Identification and Pyramid of QTLs Based on Rice Short-wide Grain CSSL-Z414, SSSL, DSSL and Candidate Gene Analysis of qGL11 and qGW5

Juan Li

Southwest University

Hongxia Yang

Southwest University

Guangyi Xu

Southwest University

Keli Deng

Southwest University

Jinjin Yu

Southwest University

Siqian Xiang

Southwest University

Kai Zhou

Southwest University

Qiuli Zhang

Southwest University

Ruxiang Li

Southwest University

Miaomiao Li

Southwest University

Yinghua Ling

Southwest University

Zhenglin Yang

Southwest University

Guanghua He

Southwest University

Fangming Zhao (✉ zhaofangming2004@163.com)

Southwest University <https://orcid.org/0000-0003-2781-0452>

Keywords: Rice, Chromosome segment substitution line, Grain size, QTL, CycT1, 3

Posted Date: September 21st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-889515/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Most of rice agronomic traits as grain length etc. are complex traits controlled by multiple genes. Chromosome segment substitution lines (CSSLs) are ideal materials for dissecting and studying of these complex traits.

Results

A rice short-wide grain CSSL Z414 was identified among progeny of the recipient parent Xihui 18 (an *indica* restorer line) and the donor parent Huhan 3 (a *japonica* cultivar). Z414 carried 4 substitution segments (average length was 3.04 Mb), and displayed shorter panicle length and less number of primary branches, shorter, wider and larger grain, higher brown rice rate and chalkiness degree when compared with Xihui 18. Then, 9 quantitative trait loci (QTLs) for associated traits were identified using the secondary F₂ population from Xihui 18 / Z414. Among them, 6 QTLs (*qPL3*, *qGW5*, *qGL11*, *qRLW5*, *qRLW11*, *qGWT5*) could be verified by corresponding single segment substitution lines (SSSLs, S1-S6). In addition, 4 QTLs (*qGL3*, *qGL5*, *qCD3* and *qCD5*) were detected by S1 and S5, which was not detected by the F₂ population. Thus, the grain length of Z414 was controlled by *qGL11*, *qGL3* and *qGL5*, and the grain width of Z414 was answered by *qGW5*. Then by substitution mapping, *qGL11* and *qGW5* were delimited within the estimated substitution length of 1.42 and 1.14 Mb on chromosomes 11 and 5, and 4 and 2 candidate genes were found respectively for *qGL11* and *qGW5* by sequencing. However, only two had expression differences by qRT-PCR analysis. Finally, Analysis of QTL epistatic effects revealed that pyramid of *qGL3* (a= 0.22) and *qGL11* (a=-0.19) caused grain length of double segment substitution line (DSSL, D2) shorter than that of S5 (*qGL11*).

Conclusions

We developed a rice short –wide grain CSSL with 4 substitution segments from Huhan 3 based on the genetic backgrounds of Xihui 18. The grain width of Z414 was controlled by *qGW5*, and *GS5* should be the candidate gene for *qGW5* by sequencing and qRT-PCR analysis. The grain length of Z414 was controlled by *qGL11*, *qGL3*, and *qGL5*, and *CycT1;3* should be the best candidate gene of *qGL11*, whose specific function of regulating grain length was still unknown, and *qGL11* is epistatic to *qGL3*.

Background

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world, providing staple food for more than half of the world's population (Li et al. 2014). It is necessary to continue to improve rice breeding efficiency. At the beginning of the post-genomic era, a new concept of “breeding by design” was proposed, which aims to control all allelic variation for all genes of plant agronomic importance (Zhang et al. 2021a). The core of rice design breeding is the use of naturally occurring variation (Zhang et al. 2021a). First of all, identification of favorable alleles is principle. Here, a rice short –wide grain

chromosome segment substitution line (CSSL) was identified from naturally occurring variation. To detect the alleles for grain size in the CSSL are important for our carrying out the plan of design breeding. Grain size and weight are important target traits determining grain yield and quality in rice (Feng et al, 2021).

At present, more than 400 QTLs related to grain size have been mapped (Huang et al. 2013). Some of them have been cloned. Several signaling pathways that determine grain size have been identified by these cloned genes, including phytohormones pathway, G protein signal pathway, ubiquitin-proteasome pathway, mitogen-activated protein kinase (MAPK) signal transduction pathway, and transcription factor regulation (Li et al. 2016). Several phytohormones such as brassinosteroids (BRs) and auxins (IAA) have been suggested to play an important role in rice grain size. In rice, Three QTLs (*GS5*, *qGL3/GL3.1* and *GW5*) for grain size might be involved in BR signaling. *GS5* encodes a putative serine carboxypeptidase, and competitively inhibits the interaction between *OsBAK1-7* and *OsMSBP1* by influencing BR signaling. Higher expression of *GS5* results in wide and heavy grains as a result of increased cell proliferation and expansion in spikelet hulls (Li et al. 2011; Xu et al. 2015a). *GL3.1* suppresses BR signaling by regulating the phosphorylation and stability of *OsGSK3* (Gao et al. 2019). *GW5* can repress the kinase activity of *GSK2*, resulting in accumulation of unphosphorylated *OsBZR1* and *DLT* proteins in the nucleus to mediate BR responsive gene expression and growth responses, thus affect the grain width (Liu et al. 2017). Two QTLs (*TGW6* and *BG1*) for rice grain size might participate in IAA signaling. *TGW6* encodes protein with indole-3-acetic acid (IAA)-glucose hydrolase activity. In sink organs, the Nipponbare *tgw6* allele limits cell number and grain length by controlling IAA supply. Loss of function of the Kasalath allele enhances grain weight and yield significantly (Ishimaru et al. 2013). *BG1* controls rice grain size as a positive regulator of IAA response and transport (Liu et al. 2015a). G protein signaling is involved in regulating grain size in rice. In the pathway, heterotrimeric G protein is usually composed of five subunits. G α subunits of *RGA1* provides a foundation for grain size expansion, G β subunits of *RGB1* is essential for plant survival and growth, G γ subunits of *GS3* act as a brake in this pathway, *GS3* reduces grain length by competing for binding to *RGB1* to inhibits the downstream signal of *DEP1/GGC2* (Sun et al. 2018). Ubiquitin can directly or indirectly regulate grain size by affecting protein transport, signal transduction and degradation. *GW2* encoding a RING-type protein with E3 ubiquitin ligase activity negatively regulates cell division by targeting its substrate to proteasomes for regulated proteolysis. 1 bp missing in the *GW2* allele from WY3 causes the terminate translation prematurely during the transcription process, which makes the substrates that should be degraded cannot be recognition especially, and the grain length becomes longer (Song et al. 2007). *LG1* encodes *OsUBP15* that possesses de-ubiquitination activity in vitro, loss-of-function and down-regulated expression of *OsUBP15* produced smaller grains (Shi et al. 2019). MAPK cascades transmit developmental signals to target molecules through sequential phosphorylation (Xu et al. 2015b). *OsMKKK10*, *OsMKK4* and *OsMAPK6* act in the same MAPK pathway to regulates grain size as a positive regulator (Xu et al. 2018). *GSN1* is a negative regulator of the *OsMKKK10-OsMKK4-OsMPK6* cascade, *gsn1* mutant has larger grain size (Guo et al. 2018). Transcription regulators are considered to be important factors in controlling plant seed size. *GS2* affects grain size by encoding a transcription factor *OsGRF4* (Duan et al. 2015). *GW8* is an SBP-domain transcription factor,

regulates grain width as a positive regulator, can bound directly to the *GW7* promoter and repressed its expression (Wang et al. 2015).

Although several genes for rice grain size have been cloned, it is still fragmentary for our understanding of the underlying mechanisms that regulate rice grain size. Thus, it is necessary to identify more QTLs for grain size. Here, we identified a rice short-wide grain chromosome segment substitution line (CSSL) Z414 with 4 substitution segments, derived from *indica* restorer line Xihui 18 as recipient parent and Huhan 3 as donor parent. We characterized Z414 genetically and mapped quantitative trait loci (QTLs) for associated traits by a secondary F_2 population derived from a cross between Xihui18 and Z414. In addition, we developed single-segment (SSSLs) and double-segment (DSSLs) for each QTL in the F_3 generation using marker-assisted selection (MAS). We verified the accuracy of QTL mapping using SSSLs. We conducted analysis of the additive and epistatic effects of QTLs on associated traits and assessed the effect of QTL pyramiding using DSSLs. In addition, the major QTLs *qGL11* and *qGW5* were substitution-mapped and candidate genes were analyzed by DNA sequencing and qRT-PCR.

Results

Identification of substitution segments of Z414

Based on the development of Z414, 8 polymorphic SSR markers on the substitution segments and 24 SSR markers outside the substitution segments of Z414 were used for further identification of substitution segments and purity detection of its genetic backgrounds using 10 plants of Z414. The results showed that the substitution segments of 10 plants of Z414 were consistent and no other residual segments from Huhan 3 were detected. Z414 contained 4 substitution segments from Huhan 3, which were distributed on the chromosome 3, 5 and 11. The total estimated length of substitution segments was 12.17 Mb, and the average length was 3.04 Mb (Fig. 1).

Important traits assessment of Z414

Compared with Xihui 18, Z414 displayed significant increase in grain width, 1000-grain weight, brown rice rate and chalkiness degree by 23.5%, 16.4%, 8.8% and 26.1%, respectively. While there was significant decrease in panicle length, number of primary branches, grain length and ratio of length to width of Z414, reducing by 17.5%, 17.7%, 7.7% and 25.1%, respectively. There were no significant differences in other traits, such as plant height, panicle number, number of secondary branches, spikelet number per panicle, grain number per panicle, seed setting rate, yield per plant, head rice rate, chalky rice rate and gel consistency (Fig. 2, Table 1).

Cytological analysis of the glumes in Z414 and Xihui 18

Scanning electron microscopy was used to analyze the cell morphology of glumes in Xihui 18 and Z414 at the heading stage. The cell width in the inner epidermis of the glumes of Z414 was increased significantly than that of Xihui 18 by 22.23%. The total cell number in the outer epidermis of the glume

along the longitudinal axis of Z414 was reduced significantly than that of Xihui 18 by 13.52% and the cell length in the inner epidermis of the glumes of Z414 exhibited no significant difference compared with that of Xihui 18 (Fig. 3). The results indicated that the short-wide grain of Z414 was mainly caused by the decrease of glume cell number and increase of glume cell width.

Identification of QTL using the secondary F₂ population from Xihui18/Z414

A total of 9 QTLs were detected on 3 substitution segments of Z414. They explained the phenotypic variation from 3.86% to 50.39% (Table 2). The allele *qGW5* from Huhan 3 increased the grain width of Z414 by 0.12 mm, explaining 50.39% of the phenotypic variation. The additive effect of *qGWT5* from Huhan 3 increased 1000-grain weight of Z414 by 0.63 g. Furthermore, *qGW5*, *qRLW5* and *qGWT5* all linked to the same marker RM5874, suggesting that they should belong to pleiotropy. The allele *qGL11* from Huhan 3 reduced the grain length of Z414 by 0.08 mm, explaining 9.96% of the variation in grain length. Similarly, *qGL11*, *qPL11*, *qRLW11*, *qGWT11* and *qBRR11* all linked to the same marker RM1812. However, the additive effects of *qRLW11* and *qGWT11* decreased the values of corresponding traits, while *qPL11* and *qBRR11* increased ones (Fig. 1, Table 2), indicating that these QTLs maybe belong to pleiotropy or cluster distribution. In addition, The additive effect of *qPL3* reduced panicle length of Z414 by 0.58 cm per panicle.

Verification and pyramid of QTLs using the developed SSSLs and DSSLs

On the basis of primary QTL mapping, 6 single segment substitution lines (SSSLs, S1-S6) and 2 double segment substitution lines (DSSLs, D1, D2) were developed in F₃ by molecular marker assisted selection (MAS) method. Among them, S3, S4, and S5 belonged to SSSLs with overlapping substitution segments (Fig. 4 a).

6 QTLs (*qPL3*, *qGW5*, *qGL11*, *qRLW5*, *qRLW11*, *qGWT5*) could be verified by SSSLs (Fig. 4a-f), which indicated that these QTLs could be inherited stably. 3 QTLs (*qPL11*, *qGWT11*, and *qBRR11*) were not validated by S6, suggesting that the expression of some minor QTLs might be easily influenced by the environment, whose contribution rates to phenotypic variation were only 7.33% for *qPL11*, 3.86% for *qGWT11* and 5.92% for *qBRR11*. In addition, 4 QTLs (*qGL3*, *qGL5*, *qCD3* and *qCD5*) for grain length and chalkiness degree were detected by S1 and S5, which were not detected in the secondary F₂ population (Fig. 4c, g). The results showed that SSSLs had a higher efficiency of QTL detection.

The Panicle length (25.49 cm) of S1 carrying *qPL3* (a=-0.77) was significantly shorter than that (27.03cm) of Xihui 18, while panicle length (27.28, 27.51, 27.57, 27.42 and 26.45 cm) of S2, S3, S4, S5, S6 without QTL for panicle length displayed no significant differences with that (27.03 cm) of Xihui 18 (Fig. 4a, b). Grain length (9.66 and 9.55 mm) of S5 and S6 carrying QTLs (*qGL5* and *qGL11*) with negative effects were significantly shorter than that (9.92 mm) of Xihui18, while grain length (10.35 mm) of S1 carrying *qGL3* with 0.22 mm of additive effect was significantly longer than that (9.92 mm) of Xihui 18, the grain length (9.85, 9.92 and 9.88 mm) of S2, S3 and S4 without QTL for grain length exhibited no significant differences with that (9.92 mm) of Xihui 18 (Fig. 4a, c). Grain width (3.59 and 3.52 mm) of S4

and S5 harboring *qGW5* with positive additive effects were significantly wider than that (3.12 mm) of Xihui 18, while grain width (3.22, 3.15, 3.08 and 3.20 mm) of S1, S2, S3 and S6 without QTL for grain width showed no significant differences with that (3.12 mm) of Xihui 18. The results indicated that *qGW5* was located in the substitution interval of RM405–RM5874–RM3322–RM3328 of chromosome 5 by theory of substitution mapping (Fig. 4a, d). 1000-grain weight (34.40 and 33.43 g) of S4 and S5 containing *qGWT5* with positive effects were significantly larger than that (30.75g) of Xihui 18, and 1000-grain weight (32.66, 30.22, 31.19 and 31.98 g) of S1, S2, S3, S6 without QTL for grain weight had no significant differences with that (30.75 g) of Xihui 18. Similarly, *qGWT5* should be located in the substitution interval of RM405–RM5874–RM3322–RM3328 of chromosome 5 by theory of substitution mapping (Fig. 4a, e). Ratio of length to width (2.75, 2.75 and 2.99) of S4, S5 and S6 carrying QTLs with negative additive effects were significantly less than that (3.19) of Xihui 18, and ratio of length to width (3.22, 3.13 and 3.22) of S1, S2 and S3 without QTL for the trait displayed no significant differences with that (3.19) of Xihui 18. In the same way, *qRLW5* should be located in the substitution interval of RM405–RM5874–RM3322–RM3328 of chromosome 5 (Fig. 4a, f). Chalkiness degree (19.76%) of S1 harboring *qCD3* ($a=-0.59$) was significantly lower than that of Xihui18 (20.93%), while chalkiness degree (24.02%) of S5 carrying *qCD5* ($a=1.54$) was significantly higher than that (20.93%) of Xihui 18, the chalkiness degree (20.62 %, 22.00%, 22.35% and 22.35%) of S2, S3, S4 and S6 without QTL for chalkiness degree displayed no significant differences with that (20.93%) of Xihui 18 (Fig. 4 g).

Pyramid of *qGL3* ($a= 0.22$) and *qGL11* ($a=-0.19$) yielded an epistatic effect of -0.31, which resulted in reduction 0.28 mm of the grain length in D2. The result suggested that pyramid of *qGL3* and *qGL11* resulted in shorter grains than S6 (containing *qGL11*) (Fig. 4c), indicating that *qGL11* displayed epistatic to *qGL3*. Pyramid of *qGL3* ($a= 0.22$) and a substitution segment without QTL for grain length on chromosome 3 produced no epistatic effect in D1, which was consistent with the D1 phenotype (10.25 mm), displaying no significant difference with that (10.35 mm) of S1, and significantly longer than that of Xihui18 and S2 (Fig. 4a, c). Pyramid the substitution segment without QTL for 1000-grain weight on chromosome 3 and another substitution segment on chromosome 11 yielded an epistatic effect of -2.94, resulting in decrease 2.94 g of 1000-grain weight genetically in D2, which matched with the phenotype (28.99 g) of D2, exhibiting significantly lower than that (32.65, 31.98, and 30.75 g) of S1, S6 and Xihui18 (Fig. 4a, e). The results suggested that pyramid of two substitution segments without QTL for grain weight (D2) yielded smaller 1000-grain weight than S1 and S6. In D1 and D2, no epistatic effects were detected between associated QTLs for grain width, panicle length, ratio of length to width and chalkiness degree (Fig. 4b, d, f, g) together with the other QTLs for plant height, number of primary branches, number of secondary branches, spikelet number per panicle, grain number per panicle, seed setting rate, yield per plant, head rice rate, chalky rice rate and gel consistency (data was not shown) .

Substitution mapping and candidate gene analysis of *qGL11* and *qGW5*

The *qGL11* was firstly dissected into single segment substitution line (S6), whose estimated length of substitution segment was 1.42 Mb and the maximum substitution length was 2.84 Mb (from RM7173 to RM6085), Then 11 SSR markers were newly designed at the interval and 4 displayed polymorphism

between Xihui 18 and Huhan 3. The electrophoresis bands for RM26001 and RM26038 were identical with those of Xihui 18, and RM26045 and RM26114 were same with Huhan 3. Thus, the estimated length and maximum length of the substitution segment of S6 were further shortened from the original 1.42 Mb and 2.84 Mb to 1.415 Mb and 1.66 Mb, respectively (from RM26038 to RM6085) (Fig. 5a). In the maximum substitution region of *qGL11*, there are 2 genes related to phytohormones and ubiquitin-proteasome pathway, namely *LOC_Os11g04190* (*OsPIN1c*, auxin efflux carrier component) and *LOC_Os11g04880* (ubiquitin-like protein 5). Three genes associated with cell length and number, including *LOC_Os11g04350* (cell death associated protein), *LOC_Os11g04580* (long cell-linked locus protein) and *LOC_Os11g05850* (*CycT1;3*, rice cyclin gene). And 3 genes related to GRAS family transcription factor containing protein, including *LOC_Os11g04400*, *LOC_Os11g04570* and *LOC_Os11g04590*. The other genes were not taken into account temporarily, including hypothetical proteins, unknown functional proteins, non-protein coding transcript and other family genes whose function was irrelevant to grain size. Therefore, we preliminarily selected these 8 genes as candidate genes of *qGL11*. By DNA sequencing of these genes between Xihui 18 and S6, *LOC_Os11g04350*, *LOC_Os11g04400*, *LOC_Os11g04580* and *LOC_Os11g04880* showed no differences in promoter and exon regions between them, suggesting that these 4 genes were not candidate genes for *qGL11*. Four genes displayed differences in DNA sequence between Xihui 18 and S6, including *LOC_Os11g04190* (an auxin efflux carrier component), *LOC_Os11g04570* and *LOC_Os11g04590* (GRAS family transcription factor), *LOC_Os11g05850* (*CycT1;3*, a rice cyclin gene) (Fig.5b-e). For *LOC_Os11g04190*, 5 single nucleotide polymorphism (SNP) differences in the coding DNA sequence (CDS) and 1 SNP difference in the 3' untranslated region (UTR) existed between Xihui 18 and S6. The 583rd base of the CDS was changed from A of Xihui 18 to G of S6, which resulted in a mutation from Thr of Xihui 18 to Ala of S6. The other 4 SNP differences in CDS did not cause amino acid changes (Fig. 5b). For *LOC_Os11g04570*, 14 SNP differences in the CDS were detected, of which 9 caused amino acid mutations and 5 nonsense mutations (Fig. 5c). For *LOC_Os11g04590*, 8 SNP differences in the CDS were exhibited, of which 2 caused amino acid mutations and 6 nonsense mutations. In addition, (CCG)₇ repeat after the 180th base of the CDS was in Xihui 18, while only (GGC)₆ repeat in S6, and the CCG encodes Proline (Fig. 5d). However, qRT-PCR analysis showed that the expression levels of the *LOC_Os11g04190*, *LOC_Os11g04570* and *LOC_Os11g04590* displayed no significant differences in root, stem, leaf, sheath and panicle between Xihui 18 and S6 (Fig. 5f-h). For *LOC_Os11g05850* (*CycT1;3*), there were 6 SNP differences and a 25-base insertion in the 5'UTR and 1 SNP difference in the 3'UTR, and 1 SNP difference in the CDS which did not cause amino acid change and still encoded alanine (Fig. 5e). Especially, the expression level of the *LOC_Os11g05850* was significantly higher in sheath and panicle in S6 than in Xihui18 (Fig. 5i). Thus, *LOC_Os11g05850* (*CycT1;3*) should be the best candidate gene for *qGL11*, and *LOC_Os11g04190*, *LOC_Os11g04570*, *LOC_Os11g04590* and *LOC_Os11g05850* (*CycT1;3*) were potential candidate genes.

Using substitution mapping of S3, S4 and S5, *qGW5* was delimited into 1.135 Mb of the estimated substitution length and 1.33 Mb of the maximum substitution interval between RM405 and RM17984 (Fig. 6a). At the interval, we found 6 genes involved in the reported signaling pathway related to grain

size, such as phytohormones, ubiquitin-proteasome pathway and transcription factor regulation, including *LOC_Os05g06270* (*APIP6*, RING E3 Ubiquitin Ligase), *LOC_Os05g06280* (*SRS3*, small and round seed 3), *LOC_Os05g06660* (*GS5*, regulator of grain size), *LOC_Os05g06320* (*OsERS2*, ethylene receptor), *LOC_Os05g06670* (*OsGA2ox1*, gibberellin 2-oxidase gene) and *LOC_Os05g07720* (*OsTAR1*, IAA biosynthesis gene). The other genes as hypothetical proteins, unknown functional proteins and non-protein coding transcripts were not temporarily taken into account. By DNA sequencing of these genes, only *LOC_Os05g06660* (*GS5*) and *LOC_Os05g07720* (*OsTAR1*) were found to be existed differences between Xihui 18 and S5. For *LOC_Os05g06660* (*GS5*), which was a regulator of grain size, (GGC)₇ repeat after the 90th base of the CDS were existed in S5, while only (GGC)₅ repeat in Xihui 18, and the GGC encodes Glycine (Fig. 6b). For *LOC_Os05g07720* (*OsTAR1*), which is an IAA biosynthesis gene, 4 SNP differences in the CDS were detected between Xihui18 and S5, of which 3 caused amino acid mutations and 1 nonsense mutations (Fig. 6c). Moreover, qRT-PCR analysis showed that the expression level of the *LOC_Os05g06660* (*GS5*) was significantly higher in stem, leaf, sheath and panicle in S5 than in Xihui18, while no significant expression differences of the *LOC_Os05g07720* (*OsTAR1*) were found in all organs between Xihui 18 and S5 (Fig. 6d, e). Thus, *LOC_Os05g06660* (*GS5*) should be the best candidate gene for *qGW5* and *LOC_Os05g07720* (*OsTAR1*) were potential one for *qGW5*.

Discussion

CSSL-Z414 and secondary SSSLs are ideal rice genetic study materials, and some are potential to be as restorer lines for hybrid rice breeding

Restorer line plays an important role in heterosis utilization (Wu et al. 2018), and restorer lines are very important for breeding hybrid rice varieties. Xihui 18 was an *indica* restorer line bred by Southwest University. It had the characteristics of good combining ability, large panicle and multiple grains per panicle and narrow-long grain. In this study, we identified a short-wide grain CSSL-Z414 derived from Xihui 18 as the recipient parent. Z414 carried 4 chromosome substitution segments from donor Huhan 3. Again, none of their substitution segments contained the fertility restoration genes *Rf-1* (Akagii et al. 2004), *Rf2* (Itabashi et al. 2011), *Rf3* (Cai et al. 2013) and *Rf4* (Cai et al. 2013), so Z414 are restorative. Z414 carried 9 QTLs for important traits and displayed short-wide and larger grain, shorter panicle and less number of primary branches, and higher brown rice rate and chalkiness degree. Thus, Z414 was not suited to be directly used as rice restorer line. While it is an ideal genetic study material for complex traits due to its consistent genetic background with Xihui18 except for only 4 substitution segments from Huhan 3. By genetic dissection, we obtained 6 single segment substitution lines (S1-S6) and 2 double segment substitution lines (D1-D2) harboring target QTLs. Intriguingly, S1 carried *qGL3* and *qCD3* and exhibited both long grain and lower chalkiness degree. D2 harbored both *qGL3*, *qGL11* and *qCD3* and displayed shorter grain and lower chalkiness degree. Lower chalkiness degree is one of very important factors affected rice grain quality (Yang et al. 2021) and different grain size are import to meet the requirement of various people's preferences (Feng et al. 2021; Liang et al. 2021). Thus, some secondary substitution lines with significantly lower chalkiness degree than Xihui 18, such as S1 and D1

are potential to be directly used as novel rice restorer lines to match with sterile lines. As for S5 with short-wide grain (*qGL5*, *qGW5*, *qGWT5*, *qRLW5*) and high chalkiness degree (*qCD5*) and S6 with short grain (*qGL11*, *qRLW11*), they can be used in researching the formation mechanism of these traits. Consequently, these SSSLs and DSSLs are important in both breeding and theoretical study.

QTL identification in Z414 and SSSLs and genetic model analysis together with comparison to the reported genes

Owing to Z414 still containing 8 difference traits from Xihui 18 and 4 substitution segments from donor parent Huhan 3. It is necessary for us to know which substitution loci control these traits and further dissect these loci into single segment substitution line. Finally, using the secondary F₂ population from Xihui 18/Z414 and 6 SSSLs (S1-S6), a total of 13 QTLs were found to be responsible for these difference traits, such as grain length, grain width, ratio of length to width, 1000-grain weight, panicle length, brown rice rate and chalkiness degree. Based on the analysis of these QTLs, Short grain of Z414 was controlled by *qGL11*, *qGL3* and *qGL5*, which was dissected into S6, S1 and S5, respectively. Wide grain of Z414 was responded by *qGW5*, which was then validated in S4 and S5. Large grain of Z414 was in charge of *qGWT5* (verified by S4 and S5) and *qGWT11* (verified by S6). Short panicle of Z414 was harbored by *qPL3* (validated by S1) and *qPL11*. Higher brown rice rate was explained by *qBRR1*. *qRLW5* and *qRLW11* (verified by S4, S5 and S6) were responsible for the short-wide grain shape of Z414. *qCD5* and *qCD3* (detected by S5 and S1) answered for the higher chalkiness degree. Thus, these special genetic materials could help us to understand the genetic models for different traits at specific genetic background and then to be applied in design breeding plan. As for each single homozygous segment substitution line, such as S6 carrying only *qGL11* for grain length, the genetic model of grain length at a specific environment is very simple, displaying only additive effects for the single homozygous locus at the backgrounds of Xihui 18. When for double homozygous segment substitution line, such as D2, the genetic model of grain length is the algebra sum of the additive effects (A) of the single homozygous loci *qGL11* and *qGL3* and the A×A epistatic interaction effect of the non-allelic homozygous loci (*qGL3* and *qGL11*). For single heterozygous segment substitution line, the genetic model of heading date exists only dominant effect (D). For double heterozygous segment substitution line, the genetic model of heading date is the algebra sum of dominant effect (D) for each single heterozygous loci and the D×D epistatic interaction effect of the non-allelic heterozygous loci (Yang et al. 2018a). Thus, in natural F₁ hybrid materials, the genetic model is very complex exhibiting the combination of the above models. Genetic studies have shown that overdominance, incomplete dominance, and epistasis may all contribute to heterosis (Zhang et al. 2021b). Consequently, the development of single segment substitution lines and double segment substitution lines for different QTLs are very important to predict pyramid result of target QTL for precision design breeding. Studies have shown that pyramid different QTLs yielded different epistatic interaction. In this study, pyramid of *qGL3* (a=0.22) and *qGL11* (a=-0.19) yielded shorter grains (G=-0.28) in D2 than S6 (containing *qGL11*), displaying *qGL11* was epistatic to *qGL3*. Zhang et al (2020) showed that pyramid of *qGL5* (a=0.30) and *qGL6* (a=0.13) produced longer grains (G=0.74) of D2 than S2 and S3 at the genetic backgrounds of Nipponbare. Liang et al (2021) argued that pyramiding of

different QTLs produces different epistatic interaction and their performance after pyramiding depends on the comparison between the algebraic sum of additive and epistatic effects of QTLs in the pyramidal line and the additive effect of all single QTLs. On the basis of the rule, so long as knowing the additive effects and epistatic effects of target QTLs at the specific environment, the phenotype of novel pyramided genotypes at specific environment can be predicted and then suitable genotypes can be selected according to our breeding goal, thus realizing the concept of design breeding.

Compared with the reported genes, *OsAPC6*, *PLS2* and *OspPLAIIa* can be as the candidate genes for *qPL3* according to its physical distance and biological function. *OsAPC6* interferes with the gibberellin signal pathway and leads to cell reduction (Awasthi et al. 2012). *PLS2* encodes a glycosyltransferase, which participates in rice leaf senescence, and at the same time leads to shortening of internode and ear length (Wang et al. 2018). *OspPLAIIa* encodes glycoprotein-related phospholipase A and reduces panicle length (Liu et al. 2015b). According to the results in the study, *qGW5* for grain width, *qGL5* for grain length, *qRLW5* for ratio of length to width and *qGWT5* for 1000-grain weight were within the interval of RM405 to RM17984 of chromosome 5, these QTLs should belong to pleiotropy. At the substitution interval, *GS5* and *OsTAR1* were potential candidate genes of these QTLs. Enhanced expression of *GS5* inhibits the interaction between *OsBAK1-7* and *OsMSBP1*, providing an explanation for the positive association between grain size and *GS5* expression (Xu et al. 2015a). By DNA sequencing of *GS5*, we found that two GGC repeat encoding Glycine were added in CDS sequence of S5 compared with that of Xihui18. Again, qRT-PCR analysis showed that the expression level of the *GS5* was significantly higher in stem, leaf, sheath and panicle in S5 than in Xihui18. *OsTAR1* control the production of IAA in the developing rice grain, the *ostar1* mutant has smaller grain (Xu et al. 2021). By DNA sequencing, there was 4 SNP differences in the CDS between Xihui18 and S5. While the expression of *OsTAR1* displayed no significant differences between Xihui 18 and S5. Thus, *GS5* should be the best candidate gene of *qGW5*. Whether *GS5* and *OsTAR1* were target genes of *qGW5*, the genetic complementary experiments of two genes were ongoing. Although *GS5* and *OsTAR1* have been cloned, we have found the alleles from Huhan 3 and developed the S5 based on the genetic backgrounds of Xihui 18, which can be used in our plan of molecular design breeding. *qGL3* was in the similar region with *PGL1*, *OsLG3*, *TUD1* and *OsOFP19*. *PGL1* encodes an atypical basic helix-loop-helix protein (bHLH) that does not bind DNA. Its overexpression can increase grain length (Heang et al. 2012). *OsLG3* positively regulates rice grain length and has no effect on grain quality (Yu et al. 2017). *TUD1* encodes an E3 ubiquitin ligase of the U-box family, which participates in the brassinolide response and interacts with the heterotrimeric G protein subunit *D1* to regulate brassinolide-mediated rice growth (Hu et al. 2013). *OsOFP19*, *OSH1* and *DLT* might form a functional complex that regulates cell proliferation and cell growth (Yang et al. 2018b). *qCD3* was in the similar region with *GOT1B*, the mutations in the coding sequence of *Golgi Transport 1 (GOT1B)* cause high levels of endosperm 57 kDa proglutelins and of the luminal chaperone binding protein (BiP) and increased grain chalkiness (Fukuda et al. 2016). *qCD5* was in the similar region with *Chalk5*. *Chalk5* encodes a vacuolar H⁺-translocating pyrophosphatase (V-PPase), which increases the chalkiness of the endosperm by disturbing the PH homeostasis of the endomembrane trafficking system in

developing seeds (Li et al. 2014). In addition, *qPL11*, *qRLW11*, *qGWT11* and *qBRR11* have not been reported.

***CycT1;3* should be the best candidate gene for *qGL11* whose function in regulating grain length was still unknown**

Elucidation of the molecular mechanism underlying grain size is important for rice design breeding. Considering genes associated with grain size that have been cloned, the majority involved in phytohormones pathway, G protein signal pathway, ubiquitin-proteasome pathway, Mitogen-activated protein kinase (MAPK) signal transduction pathway, and transcription factor regulation (Li et al. 2016). Within the maximum substitution interval of 1.66 Mb for *qGL11* in S6, *LOC_Os11g04190*, *LOC_Os11g04570*, *LOC_Os11g04590* and *CycT1;3* which involved in the above signaling pathways might be candidate genes for *qGL11*. *LOC_Os11g04190* is an unreported auxin efflux carrier component. IAA biosynthesis and transport is important for grain size. *LOC_Os11g04570* and *LOC_Os11g04590* are expressed proteins containing GRAS family transcription factors. GRAS family genes are usually related to plant hormones, and plant hormones play an important role in the molecular mechanism of grain size (Li et al, 2016). For example, *SMOS2*, a member of the GRAS family, can negatively regulate grain size by participating in the BR signaling pathway (Hirano et al. 2017). Moreover, By DNA sequencing, there were differences in both DNA sequence and Amino acids for *LOC_Os11g04190*, *LOC_Os11g04570* and *LOC_Os11g04590*. While qRT-PCR analysis showed that the expression of three genes displayed no differences at any organs between Xihui 18 and S6. Thus, *LOC_Os11g04190*, *LOC_Os11g04570* and *LOC_Os11g04590* can only act as potential candidate genes for *qGL11*. However, there was differences in both DNA sequence and gene expression in sheath and panicle for *CycT1;3* between Xihui 18 and S6. Thus, *CycT1;3* should be the best candidate gene for *qGL11*. *CycT1;3* encodes cyclin protein, involving in biological progress of the cell Mitosis cycle. Intriguingly, cytological analysis showed that the shorter grain of Z414 depended on the decrease of cell number of glume rather than cell length. The result indicated that *qGL11* should be related to cell mitosis cycle. There were many studies on cell cycle regulation in yeast and animals, but few studies in plants. The biological process of the cell cycle is very complicated, cell proliferation in plants is mainly controlled by a family of cyclin-dependent kinases (CDKs). The activity of CDKs is directly regulated by binding and activation of cyclins (Wang et al. 2004; Gunbin et al. 2011). Plant cyclins can be regarded as a superfamily, which consists of several small families, such as A-, B-, C-, D-, H-, L-, T-, U-, SDS and J18 type Cyclin. The conserved regions of different types of cyclins in plants have different homology. There are more reports of A, B, D and E cyclins in plants (Nieduszynski et al. 2002), but still few reports on T cyclins, and there is a lack of systematic research on their expression patterns. Moreover, the orthologous homology of plant T-type cyclin and animal T-type protein is different, plant T-type cyclin forming a separate clade related to human T-type cyclin (Nieduszynski et al. 2002; La et al. 2006). Therefore, T-type cyclin in plant is worthy to further study. Qi et al (2012) found that *GL3.1* encoding *OsPPKL1* can directly use Cyclin-T1;3 as a substrate to phosphorylate Cyclin-T1;3, which makes Cyclin-T1;3 down-regulated in rice and resulting in shorter grains. But the molecular mechanism of how *CycT1;3* affects grain development remains unknown. In this study, although we detected *qGL3* for grain length in S1, however, *OsPPKL1* was not in the institution

interval of S1. On all accounts, these results are important for the in-depth study of *qGL11* and the series molecular design breeding based on backgrounds of Xihui 18.

Conclusions

We identified a rice short-wide grain CSSL-Z414 carrying 4 substitution segments with average length of 3.04 Mb, which was derived from an *indica* restorer line Xihui18 as the recipient parent and Huhan 3 as the donor parent. Then, 9 QTLs were detected using the secondary F₂ population of Xihui 18/ Z414. And 6 QTLs (*qPL3*, *qGW5*, *qGL11*, *qRLW5*, *qRLW11*, *qGWT5*) could be validated by 6 SSSLs which were developed using MAS in F₃ population. In addition, 4 QTLs (*qGL3*, *qGL5*, *qCD3* and *qCD5*) were detected by S1 and S5, which was not detected by the F₂ population. In particular, the chalkiness degree of S1 was lower than that of Xihui 18, which is potential to be used as novel restorer lines. Pyramid of *qGL3* and *qGL11* yielded shorter grains in D2 than S6 (containing *qGL11*), displaying *qGL11* was epistatic to *qGL3*. In D1 and D2, no epistatic effects were detected between related QTLs for grain width, panicle length, ratio of length to width and chalkiness degree. The grain length of Z414 was mainly controlled by *qGL11*, *qGL3* and *qGL5*. Cytological analysis, DNA sequencing and qRT-PCR analysis showed that *CycT1;3* should be the best candidate gene for *qGL11*, whose specific function regulating grain length was still unknown. The grain width of Z414 was controlled by *qGW5*, and *GS5* was the best candidate gene for *qGW5*. These results laid a good foundation for the in-depth study for *CycT1;3* regulating rice grain length and molecular design breeding based on backgrounds of Xihui 18.

Materials And Methods

Materials

Z414 was developed from Xihui 18 as the recipient parent and Huhan 3 as the donor parent. Xihui 18 was a rice excellent *indica* restorer line bred by Southwest University, with the characteristics of good combining ability, large panicle and multiple grains, and narrow-long grain. The *japonica* variety Huhan 3 had the characteristics of strong stress resistance and short-wide grain. Firstly, 429 simple sequence repeats (SSR) markers covering the whole rice genome were used to screen the polymorphisms between Xihui 18 and Huhan 3. Then, 241 polymorphic markers were selected to develop CSSLs beginning from BC₂F₁, 20 plants in each generation were taken. Until in BC₃F₇, a short-wide grain CSSL-Z414 with 4 substitution segments was identified in the study. The identification of chromosome substitution segments refers to the method described by Ma et al (2019), and the estimated length of chromosome substitution segments was calculated according to the method of Paterson et al (1991).

Material planting

Xihui 18 was crossed with Z414 at the experimental station of Southwest University, Chongqing, China in July of 2018, and the hybrid seeds were harvested and planted at the Hainan base in September of the same year and the F₁ seeds were harvested. Seeds of Z414, Xihui 18, and the F₂ population of 150 plants

were sown on March 10, 2019 at the experimental station of Southwest University. Thirty seedlings of each parent and all F_1 seedlings were transplanted to the field on April 20, 2019, with 10 plants per row. The spacing between rows and individual plants were 26.4 and 16.5 cm, respectively. Finally, the 12 individuals selected from F_2 population for developing SSSL and DSSL, as well as Z414 and Xihui 18, were planted at the experimental station of Southwest University on March 10, and transplanted on April 20, 2020, each material for 30 individuals. Conventional management practices were applied.

Assessment of agronomic traits and quality parameters

In the maturity period, 10 plants of Xihui 18 and Z414, together with 150 plants of F_2 were harvested. 13 traits were investigated including plant height, panicle number per plant, panicle length, number of primary branches, number of secondary branches, spikelets number per panicle, grains number per panicle, seed set rate, grain length, grain width, ratio of length to width, 1000-grain weight, and the yield per plant. The specific method referred to the described in Wang et al (2020).

The quality parameters referred to the national standard GB/T5495-2008. 10g grains of Xihui 18 and Z414 and 150 F_2 individuals were ground into brown rice. The brown rice was milled into polished rice using the CLS.JNM-1 rice husker. And then brown rice rate and head rice rate were calculated. The chalky rice rate and chalkiness degree were measured using all head rice for each sample by Wanshen SC-E. And the gel consistency was measured refer to the method described in Tang et al (1991).

The mean value of each trait was used in Student's *t*-test statistical analysis for comparison between Xihui 18 and Z414, and all parameters including mean value, standard deviation, skewness, kurtosis of these traits in the F_2 population was performed using statistical functions in Microsoft Excel 2016.

Scanning electron microscope analysis of glumes in Z414 and Xihui 18

At the completion of the booting stage and before the heading period, the inner and outer epidermal cells of the glume in Xihui18 and Z414 were investigated using a Hitachi SU3500 scanning electron microscope (Hitachi, Tokyo, Japan) with a frozen stage (-40°C) under a low-vacuum environment.

QTL mapping

QTL mapping population was a secondary F_2 population consisted of 150 individuals derived from a cross between Xihui18 and Z414. The improved CTAB method described by McCouch et al (1988) was used to extract DNA from the parents and 150 F_2 individuals. PCR amplification, polyacrylamide gel electrophoresis and rapid silver staining were carried out by the method described by Zhao et al (2016). Xihui 18 lanes were scored as “-1”, Z414 lanes were scored as “1”, heterozygous lanes were scored as “0”, and the absence of marker lanes was scored as “.”. Lanes of each marker located on the substitution segment of Z414, together with the phenotypic value of each individual of the F_2 population were used to identify putative QTL using the restricted maximum likelihood (REML) method implemented in the HPMIXED procedure in SAS (SAS Institute Inc., Cary, NC, USA). The P-value <0.05 was used as the

threshold to determine whether a QTL could be linked to a certain marker on the substitution segment of Z414.

Development of SSSLs and DSSLs, and validation and epistatic interaction analysis of target QTLs

According to QTL mapping in 2019, 12 individuals carrying target QTL and no or less heterozygous markers selected from the F_2 were used to develop SSSL and DSSL by molecular marker-assisted selection (MAS) method. Each was planted as a line in 2020. Then, the leaves of 20 individuals for each line were taken to extract DNA to further develop SSSL and DSSL using the residual heterozygous markers in substitution segment by MAS. SSSL and DSSL obeyed the rule that each substitution line only carries the target substitution segment while the lanes of other markers were same with Xihui 18.

All individuals of SSSL and DSSL and 10 individuals of Xihui 18 were harvested after maturity in August of 2020. All the involved traits were measured again with the same methods in 2019. Since only one difference in single substitution segment was existed between each SSSL and Xihui18. Under certain environment (same year and same experimental field and no replicate plot designed), the genetic models for Xihui18 and SSSL carrying a specific QTL were: $P_0 = \mu + \varepsilon$ and $P_i = \mu + a_i + \varepsilon$, respectively, where, P_0 and P_i represent the phenotype value of any plant in plot of Xihui 18 and the SSSL_i carrying the 'i' substitution segment. μ represent the mean value of Xihui18 population, a_i represent additive effect of the QTL, ε represent random error. Thus, t -test was used to detect whether SSSL carry a QTL for a certain trait by comparing the statistical difference between each SSSL and Xihui 18, when the P -value was less than 0.05, a QTL was considered to be existed, and the additive effect of the QTL was calculated as half the difference between the mean phenotypic values of SSSL and Xihui18 (Wang et al. 2021). All calculations were conducted in Microsoft Excel 2016.

At the same environment, the genetic model for DSSL was: $P_{ij} = \mu + a_i + a_j + I_{ij} + \varepsilon$, where P_{ij} represents the phenotype value of any plant in plot of the DSSL_{ij}, μ represents the mean value of Xihui18 population, a_i and a_j represent the additive effect of QTL in substitution segment i and j, respectively. I_{ij} represent the $a_i a_j$ epistatic effect between QTLs in substitution segment i and j. Thus, the epistatic effect between QTLs in DSSL was tested for the significance of each trait between (Xihui18+DSSL_{ij}) and (SSSL_i+SSSL_j) using t -test, when the P -value is less than 0.05, it is considered that there is an epistatic effect between the QTLs. The epistatic effects between non-allele QTLs were estimated as half of the mean phenotypic values of (Xihui18+DSSL_{ij})-(SSSL_i+SSSL_j) (Wang et al. 2021). Finally, we used IBM SPSS Statistics 25.0 to conduct multiple comparison.

Candidate gene prediction and sequencing of *qGL11* and *qGW5*

Based on the result of QTL verification by SSSL, we synthesized new molecular markers to delimited the substitution interval. Candidate gene information within the maximum substitution of *qGL11* and *qGW5* was predicted and combined with gene annotations to select possible candidate genes with Gramene (http://www.gramene.org/rice_mutant/) and the China National Rice Database Center

(<http://www.ricedata.cn/>). The candidate gene sequence, including promoter, 5'UTR, exons, introns and 3'UTR was downloaded, and the primers were designed on Vector NTI to amplify the target fragments using DNA of Xihui 18 and corresponding SSSL as templates, respectively. The amplicons were sequenced by Tsingke Biological Technology Co., Ltd. (Chongqing, China).

Total RNA extraction and qRT-PCR analysis

Total RNA was extracted from root, stem, leaf, sheath and panicle of Xihui 18 and SSSLs using the RNAPrep Pure Plant RNA Purification Kit (Tiangen, Binjing, China) in the booting stage, and reverse transcribed using the GoScript Reverse Transcription System, and then analyzed quantitatively on a Bio-Rad CYF96 using real-time PCR Master Mix (TaKaRa Biotechnology (Dalian, China) Co. Ltd.).

Abbreviations

QTL: Quantitative trait loci; CSSLs: Chromosome segment substitution lines; SSSL: Single-segment substitution lines; DSSL: Double-segment substitution lines; SSR: Simple sequence repeat; MAS: Marker-assisted selection; PL: panicle length; GL: Grain length; GW: Grain width; RLW: Grain length-to-width ratio; GWT: 1000-grain weight; BRR: Brown rice rate ; CD: Chalkiness degree; SNP: Single nucleotide polymorphism; UTR: Untranslated region; CDS: Coding DNA sequence

Declarations

Acknowledgments

Professor Shizhong Xu (University of California, Riverside, USA) wrote the stem program for QTL mapping.

Authors' Contribution

FMZ designed the experiment and proposed the structure and content. JL, HXY, GYX, KLD, JJY, SQX, KZ, QLZ, RXL, MML and YHL performed experiment. FMZ, JL and GHH analyzed the data. ZLY was responsible for field management. JL and FMZ wrote the paper. All authors read and approved the final version.

Funding

The study was supported by National natural science foundation of China (31871593), the Chongqing technical innovation and application development Project (cstc2019jscx-msxmX0392).

Availability of Data and Material

The datasets supporting the conclusions of this article are included within the article.

Ethics Approval and Consent to Participate

This study complied with the ethical standards of China, where this research work was conducted.

Consent for Publication

All authors provide their consent for publication.

Competing Interests

The authors declare that they have no conflict of interest.

References

- Akagi H, Nakamura A, Yokozeki-Misono Y, Inagaki A, Takahashi H, Mori K, Fujimura T (2004) Positional cloning of the rice *Rf-1* gene, a restorer of BT-type cytoplasmic male sterility that encodes a mitochondria-targeting PPR protein. *Theor Appl Genet* 108(8):1449-1457. doi:10.1007/s00122-004-1591-2
- Awasthi A, Paul P, Kumar S, Verma SK, Prasad R, Dhaliwal HS (2012) Abnormal endosperm development causes female sterility in rice insertional mutant *OsAPC6*. *Plant Sci* 183:167-174. doi:10.1016/j.plantsci.2011.08.007
- Cai J, Liao QP, Dai ZJ, Zhu HT, Zeng RZ, Zhang ZM, Zhang GQ (2013) Allelic differentiations and effects of the *Rf3* and *Rf4* genes on fertility restoration in rice with wild abortive cytoplasmic male sterility. *Biol Plant* 57(2):274-28. doi:10.1007/s10535-012-0294-9
- Duan PG, Ni S, Wang JM, Zhang BL, Xu R, Wang YX, Chen HQ, Zhu XD, Li YH (2015) Regulation of *OsGRF4* by *OsmiR396* controls grain size and yield in rice. *Nat Plants* 2(1):15203. doi:10.1111/pbi.12569
- Feng Y, Yuan XP, Wang YP, Yang YL, Zhang MC, Yu HY, Xu Q, Wang S, Niu XJ, Wei XH (2021) Validation of a QTL for grain size and weight using an introgression line from a cross between *Oryza sativa* and *Oryza minuta*. *Rice*, 14(1): 43. Doi:10.1186/s12284-021-00472-1
- Fukuda M, Kawagoe Y, Murakami T, Washida H, Sugino A, Nagamine A, Okita TW, Ogawa W, Kumama T (2016) The Dual Roles of the Golgi Transport 1 (GOT1B): RNA localization to the cortical endoplasmic reticulum and the export of proglutelin and α -Globulin from the cortical ER to the colgi. *Plant Cell Physiol* 57(11): 2380-2391
- Gao XY, Zhang JQ, Zhang XJ, Zhou J, Jiang ZH, Huang P, Tang ZB, Bao YM, Cheng JP, Tang HJ, Zhang WH, Zhang HS, Huang J (2019) Rice *qGL3/OsPPKL1* functions with the *GSK3/SHAGGY*-Like Kinase *OsGSK3* to modulate brassinosteroid signaling. *Plant Cell* 31(5):1077-1093. doi:10.1105/tpc.18.00836
- Gunbin KV, Suslov VV, Turnaev II, Afonnikov DA, Kolchanov NA (2011) Molecular evolution of cyclin proteins in animals and fungi, *BMC Evol Biol* 11(1):224. doi:10.1186/1471-2148-11-224

- Guo T, Chen K, Dong NQ, Shi CL, Ye WW, Gao JP, Shan JX, Lin HX (2018) Grain size and number negatively regulates the *OsMKKK10-OsMKK4-OsMPK6* cascade to coordinate the trade-off between grain number per panicle and grain size in rice. *Plant Cell* 30(4):871-888. doi:10.1105/tpc.17.00959
- Heang D, Sassa H (2012) Antagonistic actions of HLH/bHLH proteins are involved in grain length and weight in rice. *PLoS ONE* 7(2): e31325. doi:10.1371/journal.pone.0031325
- Hirano K, Yoshida H, Aya K, Kawamura M, Hayashi M, Hobo T, Sato-Izawa K, Kitano H, Ueguchi-Tanaka M, Matsuoka M (2017) *SMALL ORGAN SIZE 1* and *SMALL ORGAN SIZE 2/DWARF AND LOW-TILLERING* form a complex to integrate auxin and brassinosteroid signaling in rice. *Mol Plant* 10(4): 590-604. doi:10.1016/j.molp.2016.12.013
- Hu XM, Qian Q, Xu T, Zhang Y, Dong GJ, Gao T, Xie Q, Xue YB (2013) The U-Box E3 ubiquitin ligase *TUD1* functions with a heterotrimeric Ga subunit to regulate brassinosteroid-mediated growth in rice. *PLoS Genet* 9(3): e1003391. doi:10.1371/journal.pgen.1003391
- Huang RY, Jiang LG, Zheng JS, Wang TS, Wang HC, Huang YM, Hong ZL (2013) Genetic bases of rice grain shape: so many genes, so little known. *Trends Plant Sci* 18(4):218-226. doi:10.1016/j.tplants.2012.11.001
- Ishimaru K, Hirotsu N, Madoka Y, Murakami N, Hara N, Onodera H, Kashiwagi T, Ujiie K, Shimizu B, Onishi A, Miyagawa H, Katoh E (2013) Loss of function of the IAA-glucose hydrolase gene *TGW6* enhances rice grain weight and increases yield. *Nat Genet* 45(6):707-711. doi:10.1038/ng.2612
- Itabashi E, Iwata N, Fujii S, Kazama T, Toriyama K (2011) The fertility restorer gene, *Rf2*, for lead rice-type cytoplasmic male sterility of rice encodes a mitochondrial glycine-rich protein. *Plant J* 65(3):359-367. doi:10.1111/j.1365-313X.2010.04427.x
- La HG, Li J, Ji ZD, Cheng YJ, Li XL, Jiang SY, Venkatesh PN, Ramachandran S (2006) Genome-wide analysis of cyclin family in rice (*Oryza Sativa* L.). *Mol Genet Genomics* 275(4):374-386. doi:10.1007/s00438-005-0093-5
- Li N, Li YH (2016) Signaling pathways of seed size control in plants. *Curr Opin Plant Biol* 33: 23-32. doi:10.1016/j.pbi.2016.05.008
- Li YB, Fan CC, Xing YZ, Jiang YH, Luo LJ, Sun L, Shao D, Xu CJ, Li XH, Xiao JH, HeYQ, Zhang QF (2011) Natural variation in *GS5* plays an important role in regulating grain size and yield in rice. *Nature Genetics*, 2011, 43(12): 1266-1269 doi: 10.1038/ng.977
- Li YB, Fan CC, Xing YZ, Yun P, Luo LJ, Yan B, Peng B, Xie WB, Wang GW, Li XH, Xiao JH, Xu CG, He YQ (2014) *Chalk5* encodes a vacuolar H⁺-translocating pyrophosphatase influencing grain chalkiness in rice. *Nat Genet* 46(4): 398-404.

- Liang PX, Wang H, Zhang QL, Zhou K, Li MM, Li RX, Xiang SQ, Zhang T, Ling YH, Yang ZL, He GH, Zhao FM (2021) Identification and pyramiding of QTLs for rice grain size based on short-wide grain CSSL-Z563 and fine-mapping of *qGL3-2*. *Rice*, 14(1): 35. doi:10.1186/s12284-021-00477-w
- Liu GM, Zhang K, Ai J, Deng XJ, Hong YY, Wang XM (2015b) *Patatin-related phospholipase A, pPLAIIIa*, modulates the longitudinal growth of vegetative tissues and seeds in rice. *J Exp Bot* 66(21):6945-6955. doi:10.1093/jxb/erv402
- Liu JF, Chen J, Zheng XM, Wu FQ, Lin QB, Heng YQ, Tian P, Cheng ZJ, Yu XW, Zhou KN, Zhang X, Guo XP, Wang JL, Wang HY, Wan JM (2017) *GW5* acts in the brassinosteroid signalling pathway to regulate grain width and weight in rice. *Nat Plants* 3(5): 17043. doi:10.1038/nplants.2017.43
- Liu L, Tong HN, Xiao YH, Che RH, Xu F, Hu B, Liang CZ, Chu J F, Li JY, Chu CC (2015a) Activation of *Big Grain1* significantly improves grain size by regulating auxin transport in rice. *Proc Natl Acad Sci USA* 112(35):11102-11107. doi:10.1073/pnas.1517098112
- Ma F Y, Zhu XY, Wang H, Wang SM, Cui GQ, Zhang T, Yang ZL, He GH, Ling YH, Wang N, Zhao FM (2019) Identification of QTL for kernel number-related traits in a rice chromosome segment substitution line and fine mapping of *qSP1*. *Crop J* 7(4):494-503. doi:10.1016/j.cj.2018.12.009
- McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosome. *Theor Appl Genet* 76(6):815-829. doi:10.1007/BF00273666
- Nieduszynski CA, Murray J, Carrington M (2002) Whole-genome analysis of animal A- and B-type cyclins. *Genome Biol* 3(12):1164-1176. doi:10.1186/gb-2002-3-12-research0070
- Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. *Genetics* 127(1):181-197.
- Qi P, Lin YS, Song XJ, Shen JB, Huang W, Shan JX, Zhu MZ, Jiang LW, Gao JP, Lin HX (2012) The novel quantitative trait locus *GL3.1* controls rice grain size and yield by regulating *Cyclin-T1;3*. *Cell Res* 22(12):1666-1680. doi:10.1038/cr.2012.151
- Shi CL, Ren YL, Liu LL, Wang F, Zhang H, Tian P, Pan T, Wang YF, Jing RN, Liu TZ, Wu FQ, Lin QB, Lei CL, Zhang X, Zhu SH, Guo XP, Wang JL, Zhao ZC, Wang J, Zhai HQ, Cheng ZJ, Wan JM (2019) Ubiquitin specific protease 15 has an important role in regulating grain width and size in rice. *Plant Physiol* 180(1): 381-391. doi:10.1104/pp.19.00065
- Song XJ, Huang W, Shi M, Zhu MZ, Lin HX (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nat Genet* 39(5): 623-63. doi:10.1038/ng2014
- Sun SY, Wang L, Mao HL, Shao L, Li XH, Xiao JH, Ouyang YD, Zhang QF (2018) A G-protein pathway determines grain size in rice. *Nat Commun* 9(1): 851. doi:10.1038/s41467-018-03141-y

- Tang SX, Khush GS, Juliano BO (1991) Genetics of gel consistency in rice (*Oryza sativa* L.). J Genet 70(2):69-78. doi:10.1007/BF02927807
- Wang DC, Zhou K, Xiang SQ, Zhang QL, Li RX, Li MM, Liang PX, Farkhanda N, He GH, Ling YH, Zhao FM(2021) Identification, pyramid and candidate genes of QTLs for associated traits based on a dense erect panicle rice CSSL-Z749 and five SSSLs, three DSSLs and One TSSL. Rice 149(1): 55. doi:10.1186/s12284-021-00496-7
- Wang GF, Kong HZ, Sun YJ, Zhang XH, Zhang W, Altman NM, dePamphilis CW, Ma H (2004) Genome-wide analysis of the cyclin family in arabidopsis and comparative phylogenetic analysis of plant cyclin-like proteins. Plant Physiol 135(2):1084-1099. doi:10.1104/pp.104.040436
- Wang H, Zhang JY, Farkhanda N, Li J, Sun SF, He GH, Zhang T, Ling YH, Zhao FM (2020) Identification of rice QTLs for important agronomic traits with long-kernel CSSL-Z741 and three SSSLs. Rice Sci 27(5)414-423. doi:10.1016/j.rsci.2020.04.008
- Wang M, Zhang T, Peng H, Luo S, Tan JJ, Jiang KF, Heng YQ, Zhang X, Guo XP, Zheng JK, Cheng ZJ (2018) Rice premature leaf senescence 2, encoding a glycosyltransferase (GT), is involved in leaf senescence. Front Plant Sci 9: 560. doi:10.3389/fpls.2018.00560
- Wang SK, Li S, Liu Q, Wu K, Zhang JQ, Wang SS, Wang Y, Chen XB, Zhang Y, Gao CX, Wang F, Huang HX, Fu XD (2015) The *OsSPL16-GW7* regulatory module determines grain shape and simultaneously improves rice yield and grain quality. Nat Genet 47(8):949-954. doi:10.1038/ng.3352
- Wu B, Hu W, Xing YZ (2018) The history and prospect of rice genetic breeding in China. Hereditas 40(10):841-857. (in Chinese with English abstract). doi:10.16288/j.ycz.18-213
- Xu CJ, Liu Y, Li YB, Xu XD, Xu CG, Li XH, Xiao JH, Zhang QF (2015a) Differential expression of *GS5* regulates grain size in rice. J Exp Bot 66(9):2611-2623. doi:10.1093/jxb/erv058
- Xu J, Zhang SQ (2015b) Mitogen-activated protein kinase cascades in signaling plant growth and development. Trends Plant Sci 20(1):56-64. doi:10.1016/j.tplants.2014.10.001
- Xu R, Duan PG, Yu HY, Zhou ZK, Zhang BL, Wang RC, Li J, Zhang GZ, Huang SS, Lyu J, Li N, Chai TY, Tian ZX, Yao SG, Li YH (2018) Control of grain size and weight by the *OsMKKK10-OsMKK4-OsMAPK6* signaling pathway in rice. Mol Plant 11(6):860-873. doi:10.1016/j.molp.2018.04.004
- Xu XY, E ZG, Zhang DP, Yun QB, Zhou Y, Niu BX, Chen C (2021) *OsYUC11*-mediated auxin biosynthesis is essential for endosperm development of rice. Plant Physiol 185(3): 934-950. doi:10.1093/plphys/kiaa057
- Yang C, Ma YM, He Y, Tian ZH, Li JX (2018b) *OsOFP19* modulates plant architecture by integrating the cell division pattern and brassinosteroid signaling. Plant J 93(3):489-501. doi:10.1111/tpj.13793

- Yang WF, Liang JY, Hao QW, Luan X, Tan QY, Lin SW, Zhu HT, Liu GF, Liu ZP, Bu SH, Wang SK, Zhang GQ (2021) Fine mapping of two grain chalkiness QTLs sensitive to high temperature in rice. *Rice* 14(1):33. doi:10.1186/s12284-021-00476-x
- Yang ZF, Jin LL, Zhu HT, Wang SK, Zhang GQ, Liu GF (2018a) Analysis of epistasis among QTLs on heading date based on single segment substitution lines in rice. *Sci Rep* 8(1): 3059. doi:10.1038/s41598-018-20690-w
- Yu J, Xiong HY, Zhu XY, Zhang HL, Li HH, Miao JL, Wang WS, Tang ZS, Zhang ZY, Yao GX, Zhang Q, Pan YH, Wang X, Rashid MAR, Li JJ, Gao YM, Li ZK, Yang WC, Fu XD, Li ZC (2017) *OsLG3* contributing to rice grain length and yield was mined by Ho-LAMap. *BMC Biol* 15(1):28. doi:10.1186/s12915-017-0365-7
- Zhang GQ(2021a) Target chromosome-segment substitution: A way to breeding by design in rice. *Crop J* 9(03): 658-668. doi:10.1016/j.cj.2021.03.001
- Zhang SN, Huang XH, Han B (2021b) Understanding the genetic basis of rice heterosis: Advances and prospects, *Crop Journal* 9(03): 688-692. doi:10.1016/j.cj.2021.03.011
- Zhang T, Wang SM, Sun SF, Zhang Y, Li J, You J, Su T, Chen WB, Ling YH, He GH, Zhao FM (2020) Analysis of QTL for grain size in a rice chromosome segment substitution line Z1392 with long grains and fine mapping of *qGL-6*. *Rice* 13(1): 30. Doi:10.1186/s12284-020-00399-z
- Zhao FM, Tan Y, Zheng LY, Zhou K, He GH, Ling YH, Zhang LH, Xu SZ (2016) Identification of rice chromosome segment substitution line Z322-1-10 and mapping QTLs for agronomic traits from the F_3 population. *Cereal Res Commun* 44(3):370-380. doi:10.1556/0806.44.2016.022

Tables

Table 1 -Statistical parameters of different traits in Xihui18, Z414, and the F_2 population

Trait	Mean±SD (parents)		F ₂ population			
	Xihui 18	Z414	Mean±SD	Range	Skew	Kurt
Plant height (cm)	133.87±3.48	130.90±4.24	132.67±5.88	118.70-147.80	-0.07	0.14
Panicle number	4.22±0.66	4.13±0.93	5.22±2.45	2.00-25.00	4.59	32.83
Panicle length (cm)	27.03±1.1	25.03±0.73**	25.85±1.70	19.78-31.06	-0.05	1.19
Number of primary branches	16.62±0.73	13.67±0.56**	14.69±1.22	10.00-17.67	-0.49	1.73
Number of secondary branches	42.28±6.14	36.90±4.18	36.66±8.66	18.60-62.67	0.28	0.32
Spikelet number per panicle	201.68±25.66	177.16±22.84	182.08±34.02	110.33-307.89	0.32	0.81
Grain number per panicle	229.41±27.33	201.49±23.15	203.72±37.06	124.60-338.78	0.28	0.84
Seed setting rate (%)	88.21±0.20	89.05±0.10	89.23±3.62	69.03-95.32	-2.33	8.93
Grain length (mm)	10.22±0.08	9.43±0.11**	9.74±0.17	9.17-10.27	0.07	0.47
Grain width (mm)	3.02±0.02	3.73±0.07**	3.37±0.17	2.93 - 3.70	-0.22	-0.19
Ratio of Length to width	3.38±0.03	2.53±0.06**	2.90±0.16	2.56-3.29	0.33	-0.17
1000-grain weight (g)	28.96±0.86	33.63±0.49**	31.69±1.77	27.40-36.37	0.29	-0.31
Yield per plant (g)	24.82±4.82	21.82±4.84	30.69±17.07	12.40-147.37	3.56	18.42
Brown rice rate (%)	69.03±2.49	75.11±4.31**	74.12±4.05	69.12-83.05	3.80	28.95
Head rice rate (%)	28.13±2.36	25.21±1.78	25.21±6.38	10.11-y the 43.22	0.38	0.15
Chalky rice rate (%)	99.19±1.60	100.00±0.00	99.01±1.78	92.00-100.00	-1.92	3.30
Chalkiness degree (%)	19.98±1.76	25.29±1.28**	24.18±2.04	18.84-29.39	-0.19	-0.13

Gel consistency (mm)	10.22±0.44	10.62±0.45	9.08±0.76	6.60-11.50	-0.23	0.95
----------------------	------------	------------	-----------	------------	-------	------

* and ** indicate significant difference between traits of Xihui 18 and Z414 at $P<0.05$ and $P<0.01$, respectively.

Table 2-QTL for agronomic and quality traits identified in substitution segments of Z414

Trait	QTL	Chr.	Linked marker	Additive effect	R ² (%)	P-value
Panicle length (cm)	<i>qPL3</i>	3	RM3766	-0.58	9.02	0.0295
Panicle length (cm)	<i>qPL11</i>	11	RM1812	0.51	7.33	0.0063
Grain width(mm)	<i>qGW5</i>	5	RM5874	0.12	50.39	0.0013
Grain length (mm)	<i>qGL11</i>	11	RM1812	-0.08	9.96	0.0138
Ratio of length to width	<i>qRLW5</i>	5	RM5874	-0.10	38.38	0.0038
Ratio of length to width	<i>qRLW11</i>	11	RM1812	-0.04	7.15	0.0069
1000-grain weight (g)	<i>qGWT5</i>	5	RM5874	0.63	10.47	0.0450
1000-grain weight (g)	<i>qGWT11</i>	11	RM1812	-0.37	3.86	0.0457
Brown rice rate (%)	<i>qBRR11</i>	11	RM1812	1.19	5.92	0.0309

Figures

Fig.1. Chromosome substitution segments of Z414

Figure 1

Chromosome substitution segments of Z414. Physical distances (Mb) and mapped QTL are marked at left, markers and substitution segment length are displayed at the right. Black section on each chromosome are substitution segments. PL, Panicle length; GW, Grain width; GL, Grain length; RLW, ratio of length to width; GWT, 1000-Grain weight; BRR, Brown rice rate.

Figure 2

Phenotype of Xihui 18 and Z414. a Plant type of Xihui 18 and Z414. b Panicle of Xihui 18 and Z414. c Grain, brown rice and polished rice of Xihui 18 and Z414. Bars in a 20 cm, b 5 cm; c 5mm.

Figure 3

Scanning electron microscopy observation and analysis of glume in Xihui 18 and Z414. a-f Scanning electron microscope of the lemma (a, d), outer epidermis (b, e), and inner epidermis (c, f) of the glume of Xihui 18(a-c) and Z414(d-f). g, h and i represent cell length, width and total number of cells in outer epidermis of lemma at 200x magnification, respectively. ** and * respectively indicates difference at 0.01 and 0.05 level between Xihui 18 and Z414. Bars in a and d 10 mm, b and e 500 μm, c and f 500 μm.

Figure 4

Additive and epistatic effect of QTLs for related traits in SSSL, DSSL and their phenotype. a Schematic diagram of substitution segment and QTL located on them of S1-S4, and D1 and D2; b-f Parameters of QTLs in different SSSLs and DSSLs for panicle length (b), Grain length(c), Grain width (d), 1000-grain weight (e), ratio of grain length to width(f) and Chalkiness degree (g). Different lower-case letters indicate a significant difference ($P < 0.05$) as determined by Duncan's multiple comparison. μ : mean value of according trait; ai: additive effect for each QTL controlling according trait. I: epistatic effect between QTLs. P: P-value in SSSL indicated between SSSL and Xihui18 for t-test; P-value in DSSL indicated

between (DSSLij+ Xihui18) and (SSSLi+ SSSLj) for t-test. S1:(Chr.3 RM3417–RM3766–RM14809–RM7425); S2(Chr.3 RM5813–RM3346–RM1221); S3(Chr.5 RM2010–RM405–RM5874); S4(Chr.5 RM2010–RM405–RM5874–RM3322–RM3328); S5(Chr.5 RM405–RM5874–RM3322–RM3328); S6(Chr.11 RM7173–RM1812–RM6085); D1(Chr.3 RM3417–RM3766–RM14809–RM7425, Chr.3 RM5813–RM3346–RM1221); D2(Chr.3 RM3417–RM3766–RM14809–RM7425, Chr.11 RM7173–RM1812–RM6085); The internal markers connected with hyphens indicate the substitution segment from donor, whereas the markers at each end of the substitution segment linked with ‘-’ indicate whether segment recombination might occur.

Figure 5

Sequence analysis and relative expression level of candidate genes for qGL11 between Xihui 18 and S6. a QTL mapping of qGL11. Black regions indicate the estimated length of substitution segment. b-e The DNA sequence of LOC_Os11g04190 (b), LOC_Os11g04570 (c), LOC_Os11g04590 (d) and CycT1;3 (e) in S6 compared with Xihui 18. In candidate gene sequence, the red box represents the coding sequences region, the white box represents 5'UTR and 3'UTR, the solid red line represents introns, the black line in the gene sequence presents the mutation site, and arrow represents sequence change from Xihui18 to S6. f-i Relative expression level of candidate genes LOC_Os11g04190 (f), LOC_Os11g04570 (g), LOC_Os11g04590 (h) and CycT1;3 (i) in root, stem, leaf, sheath and panicle between Xihui 18 and S6.

Figure 6

Sequence analysis and relative expression level of candidate genes for qGW5 between Xihui 18 and S5. a QTL mapping of qGW5. Black regions indicate the estimated length of substitution segment. b-c The DNA sequence of GS5 (b) and OsTAR1 (c) in S5 compared with Xihui 18. In candidate gene sequence, the red box represents the coding sequences region, the white box represents 5'UTR and 3'UTR, the solid red line represents introns, the black line in the gene sequence presents the mutation site, and arrow represents

sequence change from Xihui18 to S5. d-e Relative expression level of candidate genes GS5 (d) and OsTAR1 (e) in root, stem, leaf, sheath and panicle between Xihui 18 and S5.