Dissection of Closely Linked QTLs Controlling Grain Size in Rice

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Dissection of closely linked QTLs controlling grain size in rice

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Key message
Two small-effect QTLs, qTGW7.2a for grain width consequently affect grain weight was fine mapped to a 21.10-kb interval, and qTGW7.2b was limited within a 52.71-kb interval for grain length and width with opposite allelic directions, exhibiting little influence on grain weight.

Abstract
Grain size is a key constituent of grain weight and appearance in rice. However, insufficient attention has been paid to the small-effect QTLs on grain size. In the present study, residual heterozygous populations were developed for mapping two genetically linked small-effect QTLs for grain size. After genotyping and phenotyping of five successive generations, qGS7.1 was dissected into three QTLs and two were selected for further analysis. qTGW7.2a was finally mapped into a 21.10-kb interval containing four annotated candidate genes. Transcript levels assay showed that the expression of candidates LOC_Os07g39490 and LOC_Os07g39500 were significantly reduced in the NIL-qTGW7.2aBG1. Cytological observation indicated that qTGW7.2a regulated grain width through controlling cell expansion. Use the same strategy, qTGW7.2b was fine mapped into a 52.71-kb interval, showing a significant effect on grain length and width with opposite allelic directions but little on grain weight. Our study provides new genetic resources for yield improvement and fine-tunes of grain size in rice.

Keywords
Rice, Quantitative trait locus, Small effect, Residual heterozygous population

Introduction
Rice (Oryza sativa L.) is one of the most important staple crops which feeding half of the world’s population. Therefore, grain yield became a prime target for breeders. Grain yield is characterized by three components: panicle number, filled grain number
per panicle, and grain weight. Grain weight is mainly determined by grain size, which simultaneously affects appearance (Zuo and Li 2014). Thus, grain size is a primary target for yield improvement.

Grain length and grain width determine grain size, and both are complex traits controlled by quantitative trait locus (QTL). To date, 20 grain size related QTLs with large-effect have been cloned and characterized. Several signals and regulatory pathways controlling grain size have been identified in rice, such as the G-protein signaling pathway, the ubiquitin-proteasome pathway, the mitogen-activated protein kinase (MAPK) signaling pathway, the phytohormone signaling, and transcriptional regulators (Fan and Li 2019; Li and Li 2016). GS3 and DEP1 encode G-protein γ-subunits and regulate grain size and weight (Fan et al. 2006; Huang et al. 2009). OsLG3b encodes a MADS-domain transcription factor OsMADS1 which acts as a key downstream effector of G-protein βγ dimers in controlling grain size and appearance (Yu et al. 2018). HGW, GW2, WTG1, and OsUBP15 regulate grain size and weight via the ubiquitin-proteasome pathway (Huang et al. 2017; Li et al. 2012; Shi et al. 2019; Song et al. 2007). OsMKK10, OsMKK4/SMG1, and OsMAPK6 are involved in the MAPK signaling pathway (Duan et al. 2014; Guo et al. 2018; Liu et al. 2015a). OsRac1 and GSN1 directly interact with OsMAPK6, inactivate and activate OsMAPK6 via dephosphorylation and phosphorylation (Guo et al. 2018; Zhang et al. 2019). Furthermore, ERECTA1 acts upstream of the OsMKK10-OsM KK4-OsMPK6 cascade to control spikelet number by regulating cytokinin metabolism in rice (Guo et al. 2020). Some proteins participate in the brassinosteroids (BR) signal pathway: GW5 encodes a calmodulin-binding protein, GS5 encodes a putative serine carboxypeptidase, GL3.1 encodes a protein phosphatase kelch (PPKL), GS2 encodes transcription factor OsGRF4, and GSK2 kinase has multiple substrates that carry out various BR responses (Hu et al. 2015; Li et al. 2011; Liu et al. 2017; Qi et al. 2012). Besides, TGW6, BG1, GL3.3/TGW3/qTGW3, GSA1, and RBG1 are involved in the auxins signaling pathway (Dong et al. 2020; Hu et al. 2018; Ishimaru et al. 2013; Liu et al. 2015b; Lo et al. 2020; Xia et al. 2018; Ying et al. 2018). GNP1 encodes GA20ox1, increased grain number and yield by increasing cytokinin activity. GW6 encodes a GA-regulated GAST family protein, positively regulates grain width and weight through the gibberellins pathway (Shi et al. 2020; Wu et al. 2016). Additionally, many other major QTLs regulate grain size and weight through the transcriptional levels, such as GW8, GL7/GW7, GW6a, GLW7, GL4, OsLG3, GS9, GL6, TGW2, and SG3 (Li et al. 2020; Ruan et al. 2020; Si et al. 2016; Song et al. 2015; Wang et al. 2012; Wang et al. 2015a; Wang et al. 2015b; Wang et al. 2019a; Wu et al. 2017; Xiong et al. 2018; Yu et al. 2017; Zhao et al. 2018).

Small-effect QTLs also play important roles in regulating grain size and are widely utilized in commercial rice varieties (Kinoshita et al. 2017). Many QTLs with small-effect are responsible for quantitative genetic variation, these QTLs are often unexpected based on prior knowledge of the trait or correspond to computationally predicted genes (Mackay et al. 2009). Therefore, it is beneficial to validate these small-effect QTLs for breeding. In recent years, more than 400 small-effect QTLs for grain size and weight were reported (Huang et al. 2013). However, only a few were
fine-mapped or cloned. *DTH2* encodes a CONSTANS-like protein that promotes heading by inducing the florigen genes *Hd3a* and *RFLT1* (Wu et al. 2013). *qTGW1.2b* regulates grain weight through encodes a VQ-motif protein OsVQ4 (Chan et al. 2020). A naturally varying QTL, *qTGW12a*, which encodes the multidrug and toxic compound extrusion (MATE) transporter, regulates grain weight in rice (Du et al. 2021).

The residual heterozygous method (Du et al. 2008) was mainly used for QTL mapping in this study. Residual heterozygote, which shows heterozygosity of the target region and high homozygosity in the background. The progeny population obtained by selfing is equal to the natural near-isogenic line (NIL)-F2 population, which applies to validating, resolving, and fine mapping of QTL. To date, a series of small-effect QTLs have been fine mapped using this method (Dong et al. 2018; Wang et al. 2019b; Zhang et al. 2020; Zhu et al. 2019).

In a previous study, a grain size QTL, *qGS7.1* has been identified on chromosome 7 (Xue et al. 2019). Then, *qGS7.1* was dissected into two QTLs, named *qTGW7.1* and *qTGW7.2*. In the present study, we aimed to fine map the *qTGW7.2* using a set of backcross recombinant inbred lines between BG1 (Big Grain 1) and XLJ (Xiaolijing). Two independent QTLs named *qTGW7.2a* and *qTGW7.2b* regulate grain size were genetically dissected in the target region. Finally, *qTGW7.2a* was located into a 21.10-kb region controlling grain width and weight, while *qTGW7.2b* was mapped to a 52.71-kb interval affecting grain length and width, not the grain weight.

**Materials and methods**

**Plant materials**

Five runs and a total of 23 residual heterozygous populations were used to map the target QTL in this study. The populations were derived from two BC4F6 individuals from the cross of XLJ///XLJ///XLJ///XLJ///XLJ//BG1(Fig.S1).

In the first run, two single plants with heterozygous regions of *qGS7.1* were selected and developed two BC4F7 populations consisting of 137 plants (R7) and 142 plants (R8) used for QTL validation and mapping. New polymorphic markers were designed and used to test genotypes of these populations.

In the second run, six resultant BC4F8 populations, R9 to R14, consisting of 189, 193, 198, 151, 116, and 213 plants respectively were developed from six residual heterozygous BC4F7 single plants with updated target regions. Then, the BC4F9 population contains 3989 individuals derived from the R9 population was constructed and used for selecting recombinants.

In the third run for QTL validation and mapping, eleven single plants were selected from the BC4F9 generation to develop eleven BC4F10 populations, R15 to R25, totally consisting of 794 plants.

In the fourth run, three NIL populations with homozygous in the segregating region, namely N1 to N3, were developed to validate the QTL. Two single plants
without qTGW7.2b target region were selected and selfed to develop populations named R26 and R27, made up of 209 and 223 plants, respectively. Meanwhile, a BC4F11 population including 6128 individuals derived from the R23 population was constructed and used for further mapping.

In the fifth run, two single plants were selected from BC4F11 plants in the XP7-12-XP7-23 interval to develop progeny populations consisting of 233 (R28) and 98 plants (R29) for validation and fine-mapping of qTGW7.2a.

Field experiments and traits measurement

Plants were grown at the field stations of the China National Rice Research Institute in Lingshui, Hainan province, and Fuyang, Zhejiang province. After harvesting, 300 dry seeds were randomly selected for measuring thousand-grain weight (TGW, g), grain length (GL, mm), grain width (GW, mm), and the ratio of grain length to width (RLW) using an automatic seed counting and analyzing instrument (Model SC-G, Wanshen Ltd., Hangzhou, China).

DNA extraction and molecular markers development

Total DNA was extracted from fresh leaf samples by the CTAB method (Murray and Thompson 1980). The PCR products were visualized on 8% non-denaturing polyacrylamide gels by silver staining. A total of 31 polymorphic DNA markers were used (Table S1).

RNA extraction and qRT-PCR

Total RNA was extracted from rice panicles using RNAprep pure Plant Kit (TIANGEN). Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) was performed using SYBR Premix Ex Taq II (TAKARA). Data analysis used the 2−ΔΔCt method and the UBQ10 was used as the internal reference to normalize the gene expression (Livak and Schmittgen 2001). The qRT-PCR primers used in this study are listed in Table S1.

Cytological observation

During the heading stage, young spikelet hulls of NIL-qTGW7.2aBG1 and NIL-qTGW7.2aXL2 were fixed in 2.5% glutaraldehyde for 12 hours at 4°C and then dehydrated in serial graded ethanol (30%, 50%, 70%, 80%, 90%, 95%, and 100%), and last preserved in 100% ethanol. The samples were dried in a Hitachi HCP-2 critical point drier, and cell length and width of the inner glumes were observed by scanning electron microscopy (Hitachi SU-8010). ImageJ software was used to measure cell numbers and cell size.

Data analysis
Three genotypes could obtain after genotyping this population. Two homozygous genotype plants which carried alleles from XLJ and BG1 were used to detect the phenotypic differences by student’s t-test. We deduce there was a QTL when \( p < 0.05 \). Subsequently, the heterozygous individual harboring target QTL was used for developing a new residual heterozygous population.

All of the analysis data, including the additive effect (\( A \)) and the proportion of phenotypic variance explained by the QTL (\( R^2 \)) were obtained from the Windows QTL Cartographer Version 2.5 software to estimate the genetic effects.

**Results**

**Validation and mapping of \( qGS7.1 \)**

We have identified a grain size QTL, \( qGS7.1 \), in the X7-9-RM351 interval on chromosome 7 (Fig. 1a). To narrow down the target region, 12 polymorphic markers were designed based on the sequence differences between BG1 and XLJ. RM21758 became the new boundary when all plants were homozygous for it. R7 and R8 populations were derived from two segregated single plants selected from the R6 population (Fig. 1b) to validate \( qGS7.1 \) and exclude the non-target interval. Both were showed significant enhancement of GW, GL, and TGW from XLJ alleles. In the R7 population, the additive effects were -0.445g for TGW, -0.082mm for GL, and -0.011mm for GW, explaining 21.95%, 30.35%, and 8.31% of the phenotypic variance, respectively. In the R8 population, the additive effects were -0.439g for TGW, -0.075mm for GL, and -0.010mm for GW, having \( R^2 \) of 28.01%, 25.40%, and 8.91%, respectively (Table 1). The effects detected in the two populations were comparable indicated that \( qGS7.1 \) was located in the region between RM21758 and Chr07MM3011.

**Dissection of \( qGS7.1 \) into three QTLs controlling grain size**

To validate and narrow down the update region, six progeny populations with sequential segregating regions jointly covering the entire QTL region (Fig. 1c) were developed. In the R9, R10, and R11 populations, significant enhancements were discovered in XLJ alleles for TGW, GW, and GL. The additive effects for TGW were -0.477g, -0.670g and -0.531g, for GL were -0.051mm, -0.114mm and -0.063mm, for GW were -0.014mm, -0.029mm and -0.021mm, respectively. The additive effects for TGW in R10 and R11 populations were higher than that in the R9 population. Meanwhile, significant genotypic variances were detected for TGW and GW in the R13 and R14 populations that enhancing alleles derived from XLJ. The additive effects for TGW in R13 and R14 populations were -0.265g and -0.180g, for GW were -0.031mm and -0.018mm, respectively. There were no significant differences in the R12 population (Table 2).

The above results showed that \( qGS7.1 \) was a composite of two independent QTLs (Fig. 1c). The first QTL, named \( qTGW7.1 \), explaining 11.19% and 4.06% of the phenotypic variance for TGW, 11.28% and 7.25% for GW in the R13 and R14 populations, respectively. The second QTL, named \( qTGW7.2 \), with the \( R^2 \) values of
32.37%, 26.54% and 21.55% for TGW, 18.92%, 14.27% and 13.96% for GL, 7.63%, 12.63% and 11.95% for GW in the R9-R11 populations, respectively.

qTGW7.2 was selected for further analysis. Eleven populations (R15-R25) were developed from 11 heterozygous individuals in BC4F9 populations (Fig. 1d). In the R15 population, significant genotypic effects were detected in TGW and GW with the positive allele from XLJ. The additive effects for TGW and GW in the R15 population were -0.604g and -0.034mm, with the R² values of 42.48% and 49.12%. There were no significant differences in R16, R17, R18, R19, and R20 populations. Similarly, significant genotypic variances were detected for GL and RLW in R21 and R22 populations. The additive effects for GL were -0.050mm and -0.053mm, explaining 8.32% and 20.81% of the genotypic variance in both populations. For RLW, the additive effects were -0.030 and -0.018, having R² of 19.61% and 6.62%, respectively; in R23, R24, and R25 populations, significant genotypic effects were showed for TGW, GL, and GW. The additive effects for TGW were -0.558g, -0.505g and -0.504g, for GL were -0.087mm, -0.065mm and -0.095mm, for GW were -0.011mm, -0.020mm and -0.020mm, respectively (Table 3).

To sum up, qTGW7.2 was dissected into two separate QTLs (Fig. 1d). The first QTL, qTGW7.2a had considerable effects on TGW and GW within a 53.96-kb region spanning XP7-12 to XP7-16. The second QTL, qTGW7.2b, was located between Chr07MM2985 and RM21891, a 52.71-kb interval and affected GL and RLW but had little effect on TGW.

Three NIL populations (N1-N3) derived from the R15, R16, and R21 populations were developed to validate the function of qTGW7.2a and qTGW7.2b (Table S2); meanwhile, two progeny populations, R26 and R27, derived from two recombinants containing qTGW7.2a only were used in this study (Fig. S2). In the N1 population, significant genotypic effects were showed for TGW and GW, the additive effects for TGW and GW were -0.421g and -0.021mm, explaining 31.59% and 40.05% of phenotypic variance, respectively, and which was coincident with R26 and R27 populations. However, in the N3 population, highly significant genotypic effects were detected for GL, GW, and RLW, the additive effects were -0.070 mm, 0.020 mm, and -0.051, explaining 25.78%, 34.30%, and 54.26% of phenotypic variance, respectively, and which was not coincident with the results of the R21 population. There was no significant difference in the N2 population (Table S2).

qTGW7.2a mainly controls TGW through regulating GW, with enhancing alleles derived from XLJ. qTGW7.2b simultaneously affected GL and GW in opposite ways with no significant effect on TGW (Fig. S3). The former was selected for further analysis for the stable function and considerable effect.

Fine-mapping qTGW7.2a into a 21.10-kb region

For further mapping of qTGW7.2a, we constructed a BC4F11 population consisting of 6128 individuals. Two recombinants in the RM21871-XP7-23 interval were utilized to develop two progeny populations, R28 and R29. Highly significant phenotypic effects were detected in TGW and GW in the R28 population. The additive effects were
-0.213g and -0.013mm, having $R^2$ of 11.29% and 9.70%, respectively. There were no significant differences in the R29 population (Table 4). According to the mapping results of the BC4F12 population, we mapped $qTGW7.2a$ to the 21.10-kb interval between Chr07MM2954 and XP7-16 (Fig. 1e). $qTGW7.2a$ increased TGW and GW with the allele from XLI as compared grain size and weight between NIL-$qTGW7.2a^{XLJ}$ and NIL-$qTGW7.2a^{BG1}$ (Fig. 2), was same as the R28 population.

Grain size is restricted by the size of the spikelet hull in rice, which is determined by both cell proliferation and expansion. Therefore, we compared the cell number and cell size of the outer glume epidermal cells between NIL-$qTGW7.2a^{XLJ}$ and NIL-$qTGW7.2a^{BG1}$ (Fig. 3a). There was no significant difference in cell number or cell length between NIL-$qTGW7.2a^{XLJ}$ and NIL-$qTGW7.2a^{BG1}$ (Fig. 3b, c). However, the cell width of NIL-$qTGW7.2a^{XLJ}$ was greater than NIL-$qTGW7.2a^{BG1}$ (Fig. 3d). These findings suggest that the grain size increase in NIL-$qTGW7.2a^{XLJ}$ is predominantly due to cell width expansion.

### Candidate genes of $qTGW7.2a$

There are four ORFs located in the region spanning $qTGW7.2a$. $LOC_{Os07g39470}$ encodes a rice GRAS family protein, CIGR2, which suppresses cell death in rice inoculated with rice blast via activation of a Heat Shock Transcription Factor, OsHsf23 (Tanabe et al. 2016). $LOC_{Os07g39480}$ encodes WRKY78, a transcriptional factor that is involved in regulating plant height and seed size (Zhang et al. 2011). $LOC_{Os07g39490}$ and $LOC_{Os07g39500}$ are unknown functional proteins (Table S3).

Sequences of the coding domain sequence (CDS) in four genes between the NIL-$qTGW7.2a^{XLJ}$ and NIL-$qTGW7.2a^{BG1}$ were compared (Fig. 4a). Two synonymous SNPs were detected in $LOC_{Os07g39470}$, indicating that there were no differences between the two alleles. For $LOC_{Os07g39480}$, there were four polymorphism sites, three of which were synonymous and one 3-bp deletion in the XLI allele, resulting in a serine deletion. For $LOC_{Os07g39490}$, three SNPs include one synonymous and two non-synonymous resulting in two amino acids substituted; especially, a 2-bp deletion in NIL-$qTGW7.2a^{BG1}$ resulting in NIL-$qTGW7.2a^{BG1}$ producing an alternatively spliced protein, in which the terminal 62 residues were truncated. Finally, there were 18 SNP variations in $LOC_{Os07g39500}$ between two NILs, including thirteen non-synonymous mutations and a premature stop codon at T784C in the BG1 allele. These results suggest that either $LOC_{Os07g39490}$ or $LOC_{Os07g39500}$ is the candidate gene for $qTGW7.2a$.

Subsequently, the expression levels of four candidates in panicles of NIL-$qTGW7.2a^{XLJ}$ and NIL-$qTGW7.2a^{BG1}$ were analyzed (Fig. 4b). The expression levels of $LOC_{Os07g39490}$ and $LOC_{Os07g39500}$ were significantly higher in NIL-$qTGW7.2a^{XLJ}$ than that in NIL-$qTGW7.2a^{BG1}$, while there were no significant differences in $LOC_{Os07g39470}$ and $LOC_{Os07g39480}$. These results repeatedly indicated that the candidate gene of $qTGW7.2a$ was more likely to be either $LOC_{Os07g39490}$ or $LOC_{Os07g39500}$.
Discussion

Remarkable progress has been achieved by the discovery of large-effect QTLs affecting yield and quality in recent years, however, rarely small-effect QTLs have been cloned in rice (Chan et al. 2020; Du et al. 2021). In our study, two small-effect QTLs regulating grain size were identified and fine mapped. \( q_{TGW7.2a} \) was limited between Chr07MM2954 and XP7-16 with a 21.10-kb interval, affecting grain width and weight. \( q_{TGW7.2b} \) inversely affects the ratio of grain length to width was mapped into the 52.71-kb region between Chr07MM2985 and RM21891.

All the populations used in this study were derived from a single plant with the same background and were cultivated in Fuyang and Lingshui followed the chronological order. \( q_{TGW7.2a} \) could be detected in both environments, but the effects on TGW and GW were not stable. The additive effects on TGW and GW increased by XLJ allele were in the range of -0.604 to -0.213g, and -0.034 to -0.013mm, respectively (Table 3, 4 and Table S2). Especially for \( q_{TGW7.2b} \), in R21 and R22 populations, \( q_{TGW7.2b} \) regulates grain length, has little influence on grain width and grain weight, but in the N3 population, \( q_{TGW7.2b} \) was detected affecting grain length and grain width with opposite allelic directions and had little effect on grain weight (Table 3 and Table S2). These results suggested that small-effect QTLs could be steadily detected using the residual heterozygous method, but the effects of QTL could be affected by environmental interaction.

In the present study, four annotated genes were found in the 21.1-kb interval covering \( q_{TGW7.2a} \). Firstly, \( LOC\_Os07g39470 \) encodes CIGR2 belonging to the rice GRAS family, and members of this family encode transcriptional regulators with functions in a wide range of signaling mechanisms such as growth and development, hormone signaling, and plant defense (Tanabe et al. 2016). However, there were only two synonymous SNPs between the \( CIGR2 \) alleles. Secondly, \( LOC\_Os07g39480 \) encodes a transcriptional factor, WRKY78, which was involved in regulating plant height and seed size. Knocking-down of \( WRKY78 \) led to a semi-dwarf and small seed phenotype by reducing cell length (Zhang et al. 2011). However, except for three SNPs showing synonymous mutation, there was just one serine deletion in the CDS of NIL-\( q_{TGW7.2a}^{XLJ} \). The expression level of \( WRKY78 \) was comparable between the two NILs. These results suggest that \( CIGR2 \) and \( WRKY78 \) may not be candidate genes for \( q_{TGW7.2a} \). Previous studies showed that introducing a premature stop codon and preventing transcription of a mature protein influencing grain size, such as \( GW2 \), \( GS3 \), \( qLGY3/GW3p6 \), \( WTG1 \), \( OsMAPK6 \), \( TGW6 \), and \( GL6 \) (Fan et al. 2006; Huang et al. 2017; Ishimaru et al. 2013; Liu et al. 2015a; Liu et al. 2018; Song et al. 2007; Wang et al. 2019a; Wang et al. 2019c). In our study, \( LOC\_Os07g39490 \) and \( LOC\_Os07g39500 \) encode hypothetical proteins. In its coding region, a non-synonymous mutation existed as a premature stop codon and preventing transcription of a mature protein in NIL-\( q_{TGW7.2a}^{BG1} \). Therefore, more studies in gene editing such as CRISPR/Cas9-targeted mutagenesis and gene overexpression need to be done to confirm the gene for \( q_{TGW7.2a} \).

Among QTLs with large effect, \( GW2 \), \( GS5 \), \( GW5/GSE5 \), and \( GW6 \), those regulate
grain weight through controlling grain width (Duan et al. 2017; Li et al. 2011; Liu et al. 2017; Shi et al. 2020; Song et al. 2007; Xu et al. 2015). In our study, \( q_{TGW7.2a} \) increased grain width and weight. These suggest that \( q_{TGW7.2a} \) could be used for yield improvement. For large-effect QTLs such as \( GL7/GW7, GW8, \) and \( GS9 \), those have similar effects with \( q_{TGW7.2b} \) on grain length and width that regulate grain size (Wang et al. 2012; Wang et al. 2015a; Wang et al. 2015b; Zhao et al. 2018). \( q_{TGW7.2b} \) in the XLJ allele increased grain length but decreased grain width, which has little effect on grain weight indicated that \( q_{TGW7.2b} \) could be used to fine-tune grain size.

**Conclusion**

Two small-effect QTLs for grain size and grain shape, \( q_{TGW7.2a} \) and \( q_{TGW7.2b} \), were fine-mapped in this study. \( q_{TGW7.2a} \) was limited to a 21.10-kb region containing four genes. This QTL regulates grain width and weight, which has potential for yield improvement. \( q_{TGW7.2b} \) which inversely regulates grain length and width was within a 52.71-kb interval. This QTL has potential for fine-tuning grain shape and grain appearance. These results provide a basis for QTL cloning and offer new resources for yield and quality improvement.

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**Author contribution statement**

PX performed most of the experiments, analyzed the data, and wrote the manuscript; YYC and contributed to sequencing and constructed the populations; XXW, BFW, QQY analyzed the data collection the phenotypes, and revised this manuscript; KG and YWK conducted the field trials; LPS, PY, LYC, YXZ, XDZ, and SHC designed the experiments, supervised and completed the writing, and reviewed the manuscript. All authors read and approved the final manuscript.

**Conflict of interest**

The authors declare no conflict of interests regarding the publication of this paper. All authors have read the manuscript carefully.

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Figures

Figure 1

Genotypic compositions of residual heterozygous populations in the target regions. a Composition of R1 population in previous study. b Two residual heterozygous populations in BC4F7. c Six residual
heterozygous populations in BC4F8. d Eleven residual heterozygous populations in BC4F10. e Two residual heterozygous populations in BC4F12.

Figure 2

qTGW7.2a regulates grain weight and width. a Grain phenotypes of rice NIL plants. Bar, 1 cm. b Comparison of thousand grain weight. c Comparison of grain length. d Comparison of grain width. e Comparison of the ratio of grain length to width. Data are given as mean ± SD. Student’s t-test was used to generate P value.
Scanning electron microscopic observation and analysis of the glume. a Scanning electron micrograph of the outer glume epidermal cells between NIL-qTGW7.2a XLJ and NIL-qTGW7.2a BG1. Bar, 100 μm. b Cell number of outer epidermal cells. c Cell length of outer epidermal cells. d Cell width of outer epidermal cells. Data are given as mean ± SD. Student’s t-test was used to generate P value; *, P < 0.05; **, P < 0.01.
The coding domain sequence alignment and transcript levels of annotated genes between NIL-qTGW7.2aXLJ and NIL-qTGW7.2aBG1. a The red words represent variants, the red frame represent premature stop, +, the variant sites in the coding domain sequence. Bar, 200bp. b The experiment was performed using panicles of $1 \leq P < 3$ cm (P3) and $5 \leq P < 8$ cm (P8) collected from NIL-qTGW7.2aXLJ and NIL-qTGW7.2aBG1. Data are given as mean ± SD. Student’s t-test was used to generate P value; *, $P < 0.05$; **, $P < 0.01$.

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