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# Identification of *qGL4.1* and *qGL4.2*, two closely linked QTL controlling grain length in rice

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## Abstract

Grain size is an important appearance quality trait in rice, which also affects grain yield. In this study, a recombinant inbred line (RIL) population derived from a cross between *indica* variety 9311 and *japonica* variety Cypress was constructed. And 181 out of 600 RILs were sequenced, and a high-density genetic map containing 2842 bin markers was constructed, with a total map length of 1500.6 cM. A total of 10 quantitative trait loci (QTL) related to grain length (GL), grain width (GW), grain length-to-width ratio (LWR), and 1000-grain weight (TGW) were detected under two environments. The genetic effect of *qGL4*, a minor QTL for GL and TGW, was validated using three heterogeneous inbred family (HIF) segregation populations. It was further dissected into two closed linked QTL, *qGL4.1* and *qGL4.2*. By progeny testing, *qGL4.1* and *qGL4.2* were successfully delimited to intervals of 1304-kb and 423-kb, respectively. Our results lay the foundation for the map-based cloning of *qGL4.1* and *qGL4.2* and provide new gene resources for the improvement of grain yield and quality in rice.

**Keywords** Rice · RIL · Grain size · QTL · HIF · *qGL4.1* · *qGL4.2*

## Introduction

Rice is one of the most important food crops and provides energy for about half of the world's population. Grain weight, a major component of rice yield, is directly influenced by grain size/shape, which can be further divided by grain length (GL), grain width (GW), grain thickness (GT), and length-to-width ratio (LWR) (Xing and

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Zhang 2010). Grain size is also an important appearance quality. Rice with good appearance quality will be accepted by more consumers and increase its market value.

Lots of previous studies demonstrated that grain size is a complex quantitative trait regulated by many quantitative trait loci (QTL). Till now, large numbers of QTL for grain size have been mapped, and some major QTL have been successfully cloned and functionally characterized. These genes are involved in multiple pathways in regulating spikelet hull cell proliferation and/or expansion and then influencing grain size. Among those, G protein signaling plays a role in controlling grain size. Such as GS3 and DEP1, which both encode G protein γ-subunits and antagonistically regulate grain size (Fan et al. 2006; Huang et al. 2009b; Sun et al. 2018; Takano-Kai et al. 2009). The ubiquitin–proteasome pathway is also involved in grain size control. Both *GW2* and *CLG1* encode E3 ubiquitin ligase and oppositely regulate grain size (Song et al. 2007; Yang et al. 2021). Phytohormone biosynthesis and signaling-related genes modulate grain size by controlling cell expansion and proliferation. *GS5*, *GW5*/*GSE5*, *qGL3*/*qGL3.1*, and *GS9* all affect the BR signaling and consequently regulate grain size (Hu et al. 2012; Li et al. 2011; Liu et al. 2017; Qi et al. 2012; Shomura et al. 2008; Weng et al. 2008; Zhang et al. 2012; Zhao et al. 2018). *BG1* and *TGW6* affect grain size through the auxin signaling pathway (Ishimaru et al. 2013; Liu et al. 2015). *GW6* regulates GA responses and biosynthesis and positively regulates grain width (Shi et al. 2020). Besides, several transcription factors have been shown to regulate grain size, such as the SQUAMOSA promotor binding protein-like (SPL) family members: *GL7*/*GLW7*/*OsSPL13*, *GW8*/*OsSPL16*, and *OsSPL18* (Si et al. 2016; Wang et al. 2012, 2015b, 2015c; Yuan et al. 2019; Zhou et al. 2015), and *GS2*/*GL2*, which encodes the plant-specific transcription factor OsGRF4 (Che et al. 2015; Duan et al. 2015; Hu et al. 2015).

In this study, QTL mapping of rice grain size-related traits was conducted in 2 years using a RIL population derived from variety 9311 and Cypress. And 10 QTL affecting grain size and weight were identified. *qGL4*, a minor QTL for GL and 1000-grain weight (TGW), was repeatedly detected in 2 years. The genetic effect of *qGL4* was confirmed using three HIF-$F_2$ populations. It was further dissected into two closed linked minor QTL, *qGL4.1* and *qGL4.2*. By progeny testing, *qGL4.1* and *qGL4.2* were successfully delimited to intervals of 1304-kb and 423-kb, respectively. The results of this study laid a foundation for cloning of *qGL4.1* and *qGL4.2* and would be helpful for breeding applications.

## Materials and methods

### Population development and field experiments

*Indica* variety 9311 is an elite high-yield restorer line in China, which has reference genome (Gao et al. 2013; Yu et al. 2002). Cypress is a heat-resisting and good grain quality tropical *japonica* variety from the Philippines (Cooper et al. 2008). A RIL population consisting of 600 lines was derived from 9311 and Cypress by single-seed descent method. The parents and RILs of $F_8$ and $F_9$ generations were planted at

the experiment paddy field of the Rice Institute of Anhui Academy of Agricultural Sciences in the summer of 2018 (Hefei, Anhui) and the spring of 2019 (Lingshui, Hainan).

In order to validate the genetic effect of *qGL4*, three independent HIFs of *qGL4* were identified from the RILs. And the HIF-$F_2$ populations were planted at the Lingshui experimental station in the spring of 2020.

To fine-map *qGL4*, progeny testing of 14 and 17 recombinants from HIF1 and HIF2 segregating populations, respectively, were conducted at the Hefei experimental station in the summer of 2020, with each line consisting of 36 individuals.

In order to prove that qGL4 region contained two closely linked minor QTL, 4 HIF-F2 populations (HIF1 qGL4.1, HIF1 qGL4.2, HIF2 qGL4.1, and HIF2 qGL4.2) each with 192 individuals were planted at the Lingshui experimental station in the spring of 2021. And progeny testing of 12 and 13 recombinants of *qGL4.1* and *qGL4.2*, respectively, were conducted at the Hefei experimental station in the summer of 2021.

Seedlings of about 30 days after sowing were transplanted with a single plant spacing of 16.7 cm and 26.6 cm between rows in the field. Field cultivation and management followed normal local practices.

### Trait evaluation

For the RILs, 181 out of 600 lines were randomly selected for phenotyping and genotyping. Nine individuals of each line with a similar field performance were mixed and harvested for measuring grain size and weight. For the HIF-$F_2$ populations and recombinant progeny lines, each plant was genotyped and harvested for phenotyping.

Before phenotyping, harvested seeds were air-dried at room temperature for approximately 3 months to maintain a consistent water balance. At least 300 mature grains were used to measure the GL, GW, LWR, and TGW via a rice yield trait scorer (Yang et al. 2014).

### Genotyping, genetic map construction, and QTL analysis

The 9311 genome sequences were downloaded from the Rice Information System database (http://rice.genomics.org.cn/rice). Genotyping of Cypress and the 181 RILs ($F_8$) was carried out using genotyping-by-sequencing (GBS) technology on the Illumina HiSeq 2500 platform by the Beijing Genomics Institute (BGI). For Cypress, a total of 8.3 Gb of clean data with 19.1 × genome coverage was obtained. And the 181 RILs obtained a total of 53.8 Gb of clean data with an average of 0.7 × of genome coverage. The sequencing data were aligned to the Nipponbare reference genome (MSU v7.0, http://rice.uga.edu/). After SNP calling and filtering, a total of 33,799 high-quality and evenly distributed SNPs were selected to construct the genetic map. All the SNPs were used to identify the bin blocks by the sliding window method (Huang et al. 2009a). And a bin-based genetic linkage map was generated using R/qtl (Broman et al. 2003). QTL analysis was conducted by composite

interval mapping (CIM) method using WinQTLCart2.5 (Wang et al. 2007) with logarithm of odds (LOD) values of 2.5; the QTL intervals were calculated by WinQTLCart2.5 with a confidence interval of 95%.

In the HIF-$F_2$ populations, the genotyping was performed using Kompetitive Allele-Specific PCR (KASP) markers (LGC Group) and InDel markers, which were designed according to the variations between the parents. The genetic maps were constructed by MapMaker/Exp3.0 (Lander et al. 1987). And QTL analysis was performed by WinQTLCart2.5 (Wang et al. 2007).

## Results

### Phenotypic performance of grain size traits

The GL, GW, LWR, and TGW of the parents and 181 RILs were investigated in two environments. Compared with Cypress, 9311 showed larger values in GL, GW, and TGW, but smaller values in LWR (Fig. 1a–d). All these traits in the RIL population showed significant transgressive segregation and followed normal distribution in two years (Fig. 1a–d), indicating that these traits were controlled by multiple genes.

Correlation analysis was conducted for these traits. As expected, TGW was highly positively correlated with GL and GW. LWR displayed a significantly positive correlation with GL and a significantly negative correlation with GW (Table S1).

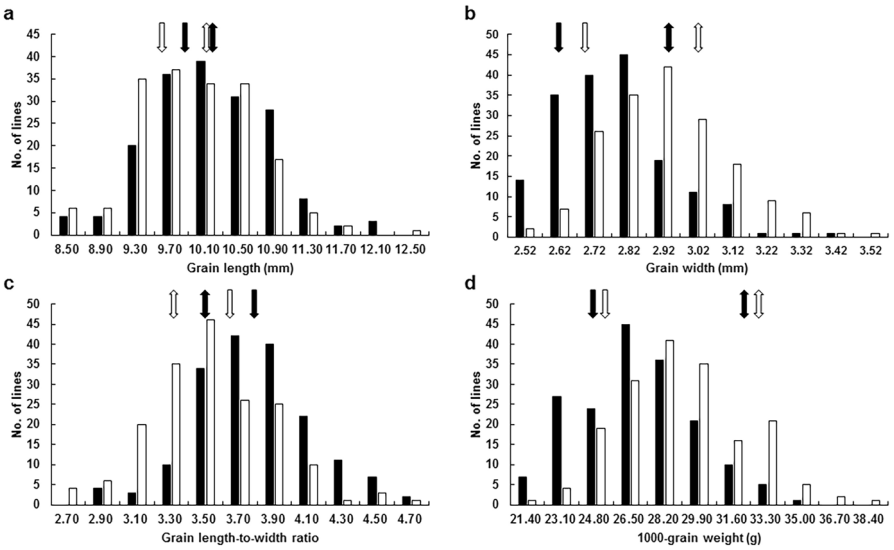


**Fig. 1** Performance of grain size-related traits in the RILs. **a–d** Frequency distribution of grain length, grain width, length–width ratio, and 1000-grain weight. Black bars and arrows, year of 2018. White bars and arrows, year of 2019. Single-headed and double-headed arrows indicate the trait values of Cypress and 9311, respectively

## Construction of genetic linkage map

A total of 33,799 SNPs with polymorphism between 9311 and Cypress were employed to construct the linkage map. We constructed a linkage map that contained 2842 bins for the RIL population with a total length of 1500.6 cM and an average maker density of 0.53 bin per cM in the rice genome (Fig. 2a, Table S2). The chromosome length ranged from 60.2 cM (chromosome 11 with 112 bins) to 230.0 cM (chromosome 1 with 419 bins) (Fig. 2b, Table S2).

## QTL for grain size in the RIL population

In total, ten QTL for grain size and weight were identified on 5 chromosomes in the RIL population in 2 years. The phenotypic variation explained by each QTL ranged from 4.6 to 38.1% (Fig. 2b, Table 1).

For GL, four QTL were identified. And all of them were repeatedly detected in 2 years. A major QTL (*qGL7b*), located between 94.6 and 96.4 cM on chromosome 7, showed a high LOD score of 12.59 and 10.45 and phenotypic variation of 21.2% and 18.0% in 2018 and 2019, respectively. *qGL7a* was a minor QTL located at the end of the short arm of chromosome 7 and explained 5.8% and 6.5% of the phenotypic variation in 2018 and 2019, respectively. *qGL4* had a LOD score of 6.82 in 2018 and 4.25 in 2019 and explained 10.8% and 6.7% of the phenotypic variation, respectively. *qGL5* was located between 122.0 and 125.1 cM of chromosome 5 and contributed 6.8% and 6.6% of phenotypic variation in 2018 and 2019, respectively. *qGL5* and *qGL7b* showed negative additive effects, indicating that alleles from Cypress increased GL, while *qGL4* and *qGL7a* had positive additive effects, indicating that alleles from 9311 enhanced GL.

Three QTL for GW were detected, of which two were repeatedly detected in both years. The major QTL *qGW7* had a LOD score of 10.66 and 15.44, an additive effect of 0.08 mm and 0.11 mm, and explained 21.1% and 31.0% of the phenotypic variation in 2018 and 2019, respectively. And the 9311 allele of *qGW7* enhanced GW. *qGW2*, a minor QTL, explained 4.6% and 5.3% of the phenotypic variation in 2018 and 2019, respectively. *qGW3* was only identified in 2018 with a LOD score of 2.54.

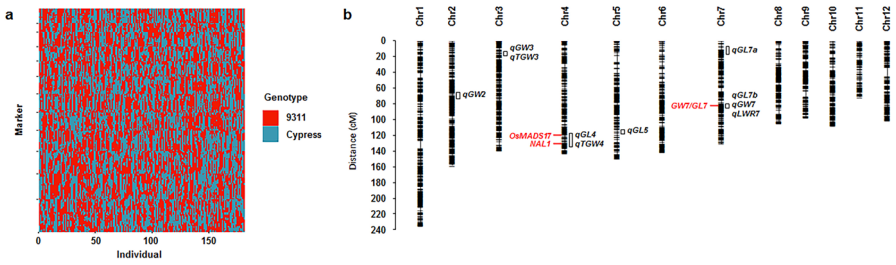


**Fig. 2** Bin map, genetic linkage map, and QTL of grain size-related traits in the RILs. **a** Bin map of 181 RILs. The *x* axis shows the 181 lines. The *y* axis shows the bins along the chromosomes (from bottom to top, chromosome 1 to chromosome 12). **b** Genetic linkage map and QTL of grain size-related traits. Red font, known genes. Black font, QTL detected in this study

**Table 1** QTL for grain size and weight detected in the RIL population derived from the cross between 9311 and Cypress

| Trait | QTL | Interval (cM) | Position | 2018 Hefei | | | 2019 Lingshui | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | LOD | A | V (%) | LOD | A | V (%) |
| GL | *qGL4* | 126.4–137.2 | Chr4 30,846,814–33571994 | 6.82 | 0.23 | 10.8 | 4.25 | 0.18 | 6.7 |
| (mm) | *qGL5* | 122.0–125.1 | Chr5 25,580,666–26969410 | 5.04 | −0.18 | 6.8 | 4.17 | −0.19 | 6.6 |
| | *qGL7a* | 2.0–9.1 | Chr7 2,760,544–5133687 | 3.66 | 0.17 | 5.8 | 4.00 | 0.18 | 6.5 |
| | *qGL7b* | 94.6–96.4 | Chr7 24,559,911–24,800,672 | 12.59 | −0.33 | 21.2 | 10.45 | −0.31 | 18.0 |
| GW | *qGW2* | 66.5–70.5 | Chr2 12,689,979–19,786,073 | 2.63 | −0.04 | 4.6 | 3.09 | −0.05 | 5.3 |
| (mm) | *qGW3* | 16.2–18.5 | Chr3 3,535,224–3,833,034 | 2.54 | 0.04 | 4.6 | | | |
| | *qGW7* | 94.6–96.4 | Chr7 24,559,911–24,800,672 | 10.66 | 0.08 | 21.1 | 15.44 | 0.11 | 31.0 |
| LWR | *qLWR7* | 94.6–96.4 | Chr7 24,559,911–24,800,672 | 20.35 | −0.22 | 38.1 | 19.68 | −0.22 | 35.5 |
| TGW | *qTGW3* | 16.2–18.5 | Chr3 3,535,224–3,833,034 | | | | 2.75 | 0.73 | 4.8 |
| (g) | *qTGW4* | 126.4–137.2 | Chr4 30,846,814–33571994 | 7.80 | 1.40 | 23.1 | 8.24 | 1.29 | 15.5 |

*QTL*, quantitative trait locus; *GL*, grain length; *GW*, grain width; *LWR*, grain length-to-width ratio; *TGW* , 1000-grain weight; *LOD*, logarithm of odds; *A*, additive effect (the positive value means that 9311 allele increases the trait value); *V*, phenotypic variation explained by the QTL

*qLWR7* was the only QTL controlling LWR. It shared the same interval with the major QTL *qGL7b* and *qGW7*. *qLWR7* had a LOD score of 20.35 and 19.68 and explained 38.1% and 35.5% of the phenotypic variation in 2018 and 2019, respectively. And the Cypress allele of *qGW7* increased LWR.

For TGW, two QTL were identified. *qTGW4* was repeatedly detected in 2 years. It had an additive effect of 1.40 g in 2018 and 1.29 g in 2019 and explained 23.1% and 15.5% of the phenotypic variation, respectively. *qTGW4* shared the same interval with the GL QTL *qGL4*, and the 9311 alleles enhanced trait values. *qTGW3* was only identified in 2019; nevertheless, it shared the same interval with the GW QTL *qGW3*.

The 94.6–96.4 cM interval on chromosome 7 had significant effects on GL, GW, and LWR in 2 years. The cloned gene *GW7*/*GL7* was located to the interval of this major QTL (Wang et al. 2015b, 2015c; Zhou et al. 2015) (Fig. 2b). Three novel minor QTL for GL, *qGL4*, *qGL5*, and *qGL7a*, were identified in this study. And *qGL4* co-located to the interval of grain weight QTL, *qTGW4*. Therefore, *qGL4* was selected for further validation and fine mapping.

### Validation of *qGL4* in HIFs

Based on the results of QTL mapping, three HIFs (HIF1, HIF2, and HIF3) of *qGL4* were identified. HIF1 came from the 181 RILs for preliminary mapping, and HIF2 and HIF3 were identified from the rest 419 RILs. HIF1 and HIF2 were heterozygous in the primary mapping region of *qGL4* (chromosome 4, M350-M363), while HIF3 was heterozygous from marker M350 to K4-4 and 9311 homozygous from marker K4-5 to M363 (Fig. 3a, Table S3). HIF1, HIF2, and HIF3 segregating populations consisted of 176, 173, and 166 individuals, respectively, were planted to validate the genetic effect of *qGL4*. The effects of *qGL4* in HIF1 and HIF2 populations were similar, explained 34.2–56.1% of the TGW and GL variations, with the 9311 allele increasing TGW and GL by 0.87 g and 0.75 g, and 0.23 mm and 0.28 mm, respectively. In HIF3, the additive effects of *qGL4* were 0.59 g for TGW and 0.12 mm for GL, respectively, which were much lower than those in HIF1 and HIF2 (Fig. 3b–d, Table 2). The varied effects were supposed to be caused by the differences in genetic backgrounds. No QTL for GW was detected in HIF1, HIF2, and HIF3 populations (Table 2), indicating that *qGL4* influenced TGW by controlling GL. The frequency distribution of GL and TGW in HIF1 and HIF2 segregating populations were nearly bimodal, and GL and TGW of heterozygous plants were between those of Cypress and 9311 homozygous, indicating that a semidominant allele of *qGL4* from 9311 controlled GL and TGW (Fig. 3e–h, Table 2).

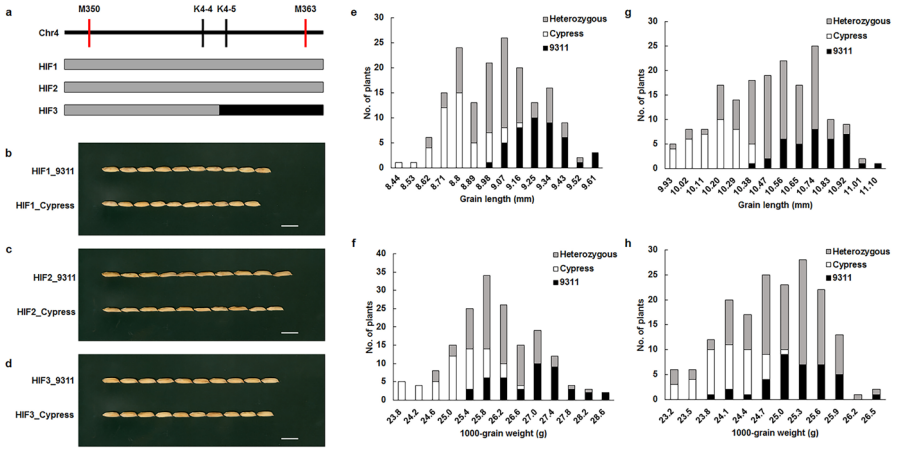


**Fig. 3** Validation of *qGL4* in three HIF-$F_2$ populations. **a** Genotypes of *qGL4* region in the HIFs. Black and gray blocks indicate 9311 homozygous and heterozygous genotypes, respectively. **b–d** Observation of grain length of 9311 and Cypress homozygous plants in the HIFs. Bar = 1 cm. **e**, **f** Frequency distribution of grain length and 1000-grain weight in HIF1-$F_2$ population. **g**, **h** Frequency distribution of grain length and 1000-grain weight in HIF2-$F_2$ population. Black, white, and gray columns indicate 9311 homozygous, Cypress homozygous, and heterozygous genotypes, respectively

**Table 2** QTL effects of *qGL4* detected in the three HIF populations

| | Trait | Interval | LOD | A | D | V (%) |
|---|---|---|---|---|---|---|
| HIF1 | TGW (g) | M350-M363 | 20.56 | 0.87 | 0.10 | 41.8 |
| | GL (mm) | M350-M363 | 26.14 | 0.23 | 0.00 | 50.3 |
| HIF2 | TGW (g) | M350-M363 | 15.70 | 0.75 | 0.52 | 34.2 |
| | GL (mm) | M350-M363 | 29.12 | 0.28 | 0.07 | 56.1 |
| HIF3 | TGW (g) | M350-M363 | 12.88 | 0.59 | 0.19 | 30.3 |
| | GL (mm) | M350-M363 | 15.64 | 0.12 | 0.03 | 42.6 |

*HIF*, heterogeneous inbred family; *GL*, grain length; *TGW*, 1000-grain weight; *LOD*, logarithm of odds; *A*, additive effect (the positive value means that 9311 allele increases the trait value); *D*, dominant effect; *V*, phenotypic variation explained by the QTL

### *qGL4* contained two closely linked QTL, *qGL4.1* and *qGL4.2*

As GL explained higher phenotypic variation than TGW in the HIFs (Table 2), we used GL as the target trait to fine-map *qGL4*. Due to a much lower genetic effect in HIF3, 14 and 17 recombinants from HIF1 and HIF2 segregating populations were selected to conduct fine mapping of *qGL4*. Thirty-six progenies of each recombinant were planted, genotyped, and phenotyped. Student's *t*-test was performed to compare the GL differences between two types of homozygous progenies isolated from the recombinant. If there was a significant difference, the candidate gene was considered to be heterozygous; otherwise, it was considered to be homozygous. Results of progeny testing showed that the GL was segregated in recombinants HIF1-7, HIF1-42, HIF1-131, HIF2-78, and HIF2-172, indicating that *qGL4* was heterozygous, and it should be located in the region downstream of marker M350 (Fig. 4). However, progeny testing of recombinants HIF1-95, HIF2-37, and HIF2-112 also proved that *qGL4* was heterozygous, and it should be located in the region upstream of marker K4-5 (Fig. 4). For each recombinant, the average value of GL of 9311 homozygous progenies was about 0.2 mm longer that of Cypress homozygous progenies (Fig. 4), indicating that the additive effect of the candidate gene on GL was about 0.1 mm, which was the same level in HIF3 (0.12 mm), and about half of those in HIF1 (0.23 mm) and HIF2 (0.28 mm) (Table 2). Moreover, grains of line HIF1-42 (9311 homozygous in the region upstream of marker K4-5) were longer than those of line HIF1-7 (Cypress homozygous in the region upstream of marker K4-5); grains of line HIF2-112 (9311 homozygous in the region downstream of marker M350) were also longer than those of line HIF2-37 (Cypress homozygous in the region downstream of marker M350) (Fig. 4). These results indicated that *qGL4* might contained two closely linked minor QTL, one was located downstream of marker M350 (named *qGL4.1*), and the other was located upstream of marker K4-5 (named *qGL4.2*) (Fig. 4).

In order to separately confirm the genetic effects of *qGL4.1* and *qGL4.2*, HIF1 *qGL4.1*, HIF1 *qGL4.2*, HIF2 *qGL4.1*, and HIF2 *qGL4.2* were developed from HIF1-42, HIF1-95, HIF2-172, and HIF2-112, respectively. In these HIFs, one locus was heterozygous, and the other was homozygous (Fig. 4). And segregating populations of

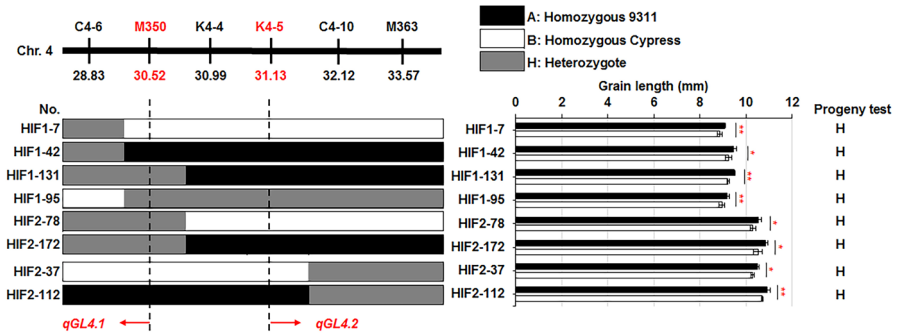


**Fig. 4** Fine mapping of *qGL4*. The progeny test results represented the genotypes reflected by the grain length differences between two types of homozygous progenies isolated from the recombinant; if the *P* value was less than 0.05, the candidate gene was considered to be located in the heterozygous fragment; otherwise, it was considered to be located in the homozygous fragment. HIF, heterogeneous inbred family. Black, white, and grey blocks represent the genotypes of homozygous 9311, homozygous Cypress, and heterozygous genotype, respectively. Data are means ± SD. $^{**}P < 0.01$, $^{*}P < 0.05$, based on Student's two-tailed *t*-test

each HIFs consisting of 192 individuals were planted. A QTL analysis showed that the genetic effects of *qGL4.1* and *qGL4.2* for GL were similar, had an additive effect of 0.10–0.14 mm, and explained 31.3–39.6% of the variations in the HIF segregating populations (Table 3). *qGL4.1* had an additive effect of 0.26 g and 0.43 g for TGW in HIF1 *qGL4.1* and HIF2 *qGL4.1*, respectively. And the additive effects of *qGL4.2* for TGW were 0.74 g and 0.61 g in HIF1 *qGL4.2* and HIF2 *qGL4.2*, respectively (Table 3). For *qGL4.1* and *qGL4.2*, the alleles from 9311 increased both GL and TGW (Table 3). Furthermore, significant differences were found in GL and TGW between the 9311 and Cypress homozygous plants in all the HIF populations, and both *qGL4.1* and *qGL4.2* were semidominant on GL and TGW (Fig. 5a–h, Table 3). These results proved that *qGL4* contained two closely linked minor QTL, *qGL4.1* and *qGL4.2*. And *qGL4.1* was

**Table 3** QTL effects of *qGL4.1* and *qGL4.2* detected in the HIF populations

| Population | Trait | Interval | LOD | A | D | V (%) |
|---|---|---|---|---|---|---|
| HIF1 *qGL4.1* | TGW (g) | C4-6 ~ M350 | 4.72 | 0.26 | 0.14 | 10.7 |
| | GL (mm) | C4-6 ~ M350 | 17.46 | 0.10 | 0.00 | 34.2 |
| HIF1 *qGL4.2* | TGW (g) | K4-5 ~ M363 | 15.89 | 0.74 | 0.13 | 32.4 |
| | GL (mm) | K4-5 ~ M363 | 20.46 | 0.13 | 0.03 | 39.6 |
| HIF2 *qGL4.1* | TGW (g) | C4-6 ~ M350 | 5.83 | 0.43 | 0.04 | 14.64 |
| | GL (mm) | C4-6 ~ M350 | 17.73 | 0.13 | 0.02 | 35.4 |
| HIF2 *qGL4.2* | TGW (g) | K4-5 ~ M363 | 4.65 | 0.61 | 0.30 | 12.1 |
| | GL (mm) | K4-5 ~ M363 | 13.49 | 0.14 | 0.01 | 31.3 |

*HIF*, heterogeneous inbred family; *GL*, grain length; *TGW*, 1000-grain weight; *LOD*, logarithm of odds; *A*, additive effect (the positive value means that 9311 allele increases the trait value); *D*, dominant effect; *V*, phenotypic variation explained by the QTL

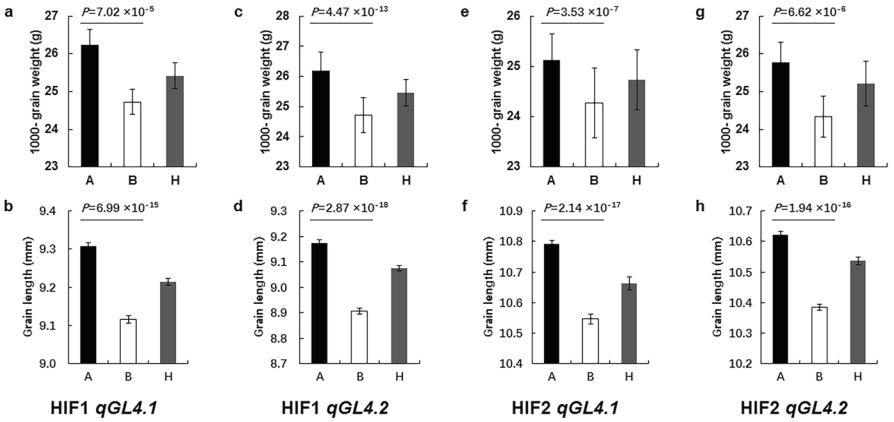


**Fig. 5** Grain size difference among three genotype plants in the HIF-$F_2$ populations of *qGL4.1* and *qGL4.2*. **a**, **b** 1000-grain weight and grain length differences in HIF1 *qGL4.1*. **c**, **d** 1000-grain weight and grain length differences in HIF1 *qGL4.2*. **e**, **f** 1000-grain weight and grain length differences in HIF2 *qGL4.1*. **g**, **h** 1000-grain weight and grain length differences in HIF2 *qGL4.2*. A, B, and H represent 9311 homozygous, Cypress homozygous, and heterozygous genotypes, respectively. Data are means ± SD. *P*, Student's *t*-test

heterozygous, and *qGL4.2* was homozygous in HIF3, which could explain why the genetic effect of *qGL4* in HIF3 was much lower than in HIF1 and HIF2 (Fig. 3a–d, Table 2).

### Fine mapping of *qGL4.1* and *qGL4.2*

To fine-map *qGL4.1*, three new polymorphic markers (C4-1, C4-3, and C4-7) were developed, and the progeny testing of 12 recombinants from HIF1 *qGL4.1* and HIF2 *qGL4.1* segregating populations were conducted. Results of progeny testing showed that recombinants YP27, YP19, and YP22 carried homozygous genotype of *qGL4.1*, proving that *qGL4.1* should be located upstream of marker C4-1. YP26 carried Cypress homozygous, and YP18 carried heterozygous genotype of *qGL4.1*, indicating that *qGL4.1* should be located downstream of C4-7. Therefore, *qGL4.1* should be located in the 1304-kb region flanked by markers C4-1 and C4-7 (Fig. 6a, Table S3).

To fine-map *qGL4.2*, the progeny testing of 13 recombinants from HIF1 *qGL4.2* and HIF2 *qGL4.2* segregating populations were conducted. Genotyping of newly designed polymorphic markers finally narrowed down *qGL4.2* to a 423-kb region between markers C4-17 and C4-10 (Fig. 6b, Table S3).

## Discussion

### QTL for grain size and weight

Rice grain size is a trait of great application value. In the past two decades, significant progress has been achieved in the map-based cloning of QTL for grain size and

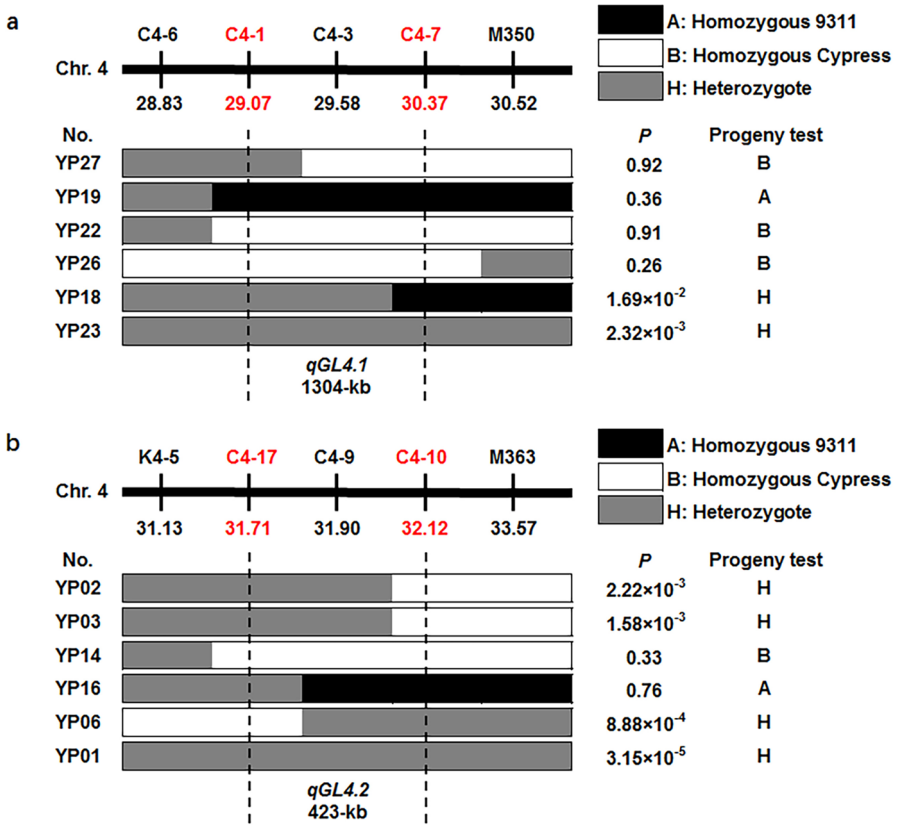


**Fig. 6** Fine mapping of *qGL4.1* and *qGL4.2*. **a** Fine mapping of *qGL4.1*. **b** Fine mapping of *qGL4.2*. The progeny test results represented the genotypes reflected by the grain length differences between two types of homozygous progenies isolated from the recombinant; if the *P* value was less than 0.05, the candidate gene was considered to be heterozygous; otherwise, it was considered to be homozygous. Black, white, and gray blocks represent 9311 homozygous, Cypress homozygous, and heterozygous genotypes, respectively

weight (Fan and Li 2019; Li et al. 2018; Ren et al. 2023). In this study, QTL mapping for grain size and weight was carried out using a RIL population derived from a cross between *indica* variety 9311 and *japonica* variety Cypress. A total of 10 QTL on 5 chromosomes were found to control grain size-related traits, and 8 of them were repeatedly detected in 2 years. Three major QTL, *qGL7b*, *qGW7*, and *qLWR7*, were co-located at the same locus, and the allele from Cypress increased GL and LWR and decreased GW (Table 1). And the cloned *GL7*/*GW7*/*SLG7* was found to be located in this interval (Fig. 2b). Highly-expressed alleles of *GL7*/*GW7*/*SLG7* produce slender grains with low chalkiness. And it has few negative effects on grain yield-related traits (Wang et al. 2015b, 2015c; Zhou et al. 2015). Qiu et al. (2012) identified a QTL (*qSS7*) for GL, GW, and LWR, using a segregating population derived from a cross between an *indica* variety Zhenshan97 and a chromosomal

segment substitution line of a *japonica* variety Cypress within the genetic background of Zhenshan97. And *qSS7* was fine-mapped to a 23-kb region containing the *GL7*/*GW7*/*SLG7* gene. These indicated that *qGL7b*, *qGW7*, and *qLWR7* were *GL7*/*GW7*/*SLG7*. It positively controls grain length and negatively regulates grain width and, as a result, does not influence grain weight (Qiu et al. 2012; Wang et al. 2015b, 2015c; Zhou et al. 2015). In addition, several minor QTL for grain size and weight were found on chromosomes 2, 3, 4, 5, and 7. *qGW2*, *qGL5*, and *qGL7a* only involved grain shape changes without affecting grain weight in primary mapping population (Table 1). *qGL4* had a relatively large effect on GL and TGW, and it was further validated and dissected. The genetic effects of other QTL need to be further verified. It is worth noticing that the GL of 9311 and Cypress were similar (Fig. 1a). However, four QTL for GL were identified, and both the parents carried two QTL that enhanced GL (Table 1).

### Validation of QTL in HIFs

Many traits are controlled by multiple QTL and affected by the environment. In order to verify the genetic effect of QTL and conduct fine-mapping, it is required to produce as isogenic a background as possible to avoid the statistical noise caused by variations in non-critical parts of the genome. Near isogenic lines (NILs) are ideal genetic materials for this purpose. However, their generation is time-consuming, especially for the minor QTL. NIL-$F_2$ populations developed from HIFs of the RIL population are also ideal materials for validating minor QTL (Tuinstra et al. 1997). In this study, a total of 600 RILs were developed. And 181 lines were randomly selected for primary mapping ($F_8$ for genotyping, $F_8$ and $F_9$ for phenotyping). Based on the results of QTL mapping, three HIFs (HIF1, HIF2, and HIF3) of *qGL4* were identified. HIF1 came from the 181 RILs for preliminary mapping, and HIF2 and HIF3 were identified from the rest 419 RILs. The genetic effect of *qGL4* was validated independently using the HIF-$F_2$ populations. We believe developing a relatively large RIL population and conducting primary mapping in appropriately early generations would ensure the identification of HIFs.

### Candidate genes underlying *qGL4.1* and *qGL4.2*

The genetic effect of *qGL4* was confirmed using three HIF-$F_2$ populations. *qGL4* was a stably expressed QTL with relatively large effects on GL and TGW. It was further dissected into two closed linked minor QTL, *qGL4.1* and *qGL4.2*. Clustering distribution of QTL controlling grain size and weight has been frequently reported (Cheng et al. 2021; Guo et al. 2013; Li et al. 2011; Wang et al. 2015a; Zhang et al. 2016). *qGL4.1* was further delimited to the 1304-kb region flanked by markers C4-1 and C4-7 (Chr4, 29.07–30.37 Mb) (Fig. 6a). And *qGL4.2* was narrowed down to a 423-kb region between markers C4-17 and C4-10 (Chr4, 31.71–32.12 Mb) (Fig. 6b).

Rice *NARROW LEAF 1* (*NAL1*) is a key gene regulating leaf size, chlorophyll content, adventitious root formation, grain number per panicle, and grain yield (Fujita et al. 2013; Jiang et al. 2015; Xu et al. 2015; Zhai et al. 2023;

Zhang et al. 2014). *NAL1* (Chr4, 31.20 Mb) is located between *qGL4.1* (Chr4, 29.07–30.37 Mb) and *qGL4.2* (Chr4, 31.71–32.12 Mb) (Fig. 6a, b). It was reported that the grain weight of 9311 was significantly higher than a near isogenic line carrying the *NAL1* region from Nipponbare (Zhang et al. 2014). An integrated genome-wide association study also regarded *NAL1* as the gene that underlay a GL QTL on chromosome 4 (Wei et al. 2021). However, grain weight alterations were not reported in *nal1* mutants, overexpression lines, and knockdown lines (Fujita et al. 2013; Jiang et al. 2015; Xu et al. 2015; Zhai et al. 2023; Zhang et al. 2014). From others and our data, we concluded that *NAL1* was not a QTL for grain size and weight, and the grain weight difference between the NILs reported by Zhang et al. (2014) was possibility caused by the introduction of *qGL4*, but not the closed linked *NAL1*.

A recent study indicated that *OsMADS17* simultaneously increasing grain number, GL, GW, and TGW through decreased translation efficiency caused by a 65-bp deletion in the 5' UTR region (Li et al. 2023). *OsMADS17* (Chr4, 29.31 Mb) was located in the 1304-kb interval of *qGL4.1* (Chr4, 29.07–30.37 Mb) (Fig. 6a). However, the 65-bp deletion in the 5' UTR region of *OsMADS17* was not found in 9311 and Cypress. And *qGL4.1* only affected GL, but not GW, which was different from *OsMADS17* (Li et al. 2023). So, we speculated that *OsMADS17* should not be the functional gene underlying *qGL4.1*.

As the mapping interval of *qGL4.1* was relatively large, we checked the candidate genes of *qGL4.2*. According to the Rice Genome Annotation Project (RGAP) website, 38 expressed genes were identified in the 423-kb region of *qGL4.2*. Based on the gene annotation information and expression pattern, three genes were preferentially selected as the candidates: *LOC_Os04g53310* encodes a soluble starch synthase, *LOC_Os04g53510* encodes an F-box domain containing protein, and *LOC_Os04g53700* encodes a zinc finger protein. Further fine mapping and transgenic studies are required to identify the candidate genes of *qGL4.1* and *qGL4.2*.

## Conclusion

Rice grain size is a key quality and yield trait. In this study, using a RIL population derived from a cross between *indica* variety 9311 and *japonica* variety Cypress, we identified 10 QTL affecting grain size and weight in 2 years. Including a major QTL simultaneously controlled GL, GW, and LWR, and it should be the cloned gene GL7/GW7/SLG7. In addition, several minor QTL that affect grain size and weight were identified. And the genetic effect of *qGL4* was confirmed using three HIF-$F_2$ populations. It was further dissected into two closed linked minor QTL, *qGL4.1* and *qGL4.2*. By progeny testing, *qGL4.1* and *qGL4.2* were successfully delimited to the intervals of C4-1 ~ C4-7 (1304-kb) and C4-17 ~ C4-10 (423-kb), respectively. The closely linked InDel markers, such as C4-1, C4-7, C4-17, and C4-10, could be used in the marker assistant selection of *qGL4.1* and *qGL4.2* to improve rice yield and quality. These findings laid a foundation for map-based cloning of *qGL4.1* and *qGL4.2*.

**Abbreviations** *QTL*: Quantitative trait loci; *RIL*: Recombinant inbred line; *GL*: Grain length; *GW*: Grain width; *LWR*: Grain length-to-width ratio; *TGW*: 1000-Grain weight; *SNP*: Single nucleotide polymorphism; *GBS*: Genotyping-by-sequencing; *HIF*: Heterogeneous inbred family; *KASP*: Kompetitive allele-specific PCR; *LOD*: Logarithm of odds

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**Author contribution** PY and ZFL designed the research; PY and CJZ performed most of the experiments; TCM developed the RILs; JFX, KNZ, and YLW participated in part of agronomic trait statistics and field experiments. PY, CJZ, and ZFL performed data analysis and manuscript writing; all authors read and approved the final manuscript.

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**Data availability** The data used in this study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval** All authors approved the submission.

**Consent to participate** N/A.

**Consent for publication** Yes.

**Conflict of interest** The authors declare no competing interests.

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