mapping of two QTL for grain size linked in a 460-kb region on chromosome 1 of rice. *Rice* 11: 44
12. Fletcher Richard S, Mullen Jack L, Heiliger Annie, McKay John K (2015) QTL analysis of root morphology, flowering time, and yield reveals trade-offs in response to drought in *Brassica napus*. *J Exp Bot* 1:245–256
13. Guo T, Chen K, Dong NQ, Shi CL, Ye WW, Gao JP, Shan JX, Lin HX (2018) GRAIN SIZE AND NUMBER1 negatively regulates the OsMKKK10-OsMKK4-OsMPK6 cascade to coordinate the trade-off between grain number per panicle and grain size in rice. *Plant Cell* 30:871–888
14. Guo T, Lu ZQ, Shan JX, Ye WW, Dong NQ, Lin HX (2020) ERECTA1 acts upstream of the OsMKK10-OsMKK4-OsMPK6 cascade to control spikelet number by regulating cytokinin metabolism in rice. *Plant Cell* 32:2763-2779
15. Ishimaru K, Hirotsu N, Madoka Y, Murakami N, Hara N, Onodera H, Kashiwagi T, Ujiie K, Shimizu Bun-ichi, Onishi A, Miyagawa H, Katoh E (2013) Loss of function of the IAA-glucose hydrolase gene *TGW6* enhances rice grain weight and increases yield. *Nat Genet* 45: 707-711
16. Kobayashi T, Nagasaka S, Senoura T, Nakanishi Itai R, Nishizawa N (2013) Iron-binding haemerythrin RING ubiquitin ligases regulate plant iron responses and accumulation. *Nat Commun* 4:2792
17. Lander E, Green P, Abrahamson J, Barlow A, Daly M, Lincoln S, Newberg L (1987) MAPMARKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174–181
18. Li N, Xu R, Li Y (2019) Molecular networks of seed size control in plants. *Annu Rev Plant Biol* 70:435-463
19. Li Q, Lu L, Liu H, Bai X, Zhou X, Wu B, Yuan M, Yang L, Xing Y. A minor QTL, *SG3*, encoding an R2R3-MYB protein, negatively controls grain length in rice (2020). *Theor Appl Genet* 133:2387-2399
20. Matsubara K, Ogiso-Tanaka E, Hori K, Ebana K, Ando T, Yano M (2012) Natural variation in *Hd17*, a homolog of *Arabidopsis ELF3* that is involved in rice photoperiodic flowering. *Plant Cell Physiol* 53:709–716
21. Monna L, Lin X, Kojima S, Sasaki T, Yano M (2002) Genetic dissection of a genomic region for a quantitative trait locus, *Hd3*, into two loci, *Hd3a* and *Hd3b*, controlling heading date in rice. *Theor Appl Genet* 104:772-778
22. Qi P, Lin YS, Song XJ, Shen JB, Huang W, Shan JX, Zhu MZ, Jiang L, Gao JP, Lin HX (2012) The novel quantitative trait locus *GL3.1*, controls rice grain size and yield by regulating Cyclin-T1; β 3. *Cell Res* 22: 1666-1680
23. Ruan B, Shang L, Zhang B, Hu J, Wang Y, Lin H, Zhang A, Liu C, Peng Y, Zhu L, Ren D, Shen L, Dong G, Zhang G, Zeng D, Guo L, Qian Q, Gao Z (2020) Natural variation in the promoter of *TGW2* determines grain width and weight in rice. *New Phytol* 227:629-640
24. Sadras VQ (2007) Evolutionary aspects of the trade-off between seed size and number in crops. *Field Crops Research* 100: 125-138

25. Sakamoto T, Sakakibara H, Kojima M, Yamamoto Y, Nagasaki H, Inukai Y, Sato Y, Matsuoka M (2006) Ectopic Expression of KNOTTED1-Like Homeobox Protein Induces Expression of Cytokinin Biosynthesis Genes in Rice. *Plant Physiol* 142:54-62
26. Shi C, Dong N, Guo T, Ye W, Shan J, Lin H (2020) A quantitative trait locus *GW6* controls rice grain size and yield through the gibberellin pathway. *Plant J* 103:1174-1188
27. Shibaya T, Hori K, Ogiso-Tanaka E, Yamanouchi U, Shu K, Kitazawa N, Shomura A, Ando T, Ebana K, Wu J, Yamazaki T, Yano M (2016) *Hd18*, encoding histone acetylase related to Arabidopsis FLOWERING LOCUS D, is involved in the control of flowering time in rice. *Plant Cell Physiol* 57:1828–1838
28. Song X, Huang W, Shi M, Zhu M, Lin H (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nat Genet* 39: 623-630
29. Song X, Kuroha T, Ayano M, Furuta T, Nagai K, Komeda N, Segami S, Miura K, Ogawa D, Kamura T, Suzuki T, Higashiyama T, Yamasaki M, Mori H, Inukai Y, Wu J, Kitano H, Sakakibara H, Jacobsen SE, Ashikari M (2015) Rare allele of a previously unidentified histone H4 acetyltransferase enhances grain weight, yield, and plant biomass in rice. *Proc Natl Acad Sci USA* 112: 76-81
30. Sukumaran S, Lopes M, Dreisigacker S, Reynolds M (2018) Genetic analysis of multi-environmental spring wheat trials identifies genomic regions for locus-specific trade-offs for grain weight and grain number. *Theor Appl Genet* 131:985-998
31. Taya Y, Tanaka Y, Nishimura S (1978) 5'-AMP is a direct precursor of cytokinin in Dictyostelium discoideum. *Nature* 271: 545-7
32. Wang A, Hou Q, Si L, Huang X, Luo J, Lu D, Zhu J, Shang Y, Miao J, Xie Y, Wang Y, Zhao Q, Feng Q, Zhou C, Li Y, Fan D, Lu Y, Tian Q, Wang Z, Han B. The PLATZ transcription factor *GL6* affect grain length and number in rice (2019) *Plant Physiol* 180: 2077-2090.
33. Wang L-L, Chen Y-Y, Guo L, Zhang H-W, Fan Y-Y, Zhuang J-Y (2015) Dissection of *qTGW1.2* to three QTLs for grain weight and grain size in rice (*Oryza sativa* L.). *Euphytica* 202:119–127
34. Wang S, Basten J, Zeng Z (2012) Windows QTL Cartographer 2.5, Department of Statistics; North Carolina State University: Raleigh, NC, USA
35. Wu W, Zheng X, Lu G, Zhong Z, Gao H, Chen L, Wu C, Wang H, Wang Q, Zhou K, Wang J, Wu F, Zhang X, Guo X, Cheng Z, Lei C, Lin Q, Jiang L, Wang H, Ge S, Wan J (2013) Association of functional nucleotide polymorphisms at *DTH2* with the northward expansion of rice cultivation in Asia. *Proc Natl Acad Sci USA* 110:2775–2780
36. Xing Y, Zhang Q (2010) Genetic and molecular bases of rice yield. *Annu. Rev. Plant Biol.* 61: 421–442.
37. Xu R, Duan P, Yu H, Zhou Z, Zhang b, Wang R, Li J, Zhang G, Zhuang S, Lyu J, Li N, Chai T, Tian Z, Yao S, Li Y (2018) Control of grain size and weight by the OsMKKK10-OsMKK4-OsMAPK6 signaling pathway in rice. *Mol Plant* 11:860–873
38. Yamamoto E, Iwata H, Tanabata T, Mizobuchi R, Yonemaru J-I, Yamamoto T (2014) Effect of advanced intercrossing on genome structure and on the power to detect linked quantitative trait loci in a multi-parent population: a simulation study in rice. *BMC Genet* 15:50
39. Yamamoto T, Yonemaru J, Yano M (2009) Towards the understanding of complex traits in rice: substantially or superficially? *DNA Res.* 16:141-154
40. Zhang H, Fan Y, Zhu Y, Chen J, Yu S, Zhuang J (2016) Dissection of the *qTGW1.1* region into two tightly-linked minor QTLs having stable effects for grain weight in rice. *BMC Genet* 17:98–107
41. Zheng KL, Huang N, Bennett J, Khush GS (1995) PCR-based marker-assisted selection in rice breeding: IRRI Discussion Paper Series No. 12. Los Banos: International Rice Research Institute
42. Zhu A, Sun Z, Zhu Y, Zhang H, Niu X, Fan Y, Zhang Z, Zhuang J (2019a) Identification of QTL for grain weight and grain shape using populations derived from residual heterozygous lines of *indica* rice. *Chin J Rice sci* 33:144-151

43. Zhu YJ, Sun ZC, Niu XJ, Ying JZ, Fan YY, Mou TM, Tang SQ, Zhuang JY (2019b) Dissection of three quantitative trait loci for grain size on the long arm of chromosome 10 in rice. *Peer J* 7:e6966

Tables

Table 1 Rice populations and field experiments

Generation	Name	Segregation region	Samples ^a	Location and growing season
F ₁₃	XJ1	Fi24431–Fi27642	300 plants	HZ: May-Sep. 2018
	XJ2	Fi24431–Fi27642	300 plants	HZ: May-Sep. 2018
F _{14:15}	N1	Fi24431–RM18927	TQ: 35 lines; IRBB52: 35 lines	HZ: May-Sep. 2019
	N2	Fi25273–Fi27642	TQ: 35 lines; IRBB52: 35 lines	HZ: May-Sep. 2019
	N3	RM274–Fi27642	TQ: 35 lines; IRBB52: 35 lines	HZ: May-Sep. 2019
	N4	Fi27369–Fi27642	TQ: 35 lines; IRBB52: 35 lines	HZ: May-Sep. 2019
F ₁₄	J1	Fi27369–Fi27642	240 plants	LS: Dec.2018-Apr.2019
	J2	Fi27369–Fi27642	240 plants	LS: Dec.2018-Apr.2019
	J3	Fi27438–Fi27642	240 plants	LS: Dec.2018-Apr.2019
F ₁₅	K1	Fi27369	240 plants	HZ: May-Sep. 2020
	K2	Fi27369–Fi27390	240 plants	HZ: May-Sep. 2020
	K3	Fi27369–Fi27438	240 plants	HZ: May-Sep. 2020
	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-F₂ populations in F₁₂

Population	Trait	Phenotype $\bar{\text{Mean}} \pm \text{SD}$			LOD	A ^a	D ^b	R ² (%) ^c
		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
	GL	8.047±0.075	8.209±0.081	8.105±0.088	26.6	0.073	-0.023	36.5
	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
	GL	7.859±0.076	8.053±0.084	7.958±0.066	42.81	0.094	0.017	49.9
	GW	2.796±0.021	2.817±0.022	2.812±0.019	11.16	0.011	0.008	16.9

^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

Table 3 Dissection of qTGW5 into two QTL using four sets of near isogenic lines in F13:14

Population	Segregation region	Trait	Phenotype $\bar{\text{Mean}} \pm \text{SD}$		<i>P</i>	<i>A</i> ^a	<i>R</i> ² (%) ^b
			NIL ^{TQ}	NIL ^{IRBB52}			
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	0.7794		
		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

	NSP	204.7±10.5	196.9±10.2	0.0014	-3.9	7.1
	GY	36.00±3.04	36.59±3.50	0.3406		
	SF	87.2±1.5	88.4±1.7	0.4202		
	HD	88.3±0.9	88.4±1.5	0.6440		

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

^b 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

Generation	Population	Trait	Phenotype [Mean±SD]			LOD	A ^a	D ^b	R ² (%) ^c
			Teqing	IRBB52	heterozygote				
F ₁₄	J1	TGW	25.59±0.49	25.69±0.50	25.62±0.51	0.21			
		GL	7.914±0.060	7.961±0.061	7.935±0.070	3.51	0.023	-0.003	6.6
		GW	3.030±0.029	3.027±0.025	3.026±0.026	0.50			
	J2	TGW	25.34±0.37	25.42±0.41	25.43±0.45	0.49			
		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			
	J3	TGW	25.56±0.44	25.65±0.40	25.60±0.43	0.29			
		GL	7.905±0.044	7.910±0.041	7.898±0.044	0.72			
		GW	3.042±0.029	3.045±0.030	3.043±0.030	0.05			
F ₁₅	K1	TGW	22.94±0.47	22.84±0.39	22.87±0.45	0.37			
		GL	7.796±0.066	7.800±0.061	7.791±0.059	0.15			
		GW	2.783±0.029	2.780±0.027	2.779±0.028	0.10			
	K2	TGW	23.38±0.55	23.50±0.53	23.35±0.54	0.64			
		GL	7.876±0.072	7.937±0.087	7.906±0.066	4.25	0.030	-0.001	8.2
		GW	2.810±0.035	2.802±0.034	2.800±0.032	0.74			
	K3	TGW	23.32±0.55	23.32±0.46	23.37±0.46	0.19			
		GL	7.809±0.066	7.854±0.071	7.837±0.071	2.82	0.024	0.007	5.4
		GW	2.822±0.034	2.804±0.028	2.814±0.030	2.12			
	K4	TGW	23.11±0.45	23.21±0.45	23.11±0.37	0.70			
		GL	7.856±0.075	7.905±0.071	7.865±0.062	3.88	0.024	-0.017	7.3
		GW	2.807±0.033	2.799±0.029	2.803±0.025	0.32			

- ^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

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 F_{11:12}. b Four sets of NILs in F_{13:14}. c Three NIL-F₂ populations in F₁₄. d Four NIL-F₂ populations in F₁₅.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS15.docx](#)
- [figS1.jpg](#)