



Identification and Pyramiding of QTLs for Rice Grain Size Based on Short-Wide Grain CSSL-Z436 Seven SSSLs & Eight DSSLs

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Abstract

Grain size is essential in rice agronomic traits. Rice chromosomal segment substitution lines (CSSLs) are perfect resources for QTL mapping and gene pyramid breeding of important rice traits. Here, a novel rice short-wide grain CSSL-Z436 was isolated from advanced-generation backcross population BC₃F₇ based on the hereditary backgrounds of *indica* restorer line Xihui 18. Z436 contained 8 substitution segments (average length 4.49Mb) from donor Huhan 3. Eleven (QTLs) for grain size-associated traits were identified using a secondary F₂ segregation population constructed by the cross between Xihui 18/Z436. The grain length in Z436 was mainly controlled by two major QTLs, *qGL5* and *qGL11*. The additive effects of *qGL5* and *qGL11* reduced the grain length. The grain width in Z436 was mainly controlled by *qGW2*, *qGW5*, and *qGW9*. The QTL *qGW2* had an additive effect of reducing the grain width, while *qGW5* and *qGW9* had an additive effect of increasing the grain width. Then, seven SSSLs and eight DSSLs were generated by MAS containing the target QTLs. Five QTLs (*qGL11*, *qGW5*, *qRLW5*, *qRLW11*, and *qGWT5*) were verified by the SSSLs, indicating that these QTLs are stable genetically. Distinct epistatic effects and phenotypes were created by pyramiding different QTLs. In general, the additive effect value of a single QTL and the numerical total of the additive and epistatic effects of QTLs in the pyramidal line determined gene efficiency after pyramiding. We may use this approach to anticipate the phenotype of novel pyramided genotypes and choose appropriate genotypes for molecular breeding based on specific breeding. Also, the fertility-restoring genes *Rf1~Rf4* were not altered in either Z436 or the seven SSSLs, enabling them to be used as new restorer lines to create innovative hybrid rice cultivars.

Disciplinary: Agriculture & Agronomy (Rice Research), Biotechnology.

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1 Introduction

More than half of the global population eats rice (*Oryza sativa* L.), a major food crop. Nearly 90% of global rice production and consumption came from Asian countries, with India and China alone accounting for 55% [1]. The use of morphological diversity and heterosis in breedings has shown increased harvest yield. Dietary needs are growing as the world's population grows, and the demand for arable land for food and energy crops rises. Public and worldwide rice reproduction initiatives sought to improve harvest creation by selecting grain yield sections and upgrading plant layout. In 2050, the global population is expected to reach 9 billion. Not only should paddy yields be raised, but grain quality criteria should be met to assure market acceptability [2].

The type of rice grain is measurable. QTLs for rice grain classifications have been duplicated more or less [3]. In the transcription, ubiquitination, hormone, and G protein pathways. The regulation of grain shape and size involves 14 major genes, four for width (*GW8*, *GW2*, *GS5*, and *GW5/qSW5*), eight for length (*GS2/GL2*, *GL3.1/qGL3*, *GL4*, *OsLG3*, *GLW7*, *GS3*, *OsLG3b/qLGY3*, and *TGW6*), and two for parallel effects (*GL7/GW7* and *GW6a*) [4]. There are three types of rice (*Oryza sativa* L.) based on kernel size and grain length: short, medium, and long grain. It is influenced by multiple quantitative trait loci (QTLs) and is important in rice harvest. There are several QTLs for harvest and its elements [6]. The rice chromosomes include around 500 QTLs for grain type. *GW2* encrypts an anonymous RING-type protein through E3 ubiquitin ligase movement. Grain milk production is increased, resulting in increased grain breadth, weight, and yield due to *GW2* function deficiency. Dephosphorylation activity of the *gl3.1/qgl3* allele accelerates cell division, leading to increased grain size and yield. Due to the fact that the *TGW6* shortage increases *GW*, *GL*, and grain yield [8], it is important to understand how *TGW6* works. Although many rice grain type characteristic genes have been identified, their molecular structure is unknown. For the study of grain categories, it is important to capture additional genes and QTLs.

To create chromosomal segment lines, a backcross to the receiver parent genome is used, but one or more chromosome portions from the donor parent are substituted in place. Also, CSSL has been shown to help detect target QTLs [9]. To map QTLs, chromosomal substitution segments (CSSL) may convert complex features into a Mendelian element [10, 11]. To make QTL mapping more exact, the chromosomal segment substitution line includes just one or two exchange sections from the donor parent to the receiver parent [9], [12]. Rice needs a restorative line for heterosis consumption. Southwestern University's Rice Research Institute developed the outstanding *indica* rice restorer line "Xihui 18". Also, it contains large panicles, plentiful grains, and long, thin grains. Intense stress tolerance and small, broad grains characterize Huhan 3, a japonica rice cultivar. Our study used the F₂ population of Xihui 18/Z436 to map QTLs linked with grain size, and advanced secondary SSSLs and double section substitution lines DSSLs to map target QTLs for grain dimension. A lot of future genetic research depends on this study.

2 Materials and Methods

2.1 Plant Material

This experiment used the CSSL-Z436 with eight substitution segments. CSSL-Z436 was derived from Xihui 18 and Huhan 3. Using SSR molecular marker-assisted collection, a rice CSSL-Z436 with eight substitution segments was established. First, the genome's 429 SSR markers were used to exhibit Xihui 18 and Huhan 3 polymorphism, and a total of 241 polymorphic markers were divided between the two. Then the 241 markers from BC₂F₁ through BC₃F₇ generation were used to gather molecular markers. Finally, the rice chromosomal segment substitution line Z436 was identified. CSSL-Z436 employed the same approach as [13] to identify substitution fragments. Paterson's approach [14] predicted the length of the substitution segment. (<https://www.wur.nl/en/show/Mapchart.htm>) was used to create the chromosomal map.

2.2 Material Planting Method

In 2018, the seeds of the hybrid were assembled and embedded at the Hainan base in September of the same year, Xihui 18 was crossed with Z436 at the Southwest University's experimental station in Chongqing, China. On 10 March 2019, seeds of Z436, Xihui 18, and 150 F₂ plants were planted at Southwest University's experimental station. A row of 10 plants per parent, all F₁ seedlings, were planted in the field on 20 April 2019. Plants were spaced 26.4 cm apart in rows and 16.5 cm apart. Seven SSSLs and eight DSSLs, coupled with Xihui 18 and Z436, each material for 30 individuals, were implanted at Southwest University's experimental station on 10 March 2020, and 10 March 2021. Conventional field management procedures were smeared.

2.3 Observation of Grain Size-Related Traits of Z436

On maturation, 10 Xihui18 and 150 F₂ plants were harvested. GL, GW, RLW, and 1000-GWT were evaluated as traits linked with grain dimension. The measuring methods were comparable to Fuying Ma's [13]. Using Microsoft Excel 2016, the average phenotypic value, standard deviation, and t-test of the above traits per plant for Xihui 18 and Z436 were computed.

2.4 QTL Mapping

150 plants from the secondary F₂ population were examined for QTLs. The CTAB technique was used to extract DNA from each specimen. Used for PCR amplification, quicksilver staining, and polyacrylamide gel electrophoresis. It scored “-1” for Xihui 18, “1” for Z436, “0” for heterozygote, and 0 “.” for the missing band. The QTLs were mapped using the marker assignment value and the mean of 150 F₂ plants. The QTL was discovered using SAS statistical program, and the use Mixed Linear Model (MLM) program written by professor Xu Shizhong of the University of California, Riverside, the restricted maximum likelihood (REML) method (with slight modification), To see, whether the QTL was linked to the marker on the substitution segment, the significant level P<0.05 was used as a threshold.

2.5 Analysis of Additive and Epistatic Interaction and Pyramiding of QTLs using SSSLs and DSSLs

Afterward ripeness in 2020, ten Xihui 18 plants, along with individually SSSL and DSSL were verified. Three replica measurements were occupied per plant to conclude grain size-relevant traits. Allowing for that individual substitution segments differed among individually SSSL and the receiver parent Xihui 18, the genetic simulation for Xihui 18 was, $P_0 = \mu_0 + \varepsilon$ and on behalf of the SSSL having a precise QTL was $P_i = \mu_0 + a_i + \varepsilon$ where P_0 and P_i signify the phenotype worth of any plant in the plot of Xihui 18 and the SSSL_i having the substitution section I correspondingly, and μ signifies the complete average. The preservative effect of the QTL is signified by a_i , although the random error is signified through ε . The Student's t-test was utilized to inspect statistically significant differences among each SSSL and Xihui 18, and a QTL was verified to exhibit when the P-value was a lesser amount of 0.05. Partial differences among the SSSL and Xihui 18 mean phenotypic values were employed to approximate the QTL's additive consequence. Microsoft Excel 2016 was employed to do entirely computations.

Assumed the theoretic hypothesis H_0 , seeing each DSSL_{ij}, Liberated inheritance was originated at two loci for a precise characteristic situated in the I and "j" substitution segments, revealed as " $2 + 0 = 1 + 1$ ". We acknowledged the supposition that two loci went to liberated inheritance while the P-value was further than 0.05 while equating (Xihui18 + DSSL_{ij}) and (SSSL_i+ SSSL_j) by means of a student t-test. The phenotypic assessment of (Xihui18 + DSSL_{ij}) was identical to (SSSL_i+ SSSL_j) at the time. As an alternative, When the P-value was a lesser amount of 0.05 by student t-test, yet, we excluded the assumption and expected that epistatic collaboration among the two allelic loci had happened, i.e. " $2 + 0 \neq 1 + 1$ ". The genetic simulations recommend that $P_0 = \mu_0 + \varepsilon$ for Xihui18, $P_i = \mu_0 + a_i + \varepsilon$ for SSSL_i, $P_j = \mu_0 + a_j + \varepsilon$ for SSSL_j and $P_{ij} = \mu_0 + a_i + a_j + \varepsilon$ for DSSL_{ij}, where P_{ij} represented the phenotype value of some plant in the plot of the DSSL_{ij}, Xihui18 population's mean value was designated by μ_0 , a_i and a_j signify the additive consequence of QTL in substitution segments I and j, correspondingly. The a_i and a_j signify the epistatic consequence among QTLs in substitution sections i and j were symbolized by I_{ij} . As a consequence, non-allele QTL epistatic outcomes were analyzed to be part of the mean phenotypic values of (Xihui18+DSSL_{ij}) - (SSSL_i+SSSL_j).

3 Experimental Results and Analysis

3.1 Identification of Z436 Substitution Fragments

Based on the Z436 genome-wide molecular collection in the initial stage, 17 polymorphic SSR indicators on the Z436 substitution fragments and 241 SSR marker exterior to the substitution segment were applied for additional substitution segment identification and genetic background purity revealing of Z436 consuming 10, individuals. As a consequence, it was established that the substitution segment of 10, Z436 individuals was reliable, and no further residual fragments of Xihui 18 excluding the substitution fragments were distinguished, demonstrating that the Z436

genotype has been stabilizing. Z436 confined 8 chromosome substitution fragments from 'Huhan 3', which was distributed on rice of chromosomes 2,4,5,6,7,9, and 11. Between them, the 2nd chromosome comprises two substitution fragments, and the chromosomes 4,5,6,7, 9, and 11 have a single substitution fragment. The substitution intermission was displayed in Figure 1. The total substitution fragment length was 35.97Mb, between them, the size of the extended fragment one was 14.7Mb (on chr.4), the size of the smallest one was 0.56Mb, and the average substitution fragment size was 4.49 Mb (see Figure 1).

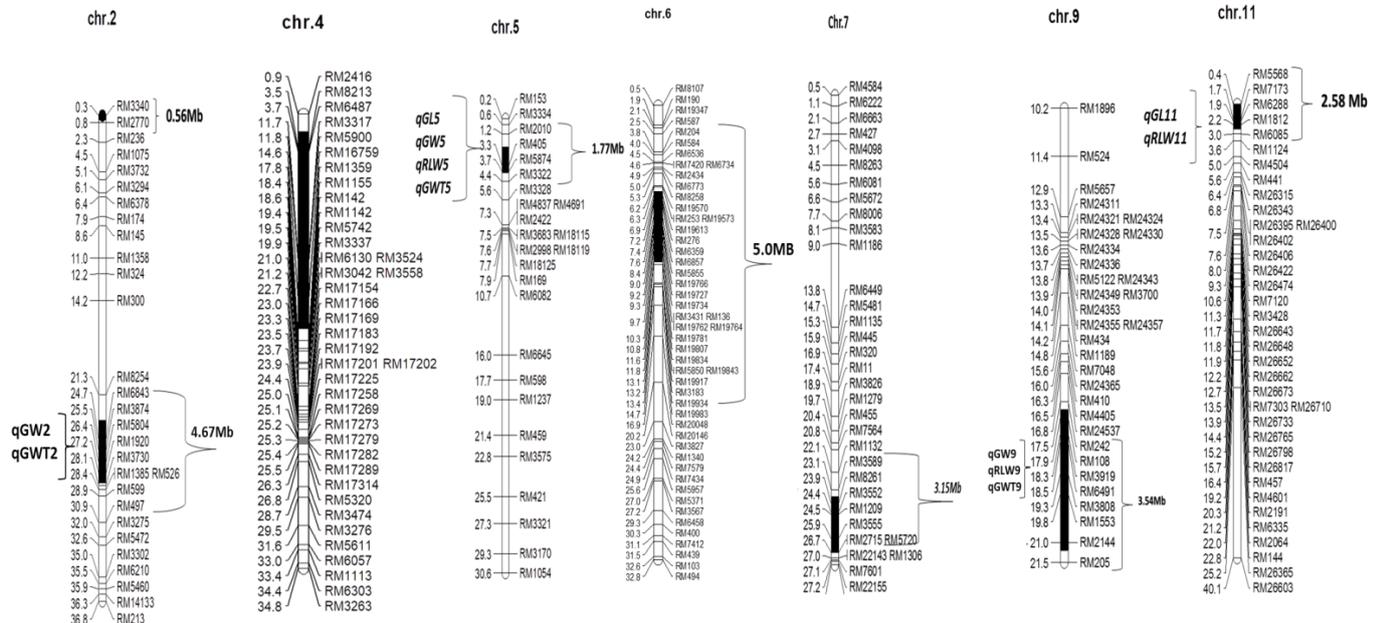


Figure 1: Z436 has QTLs and substitution segments. The reference genome was indica rice ‘9311. Each chromosome's physical distance (Mb) and mapped QTL are shown left, markers, and substitution segment lengths right. Substitution segments are black on each chromosome RLW: ratio of length-to-width, GL: grain length, GWT: thousand-grain weight, GW: grain width.

3.2 Analysis of Z436 Grain Size-Related Traits

The exceptional characteristics of Z436 were grain size diverse from Xihui 18 (Figure 2a). Compared with Xihui18, grain width and 1000-grain weight of Z436 increased more meaningfully than those of Xihui 18 (25.6% and 16.4%), correspondingly (Figure 2c, e). The grain length and ratio of length to width of Z436 reduced considerably from those of Xihui 18 (7.6% and 26.4%), correspondingly (Figure 2a, d).

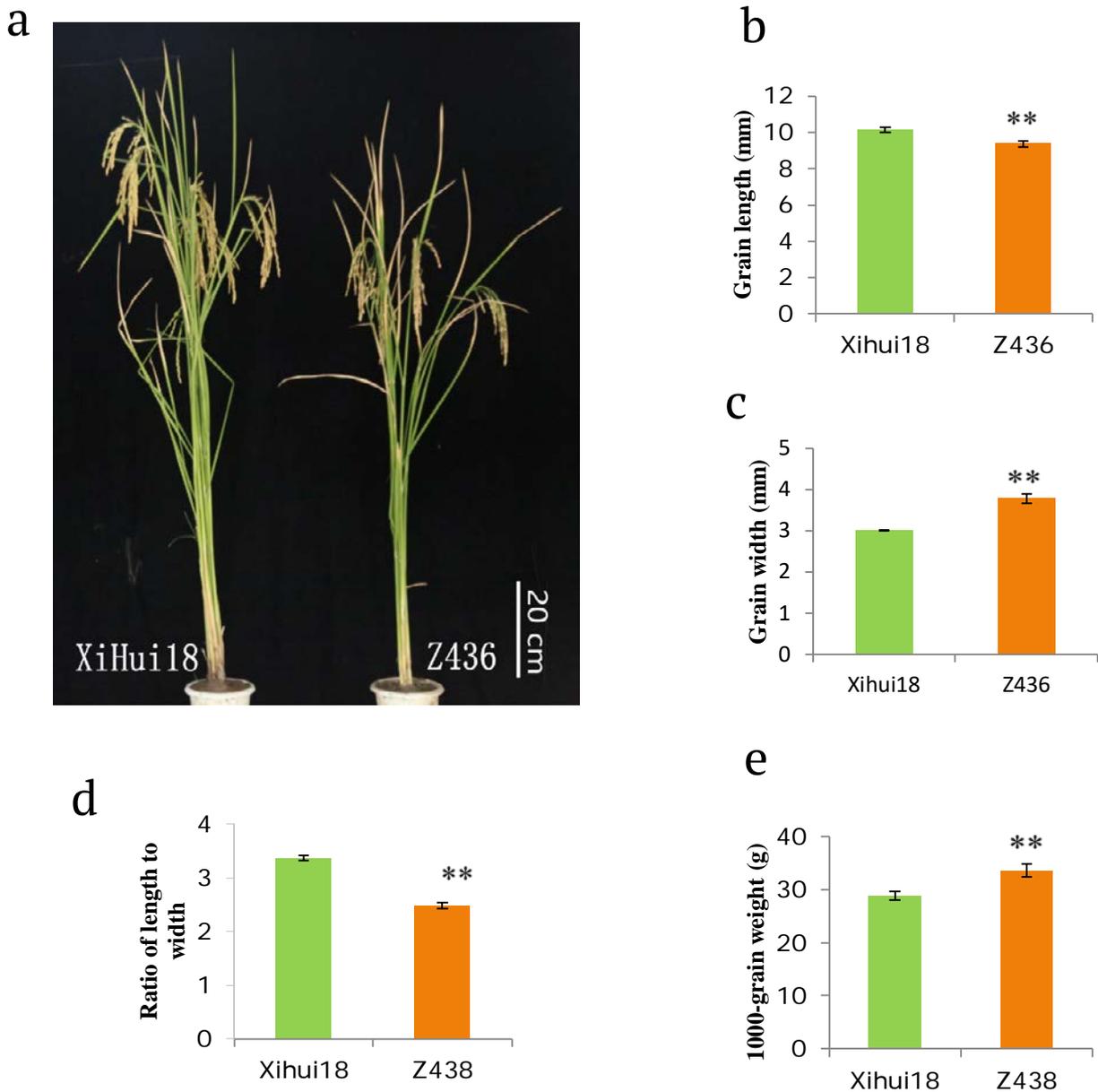


Figure 2: Phenotype of Xihui18 and Z436. a Plant type of Xihui18 and Z436, b grain length (cm), c, grain width (cm) d ratio of length to width, e 1000-grain weight (g)

3.3 QTL Mapping for Grain Size-Related Traits Carried by Substitution Segment of Z436

Conferring to the mapping of QTL, Z436 conceded 12 QTLs on behalf of grain size-related traits as of the secondary F_2 population assembled from the cross among Xihui18 and Z436, scattered on chromosomes 2, 5, 9, and 11 with the contribution rate of 1.59%-82.1% (Table 1). The grain length of Z436 was predominantly controlled by 2 QTLs *qGL5* and *qGL11*. The additive effect of *qGL5* and *qGL11* from 'Huhan 3' decreased the length of grain by 0.16mm and 0.10mm correspondingly and explained 8.60% plus 3.30% of phenotypic variation. The Z436 grain width was controlled by 3 QTLs *qGW5*, *qGW2*, and *qGW9*. The QTL *qGW2* from 'Huhan 3' had an additive effect to reduce the width of grain by 0.09mm and explained 6.08% of phenotypic variation. Although on another hand, *qGW5* and *qGW9* from 'Huhan 3' had an additive effect to increase the grain width via

0.16mm, and 0.05mm correspondingly, and described 17.66%, plus 1.59% of the phenotypic variation. The (length-width) ratio was controlled by 3 QTLs *qRLW9*, *qRLW5*, and *qRLW11*. The additive effect of *qRLW5*, *qRLW9*, and *qRLW11* from 'Huhan 3' reduced the ratio of length-width via 0.15, 0.44, and 0.04 correspondingly, and enlightened 82.31%, 7.69%, plus 7.46% of the phenotypic variation. The 1000-grain weight of Z436 was primarily controlled by three foremost QTL (*qGWT2*, *qGWT5* plus *qGWT9*) and the additive effect of the QTL *qGWT2* from 'Huhan 3' diminished the 1000-grain weight of Z436 via 1.64 g explained the 10.26% of phenotypic variation, although, on the other side, the QTLs *qGWT5* plus *qGWT9* increased the 1000-grain weight via 1.71 g, and 0.82 g, correspondingly, and enlightened 11.17% and 2.60% of phenotypic variation (Table. 1).

Table.1: QTL for grain size-related traits identified in Z436

Trait	QTL	Chromosome	Linked marker	Additive effect	Variance (%)	P-value
grain length (mm)	<i>qGL5</i>	5	RM405	-0.16	8.60	0.0041
	<i>qGL11</i>	11	RM1812	-0.10	3.30	0.0350
grain width (mm)	<i>qGW2</i>	2	RM3874	-0.09	6.08	0.0423
	<i>qGW5</i>	5	RM405	0.16	17.66	<.0001
	<i>qGW9</i>	9	RM108	0.05	1.59	0.0292
ratio of length to width	<i>qRLW5</i>	5	RM405	-0.15	82.31	<.0001
	<i>qRLW9</i>	9	RM108	-0.44	7.69	0.0244
	<i>qRLW11</i>	11	RM1812	-0.04	7.46	0.0261
1000 grain weight (g)	<i>qGWT2</i>	2	RM3874	-1.64	10.26	0.0260
	<i>qGWT5</i>	5	RM405	1.71	11.17	<.0001
	<i>qGWT9</i>	9	RM108	0.82	2.60	0.0193

3.4 Validation of QTLs by Using SSSLs and DSSSLs

Based on the results found from the QTL mapping, we established seven SSSLs (S1~S7) plus eight DSSSLs (D1~D8) by means of MAS in the F₃ population (Figure 3). Between them, 12 QTLs were distinguished on behalf of grain size-related traits in 2020, and 5 QTLs (*qGL11*, *qGW5*, *qRLW11*, *qRLW5*, and *qGWT5*) were validated in equivalent SSSLs S4 and S7, which showed that these QTLs are stable genetically (Figure 3a-f). The substitution segment of S1 plus S2 was situated on chromosome number 2. Six QTLs (*qGW2-1*, *qGW2-2*, *qGWT2-1*, *qGWT2-2*, *qRLW2-1* and *qRLW2*) were validated by S1 plus S2. The substitution segment of S3 was situated on chromosome number 4, on which one QTL *qGWT4* was detected. The substitution segment of S4 was situated on chromosome number 5, on which three QTLs (*qGW5*, *qRLW5*, *qGWT5*) were identified. The S5 substitution segment was located on the 6th chromosome number, upon which three QTLs (*qGL6*, *qGW6*, and *qRLW6*) were distinguished. The substitution segment of S6 was situated on the 7th chromosome number, upon which 4 QTLs (*qGL7*, *qGW7*, *qRLW7*, *qGWT7*) were identified. The S7 substitution segment was positioned on the 11th chromosome number, upon which 3 QTLs (*qGL11*, *qRLW11*, *qGWT11*) were identified. The QTLs (*qGL6*, *qGL7*, *qGW6*, *qGW7*, *qGWT2-1*, *qGWT2-2*, *qGWT4*, *qGWT7*, *qRLW2-1*, *qRLW2-2*, *qRLW6*, and *qRLW7*) were recognized in the equivalent SSSLs (S1~S7) correspondingly, while not identified in the secondary F₂ population of Xihui18/Z436

(Table 1). These findings showed that the SSSL displayed a higher efficiency of QTL detection. *qGL5*, *qGW9*, *qGW2*, *qRLW9*, *qGWT2*, and *qGWT9* might not be shown for the reason that none of the equivalent SSSLs was established.

3.5 Pyramiding of QTLs for Grain Size Related Traits by Using DSSLs

QTLs pyramiding on behalf of grain size-related qualities showed that several QTLs for the same trait had diverse epistatic properties. The DSSL D7 approved the substitution segment of chromosomes 6 and 7. The pyramiding of *qGL6* ($a = -0.2$) and *qGL7* ($a = 0.175$) yielded an epistatic effect of 0.18, increasing the GL genetically hereditarily by 0.16mm. Due to $-0.2 < 0.16 < 0.175$, Thus, the GL (10.30mm) of D7 was intermediated between S5 (9.60mm) plus S6 (10.35mm), Namely, a pyramiding of *qGL6* and *qGL7* also produced intermediate grain size, however in favor of long-grain of *qGL7*. The DSSL D8 carried the substitution segment of chromosomes 7 and 11. The pyramiding of *qGL7* ($a = 0.175$) in addition to *qGL11* ($a = -0.11$) produced an epistatic effect of 0.04, increased the GL genetically by 0.11mm. Because of $-0.11 < 0.11 < 0.175$. Thus, the grain length of D8 (10.2mm) was longer than that (10.35mm) of S6 (*qGL7*), while shorter than that of 9.78mm of S7 (*qGL11*). These results indicate that pyramiding of *qGL7* and *qGL11* also produced intermediate grain sizes. However, the loci for GL on D1, D2, D3, and D6 exhibited no epistatic effect. Thus, belonged to independent inheritance. (Figure 3c).

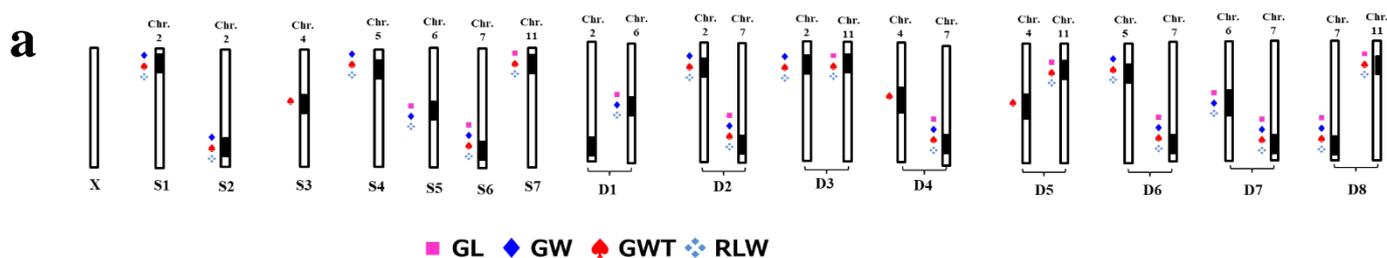
The DSSL D2 harbored the substitution segments of chromosomes 2 and 7. The pyramiding of *qGW2-1* ($a = -0.1$) and *qGW7* ($a = 0.20$) yielded an epistatic effect of -0.18, which reduced the GW of D2 genetically by -0.08. Owing to $-0.07 < -0.1 < 0.21$, the Grain width of D2 (3.05mm) was displayed as thinner than that (3.20mm) of S6 (*qGW7*) and that (3.0mm) of S1 (*qGW2-1*). That is to say, a pyramiding of *qGW2-1* and *qGW7* produced narrower grains than the genome of *qGW2-1* or *qGW7*, and *qGW2-1* was epistatic to *qGW7* in D2. The DSSL D6 carried the substitution segment of chromosomes 5 and 7. The pyramiding of *qGW5* ($a = 0.28$) and *qGW7* ($a = 0.20$) yielded an epistatic effect of -0.32, which increased the GW genetically by 0.16mm. Thus, the Grain Width of 3.11mm in D6 produced thinner grains (3.35mm) than that of both S4 (*qGW5*) and that of 3.20mm of S6 (*qGW7*). Namely, a pyramiding of wide grain *qGW5* and *qGW7* formed thinner grains and displayed *qGW7* was epistatic to *qGW5*. The DSSL D7 carried the substitution segment of chromosomes 6 and 7. The pyramiding of *qGW6* ($a = 0.07$) and *qGW7* ($a = 0.2$) yielded an epistatic effect of -0.17, which increased the GW genetically by 0.1mm. Thanks to $0.07 < 0.1 < 0.2$, Thus, the Grain width of 3.0mm in D7 became thinner than that (3.20mm) of S6 (*qGW7*), while wider than that of 2.93mm of S5 (*qGW6*). That is, a pyramiding of wide grain *qGW6* and *qGW7* yielded intermediate grain width in D7. These results indicated that pyramiding different QTL yield different epistatic effects and displayed different grain width sizes. However, the pyramiding of *qGW2-2* and *qGW6* in D1 exhibited no epistatic effect, whereas pyramiding of *qGW2-1* ($a = -0.1$) and substitution segment without QTL for GW on chromosome 11 in D3 showed no epistatic effect indicated that the two loci

belonged to independent inheritance. In other words, loci for GW on D1, D3, and D5 exhibited no epistatic effect, thus, belonging to independent inheritance (Figure 3d).

The DSSL D2 carried the substitution segment of chromosomes 2 and 7. Pyramiding of *qRLW2-1* ($a = -0.18$) and *qRLW7* ($a = -0.19$) yielded an epistatic effect of 0.17, which reduced the RLW genetically by -0.2. Owing to $-0.2 < -0.19 < -0.18$, Thus, the Ratio of Length to the width of D2 (3.23) produced a lower RLW than that (3.25) of S1 (*qRLW2-1*), while showing no phenotypic difference with 3.23 of S6 (*qRLW7*). The DSSL D7 carried the substitution segment of chromosomes 6 and 7. Pyramiding of *qRLW6* ($a = -0.17$) and *qRLW7* ($a = -0.19$) produced an epistatic effect of 0.27, reducing the RLW genetically by -0.09. Due to $-0.19 < -0.17 < -0.09$, Thus, the ratio of length to width (3.43) of D7 exhibited higher than that (3.27) of S5 (*qRLW6*), and 3.23 of S6 (*qRLW7*). In other words, a pyramiding of *qRLW6* and *qRLW7* with lower effects produced a higher RLW type and displayed *qRLW6* epistatic to *qRLW7* in D7. These results indicate that the pyramiding of different QTL for RLW produced various epistatic effects and showed various phenotypes of RLW, which increase the complexity of inheritance. However, a pyramiding of *qRLW2-2* and *qRLW7* in D1, pyramiding of *qRLW2-1* and *qRLW11* in D3, pyramiding of *qRLW5* and *qRLW7* in D6, and pyramiding of *qRLW7* and *qRLW11* in D8 exhibited no epistatic effect, whereas pyramiding of *qRLW7* ($a = -0.19$) and a substitution locus without QTL for RLW on chromosome 4 in D4 exhibited no epistatic effect. This indicated that the loci on D1, D3, D4, D6, and D8 all belonged to independent inheritance (Figure 3e).

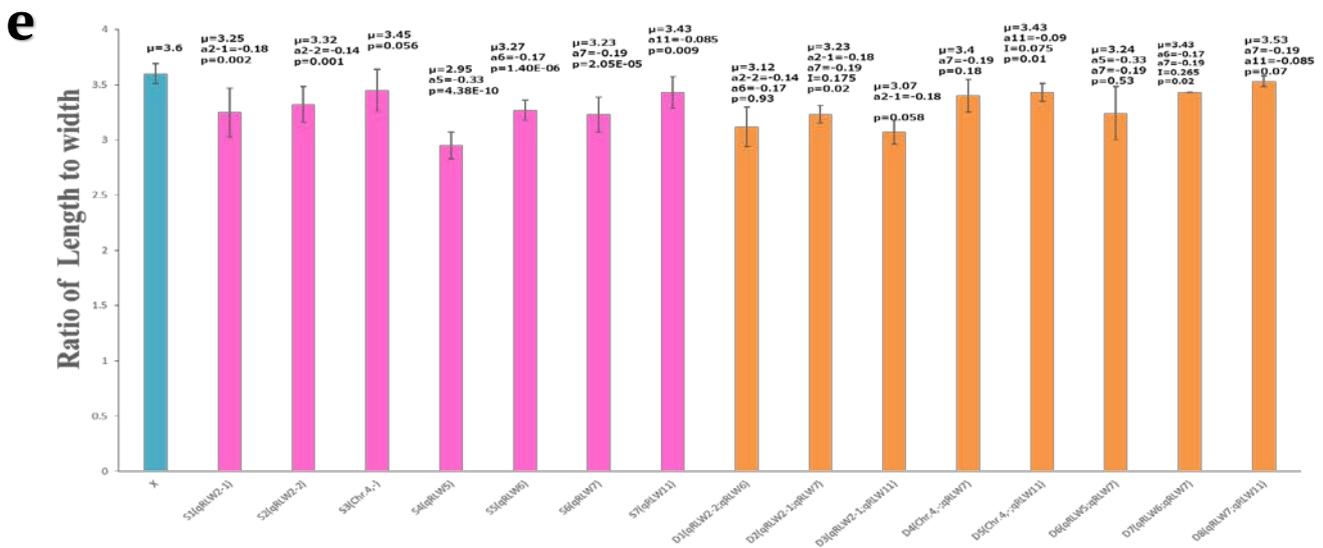
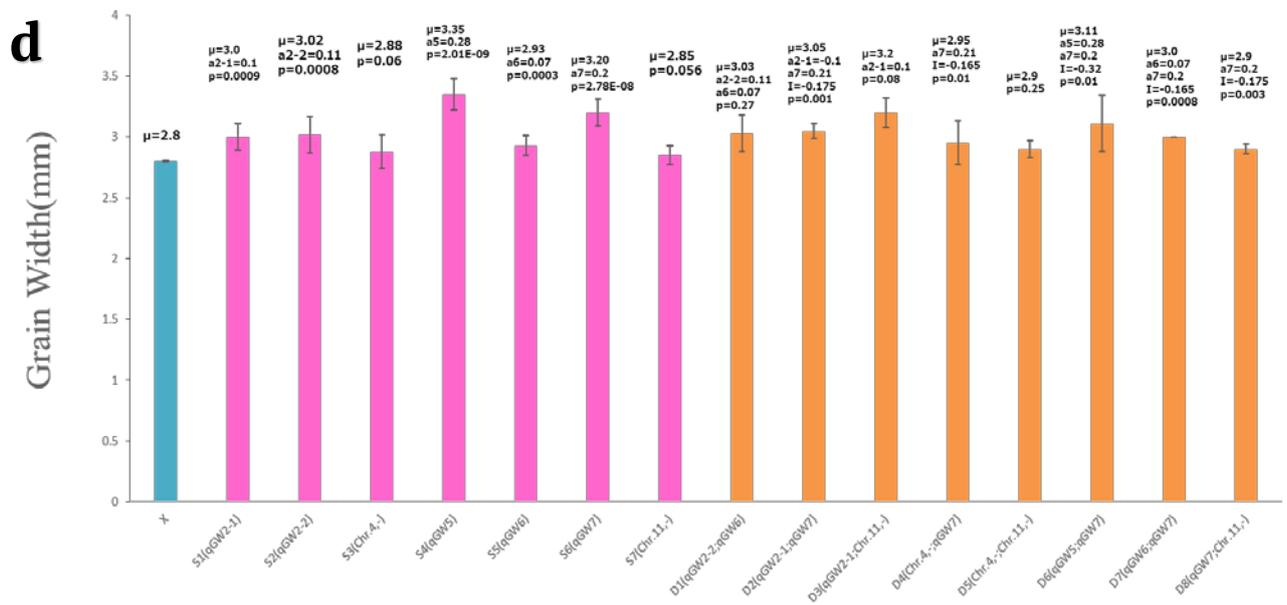
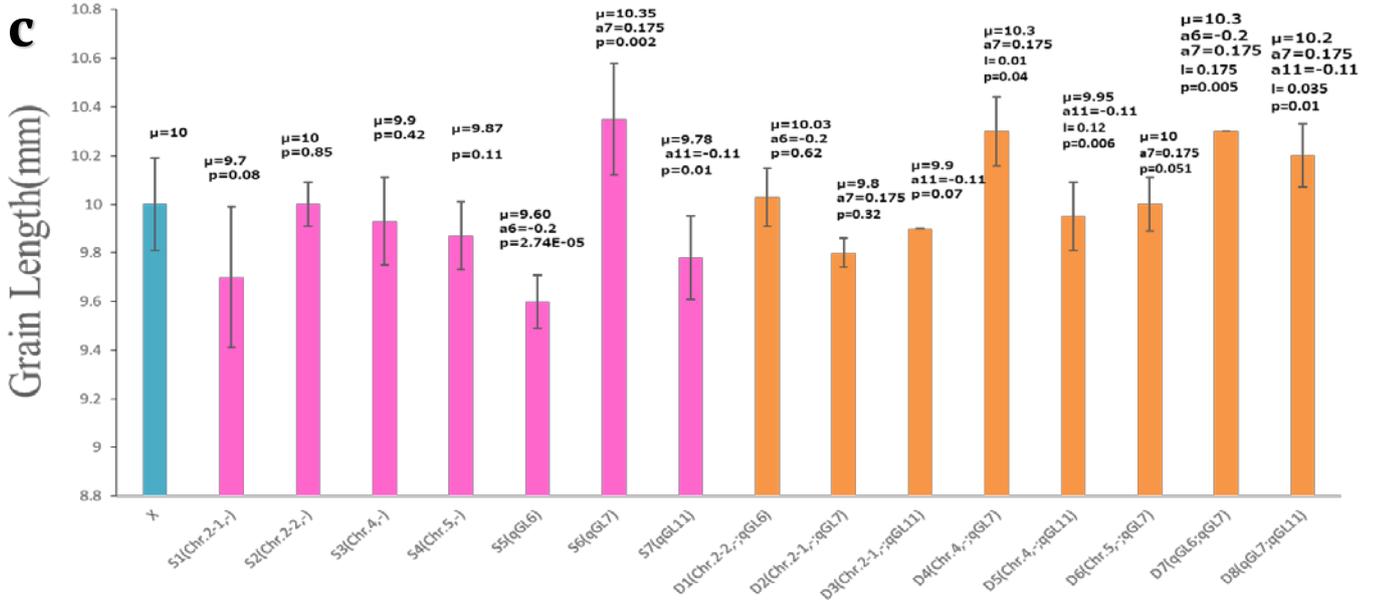
The DSSL D2 carried the substitution segments of chromosomes 2 and 7. The pyramiding of *qGWT2-1* ($a = 1.45$) and *qGWT7* ($a = 4.5$) yielded an epistatic effect of -3.9, which increased the 1000-GWT of D2 genetically by 2.05g. Owing to $1.45 < 2.05 < 4.5$, Thus, the 1000-grain weight (30.7g) of D2 displayed lighter than that (35.6g) of S6 (*qGWT7*), while heavier than that of 29.5g of S1 (*qGWT2-1*). In other words, a pyramiding of *qGWT2-1* and *qGWT7* with larger positive additive effects yielded intermediate grain weight, while the tendency of lighter grain weight and displayed *qGWT2-1* is epistatic to *qGWT7*. The result indicated that it is ineffectual to obtain larger grain weight by pyramiding *qGWT2-1* and *qGWT7*. The DSSL D4 carried the substitution segment of chromosomes 4 and 7. The pyramiding of *qGWT4* ($a = 1.75$) and *qGWT7* ($a = 4.5$) yielded an epistatic effect of -3.75, which increased the 1000-GWT weight genetically by 2.5g. Because of $1.75 < 2.5 < 4.5$, thus, 1000-grain weight (31.6g) of D4 showed lighter grains of (35.6g) than S6 (*qGWT7*), while heavier than that of 30.1g of S3 (*qGWT4*). Namely, a pyramiding of *qGWT4* and *qGWT7* with larger positive additive effects yielded intermediate grain weight, also in favor of lighter grain weight, and showed *qGWT4* epistatic to *qGWT7*. The result also indicated that it is ineffectual to obtain larger grain weight by pyramiding *qGWT4* and *qGWT7*. The DSSL D5 carried the substitution segment of

chromosomes 4 and 11. The pyramiding of *qGWT4* ($a = 1.75$) and *qGWT11* ($a = 1.55$) yielded an epistatic effect of -2.3, which increased the 1000-GWT genetically by 1.0g. Owing to $1.0 < 1.55 < 1.75$, 1000-grain weight (28.6g) of D5 produced lighter grains than that (30.1g) of S3 (*qGWT4*) and that of 29.7g of S7 (*qGWT11*). The result suggested that pyramiding of *qGWT4* and *qGWT11* with larger positive additive effects produced lighter grain weight. In another word, it is ineffectual to obtain larger grain weight by pyramiding *qGWT4* and *qGWT11*. The DSSL D6 carried the substitution segment of chromosomes 5 and 7. The pyramiding of *qGWT5* ($a = 4.95$) and *qGWT7* ($a = 4.5$) produced an epistatic effect of 5.55, which increased the 1000-GWT genetically by 3.9g. Thanks to $3.9 < 4.5 < 4.95$, thus, 1000-Grain weight (34.4g) of D6 displayed lighter grains than that (36.5g) of S4 (*qGWT5*) and that (35.6g) of S6 (*qGWT7*). Namely, a pyramiding of *qGWT5* and *qGWT7* with larger positive additive effects did not produce larger grain weight, it was discovered that pyramiding *qGWT5* and *qGWT7* does not result in larger grain. Pyramiding of QTL for 1000 grain weight caused distinct epistatic effects and revealed varied phenotypes of 1000-GWT, according to these findings. Pyramiding all of these QTLs for 1000-grain weight is ineffective in increasing 1000-grain weight, such as *qGWT2-1* and *qGWT7*, *qGWT4* and *qGWT7*, *qGWT4* and *qGWT11*, *qGWT5* and *qGWT7*. However, a pyramiding of *qGWT2-2* ($a = -1.6$) and a substitution locus without QTL for GWT on chromosome 2 in D1 exhibited no epistatic effect indicating that the two loci belonged to independent inheritance. Whereas pyramiding of *qGWT7* and *qGWT11* in D8 showed no epistatic effect. This indicated that loci on D1 and D8 belonged to independent inheritance (Figure 3f).



b





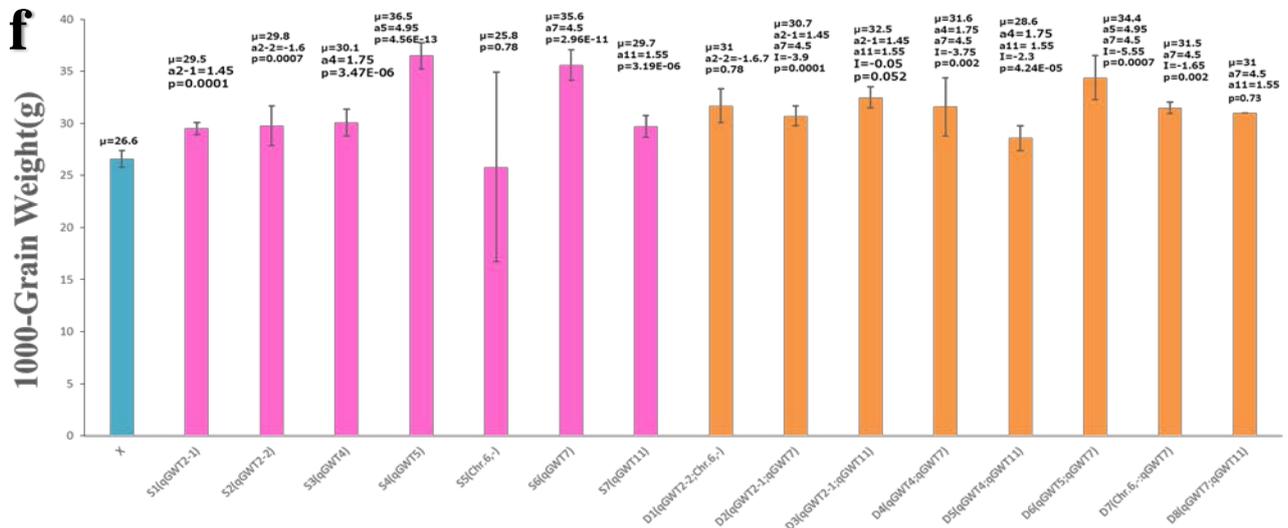


Figure 3: Analysis of additive and epistatic effect for grain size-related traits in S1~S7, D1~D8(2021). a sketch map of developed SSSLs (S1~S7), DSSLs(D1~D8). X denotes recipient Xihui18; S: SSL, D: DSSL, b denotes grain size (S1~S7) and (D1~D8), c represents grain length (GL); d represents grain width (GW); e denotes the ratio of length to width (RLW), f represents 1000 grain weight (1000-GWT. An SSSL's P-value shows the likelihood of a significant difference between the SSSL and 'Xihui 18', and the SSSL had a QT, (Student's t-test, $p < 0.05$), μ is the phenotypic value, and a represents the QTLs' additive impact, I denote the additive \times additive epistatic effect between QTLs. A DSSL's P-value reflects the likelihood of epistatic impact QTLs in the DSSL, SSSL; D, DSSL. S1: Chr2, short arm RM3340--RM2770; S2: Chr2, RM684--RM387--RM138--RM497; S3: Chr4, RM2416--RM8213-RM5900--RM1359; S4: Chr5, RM201--RM405--RM5874--3322; S5: Chr6, RM587--RM5850--RM3183;S6: Chr7, RM3589--RM8269--RM2715;S7:Chr11, RM1812--RM6085;D1: Chr2, RM684--RM387--RM138--RM497,Chr6, RM58--RM5850--RM3183; D2: Chr2, RM3340--RM2770, Chr7, RM3589--RM8269--RM2715; D3: Chr2, RM3340--RM2770, Chr11, RM1812--RM6085;D4:Chr4, RM2416--RM8213-RM5900--RM1359, Chr7, RM3589--RM8269--RM2715;D5: Chr4, RM2416--RM8213-RM5900--RM1359, Chr11, RM1812--RM6085;D6:Chr5, RM2010--RM405--RM5874--RM3322,Chr7, RM3589--RM8269--RM2715;D7: Chr6, RM587--RM5850--RM3183, Chr7, RM3589--RM8269--RM2715;D8: Chr7, RM3589--RM8269--RM2715, Chr11, RM1812--RM6085

4 Discussion

4.1 CSSL-Z436 and Secondary Substitution Lines (SSSL): a Useful Resource for Rice Breeding - an Important Application in Rice Varieties

In rice breeding, the use of heterosis is vital to the development of the breeding program. A lack of sufficient heterosis was seen because of the tight interspecies connections and modest genetic variations. Thus, heterosis between subspecies and wild species will be crucial. Therefore, Restorer lines play a critical role in the utilization of heterosis because restorer lines are quite valuable when it comes to developing hybrid rice varieties. Southwest University developed *Indica* rice restorer line Xihui18. It was considered to be the best kind because of several benefits associated with it, large panicle with multiple grains per panicle, and slender grains Using whole-genome SSR marker-assisted selection (MAS), this work developed a rice short-wide CSSL-Z436 having eight substitution segments, which alter the long slender grains of Xihui 18 into short and broad grains. In addition, the four restorer fertility genes *Rf-1* [15], *Rf2* [16], *Rf3* [17], and *Rf4* [18]

were not in the substitution interval (9.99Mb) of both Z436 and SSSLs. To some degree, these substitution lines make up for the insufficiencies of xihui18, e.g., grain width and 1000-grain weight increased significantly compared to Xihui 18. Therefore, Z436 and secondary substitution lines (SSSLs) can be utilized as direct restorer lines in the development of new rice hybrid varieties. Secondary substitution lines can also be utilized to do a genetic assessment and functional analyses on target QTLs. As a result, these materials have a promising future.

4.2 Comparison of 2020 QTLs with Known Genes

In the secondary F₂ population of Xihui 18 and Z436 there were 11 QTLs for grain size associated traits, *qGL5*, *qGW5*, *qGWT5*, and *qRLW5* were mapped in RM2010--RM405--RM5874--RM3322, and three genes *APG*, *OsPPKL2*, *SRS3*; *OsKinesin-13A*; *sar1* was located in this substitution interval. *APG* is an antagonistic interaction factor of *PGL1*, *PGL1* is a non-DNA-binding basic helix-loop-helix protein. *PGL1*, an unusual bHLH, enhanced grain length and weight in rice [19]. *OsPPKL2* encodes a protein phosphatase with the Kelch repeat domain that regulates rice grain length [20]. The *SRS3* gene is a member of the kinesin 13 subfamilies. It encodes a protein consisting of 819 amino acid residues and contains a motor domain and a coil-helix, and regulates the grain length and plant height in rice [21]. *qGL11* and *qRLW11* were mapped in RM1812--RM6085, and *CycT1;3* was located in this substitution interval. Down-regulation of Cyclin-T expression in rice results in shorter grains, suggesting that Cyclin-T plays a role in cell cycle regulation [22]. (*qGL5*, *qGW5*, *qGWT5*, and *qRLW5* were mapped in RM2010--RM405--RM5874--RM3322, and *GS5*, *OsGRF4*; *GS2*; *GL2*; *PT2*; *LGS1*; *GLW2* were mapped in this substitution interval. *GS5* encodes a serine carboxypeptidase that positively regulates rice grain size, such as grain width and grain weight [23]. Two SNPs in the promoter region of *GS5* produce variable expression of *GS5* in immature rice panicles, determining grain size. [24]. *GS2* can activate transcription and may act as a transcription activator. It inhibits cell division and growth, regulating grain size [25]. *PT2* stands for growth-regulating Factor 4 (*OsGRF4*), which governs grain shape and panicle length. *OsGRF4* expression correlates with grain size and panicle length [26]. The *GS2AA* mutation impairs the shearing of *OsmiR396*, leading to big, heavy grains and enhanced grain production. *GS2* also interacts with the transcription coactivators *OsGIF1/2/3*, increasing grain size and weight [25]. [27] identified *qGL5*, *qGW5*, *qGWT5*, *qRLW5*, *qGL11*, and *qRLW11*, *qGL5*, *qGW5*, *qGWT5*, *qRLW5*, *qGL11*, and *qRLW11*, suggesting inheritance stability. These alleles are required for biodiversity research and pyramid breeding using CSSLs with Xihui 18 genetic background.

4.3 SSSLs and DSSLs are Ideal Materials for Molecular Breeding and Analyzing Complicated Genetic Traits

SSSLs are useful for genetic investigation and breeding because they only vary in one substitution segment from the receiving parent. SSSLs are homozygous; therefore, they may be utilized permanently [28]. Using a MAS approach in the F_3 population, we constructed seven SSSLs (S1~S7) and eight DSSLs (D1~D8) based on the findings of the primary QTL mapping. Based on the QTL mapping, the SSSLs for seven target QTLs from Z436 were established, and 5 QTLs ($qGL11$, $qGW5$, $qRLW5$, $qRLW11$, and $qGWT5$) were validated, which indicates that these QTLs are genetically stable. Moreover, S1~S7 revealed several minor QTLs that were not found in the Xihui 18/Z436 secondary F_2 population, including ($qGL6$, $qGL7$, $qGW6$, $qGW7$, $qGWT2-1$, $qGWT2-2$, $qGWT4$, $qGWT7$, $qRLW2-1$, $qRLW2-2$, $qRLW6$, and $qRLW7$). These findings suggested that SSSLs had a greater sensitivity for detecting QTLs [29] and [30].

In addition, we analyzed the additive and epistatic effects and pyramid performance of $qGL6$, $qGL7$, and $qGL11$ for grain length, $qGW2-1$, $qGW5$, $qGW6$, and $qGW7$ for grain width, $qRLW6$ and $qRLW7$ for the ratio of length to width and $qGWT2-1$, $qGWT4$, $qGWT5$, $qGWT7$, and $qGWT11$ for 1000-grain weight by using eight DSSLs (D1~D8). However, the result obtained varied and indicated that some of the QTLs does not exhibit epistatic effect which showed that they belonged to independent inheritance, e.g., $qGW2-1$, $qGW2-2$, $qRLW2-2$, $qRLW7$, $qRLW2-1$, $qRLW11$, $qRLW5$, $qRLW7$, $qRLW7$, $qRLW11$, $qGWT2-2$, $qGWT7$, $qGWT11$, etc. The pyramiding of intermediate grain size QTLs $qGL6$, $qGL7$, and $qGL11$ resulted in intermediate grain sizes. Pyramiding of narrower grains QTLs $qGW7$ and $qGW2-1$ resulted in narrower grains and pyramiding of thinner grains. While pyramiding QTLs $qGW5$ and $qGW7$ resulted in thinner grains, pyramiding QTLs $qGW6$ and $qGW7$ resulted in intermediate grain width. These results indicated that pyramiding of different QTLs yielded different epistatic effects and displayed different grain width sizes. The pyramiding of higher RLW QTLs, $qRLW6$, and $qRLW7$, resulted in higher RLW. Pyramiding of 1000-grain weight QTLs $qGWT2-1$ and $qGWT7$, $qGWT4$ and $qGWT7$, $qGWT4$ and $qGWT11$, $qGWT5$ and $qGWT7$ indicated that it is ineffectual to produce a larger grain weight by using these QTLs. These results show that pyramiding the QTL for 1000 grain weight had different epistatic effects and different phenotypes of 1000-GWT. Though, The performance of genes after pyramiding was assessed by the algebraic mean of the additive and epistatic effects of QTLs in the pyramidal line.[31] Zhao showed that the difference between the DSSL's absolute genetic effect (algebraic sum of additive and epistatic effects) and the SSSL's greatest additive effect value influences whether yield-related characteristics are greater or lower. This premise underpins our results. The performance of QTLs after pyramiding is reliant on the algebraic total of additive and epistatic effects of QTLs in the pyramidal line vs the additive impact of all single QTLs.

Using this method, we may find unique pyramided genotypes and choose optimal genotypes for particular breeding objectives, bringing molecular breeding to life. For example, to get a long-grain phenotype from a short-grain line, the chosen QTLs must have an algebraic total of their additive and epistatic effects smaller than the maximal additive impact of a single QTL. If the aim is to make long grains short, the total of the additive and epistatic effects of QTLs should be less than the sum of any QTL. The algebraic total must be larger than the minimum additive impact but less than the maximum additive effect of another QTL. Thus, the present results may be used in molecular breeding for additive and epistatic QTLs.

5 Conclusion

An excellent Indica restorer line Xihui18 as the recipient parent and 'Huhan3' as the donor parent was used to identify the rice short and wide grain CSSL-Z436 12 QTLs for grain size-associated traits were also identified on Z436 substitution segments. Two key QTLs, *qGL5* and *qGL11*, controlled the grain length in Z436. The QTLs *qGL5* and *qGL11* additive effect reduced grain length. *qGW2*, *qGW5*, and *qGW9* all detected grain width in Z436. The QTL *qGW2* decreased grain width whereas *qGW5* and *qGW9* increased grain width. A total of 5 QTLs (*qGL11*, *qRLW11*, *qGW5*, *qRLW5*, and *qGWT5*) were validated by the SSSLs, indicating that these QTLs are stable genetically. SSSLs detected a number of minor QTLs in the minor F_2 population of Xihui18/Z436, indicating that the SSSL has enhanced QTL detection efficiency. 11 QTLs (*qGL5*, *qGW5*, *qRLW9* may be newly recognized. DSSLs have shown that the Pyramiding of different QTLs produced different epistatic effects and different phenotypes, while some of the QTLs do not exhibit epistatic effects which indicated that these QTLs showed independent inheritance. The pyramiding of grain size QTLs *qGL6*, *qGL7*, *qGL7*, and *qGL11* resulted in intermediate grain sizes. Pyramiding of narrower grains QTLs *qGW7* and *qGW2-1* resulted in narrower grains. While pyramiding QTLs *qGW5* and *qGW7* resulted in thinner grains, pyramiding QTLs *qGW6* and *qGW7* resulted in intermediate grain width. These results indicated that pyramiding of different QTLs yielded different epistatic effects and displayed different grain width sizes. The pyramiding of higher RLW QTLs, *qRLW6*, and *qRLW7*, resulted in higher RLW. Pyramiding of 1000-grain weight QTLs *qGWT2-1* and *qGWT7*, *qGWT4* and *qGWT7*, *qGWT4* and *qGWT11*, *qGWT5* and *qGWT7* indicated that it is ineffectual to produce a larger grain weight by using these QTLs. These results show that pyramiding the QTL for 1000 grain weight had different epistatic effects and different phenotypes of 1000-GWT. It is calculated by summing together the additive and epistatic characteristics of QTLs in each line plus the additive consequence rate of a particular QTL. Use this strategy to predict phenotypes of novel pyramided genotypes and to choose genotypes for molecular breeding. This allows them to be used as unique restorer lines to generate creative hybrid rice varieties. In general, the algebraic mean of the additive and epistatic effects of QTLs in the pyramidal line vs the additive impact value of a single QTL determined gene efficiency. This method may be used to predict the phenotype of new

pyramided genotypes and select genotypes for molecular breeding. Neither Z436 nor the seven SSSLs have changed fertility-restoring genes *Rf1-Rf4*, allowing them to be employed as new restorer lines to develop innovative hybrid rice varieties.

6 Availability of Data and Material

Data can be made available by contacting the corresponding author.

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