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Identification and Verification of *qGS11*, a QTL Controlling Grain Size and Heading Date in Rice

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Abstract: Grain size, shape and weight are important factors influencing grain yield and quality of rice. They are mostly determined by grain length (GL) and grain width (GW). A 13.2 Mb interval, RM167–RM287 on chromosome 11 of rice, was previously found to be associated with variations in 1000-grain weight (TGW). In this study, three populations derived from the *indica* rice cross Teqing/IRBB52 were used to identify and validate quantitative trait loci (QTLs) controlling GL, GW, TGW and the ratio of GL to GW (RLW) in the RM167–RM287 region. First, two QTL clusters associated with these traits were detected using two populations, segregating the RM167–RM287 interval only. One controlled GL, GW and TGW and was designated as *qGS11*. The other controlled GL and RLW. The allelic directions of the two QTL clusters on GL were opposite. Then, *qGS11* was further mapped in a 1.4 Mb interval using near-isogenic lines, showing a small effect on GL and a relatively large effect on TGW, GW and RLW. Meanwhile, a stable and small effect on the heading date was detected. The allelic direction for the heading date was the opposite for TGW and GW but the same for GL and RLW. The results suggest that *qGS11* has the potential for application in rice breeding.

Keywords: grain length; grain width; ratio of grain length to grain width; grain weight; quantitative trait locus; rice (*Oryza sativa* L.)

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1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops. Grain size, shape and weight are important traits determining grain yield and quality. Grain size is determined by grain length, width and thickness, grain shape is determined by grain length and width, and grain weight is mostly determined by grain size. These are all complex traits controlled by quantitative trait loci (QTLs).

Hundreds of QTLs controlling grain size in rice have been identified (https://www.gramene.org (accessed on 20 July 2022)). More than 20 causal genes underlying these QTLs have been cloned and functionally characterized, but few of them have shown minor effects [1]. One minor QTL, *SG3*, was isolated by using a BC_4F_3 population developed from the rice cross Nanyangzhan/Chuan 7 [2]. This QTL is closely linked to a major QTL, *GS3*, having a substantial effect on grain length and weight only when *GS3* is not functional. Another minor QTL, *qTGW1.2b*, was fine-mapped into a 44.0 kb region using seven near-isogenic line (NIL) populations that originated from the rice cross Zhenshan 97/Milyang 46. Its causal gene, *OsVQ4*, was confirmed by creating CRISPR/Cas9 knockout lines [3]. Since the QTLs underlying natural variation mostly have a subtle effect and are sensitive to variations in genetic background, environmental conditions and phenotyping

accuracy, a careful validation of the genetic effect is required before the implementation of gene cloning [3,4].

It is widely recognized that grain yield and component traits are often correlated with heading date (HD) [5,6]. *Ghd7* has major effects on HD and grain number per panicle. The enhanced expression of *Ghd7* under long-day conditions delayed heading and significantly increased panicle size [7]. For *Ghd7.1*, the transformation of the Nipponbare allele into Zhenshan 97 resulted in a 15-day delay in heading and 50% increase in grain yield [8]. For *DTH8*, *Hd1*, *Hd2* and *RFT1*, the alleles for delaying heading also acted to improve grain yield [9–12]. The isolation of more QTLs for HD would be beneficial for establishing the network regulating the HD and grain yield of rice.

In a previous study, a QTL analysis for grain size and weight was performed using an $F_{2:3}$ population, which originated from a residual heterozygote (RH) identified from an *indica* rice cross between Teqing (TQ) and IRBB52, named Ti52-3. Of the 135 DNA markers that were polymorphic between IRBB52 and TQ, 33 were segregated and 102 were homozygous in the Ti52-3 population [13]. A 13.2 Mb region of chromosome 11, RM167–RM287, was found to be associated with variations in 1000-grain weight (TGW) [14]. The additive effect was 0.22 g and 5.7 % of the phenotypic variance was explained (R^2). At the same time, this region showed no significant effect on grain length (GL) and grain width (GW). Such a large region may contain two or more QTLs for related traits, which should be dissected and delimitated into smaller regions before the implementation of gene cloning and breeding applications. The objectives of the present study are: (1) to identify QTLs for TGW, GL, GW and the ratio of GL to GW (RLW) in the interval RM167–RM287; (2) to validate and more accurately map one or more QTLs; and (3) to study the influence of these QTLs on HD.

2. Materials and Methods

2.1. Plant Materials

Three mapping populations were used. Firstly, two RH plants that were heterozygous in the interval RM167–RM287 only were identified from the Ti52-3 population (Table S1). They were selfed to produce two NIL-$F_{2:3}$ populations, including ZL1 and ZL2, consisting of 295 and 296 $F_{2:3}$ lines, respectively. Then, one plant that was heterozygous in the 1.4 Mb upper region of RM167–RM287 only was selected from the ZL1 population. It was selfed to produce an NIL-F_2 population. Plants having a homozygous genotype at RM167 were selected and selfed. An NIL population was constructed, consisting of 30 lines of NIL^{TQ} and 30 lines of NIL^{IR} that carried the TQ and IRBB52 homozygous alleles at RM167, respectively.

2.2. Field Trials and Phenotypic Evaluation

The ZL1 and ZL2 $F_{2:3}$ lines were tested in the mid-rice season in 2018. The ZL1 population was grown in Hangzhou, Zhejiang Province, from May to September, and in Jianyang, Fujian Province, from June to October. The ZL2 population was grown in Hangzhou only. The NIL population was grown in Lingshui, Hainan Province, from December 2021 to April 2022. The experiments followed a randomized complete block design with two replications. In each replication, twelve plants per line were planted in one row. Spacings of 26.7 cm between rows and 16.7 cm between plants in a row were used. Field management followed common agricultural practices. HD was scored for the ZL1 and ZL2 populations, and GL, GW, RLW and TGW were measured for all the three populations.

HD was recorded for each plant when the first panicle emerged and averaged for each line in each replication. At maturity, 5 plants from the middle 10 plants in each row were harvested in bulk and sun-dried. Two samples of approximately 10 g fully filled grains were selected for the measurement of GL, GW, RLW and TGW following the procedure reported by Zhang et al. [15].

2.3. DNA Marker Analysis

Nine polymorphic DNA markers, including six InDels and three SSRs, were used (Table S2). The InDel markers were designed according to the sequence differences between Teqing and IRBB52. The SSR markers were chosen from the Gramene database (http://www.gramene.org (accessed on 10 January 2020)). Total DNA was extracted from a 2 cm-long leaf sample collected from an F_2 plant using a mini-preparation protocol reported by Zheng et al. [16]. PCR amplification was performed following the method of Chen et al. [17]. The products were separated on 6% non-denaturing polyacrylamide gels and visualized using silver staining.

2.4. Data Analysis

Basic descriptive statistics, including mean value, standard deviation, coefficient of variation, the minimum and maximum trait values, skewness, and kurtosis, were analyzed.

For the ZL1 and ZL2 populations, QTLs were determined using the BIP (QTL mapping in the bi-parental populations) functionality of QTL IciMapping 4.1 [18]. LOD thresholds for genome-wide type I error of $p < 0.05$ were calculated with a 1000 permutation test and used to claim a putative QTL. QTLs were designed as proposed by McCouch and CGSNL [19]. For the NIL population, phenotypic differences between the two genotypes were tested using two-way ANOVA in the SAS procedure GLM following a method described previously [20].

3. Results

3.1. Phenotypic Performance of the ZL1 and ZL2 Populations

Descriptive statistics of GL, GW, RLW, TGW and HD in the ZL1 and ZL2 populations are presented in Table 1. All the traits had low skewness and kurtosis.

Table 1. Phenotypic performance of five traits in the ZL1 and ZL2 populations.

Population	Trait	Location	Mean	SD	CV	Range	Skewness	Kurtosis
ZL1	GL	Hangzhou	7.962	0.054	0.68	7.830−8.111	−0.08	−0.51
		Jianyang	7.902	0.053	0.67	7.753−8.073	0.13	−0.10
	GW	Hangzhou	2.757	0.026	0.94	2.692−2.819	0.09	−0.39
		Jianyang	2.913	0.022	0.76	2.870−2.980	0.31	−0.30
	RLW	Hangzhou	2.900	0.023	0.79	2.846−2.997	0.36	0.36
		Jianyang	2.722	0.021	0.77	2.670−2.768	0.04	−0.59
	TGW	Hangzhou	23.27	0.47	2.02	22.29−24.45	0.26	−0.61
		Jianyang	24.89	0.47	1.89	23.93−26.47	0.50	−0.24
	HD	Hangzhou	85.8	1.1	1.28	83.1−89.5	0.54	0.22
		Jianyang	85.7	1.2	1.40	83.0−88.2	0.09	−0.57
ZL2	GL	Hangzhou	8.043	0.046	0.57	7.906–8.167	0.01	−0.33
	GW	Hangzhou	2.712	0.026	0.96	2.627–2.789	−0.12	0.10
	RLW	Hangzhou	2.978	0.022	0.74	2.912–3.062	0.46	0.76
	TGW	Hangzhou	23.07	0.38	1.65	21.83–24.15	−0.25	0.19
	HD	Hangzhou	84.9	1.1	1.29	80.8–89.6	0.21	0.89

For ZL1, which was tested in two locations, two of the five traits showed consistent coefficients of variation (CV), estimated as 0.67 and 0.68 for GL and 0.77 to 0.79 for GLW. Among the other three traits, CV values in Hangzhou (HZ) were higher for GW and TGW and lower for HD compared with those in Jianyang (JY). Continuous distributions were observed for all the traits in both locations. Distributions of the same trait in HZ and JY were similar in HD, slightly different in GL and highly different in the other three traits (Figure 1).

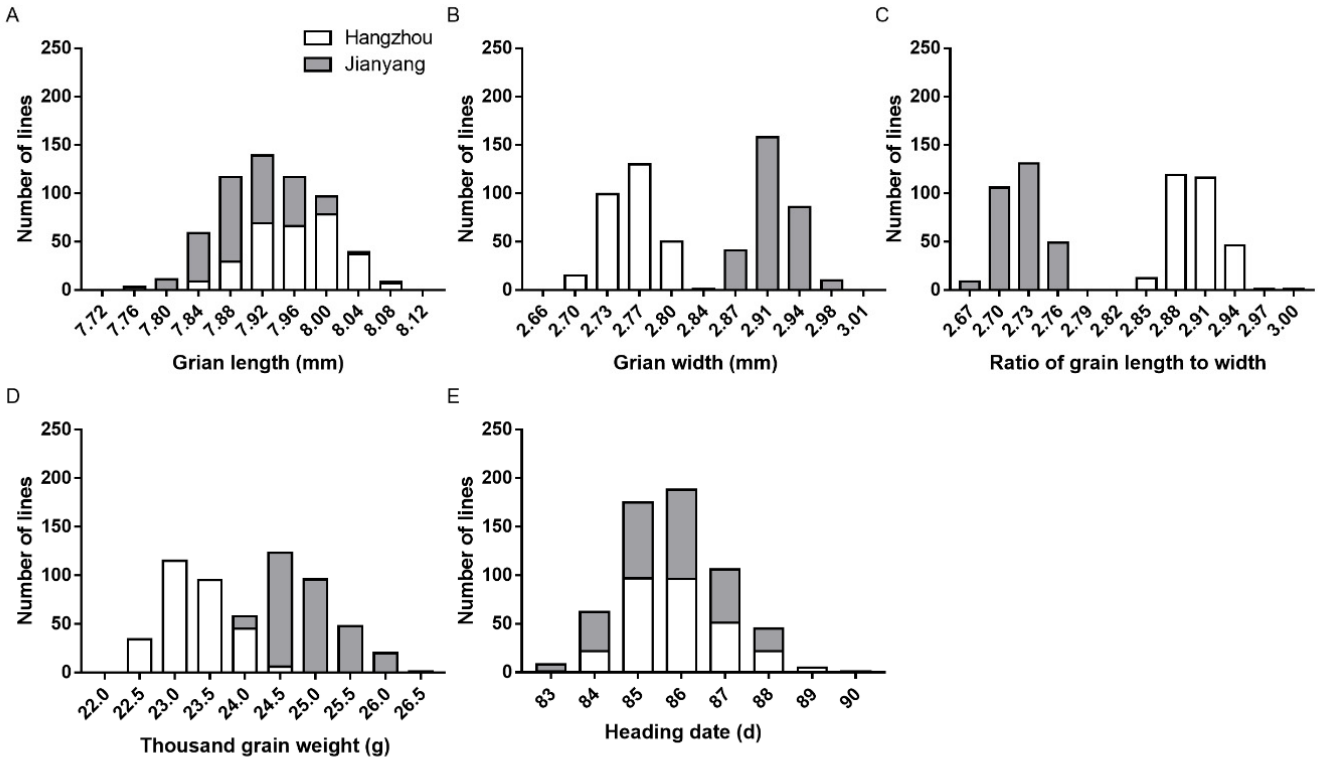

Figure 1. Frequency distribution of five traits in the ZL1 population. (**A**) Grain length. (**B**) Grain width. (**C**) Ratio of grain length to width. (**D**) Thousand grain weight. (**E**) Heading date.

Pearson's correlation analysis between the two trials conducted in different locations showed highly significant ($p < 0.001$) correlations for all five traits. The correlation coefficients were 0.476, 0.599, 0.650, 0.700 and 0.335 for GL, GW, RLW, TGW and HD, respectively. Pearson's correlation analysis was also performed between different traits in each location (Table 2). In both locations, significantly positive correlations were observed between GL, GW and TGW, while RLW showed significantly positive correlations with GL but significantly negative correlations with GW and TGW. Among these correlations, the *r* values between TGW and GW were the highest (0.896 and 0.817) in both locations. Much larger variations were observed among the correlations between HD and the other four traits. HD was negatively correlated with TGW, but positively correlated with RLW. The correlations were significant ($p < 0.001$ or $p < 0.01$) in both locations, but the coefficients were much higher in HZ (−0.468 and 0.442) than in JY (−0.180 and 0.155). Correlations between HD and two other traits, GL and GW, were either non-significant or negative significant ($p < 0.001$).

Table 2. Correlation coefficients between five traits in the ZL1 population.

Traits	GL		GW		RLW		TGW	
	Hangzhou	Jianyang	Hangzhou	Jianyang	Hangzhou	Jianyang	Hangzhou	Jianyang
GW	0.576 ***	0.434 ***						
RLW	0.175 ***	0.470 ***	−0.703 ***	−0.590 ***				
TGW	0.766 ***	0.616 ***	0.896 ***	0.817 ***	−0.406 ***	−0.244 ***		
HD	−0.194 ***	0.108	−0.504 ***	−0.058	0.442 ***	0.155 **	−0.468 ***	−0.180 **

GL (mm), grain length; GW (mm), grain width; RLW, ratio of grain length to grain width; TGW (g), 1000-grain weight; HD (d) heading date; **, $p < 0.01$; ***, $p < 0.001$.

3.2. QTLs Detected in the ZL1 Population

The results of QTL analysis for the five traits in the ZL1 population are shown in Table 3. Two QTLs were detected for GL and one QTL was detected for each of the other

four traits. The LOD scores ranged from 14.1 to 53.2 in HZ and 9.8 to 53.4 in JY. The R^2 values ranged from 13.7 to 56.6% in HZ and 6.1 to 56.7% in JY. Among these QTLs, *qTGW11*, located in the upper interval RM167–EL7110, had the highest LOD score and the largest R^2 value. The IRBB52 allele increased TGW by 0.41 g in both locations. Two other QTLs, *qGL11.1* and *qGW11*, were also located in the interval RM167–EL7110, with the enhancing alleles derived from IRBB52. The remaining three QTLs, *qGL11.2*, *qRLW11* and *qHD11*, were located in the lower interval EL7110–RM26694. They were detected with smaller LOD scores and R^2 values, with the enhancing alleles all derived from TQ.

Table 3. QTLs detected in the ZL1 population.

QTL	Interval	Hangzhou				Jianyang			
		LOD	*A*	*D*	R^2 (%)	*LOD*	*A*	*D*	R^2 (%)
qGL11.1	RM167—EL7110	17.4	0.029	0.013	18.3	26.5	0.039	0.006	16.5
qGL11.2	EL7110—RM26694	14.1	−0.028	−0.008	13.7	10.7	−0.025	−0.009	6.1
qGW11	RM167—EL7110	25.4	0.016	−0.001	45.5	32.6	0.017	0.0004	40.2
qRLW11	EL7110—RM26694	24.1	−0.016	−0.007	32.3	13.2	−0.011	−0.005	20.3
qTGW11	RM167—EL7110	53.2	0.41	0.02	56.6	53.4	0.41	0.01	56.7
qHD11	EL7110—RM26694	14.2	−0.6	−0.2	20.4	9.8	−0.6	−0.2	14.5

A, additive effect of replacing a maternal allele with a paternal allele; *D*, dominance effect; R^2, percentage of phenotypic variance explained; GL (mm), grain length; GW (mm), grain width; RLW, ratio of grain length to grain width; TGW (g), 1000-grain weight; HD (d), heading date.

The mapping results indicate that two QTL clusters for grain size may be located in the interval RM167–RM287. The upper region RM167–EL7110 had a relatively large effect on GL, GW and TGW. For ease of description, the QTLs for grain size detected in this region were integrated as *qGS11*.

For *qTGW11*, which was had the highest LOD scores, its peak LOD positions landed at the RM167 locus (Figure 2), suggesting that a Mendelian factor controlling TGW is tightly linked to RM167. To test this assumption, 70 lines having the highest values of TGW and 70 lines having the lowest values were selected from the ZL1 population. They were classified into three groups according to their genotypes at RM167. As expected, all lines having the homozygous TQ and IRBB52 genotypes were distributed in the low-value and high-value areas, respectively, while the heterozygous lines were located in both areas (Figure S1).

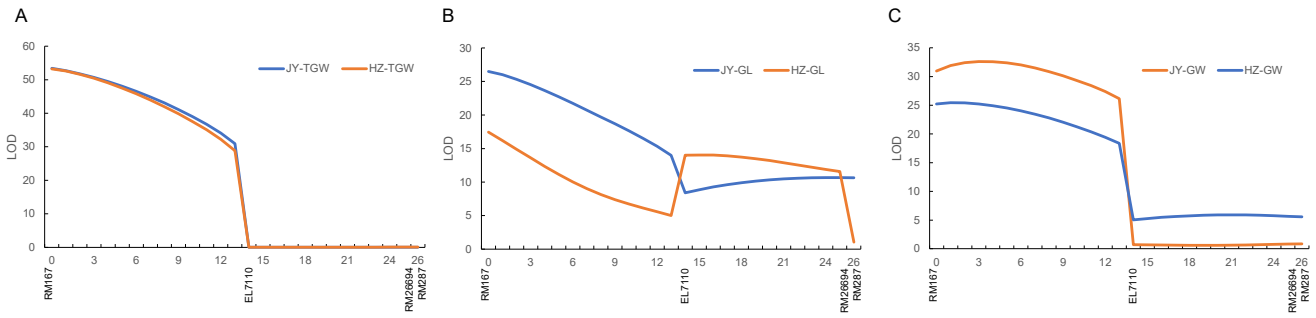

Figure 2. LOD profiles of QTLs for TGW, GL and GW detected in the ZL1 population. (**A**) TGW, 1000-grain weight. (**B**) GL, grain length. (**C**) GW, grain width.

3.3. QTLs Detected in the ZL2 Population

The results of QTL analysis for the five traits in the ZL2 population are shown in Table 4. Four QTLs, *qGW11*, *qRLW11*, *qTGW11* and *qHD11*, were detected. All of them were also detected in the ZL1 population. Two other QTLs detected in the Z1 population, *qGL11.1* and *qGL11.2*, were not found in ZL2.

Table 4. QTLs detected in the ZL2 population.

QTL	Interval	*LOD*	*A*	*D*	R^2 (%)
qGW11	RM167–EL7110	3.7	0.006	−0.009	5.6
qRLW11	EL7110–RM26694	4.1	−0.006	0.007	6.9
qTGW11	RM167–EL7110	5.6	0.14	−0.02	9.1
qHD11	RM167–EL7110	7.8	−0.5	0.2	11.3

A, additive effect of replacing a maternal allele with a paternal allele; *D*, dominance effect; R^2, percentage of phenotypic variance explained; GW (mm), grain width; RLW, ratio of grain length to grain width; TGW (g), 1000-grain weight; HD (d), heading date.

The allelic effects of the four common QTLs were consistent across the two populations, with the enhancing alleles derived from IRBB52 at *qGW11* and *qTGW11*, and from TQ at *qRLW11* and *qHD11*. Consistency was also observed for the location of *qGW11*, *qRLW11* and *qTGW11*. On the other hand, the effects of these three QTLs were much lower in ZL2 than in ZL1. In HZ, where the two populations were tested together, the LOD scores ranged from 3.7 to 5.6 in ZL2 and 24.1 to 53.2 in ZL1, and the R^2 values ranged from 5.6 to 9.1% in ZL2 and 32.3 to 56.6% in ZL2. The additive effects on GW, RLW and TGW were also decreased from 0.016 mm, 0.016 and 0.41 g in ZL1 to 0.006 mm, 0.006 and 0.14 g in ZL2, respectively. The remaining common QTL, *qHd11*, was more consistent across the two populations, especially the additive effect.

3.4. Validation of qGS11 Using NILs

Following the results of QTL mapping in the ZL1 and ZL2 populations, the upper region of the interval RM167–RM287 was targeted for further study. A plant that was heterozygous at RM167 and EL5428 but homozygous at seven other marker loci was identified and selfed to derive an NIL population segregating the 1.4 Mb interval RM167–EL5428 (Table S2).

Two-way ANOVA detected highly significant differences ($p < 0.0001$) between NIL^{TQ} and NIL^{IR} on all the four traits tested (Table 5). The enhancing alleles were derived from TQ for RLW and from IRBB52 for GL, GW and TGW. The proportions of phenotypic variance explained were 59.9% for TGW, 56.3% for GW and 20.3% for RLW, with the additive effects of 0.47 g, 0.022 mm and 0.012. All these results are in good agreement with the mapping results in the ZL1 population. For GL, the additive effect of 0.019 mm and R^2 value of 9.5% were lower than the values estimated for *qGL11.1* and *qGL11.2*, suggesting that the repulsion linkage of *qGL11.1* and *qGL11.2* in the ZL1 population may interfere with the estimation of their effects.

Table 5. The effect of *qGS11* detected in an NIL population.

Trait	Phenotypic (Mean ± SD)		*p*	*A*	R^2 (%)
	NIL^{TQ}	NIL^{IR}			
GL	7.658 ± 0.046	7.696 ± 0.051	<0.0001	0.019	9.5
GW	3.006 ± 0.014	3.049 ± 0.019	<0.0001	0.022	56.3
RLW	2.548 ± 0.020	2.524 ± 0.024	<0.0001	−0.012	20.3
TGW	23.96 ± 0.24	24.9 ± 0.34	<0.0001	0.47	59.9

A, additive effect of replacing a TQ allele with an IRBB52 allele; R^2, percentage of phenotypic variance explained. NIL^{TQ} and NIL^{IR} are near-isogenic lines carrying homozygous TQ and IRBB52 alleles in RM167–EL5428, respectively.

4. Discussion

It is generally understood that complex traits are mainly controlled by a large number of genes having small effects that interact with other genes and environmental factors [21]. However, most cloned genes/QTLs controlling grain size in rice are those having major effects, and not much attention has been paid to minor QTLs until recently [22]. In this study, the 13.2 Mb interval RM167–RM287 on chromosome 11 previously found to be

associated with genetic variation of grain weight in rice was targeted for the detection and validation of QTLs for grain weight, size and shape. Two QTL clusters were detected. One of them, *qGS11*, having a relatively large effect on grain width and weight and a smaller effect on grain length, was mapped in a 1.4 Mb interval flanked by an SSR marker, RM167, and InDel marker, EL5428. These results lay a foundation for the cloning and breeding utilization of *qGS11*.

Rice grain quality is composed of many components, among which appearance quality is one of the critical factors affecting market value [23]. The appearance quality is usually judged by GL, GW, and the percentage of chalky grain and endosperm transparency. The target region of this study exhibited significant effects on GW, RLW and TGW, with the IRBB52 allele increasing GW and TGW but reducing RLW. In previous studies using the same rice cross, this region was also reported to affect endosperm transparency with the reducing allele derived from IRBB52 [24]. It is worth noting that this region also controlled HD with the IRBB52 allele promoting heading. Pleiotropism of heading date genes on yield traits has been frequently observed, including *Ghd7*, *DTH8*/*Ghd8*, *RFT1*, *HD1* and *HD2* [7–12]. Nevertheless, no study assessing the pleiotropic effect of heading date genes on grain appearance quality in rice has been reported. The *qGS11* region is a good target for analyzing the genetic relationship between HD and appearance quality.

Four of the cloned genes for HD were located on chromosome 11, including *RCN1*, *Os11g0187200*, *OsFKF1* and *DHD1*. *RCN1* had effects on HD and grain panicles. The overexpression of *RCN1* delayed flowering and increased the spikelet number. RCN-knockdown plants exhibited small panicles with reduced branches [25]. *DHD1* interacted with *OsHAP5C/D* to delay heading by suppressing *EHd1*, *Hd3a* and *RFT1*. Compared to the wild-type lines, the plant height, panicle length, primary branching and secondary branching of overexpression lines were significantly increased, while the tiller number and grain weight were not changed [26]. *OsFKF1* delayed heading, decreased grain number, and increased panicle number, spikelet fertility and grain weight [27]. It is noteworthy that an association between delayed heading and enhanced grain yield was commonly observed [28–31], whereas early flowering is required for double-season cropping and for growing in the northernmost cultivation area [32–34]. The small but stable additive effect of the *qGS11* region on HD may be utilized for fine-tuning the growth duration.

5. Conclusions

Two QTL clusters for grain size and shape in rice were mapped in the 13.2 Mb RM167–RM287 interval on chromosome 11. One of them, *qGS11*, was located in the 1.4 Mb interval RM167–EL5428, having a relatively large effect on grain width and weight and a smaller effect on grain length. This QTL had a consistent effect across different environments, making it a good candidate for gene cloning.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12091384/s1. Table S1. Two residual heterozygous plants used to produce the ZL1 and ZL2 populations. Table S2. Polymorphic DNA markers used to map *qGS11*. Figure S1. Frequency distribution of 140 individuals with extreme thousand grain weight in ZL1 population on the RM167 locus.

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