Identification, pyramid, and candidate gene of QTL for yield-related traits based on rice CSSLs in indica Xihui18 background

Shuangfei Sun · Zongbing Wang · Siqian Xiang · Meng Lv · Kai Zhou · Juan Li · Peixuan Liang · Miaomiao Li · Ruxiang Li · Yinghua Ling · Guanhua He · Fangming Zhao

Received: 3 March 2021 / Accepted: 10 February 2022 / Published online: 24 March 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract Chromosome segment substitution line (CSSL) is important for functional analysis and design breeding of target genes. Here, a novel rice CSSL-Z431 was identified from indica restorer line Xihui18 as recipient and japonica Huhan3 as donor. Z431 contained six segments from Huhan3, with average substitution length of 2.12 Mb. Compared with Xihui18, Z431 increased panicle number per plant (PN) and displayed short-wide grains. The short-wide grain of Z431 was caused by decreased length and increased width of glume cell. Then, thirteen QTLs were identified in secondary F2 population from Xihui18/Z431. Again, eleven QTLs (qPL3, qPN3, qGPP12, qSPP12, qGL3, qGW5, qRLW2, qRLW3, qRLW5, qGWT3, qGWT5-2) were validated by six single-segment substitution lines (SSSLs, S1-S6) developed in F3. In addition, fifteen QTLs (qPN5, qGL1, qGL2, qGL5, qGW1, qGW5-1, qRLW1, qRLW5-2, qGWT1, qGWT2, qYD1, qYD2, qYD3, qYD5, qYD12) were detected by these SSSLs, while not be identified in the F2 population. Multiple panicles of Z431 were controlled by qPN3 and qPN5. OsI-AGLU should be the candidate gene for qPN3. The short-wide grain of Z431 was controlled by qGL3.
qGW5, etc. By DNA sequencing and qRT-PCR analysis, two best candidate genes for qGL3 and qGW5 were identified, respectively. In addition, pyramid of different QTLs in D1-D3 and T1-T2 showed independent inheritance or various epistatic effects. So, it is necessary to understand all genetic effects of target QTLs for designing breeding. Furthermore, these secondary substitution lines improved the deficiencies of Xihui18 to some extent, especially increasing yield per plant in S1, S3, S5, D1-D3, T1, and T2.

Keywords Rice · Restorer line · Chromosome segment substitution line · Quantitative trait loci (QTL) · Yield-related traits

Abbreviations
CSSL Chromosome segment substitution line
SSSLs Single-segment substitution lines
DSSLs Dual-segment substitution lines
TSSLs Triple-segment substitution lines
QTL Quantitative trait loci
MAS Marker-associated selection
SSR Simple sequence repeat (as markers); seed setting rate (as yield-related trait)
SNP Single-nucleotide polymorphism

Introduction

Rice (Oryza sativa L.) provides a staple food source for more than half of the world’s population (Ranjith et al. 2020). There has been a considerable improvement in rice yield by traditional breeding strategy in the past few decades. However, with the growth of population and the decreasing of available field, improving rice yield is still the main goal of rice breeding (Li and Li 2016). At the beginning of the post-genomic era, a new concept of “breeding by design” was proposed, which aims to control all allelic variations for all genes of agronomic importance. The core of the design breeding is the use of naturally occurring variation (Zhang 2021). Thus, the appropriate creation of natural variation and identification of favorable quantitative trait loci (QTLs) for agronomic traits are important.

In the last 3 decades, QTLs for yield traits in rice have been identified on almost all 12 chromosomes using primary segregating populations, such as F2 lines, recombinant inbred lines (RILs), and doubled haploid lines (DHs) (Xing and Zhang 2010). However, seldom of these QTLs were cloned due to non-accurate QTL mapping caused by excessive genetic background noise in a primary population (Liu et al. 2018). To resolve this problem, advanced populations such as chromosome segment substitution lines (CSSLs) have been used in the identification of QTL for a wide range of traits in food and commercial crops (Wu et al. 2020). CSSLs are genetic stocks representing the complete genome of any genotype in the background of a cultivar as overlapping segments. Generally, each CSSL has a single or several specific marker-defined chromosome segments from the donor with a maximum recipient parent genome recovered in the background (Balakrishnan et al. 2019). When each line only carries a single substitution segment, it can be known as single-segment substitution lines (Zhang 2021). Particularly, CSSLs or NILs (near-isogenic lines) are valuable prebreeding tools for broadening the genetic base of existing cultivars and a powerful platform for breeding by design (Balakrishnan et al. 2019; Zhang 2021). So far, the efficiency of QTL fine mapping and cloning has been improved greatly using CSSLs or NILs, such as GS3 for grain size (Sun et al. 2018), GL3.1 for grain length (Qi et al. 2012), qGL3, qGL6, and qGL3-2 for grain length (Kashif et al. 2020; Zhang et al. 2020; Liang et al. 2021), qKL3 for kernel length (Wang et al. 2020), GW7 for grain width (Wang et al. 2015), qGW1-2, qGW3-2, and qGW4-1 for grain width (Li et al. 2019), GW2 for grain weight (Song et al. 2007), GW5 for grain width and grain weight (Liu et al. 2017), GW6a for grain weight (Song et al. 2015), qGWTS and qTGW11 for 1000-grain weight (Okada et al. 2018; Wang et al. 2021b), Gn1 for grain number (Ashikari et al. 2005), qSPI for spikelets per panicle (Ma et al. 2019), qGNC7(t) for grain number per panicle (Hu et al. 2018), qSSD5 and qSSD8 for seed setting density (Wang et al. 2021a), and qPPP2 for panicles per plant (Tao et al. 2016). Although these QTLs provided some candidate genes or revealed some molecular regulatory mechanisms of rice yield traits. However, they are insufficient to explain the complexity of the genetic mechanism. Again, due to the separating of favorable alleles in different varieties (Zheng et al. 2020; Dhat et al. 2021), it is necessary to identify more QTLs for yield traits using novel CSSL developed from different elite cultivars.

Restorer line is key in hybrid rice breeding. Xihui18 as an indica rice restorer line was bred by
the Rice Research Institute of Southwest University. It had many advantages as high general combining ability, good flowering habit and long panicles, and multiple grains per panicle. However, the number of panicles per plant in Xihui18 is few and the grains are long and narrow. Huhan3, a *japonica* cultivar, is characterized by multiple panicles, short and wide grains, and strong resistance to stress. Here, we identified a rice CSSL-Z431, which is mainly characterized by short-wide grain and multiple panicles per plant, derived from Xihui18 as recipient and Huhan3 as donor parent. In this study, we will clarify the following theoretical problems: since Z431 contained 6 substitution segments, how many QTLs will respond to the different traits in Z431, and how are they distributed in these substitution segments? If QTLs controlling the same trait are more than one, will they exist independent inheritance or epistatic interaction? Which of these QTLs were reported or novel? Hence, we analyze Z431 systematically and map QTL using secondary F2 population from a cross between Xihui18 and Z431, as well as verify the QTL with the developed SSSL and analyze the inheritance model and pyramid effect of target QTLs using the DSSL and TSSL. Finally, we also analyze the candidate genes of major target QTL. It is important to understand the genetic information of target QTL for design breeding.

## Materials and methods

### Experimental materials

**Development of Z431**

The rice CSSL Z431 was developed using Xihui18 as the recipient parent and Huhan3 as the donor parent. Xihui 18 is an *indica* rice restorer line bred by Southwest University, China. Huhan3 is a *japonica* rice cultivar of China. Firstly, 241 polymorphic markers between them were screened from 429 simple sequence repeat (SSR) markers that covered the whole rice genome. Then, they were used to develop CSSLs from BC2 F1 to BC3 F7, as showed in Fig. 1. Finally, a novel CSSL Z431 with six substitution segments was identified in BC3 F7, which displayed multiple panicle numbers per plant and short-wide grain. The identification of substitution segments in Z431 was conducted according to the method of Ma et al. (2019). The estimated substitution length was calculated by the method of Paterson et al. (1991). The chromosome map was constructed using the Mapchart 2.32 software (https://www.wur.nl/en/show/Mapchart.htm).

### Field planting

In July of 2018, Xihui18 was crossed with Z431 to get a hybrid at Xiema of Chongqing experimental station of Southwest University of China. In September of the same year, the hybrid seeds were planted to get F1 seeds at the Lingshui experimental base of Hainan province. On March 10 of 2019, Xihui18, Z431, and the F2 population were planted at the experimental station of Southwest University of Chongqing, China. On April 13, thirty plants of Xihui18 and Z431 and 150 individuals of the F2 population were transplanted in the same field. The spacing between the hills and rows was 16.67 cm and 26.67 cm. On March 12 of 2020, Xihui18, Z431, and eleven individuals selected from the F2 population for the development of SSSL, DSSL, and TSSL were planted into the same experimental field and transplanted 30 plants for each material on April 15 of the same year. On March 10 of 2021, Xihui18, Z431, and 6 SSSLs (single-segment substitution lines), 3 DSSLs (dual-segment substitution lines), and 2 TSSLs (triple-segment substitution lines) developed in the F3 generation were planted into the same experimental field and transplanted 30 plants for each material on April 17 of the same year. Conventional field management practices were applied.

### Measurement of yield-related traits

Ten plants each from Xihui18 and Z431 plots, together with 150 F2 plants were harvested at the maturity stage. Ten yield-related traits were measured, including the number of panicles per plant, panicle length, number of spikelets per panicle, number of grains per panicle, grain length, grain width, ratio of length to width, 1000-grain weight, seed setting rate, and yield per plant. The methods of measurement were the same with Ma et al. (2020a). Panicle length, number of spikelets per panicle, number of grains per panicle, and seed setting rate were measured using all the effective panicles...
of the plant. The length and width of 30 grains for each plant were measured with a Vernier caliper and then calculated the mean value and ratio of grain length to width for each plant. The 1000-grain weight of Xihui18 and Z431 was measured from random samples of 3000 grains, from which 1000-grain subsets were weighed on an electronic balance, with three repetitions. The 1000-grain weight of each F₂ plant was determined as the weight of 200 grains, multiplied by 5, with three repetitions. Yield per plant was weighed by electronic balance. The mean phenotypic value and t-test of the above traits for Xihui18 and Z431 were calculated in Microsoft Excel 2016.

Scanning electron microscopy

At the completion of the booting stage and before the heading period, the phenotypic characteristics of the inner and outer epidermal cells of the glume in Xihui18 and Z431 were investigated using a Hitachi SU3500
scanning electron microscope (Hitachi, Tokyo, Japan) with a frozen stage (−40 °C) under a low-vacuum environment (Zhang et al. 2020). The total cell number between Xihui18 and Z431 in the outer epidermis of the glume along the longitudinal axis was counted in applicable pixel size (1280×960). At the same magnification (50.0 mm×320 SE), we counted the protuberances (a protuberance represents a cell) with three repetitions, except the ones on the boundary.

**QTL mapping**

A secondary F₂ population with 150 individuals was used for QTL mapping, which was derived from a cross between Xihui18 and Z431. Total genomic DNA of Xihui18, Huhan3, Z431, and the 150 individuals from the F₂ population was extracted using the cetyl-trimethylammonium-bromide (CTAB) method. PCR amplification, non-denaturing polyacrylamide gel electrophoresis, and rapid silver staining were conducted as the methods described by Zhao et al. (2016a). Bands same with Xihui18 were scored as “1,” bands same with Z431 were scored as “0,” and the absence of marker bands was scored as “.” The marker assignments of all SSR markers on the substitution segments of Z431, together with the phenotypic values of each individual in the F₂ population, were used for QTL mapping. QTL mapping was performed using the restricted maximum likelihood method by mixed linear models (REML) implemented in the HPMIXED procedure of SAS 9.3 (SAS Institute Inc, Cary, NC, USA), with significance determined at α=0.05.

**Development of SSSLs, DSSLs, TSSLs, and verification and pyramid of QTLs using SSSLs, DSSLs, and TSSLs**

According to the QTL mapping, 11 individuals from the F₂ population were selected to develop SSSLs, DSSLs, and TSSLs by MAS and grown as the lines Z801–Z811 in 2020, with 30 plants each. Then, 11 homozygous secondary segment substitution lines developed from F₃ were planted as lines, with 30 plants each. Finally, 10 individuals from the middle of each plot were sampled randomly from Xihui18 and SSSLs, DSSLs, and TSSLs after maturity, and ten yield-related traits above involved were measured.

As for each SSSLᵢ (S1-S6), given that the theoretical hypothesis H0: no QTL existed on the substitution segment of SSSLᵢ. Then, when the probability value was less than 0.05 by Student’s t test, we denied the hypothesis and considered that a QTL for a certain trait existed in SSSLᵢ. According to the genetic model under certain environment (same year and same experimental field and no replicate plot designed), \( P₀=\mu+\varepsilon \) for Xihui18, and \( Pᵢ=\mu+aᵢ+\varepsilon \) for SSSLᵢ, where \( P₀ \) and \( Pᵢ \) represent the phenotype value of any plant in the plot of Xihui18 and the SSSLᵢ carrying the “i” substitution segment. \( \mu \) represents the mean value of Xihui18, \( aᵢ \) represents the additive effect of the QTL, and \( \varepsilon \) represents the random error. The additive effect of the QTL was calculated as half the difference between the mean phenotypic values of SSSL and Xihui18 (Wang et al. 2021a; Liang et al. 2021). All calculations were conducted in Microsoft Excel 2016.

As for each DSSLᵢⱼ and TSSLᵢⱼ, given that the theoretical hypothesis H0: QT1 located in “i” substitution segment and QT2 located in “j” substitution segment and QT3 located in “k” substitution segment belonged to independent inheritance, showed as “2+0=1+1” and “3+0+0=1+1+1.” Then, when the probability value was more than 0.05 by Student’s t test, we accepted the hypothesis that QT1, QT2, and QT3 belonged to independent inheritance. At this time, the phenotype value of (Xihui18+DSSLᵢ) was the same with (SSSLᵢ+SSSLᵢ), as well as (Xihui18+Xihui18+TSSLᵢⱼ) same with (SSSLᵢ+SSSLᵢ+SSSLᵢ). However, when the probability value was less than 0.05 by Student’s t test, we denied the hypothesis and considered that epistatic interaction occurred between allelic QT1, QT2, and QT3. According to the genetic models, \( Pᵢⱼ=\mu+aᵢ+aⱼ+Iᵢⱼ+\varepsilon \) for DSSLᵢⱼ and \( Pᵢⱼk=\mu+aᵢ+aⱼ+aⱼk+Iᵢⱼk+\varepsilon \) for TSSLᵢⱼk, respectively, where \( Pᵢⱼ \) and \( Pᵢⱼk \) represented the phenotype value of any plant in the plot of the DSSLᵢⱼ and TSSLᵢⱼk. \( aᵢ, aⱼ, \) and \( aⱼk \) represent the additive effect of the QTL in “i,” “j,” and “k” substitution segments, respectively. \( Iᵢⱼ \) and \( Iᵢⱼk \) represented the \( aᵢaⱼ \) epistatic effect between QTLS in “i” and “j” substitution segments of DSSLᵢⱼ and \( aᵢaⱼak \) epistatic effect between QTLS in “i,” “j,” and “k” substitution segments of TSSLᵢⱼk, respectively. Thus, the epistatic effect between QTLS in DSSLᵢ and TSSLᵢⱼ could be estimated as half of the mean phenotypic values of [(Xihui18+DSSLᵢ)]
-\(\text{SSSL}_i + \text{SSSL}_j\)} and half of the mean phenotypic values of \((X_{i18} + X_{i18} + T_{SSL}_ij)\) and \((X_{i18} + X_{i18} + \text{SSSL}_i)\), respectively (Wang et al. 2021a; Liang et al. 2021).

**Candidate gene prediction and DNA sequencing of qPN3, qGL3, and qGW5**

At the substitution interval of three major QTLs, including qPN3, qGL3, and qGW5, we analyzed the candidate gene information by the Rice Annotation Project (https://rapdb.dna.afrc.go.jp/) and the China National Rice Database Center (http://www.ricedata.cn/) and the Gramene (http://www.grameene.org/). As for possible genes, the whole sequence was downloaded, and the primers were designed on Vector NTI to amplify the target fragments using the DNA of Xihui18 and corresponding SSSLs as templates, respectively. The PCR products were forwarded for sequencing to Tsingke biological technology Co, Ltd (Chongqing, China).

**Total RNA extraction and qRT-PCR analysis**

Total RNA was extracted from the root, stem, leaf, sheath, and panicle of Xihui18 and Z431 using the RNeasy pure plant RNA purification kit (Tiangen, Beijing, China). The first-strand complementary cDNA was synthesized from 2 µg of total RNA using oligo (dT)18 primers in 20 µL of reaction volume using the Prime script reagent kit with gDNA Eraser (Takara, Dalian, China). The qRT-PCR analysis was performed with a 7500 real-time PCR system (Applied Biosystems, Carlsbad, CA, USA) and a SYBR premix Ex Taq II kit (TaKaRa). Rice gene Actin (LOC_Os03g50885) was used as the internal control to normalize all data. Each set of experiments was repeated three times. All primer pairs used for sequencing and qRT-PCR are listed in Table S1.

**Results**

Identification of substitution segments and phenotype analysis Z431

Based on the development of Z431 using 241 polymorphic SSR markers, we further identified the substitution segments and the genetic background of 10 plants of Z431 using 21 SSR markers on the six substitution segments of Z431 and 220 SSR markers outside of the substitution segments. The results showed that the substitution segments of 10 plants of Z431 were identical and no other residual segments from Huhan3 were detected. Z431 contained six substitution segments from Huhan3, distributed on chromosomes 1, 2, 3, 5, and 12. The chromosome 5 contained two substitution segments and the other chromosomes each contained one (Fig. 2). The total length of these substitution segments in Z431 was estimated as 12.71 Mb. Among them, the length of the longest substitution segment of Z431 was estimated as 3.76 Mb, the shortest one was 0.95 Mb and the average length of these substitution segments was 2.12 Mb (Fig. 2).

Compared with Xihui18, the heading date of Z431 was earlier 8 d than that of Xihui 18 (115 d) in Chongqing, China. The panicle length, number of grains per panicle, number of spikelets per panicle, grain length, and ratio of length to width in Z431 decreased significantly by 20.37%, 38.60%, 37.77%, 1.37%, and 33.24%, respectively. The number of panicles per plant, grain width, 1000-grain weight, and yield per plant of Z431 increased significantly than Xihui18 by 80.95%, 30.00%, 16.55%, and 34.11%, respectively (Fig. 3). There was no significant difference in seed setting rate (86.40%) between Z431 and that (87.99%) of Xihui18. Thus, the increased yield per plant of Z431 was caused by the increase in the number of panicles per plant and grain weight.

**Cytological analysis of Z431**

To examine the factors responsible for the decrease in grain length and increase in width of Z431, scanning electron microscopy was used to observe the cell morphology of glumes in Xihui18 and Z431 before the heading stage. The cell length of the glume in Z431 was 88.06 µm, shorter than that (178.60 µm) of Xihui18. While the cell width in Z431 was 40.00 µm, wider than that (29.00 µm) of Xihui18 (Fig. 4a–d, g–h). There was no significant difference in the cell number of a certain glume area along the longitudinal axis between Xihui18 and Z431 (Fig. 4e–f, i). These results indicated that the short-wide grain of Z431 was caused by the increase in cell width and decrease in cell length and not by cell number of glume.
Fig. 2  Chromosome substitution segments and harboring QTLs for yield traits of Z431. The genome of indica rice ‘9311’ was used as the reference genome. Physical distance (Mb) is specified on the left of each chromosome and markers are specified on the right. The solid black segment is the substitution segment from the donor Huhan3 and the identified QTLs are listed on the left of each chromosome in italics. PN, the number of panicles per plant; PL, panicle length; GPP, number of grains per plant; SPP, number of spikelets per panicle; SSR, seed setting rate; GL, grain length; GW, grain width; RLW, ratio of length to width; GWT, 1000-grain weight.
Fig. 3 Phenotype of Xihui18 and Z431. a Plant type of Xihui18 (left) and Z431 (right). b Main panicle of Xihui18 (left) and Z431 (right). c, d Grains of Xihui18 (c) and Z431 (d). e, f Brown grains of Xihui18 (e) and Z431 (f). g–o No. of panicles per plant (g), panicle length (h), no. of spikelets per panicle (i), no. of grains per panicle (j), grain length (k), grain width (l), ratio of length to width (m), 1000-grain weight (n), yield per plant (o) of Xihui18 and Z431. Data are given as the mean and SE (n=10). * and ** indicate significant differences of traits between Xihui18 and Z431 at P<0.05 and P<0.01, respectively. Bars in a and b, 10 cm; c–f, 2 mm
Fig. 4 Scanning electron microscopy observation and analysis of glume in Z431. Scanning electron micrograph of glume, inner epidermis, and outer epidermis of glume in Xihui18 (a, c, e) and Z431 (b, d, f). g–h Cell length and cell width in the inner epidermis of Xihui18 and Z431. i The cell number in a certain area along the longitudinal axis between Xihui18 and Z431. Bars in a and b, 1 mm; c–f, 100 μm. Data are given as the mean and SE (n = 10). Asterisks (**) indicate a significant difference between the Xihui18 and Z431 at p < 0.01; ns indicates no significant difference between the Xihui18 and Z431.
Identification of QTL for yield-related traits and correlation analysis

A total of thirteen QTLs were detected in the secondary F₂ population from a cross between Xihui18 and Z431. These QTLs were distributed on five substitution segments of Z431 and explained the phenotypic variation from 2.10 to 30.75% (Table 1). Among them, there were one QTL for panicle length, number of panicles per plant, number of spikelets per panicle, number of grains per panicle, grain length, and grain width, respectively, and 3 QTLs for ratio of length to width and 1000-grain weight, respectively. The short panicle of Z431 was controlled by qPL3, which decreased the panicle length by 0.89 cm. qPN3 increased the number of panicles per plant of Z431 by 0.51, explained 6.75% of the phenotypic variation. The less number of grains and spikelets per panicle of Z431 were responded by qGPP12 and qSPP12, whose additive effects were −7.01 and −7.25, respectively. qSSR5 decreased seed setting rate by 1.19 percent point, explained 2.35% of the phenotypic variation. qGL3 for grain length from Huhan3 reduced the grain length of Z431 by 0.27 mm, explained 30.75% of the variation. qGW5 increased the grain width of Z431 by 0.09 mm, explained 2.10% of the phenotypic variation. The grain shape of Z431 was controlled by qRLW2, qRLW3, and qRLW5, while qRLW2 increased the ratio of length to width by 0.06 and qRLW3 and qRLW5 decreased the trait by 0.08 and 0.06. The 1000-grain weight of Z431 was controlled by two major QTL (qGWT3, qGWT-5–2) and one minor QTL (qGWT-5–1) and the additive effects from alleles of Huhan3 decreased the 1000-grain weight of Z431 for qGWT3 by 1.09 g, and increased it by 0.84 and 0.76 g for qGWT-5–1 and qGWT-5–2, respectively.

In addition, we also found that some QTLs were always detected in cluster, for example, qPN3, qPL3, qGL3, qRLW3, and qGWT3 linked to the same marker, so was qGPP12 and qSPP12, as well as qSSR5, qRLW5, and qGWT-5–1. Whether these traits correlated? We analyzed the Pearson correlation coefficient for 10 yield-related traits in the F₂ population by IBM SPSS Statistics 26 (Table 2). The number of panicles per plant (PN) displayed significantly positive correlations with the number of grains (GPP) and spikelets per panicle (SPP), while no correlation with panicle length (PL), grain length (GL), ratio of length to width (RLW), and 1000-grain weight (GWT) (Table 2). Panicle length (PL) showed a significantly positive correlation with the number of grains (GPP) and spikelets per panicle (SPP), while no correlation with panicle length (PL), grain length (GL), ratio of length to width (RLW), and 1000-grain weight (GWT) (Table 2). Panicle length (PL) showed a significantly positive correlation with the number of grains (GPP) and spikelets per panicle (SPP). The number of grains (GPP) exhibited a significantly positive correlation with the number of spikelets per panicle (SPP) and seed setting rate (SSR). Grain length (GL) displayed a significant negative correlation with grain width (r = −0.414**) and significantly positive correlation with ratio of length to width (r = 0.711**). Grain width also showed a significant negative correlation with the ratio of length to width.

### Table 1: QTL for yield-related traits identified in substitution segments of Z431

| Trait                  | QTL   | Chromosome | Linked marker | Additive effect | Variance (%) | P-value  |
|------------------------|-------|------------|---------------|----------------|--------------|----------|
| Panicle length (cm)    | qPL3  | 3          | RM6266        | −0.89          | 17.59        | <0.0001  |
| Number of panicles per plant | qPN3  | 3          | RM6266        | 0.51           | 6.75         | 0.0204   |
| Number of grains per panicle | qGPP12 | 12         | RM1261        | −7.01          | 4.42         | 0.0143   |
| Number of spikelets per panicle | qSPP12 | 12         | RM1261        | −7.25          | 3.85         | 0.0206   |
| Seed setting rate      | qSSR5 | 5          | RM3322        | −1.19          | 2.35         | 0.0004   |
| Grain length (mm)      | qGL3  | 3          | RM6266        | −0.27          | 30.75        | <0.0001  |
| Grain width (mm)       | qGW5  | 5          | RM169         | 0.09           | 2.10         | <0.0001  |
| Ratio of length to width | qRLW2 | 2          | RM2770        | 0.06           | 4.98         | 0.0259   |
|                        | qRLW3 | 3          | RM6266        | −0.08          | 8.78         | 0.0018   |
|                        | qRLW5 | 5          | RM3322        | −0.06          | 4.70         | 0.0390   |
| 1000-grain weight (g)  | qGWT3 | 3          | RM6266        | −1.09          | 18.86        | <0.0001  |
|                        | qGWT-5–1 | 5       | RM3322        | 0.84           | 9.38         | 0.0022   |
|                        | qGWT-5–2 | 5       | RM169         | 0.76           | 11.90        | 0.0085   |
and five QTLs (qPN5, qGW5-2, qRLW5-2, qGWT5, and qYD5) were detected by S5 (Fig. 5a, c, e, f, g, h). Among them, qRLW5-1, qGW5-2, and qGWT5-1 were detected in 2019 (Table 2) and 2021 (Fig. 5f, g, e, g, o). The substitution segment of S6 was located on chromosome 12 on which qGPP12 and qSPP12 were validated (Fig. 5a, j, k), and qYD12 (Fig. 5a, h) was detected. The substitution segment of S1 was located on chromosome 1 on which qGL1, qGW1, qRLW1, qGWT1, and qYD1 were detected; however, they were detected only once. Thus, eleven of thirteen QTLs could be validated by according SSSLs and two minor QTLs (qSSR5, qGWT5-1) were not validated by S4. In addition, fifteen QTLs were detected by according SSSLs, while not detected in the secondary F2 population. The results indicated that SSSL had higher efficiency of QTL detection (Fig. 5a).

The number of panicles per plant (7.60 and 7.20) in S3 carrying qPN3 (a = 1.63) and S5 containing qPN5 (a = 1.43) was significantly more 3.27 and 2.87 than that (4.33) of Xihui18, respectively, while those of the other SSSLs (S1, S2, S4, and S6) without QTL for PN showed no significant differences with that Xihui18 (Fig. 5c). The grain length (9.85, 9.39, 9.72, and 9.85 mm) in S1 harboring qGL1 (a = −0.24), S2 containing qGL2 (a = −0.47), S3 with qGL3 (a = −0.31), and S4 carrying qGL5 (a = −0.24) was significantly shorter than that of Xihui18 (10.33 mm), while grain length (10.41 and 10.23 mm) of S5 and S6 without QTL for GL displayed no significant differences with that of Xihui18 (Fig. 5b, d). The grain width (3.21, 3.35, 9.39, 9.72, and 9.85 mm) in S1 harboring qGL5, qGWT5-1, and qRLW5-1) were detected by S4 (Fig. 5a, d, e, f) and five QTLs (qPN5, qGW5-2, qRLW5-2, qGWT5, and qYD5) were detected by S5 (Fig. 5a, c, e, f, g, h).

Table 2 Pearson correlation coefficient among yield-related traits in the F2 population

|       | PN   | PL   | GPP  | SPP  | SSR  | GL   | GW   | RLW  | GWT  | YD   |
|-------|------|------|------|------|------|------|------|------|------|------|
| PN    | 1    |      |      |      |      |      |      |      |      |      |
| PL    | 0.110| 1    |      |      |      |      |      |      |      |      |
| GPP   | 0.893** | 0.329** | 1  |      |      |      |      |      |      |      |
| SPP   | 0.892** | 0.331** | 0.997** | 1  |      |      |      |      |      |      |
| SSR   | 0.105 | 0.042| 0.139* | 0.072| 1    |      |      |      |      |      |
| GL    | −0.053| 0.111| −0.052| −0.045| −0.102| 1    |      |      |      |      |
| GW    | −0.026| −0.054| −0.063| −0.059| −0.043| −0.414** | 1  |      |      |      |
| RLW   | −0.010| 0.122* | 0.026| 0.020| 0.062| 0.711** | −0.817** | 1  |      |      |
| GWT   | 0.037 | 0.054| −0.044| −0.034| −0.143* | 0.103| 0.185** | −0.174** | 1  |      |
| YD    | 0.886** | 0.314** | 0.964** | 0.962** | 0.131* | −0.027| −0.042| 0.011| 0.038| 1    |

*, ** indicate the coefficient of correlation between two traits existing significant difference at p = 0.01, p = 0.05 level respectively. No * indicates no significant difference at p = 0.05 level.

The development of SSSL, DSSL, and TSSL, as well as validation and pyramid analysis of QTLs using the SSSLs, DSSLs, and TSSLs

Based on the QTL mapping, six SSSLs (S1–S6), three DSSLs (D1–D3), and two TSSLs (T1 and T2) were further developed by MAS in F3 (Fig. 5a).

The substitution segment of S2 was located on chromosome 2 on which qGL2, qRLW2, qGWT2, and qYD2 were detected (Fig. 5a, d, f, g, h). Among them, qRLW2 was detected simultaneously in 2019 (Table 2) and 2021 (Fig. 5f). The substitution segment of S3 was located on chromosome 3 and six QTLs (qPN3, qGL3, qRLW3, qGWT3, qYD3, and qPL3) were validated by S3 (Fig. 5a, c, d, f, g, h, i). The substitution segments of S4 and S5 were all located on chromosome 5 and three QTLs (qGL5, qGW5-1, and qRLW5-1) were detected by S4 (Fig. 5a, d, e, f) and five QTLs (qPN5, qGW5-2, qRLW5-2, qGWT5, and qYD5) were detected by S5 (Fig. 5a, c, e, f, g, h).

Thus, qPN3 should not be controlled by the same gene with qPL3, qGL3, qRLW3, and qGWT3. While qGL3, qRLW3, and qGWT3 belonged to pleiotropy. Also, qGPP12 and qSPP12 were pleiotropy; similarly, qSSR5, qRLW5, and qGWT-5–1 should belong to pleiotropy.

Development of SSSL, DSSL, and TSSL, as well as validation and pyramid analysis of QTLs using the SSSLs, DSSLs, and TSSLs
S2 harboring $qGW2$ ($a = -1.51$) was significantly lighter than that (27.66 g) of Xihui18. And 1000-grain weight of S4 and S6 without QTL displayed no significant differences with that of Xihui18 (Fig. 5g). Panicle length (26.31 cm) of S3 with $qPL3$ ($a = -1.89$) exhibited significantly shorter than that (30.12 cm) of Xihui18, while those of the other SSSLs without QTL for panicle length had no significant difference with that of Xihui18 (Fig. 5i). The number of grains per plant (188.91) of S6 carrying $qGPP12$ ($a = -39.72$) displayed significantly less than that (268.35) of Xihui18, and those of the other SSSLs without QTL for the trait had no significant differences with that of Xihui18 (Fig. 5j). The number of spikelets per plant (189.91) of S6 carrying $qSPP12$ ($a = -44.58$) displayed significantly less than that (279.08) of Xihui18, and those of other SSSLs without QTL for the trait had no significant differences with that of Xihui18 (Fig. 5k). The yield per plant (34.82, 41.25, 42.70 g) of S1 carrying $qYD1$ ($a = 3.51$), S3 harboring $qYD3$ ($a = 6.72$), and S5 containing $qYD5$ ($a = 7.45$) was significantly higher than that (27.66 g) of Xihui18. In contrast, the yield per plant (24.41 and 23.18 g) of S2 with $qYD2$ ($a = -1.69$) and S6 containing $qYD12$ ($a = -2.31$) was significantly lower than that of Xihui18 (Fig. 5h).

From the above results, we can find that most of the yield-related traits were controlled by more than one QTL, such as PN, GL, GW, GWT, and YD. Then, did these QTLs belong to independent inheritance or epistatic interaction? We designed the experiment to clarify the effect between QTL and QTL. The yield of S5 ($a = 1.43$) in D3 yielded an epistatic effect of $-1.86$; thus, the genetic effect of PN in D3 was 1.20. Due to $1.20 < -1.86 < 1.63$, D3 had less number of panicles (6.75) than those (7.60 and 7.20) of S3 ($qPN3$) and S5 ($qPN5$), however, with no significant differences among them (Fig. 5a, c). Pyramiding of $qPN3$ ($a = 1.63$) and a substitution locus on chromosome 2 without PN in D1 yielded an epistatic effect of $-1.08$, which increased PN genotypically by 0.55. Because of $0 < 0.55 < 1.63$, the number of panicles (5.60) of D1 was more than that (4.50) of S2 (without QTL for panicles) and significantly less than that (7.60) of S3 ($qPN3$) (Fig. 5a, c). Pyramiding of $qPN5$ ($a = 1.43$) and a substitution locus without PN...
on chromosome 2 in D2 yielded an epistatic effect of 0.12, which increased PN genetically by 1.55. Owing to $0 < 1.43 < 1.55$, the number of panicles (7.60) of D2 was more than that (4.50) of S2 (without PN QTL) and that (7.20) of S5 ($qPN3$), displaying a significant difference with S2 (Fig. 5a, c). Pyramiding of $qPN3 (a = 1.63)$, $qPN5 (a = 1.43)$, and a substitution locus on chromosome 1 without PN in T1 produced an epistatic effect of −2.09, which increased the number of panicles per plant genetically by 0.97. On account of $0 < 0.97 < 1.43 < 1.63$, the number of panicles (7.20) in T1 was significantly more than that (5.25) of S1 and less than that (7.60) of S3 ($qPN3$) and equal to that (7.20) of S5 ($qPN5$), however, without significant difference with S3 and S5 (Fig. 5a, c). Pyramiding of $qPN3 (a = 1.63)$, $qPN5 (a = 1.43)$, and a substitution locus without PN on chromosome 2 in T2 produced an epistatic effect of −2.02, resulting in the increase of 1.04 number of panicles genetically. Due to $0 < 1.04 < 1.43 < 1.63$, T2 exhibited significantly more panicles (6.60) than (4.50) of S2, and less than those (7.60 and 7.20) of S3 ($qPN3$) and S5 ($qPN5$), with no significant difference with S3 and S5 (Fig. 5a, c).

Pyramiding of $qGL2 (a = −0.47)$ and $qGL3 (a = −0.31)$ in D1 yielded an epistatic effect of 0.62, which reduced grain length genetically by 0.16 mm. Due to $−0.47 < −0.31 < −0.16$, D1 showed longer grain (10.03) than those (9.39 and 9.72) of S2 ($qGL2$) and S3 ($qGL3$), displaying a significant difference with S2 (Fig. 5a, b, d). Pyramiding of $qGL3 (a = −0.31)$ and a substitution locus without GL on chromosome 5 in D3 produced an epistatic effect of −0.41, which decreased grain length genetically by 0.72. Because of $−0.72 < −0.31 < 0$, D3 exhibited significantly shorter grain length (8.97) than those (9.72 and 10.41) of S3 ($qGL3$) and S5 (no QTL) (Fig. 5a, b, d). Pyramiding of $qGL1 (a = −0.24)$, $qGL3 (a = −0.31)$, and a substitution locus without GL on chromosome 5 in T1 produced an epistatic effect of −0.18, which reduced grain length genetically by 0.73. Owing to $−0.73 < −0.31 < −0.24 < 0$, T1 displayed grain length (8.95) significantly shorter than those (9.85, 9.72, and 10.41) of S1 ($qGL1$), S3 ($qGL3$), and S5 (no QTL) (Fig. 5a, b, d). Pyramiding of $qGL2 (a = −0.47)$, $qGL3 (a = −0.31)$, and a substitution locus without GL on chromosome 5 in T2 yielded an epistatic effect of −0.05, which decreased grain length genetically by 0.83. Because of $−0.83 < −0.47 < −0.31 < 0$, T2 showed grain length (8.75) significantly shorter than those (9.39, 9.72, and 10.41) of S2 ($qGL2$), S3 ($qGL3$), and S5 (no QTL) (Fig. 5a, b, d). However, a pyramiding of $qGL2 (a = −0.47)$ and a substitution locus without GL on chromosome 5 in D2 displayed no epistatic effect, indicating that the two loci belonged to independent inheritance (Fig. 5a, b, d).

Pyramiding of $qGW5-2 (a = 0.09)$ and a substitution locus without GW on chromosome 2 in D2 yielded an epistatic effect of −0.08, which increased grain width genetically by 0.01. Due to $0 < 0.01 < 0.09$, the grain width (3.13 mm) of D2 was significantly narrower than that (3.31) of S5 ($qGW5-2$), and broader than that (3.11) of S2 (no QTL), with no significant difference (Fig. 5a, b, e). Pyramiding of $qGW5-2 (a = 0.09)$ and a substitution locus without GW on chromosome 3 in D3 produced an epistatic effect of 0.005, which increased grain width genetically by 0.095, because $0 < 0.09 < 0.095$, D3 displayed grain width (3.35 mm) significantly wider than that (3.16) of S3 (no QTL), and no significant difference with that (3.31) of S5 ($qGW5-2$) (Fig. 5a, b, e). Pyramiding of two substitution loci without GW on chromosomes 2 and 3 in D1 yielded an epistatic effect of 0.05. Because of $0 < 0.05$, D1 exhibited significantly wider grain (3.24 mm) than those (3.11 and 3.16) of S2 and S3 (Fig. 5a, b, e). However, pyramiding of $qGW1 (a = 0.04)$ and $qGW5-2 (a = 0.09)$ and a substitution locus without GW on chromosome 3 in T1 showed no epistatic effect, indicating that $qGW1$, $qGW5-2$, and the substitution locus on chromosome 3 belonged to independent inheritance. Similarly, $qGW5-2$ also displayed independent inheritance with two substitution loci without GW on chromosomes 2 and 3 in T2 (Fig. 5a, b, e).

Pyramiding of $qRLW2 (a = −0.08)$ and $qRLW3 (a = −0.14)$ in D1 yielded an epistatic effect of 0.08, which reduced the ratio of length to width genetically by 0.14. Although $−0.14 = −0.14 < −0.08$, the ratio of length to width (3.01) of D1 displayed no significant differences with that (3.13 and 3.01) of S2 ($qRLW2$) and S3 ($qRLW3$) (Fig. 5a, f). Pyramiding of $qRLW2 (a = −0.08)$ and $qRLW5-2 (a = −0.03)$ in D2 resulted in an epistatic effect of −0.04, which reduced the trait genetically by 0.15. Owing to $−0.15 < −0.08 < −0.03$, D2 exhibited significant rounder grain (2.99) than that (3.24) of S5 ($qRLW5-2$) and no significant difference with
that of (3.13) of S2 (qRLW2) (Fig. 5a, f). Pyramiding of qRLW3 (\(a = -0.14\)) and qRLW5-2 (\(a = -0.03\)) in D3 produced an epistatic effect of \(-0.25\), which reduced the trait genetically by 0.42. Because of \(-0.42 < -0.14 < -0.03\), D3 became significantly rounder grain than those (3.01 and 3.24) of S2 (qRLW5) (Fig. 5a, f). However, qRLW1, qRLW3, and qRLW5-2 displayed no epistatic effect in T1, so did qRLW2, qRLW3, and qRLW5-2 in T2 (Fig. 5a, f).

Pyramiding of qGWT3 (\(a = 1.83\)) and qGWT5 (\(a = 2.34\)) in D3 yielded an epistatic effect of \(-2.24\), which increased the 1000-grain weight genetically by \(1.93\). Although \(1.83 < 1.93 < 2.34\), D3 displayed no significant difference in 1000-grain weight (31.52 g) with that (31.32 and 32.35 g) of S3(qGWT3) and S5(qGWT5) (Fig. 5a, g). Pyramiding of qGWT1 (\(a = 1.74\)), qGWT3 (\(a = 1.83\)), and qGWT5 (\(a = 2.34\)) in T1 produced an epistatic effect of \(-4.32\), which increased the trait genetically by 1.59. Although \(1.59 < 1.74 < 1.83 < 2.34\), the 1000-grain weight (30.85 g) of T1 was no significant difference with that (31.15, 31.32, and 32.35 g) of S1 (qGWT1), S3(qGWT3), and S5(qGWT5) (Fig. 5a, g). However, pyramiding of qGWT2 and qGWT3 in D1, qGWT2 and qGWT5 in D2, and qGWT2, qGWT3, and qGWT5 in T2 all showed no epistatic effect (Fig. 5a, g), indicating that qGWT2 and qGWT3, qGWT2 and qGWT5, as well as qGWT2, qGWT3, and qGWT5 belonged to independent inheritance.

Pyramiding of qYD2 (\(a = -1.69\)) and qYD3 (\(a = 6.72\)) in D1 produced an epistatic effect of \(-3.31\), which increased the yield per plant genetically by 1.72 g. Due to \(-1.69 < 1.72 < 6.72\), D1 displayed increasing yield per plant (31.24 g) significantly less than that (24.41 g) of S2 (qYD2) and that (27.66 g) of Xihui18, and decreasing it significantly than that (24.41 g) of S2 (qYD3) (Fig. 5a, h). Pyramiding of qYD3 (\(a = 6.72\)) and qYD5 (\(a = 7.45\)) in D3 yielded an epistatic effect of \(-7.85\), which increased the yield genetically by 6.32 g. Although \(6.32 < 6.72 < 7.45\), the yield per plant (40.45 g) of D3 showed no significant difference with those (41.25 and 42.70) of S3 (qYD3) and S5 (qYD5), however, significantly higher than that (27.66 g) of Xihui18 (Fig. 5a, h). Pyramiding of qYD1 (\(a = 3.51\)), qYD3 (\(a = 6.72\)), and qYD5 (\(a = 7.45\)) in T1 resulted in an epistatic effect of \(-12.52\), which increased yield per plant genetically by 5.16 g. Due to \(3.51 < 5.16 < 6.72 < 7.45\), the yield per plant (38.12 g) of T1 was significantly higher than that (34.82) of S1 and that (27.66 g) of Xihui18, and no significant differences with those (41.25 and 42.70) of S3 (qYD3) and S5 (qYD5) (Fig. 5a, h). Pyramiding of qYD2 (\(a = -1.69\)), qYD3 (\(a = 6.72\)), and qYD5 (\(a = 7.45\)) in T2 produced an epistatic effect of \(-7.79\), which increased the yield genetically by 4.69 g. Due to \(-1.69 < 4.69 < 6.72 < 7.45\), the yield per plant (37.18 g) of T2 was significantly higher than that (24.41) of S2 and that (27.66 g) of Xihui18, and no significant differences with those (41.25 and 42.70) of S3 (qYD3) and S5 (qYD5) (Fig. 5a, h). In addition, qYD2 and qYD5 in D2 displayed independent inheritance (Fig. 5a, h).

As for panicle length, the number of grains per panicle, and the number of spikelets per panicle, all the loci in D1, D2, D3, and T1 and T2 displayed independent inheritance (Fig. 5a, i, j, k).

Candidate gene analysis of major QTLs-qPN3, qGL3, and qGW5

Considering the larger contribution rates of qPN3, qGL3, and qGW5 for major phenotypic variation of Z431, and also detecting both in F2 and SSSLs, they deserved our priority attention (Fig. 5a). qPN3 and qGL3 were all located on S3 whose estimated substitution length was 1.48 Mb (Fig. 6c, 7d). qGW5 was located on S5 whose estimated substitution length was 1.68 Mb (Fig. 8d).

About qPN3, we found a reported gene (OsIAGLU) in the substitution interval of S3, which influences the tiller formation by negatively regulating IAA. Thus, we firstly wanted to know whether the cloned OsIAGLU was the candidate gene for qPN3. By DNA sequencing, we found actually existing four differences of single-nucleotide polymorphisms (SNPs) in CDS of OsIAGLU between Xihui18 and S3. Moreover, three of four SNP differences caused changes in amino acids (Fig. 6c). Furthermore, by qRT-PCR analysis, the expression levels of OsIAGLU in root, stem, leaf, sheath, and panicle in S3 were higher than that in Xihui18, especially in root, leaf, and sheath (Fig. 6d). At the same time, the panicle number of S3 was significantly more than that of Xihui18 (Fig. 6a, b) that was consistent with the function of OsIAGLU. Thus, OsIAGLU should be the candidate gene for qPN3.

Concerning qGL3, considering that the cloned genes related to rice grain size development are
mainly involved in phytohormones such as BR, IAA, and CK signal pathway, MAPK signaling, the ubiquitin–proteasome pathway, and transcriptional

Fig. 6 Candidate gene analysis of qPN3. a Phenotype of PN in Xihui18 (left) and S3 (right) (containing qPN3). b Statistic analysis of the number of panicles per plant (PN) in Xihui18 and S3. Data were mean ± SD. ** showed 1% significant levels according to the Student t-test. c Position of qPN3 and sequence blast of OsIAGLU between Xihui18 and S3. d Relative expression levels of OsIAGLU between Xihui18 and S3 by qRT-PCR analysis.
regulatory factors (Li and Li, 2016), we checked all the genes related to the above pathways in this region of \( qGL3 \) and found 6 possible genes, including auxin-responsive protein, ABA signal transduction, and serine/threonine-protein kinase-like protein ACR4. Therefore, we preliminarily selected these 6 genes as candidate genes. Then, by the whole DNA sequencing of these genes between S3 and Xihui18, the candidate gene 1 (serine/threonine-protein kinase) and the candidate gene 5 (auxin-responsive protein) showed no differences between the two lines. The result showed that the candidate genes 1 and 5 should not be the candidate genes for \( qGL3 \). However, for the candidate gene 2 (an auxin-responsive protein),
there were 2 SNPs differences and 2 bp deletion in CDS of S3 compared with Xihui18, where the 175th base of CDS changed from C in Xihui18 to T in S3, resulting in Leu of Xihui18 to Phe of S3, and another SNP (from C to T) in the 357th of CDS without causing amino acid change. The 2 bp deletion in the 401st base of CDS in S3 caused delaying the termination of the translation (Fig. 7d). However, the qRT-PCR analysis showed that the expression level in the root, stem, leaf, sheath, and panicle displayed no significant differences between S3 and Xihui18 (Fig. 7e). These results showed that the candidate gene 2 could be temporary as the candidate gene of qGL3. With regards to the candidate gene 3 (serine/threonine-protein kinase in ABA signal transduction), there was 1 SNP difference with no resulting in amino acid change (Fig. 7d). At the same time, expression levels of the gene displayed no significant differences in the root, stem, leaf, sheath, and panicle between S3 and Xihui18 (Fig. 7f). These results indicated that candidate gene 3 should not act as the candidate gene of qGL3. For the candidate 4 (an auxin-responsive protein), we found differences of 4 SNPs between Xihui18 and S3, where the base C, T, and C in 125th, 215th, and 274th of the CDS in Xihui18 changed to A, G, and A in S3, which caused amino acid mutation from Ala, Val, and Ala in Xihui18 to Glu, Gly, and Glu in S3, respectively. And the other SNP did not cause amino acid changes (Fig. 7d). Intriguingly, the qRT-PCR analysis showed that expression levels of the gene in S3 were significantly higher than in Xihui18 in the leaf, sheath, and panicle (Fig. 7g).
About the candidate gene 6 (a serine/threonine-protein kinase-like protein ACR4), there was a deletion of base T in 1900th of CDS in S3, which caused shift mutation. In addition, there were still 3 SNP differences (Fig. 7d). Simultaneously, the qRT-PCR analysis showed that expression levels of the gene in S3 were significantly higher than in Xihui18 in the root, stem, leaf, sheath, and panicle (Fig. 7h). In conclusion, candidate genes 4 and 6 should be the best candidate genes of qGL3, and candidate gene 2 also acted as its candidate gene.

$qGW5$ was located on the substitution segment of S5, whose grain width and brown grain were significantly wider than that of Xihui18 (Fig. 8a, b, c). Since grain width also being within the grain size range, the candidate genes for $qGW5$ could be found by the above signal pathway of grain size. Firstly, we looked for all the genes within the substitution interval of 1.68 Mb for $qGW5$ by the Gramene (http://www.gramene.org/), then referred to the functions of every gene by Rice annotation project (https://rapdb.dna.affrc.go.jp/) and the China national rice database center (http://www.ricedata.cn/). Finally, we found 6 potential candidate genes according to the probable genes involved in the signal pathway of grain size (Li and Li, 2016). By DNA sequencing, we found that the candidate gene 3 (DNA-methyltransferase CMT2), candidate gene 4 (auxin-responsive protein), and candidate gene 5 (serine/threonine-protein kinase) did not exist any sequence differences between Xihui18 and S5, while the other three exhibited differences between Xihui18 and S5. The candidate gene 6 (E3 ubiquitin-protein ligase) displayed 3 SNP differences in the CDS between Xihui18 and S5. However, they all belonged to nonsense mutation (Fig. 8g). Again, the qRT-PCR analysis showed that there was also no significant difference in the expression levels for the gene in the root, stem, leaf, sheath, and panicle between S5 and Xihui18 (Fig. 8h). Thus, the candidate genes 3, 4, 5, and 6 should not be the candidate genes of $qGW5$. For the candidate 1 (RING-type E3 ubiquitin transferase), there were 2 SNP differences in the CDS between Xihui18 and S5, where the base G and T in the 62nd and 175th of CDS in Xihui18 changed to C and C in S5, which caused amino acid mutation from Gly and Met in Xihui18 to Ala and Thr in S5 (Fig. 8d). The qRT-PCR analysis showed that the expression levels of the gene were significantly higher in panicle, while lower in the root, stem, leaf, and sheath of S5 than those in Xihui18 (Fig. 8e). With regard to the candidate 2 (eukaryotic translation initiation factor 3 subunit), there were 3 SNP differences in the CDS between Xihui18 and S5. The 1500th base changed from G in Xihui18 to C in S5, resulting in amino acid mutation from Leu to Phe, whereas the other SNP did not cause amino acid changes (Fig. 8d). Furthermore, the qRT-PCR analysis showed that its expression levels were significantly higher in the stem, leaf, sheath, and panicle of S5 than that of Xihui18 (Fig. 8f). In conclusion, the candidate gene 1 (RING-type E3 ubiquitin transferase) and candidate gene 2 (eukaryotic translation initiation factor 3 subunit) should be the best candidate genes for $qGW5$.

**Discussion**

Z431 and its secondary substitution lines are potential to be used as novel rice restorer line in breeding novel hybrid rice varieties

Successful breeding of hybrid rice is an outstanding breakthrough in rice breeding history, which has dramatically improved the theory and techniques for crop genetics and breeding (Cui et al. 2020). To date, most of the hybrid rice varieties were interspecific hybrids (Li et al. 2020). Due to the close interspecies relationships and relatively small genetic differences, the heterosis was not strong enough. Thus, the use of heterosis between subspecies and wild species will be imperative. However, direct subspecies crosses often result in sterility and a reducing seed setting rate due to reproductive isolation (Nadir et al. 2018). The development of indica-japonica rice chromosome segment substitution lines can overcome the limitation (Singh et al. 2020). Excellent restorer line is an essential part of heterosis utilization, which often contains the main fertility recovery genes $Rf-1$ (Akagi et al. 2004), $Rf2$ (Etsuko et al. 2011), $Rf3$ (Cai et al. 2013), and $Rf4$ (Tang et al. 2014). Xihui18 was an elite rice indica restorer line with strong combining ability, long panicle with multiple grains, and long-narrow grain. However, its number of panicle per plant was fewer (4.26 for average in 2019 and 2021). In this study, a novel CSSL-Z431 containing six substitution segments was identified from Xihui18 as the recipient parent and Huhan3 as the donor parent. Compared with Xihui18, Z431 had
panicle number per plant of 7.56 (average value in 2019 and 2021) and short-wide grains. Again, six SSSLs (S1-S6), three DSSLs (D1-D3), and two TSSLs (T1 and T2) were developed by MAS in the F2 generation. One of the notable findings of this study was that all the fertility-restoring genes $Rf1-Rf4$ were all not substituted in Z431, SSSLs, DSSLs, and TSSLs, especially to some extent these substitution lines make up for the deficiencies of Xihui18. For example, the number of panicles per plant of S3, S5, D1, D2, D3, T1, and T2 was significantly more than that of Xihui18. Accordingly, the yield per plant (33.28, 34.82, 41.25, 42.70, 31.24, 41.74, 40.45, 38.12, and 37.18 g) of Z431, S1, S3, S5, D1, D2, D3, T1, and T2 were all significantly increased than that (27.66 g) of Xihui18. Seed setting rate of these secondary substitution lines were 87.53% for S1, 85.96% for S2, 85.14% for S3, 88.79% for S4, 91.78% for S5, 91.12% for S6, 87.44% for D1, 94.73% for D2, 93.13% for D3, 89.38% for T1, and 85.52% for T2 (calculated by the data in Fig. 5 j and k). Furthermore, the grain size of these lines displayed diversity. Therefore, Z431 and these secondary substitution lines have the potential to be used as novel restorer lines for breeding novel rice hybrid varieties.

Identification of QTL for yield-related traits of Z431 and comparison with the reported genes

Since Z431 had still six chromosomal substitution segments from donor Huhan3 and significant differences in nine yield-related traits with recipient parent Xihui18. Then, which QTLs are responsible for these different traits in these substitution segments? We further identified 13 QTLs for these traits using the secondary F2 population from crosses between Xihui18 and Z431. Among them, eleven QTLs could be validated by 6 developed SSSLs (S1-S6). In addition, fifteen QTLs could be detected by these SSSLs, while were not identified in the F2 population. Thus, there were in total 28 QTLs identified. Among them, $qGL1$, $qGW1$, $qRLW1$, and $qGWT1$ were all linked with RM283 (5.27 Mb) in S1. Again, these traits all displayed significant correlations. Thus, these QTLs should belong to pleiotropy. At this substitution interval, we found $OsOFP1$ (7.01 Mb) distancing 1.78 Mb from RM283. $OsOFP1$ negatively regulates cell reproduction and elongation by the BR signal transduction pathway. The seeds of overexpressing $OsOFP1$ transgenic plants became shorter, wider, and thicker (Yang et al. 2018). Similarly, the grains of S1 also exhibited significantly shorter, wider, and heavier than that of Xihui18. Thus, $OsOFP1$ could act as the candidate gene for $qGL1$ etc. Similar with $qGL1$, these genes as $qGL3$, $qRLW3$, $qGWT3$, $qPL3$, and $qPN3$ were all linked to RM6266 (27.64 Mb) on chromosome 3 in S3. However, PN displayed no significant correlation with PL, GL, RLW, and GWT, and the others had significant correlations with each other. Thus $qPN3$ should not be controlled by the same gene with $qPL3$, $qPN3$, $qGL3$, $qRLW3$, and $qGWT3$, and the latter should be pleiotropy. At this substitution interval, $qPN3$ should be an allele of $OsIAGLU$ (27.78 Mb). $OsIAGLU$ encodes IAA-glucose synthase, which is known to generate IAA-glucose conjugate from free IAA. The number of tillers and panicles significantly increased in the transgenic lines of overexpression of $OsIAGLU$ compared to the wild-type plants (Choi et al. 2012). By DNA sequencing of $OsIAGLU$, there were actual differences of DNA sequences between Xihui18 and S3. Especially, the expression level of $OsIAGLU$ in S3 was significantly higher than that in Xihui18, and S3 displayed significantly increasing panicles number than Xihui18. Thus, $OsIAGLU$ should be the candidate gene for $qPN3$. Although the gene has been cloned, compared with its overexpression transgenic plants, S3 are more beneficial to be used in rice design breeding. In addition, just as mentioned above, the substitution interval of S3 still contained $qGL3$, $qRLW3$, $qGWT3$, and $qPL3$. By the cloned genes for grain size, rice grain size development is mainly involved in phytohormone signal pathways such as BR, IAA, and CK, as well as MAPK signal pathway, protein kinase G protein signaling, the ubiquitin–proteasome pathway, and transcriptional regulatory factors (Li and Li. 2016). By checking all the genes with function annotations in this substitution region of S3, we found six possible genes involved in the signal pathway of grain size, including candidate gene 1 for serine/threonine-protein kinase, candidate genes 2, 4, and 5 for auxin-responsive protein, candidate 3 for abscisic acid–activated protein kinase, and candidate 6 for serine/threonine-protein kinase-like protein ACR4. Some genes for grain size have been reported involving these signaling pathways. For example, $BG1$ as a primary response gene for auxin
regulates auxin transport, positively regulating cell division and cell elongation (Liu et al. 2015). *OsPPKL1/GL3.1* encodes a protein phosphatase kelch (PPKL) family–Ser/Thr phosphatase (Zhang et al. 2012), which are involved in BR signaling (Mora-García et al. 2004). *GL3.1* controls rice seed size and yield by direct dephosphorylation of the substrate cyclin-T1;3, and down-regulation of *GL3.1* in rice results in shorter grains, suggesting that *GL3.1* might participate in BR signaling (Qi et al. 2012; Li and Li. 2016b). By sequencing, the candidate 1 (serine/threonine-protein kinase) and the candidate 5 (auxin-responsive protein) showed no difference between the two lines. The candidate gene 3 (abscisic acid–activated protein kinase) should not be the candidate genes of *qGL3* because there were no amino acid changes, although existing 1 SNP difference in CDS between Xihui18 and S3. As for candidate gene 2 (auxin-responsive protein), there were 2 SNPs differences and 2 bp deletion in CDS of S3 compared with Xihui18, while expression levels of the gene displayed no significant difference, suggesting that candidate gene 2 could temporarily act as the candidate gene of *qGL3*. Only candidate gene 4 and gene 6 existed both differences in DNA sequence and expression levels between Xihui18 and S3. Therefore, candidate gene 4 (auxin-responsive protein) or gene 6 (serine/threonine-protein kinase-like protein ACR4) should be the best candidate genes of *qGL3*. It is worth noting that these three genes were still not cloned. *qGL5*, *qRLW5*, and *qGWT5–1* were all linked to RM3322 (4.39 Mb). In the substitution interval, *OsDER1* (5.39 Mb) existed. According to the reports, suppression of expression of *OsDER1* in transgenic rice showed a decrease in grain length and grain width (Qian et al. 2018). Thus, *OsDER1* could act as a candidate gene for *qGL5*, *qRLW5*, and *qGWT5–1*. Similarly, *qGW5*, *qRLW5–2*, and *qGWT5–2* were all linked to RM169 (7.85 Mb) and they could also be detected by Zhang et al. (2020), which indicated that these two QTLs could be stably inherited. In this substitution interval of S5, we found 6 genes involved in the above signal pathway of grain size, including candidate gene 1 for RING-type E3 ubiquitin transferase, candidate gene 2 for eukaryotic translation initiation factor 3 subunit, candidate gene 3 for DNA-methyltransferase CMT2, candidate gene 4 for auxin-responsive protein, and candidate gene 5 for serine/threonine-protein kinase and candidate gene 6 for E3 ubiquitin-protein ligase. There were already some genes for grain size reported involving in these signal pathways. *GW8* codes a SBP-domain transcription factor, regulates grain width as a positive regulator, and can bound directly to the *GW7* promoter and repressed its expression (Wang et al. 2015). *GRAIN WEIGHT 2* (*GW2*) encodes a RING-type protein with E3 ubiquitin ligase activity. Loss of *GW2* increases cell number, which results in broader glumes, thus increasing grain width, weight, and yield (Song et al. 2007). However, the candidate genes 3, 4, 5, and 6 should not be the candidate genes of *qGW5* due to no substantial sequence differences between Xihui18 and S5. While candidate gene 1 (RING-type E3 ubiquitin transferase) and gene 2 (eukaryotic translation initiation factor 3 subunit) should be acted as the best candidate genes for *qGW5* owing to both differences of substantial DNA sequence and expression levels between Xihui18 and S5. Interestingly, these two genes are still not cloned. Although qRT-PCR analysis displayed difference or not, the expression pattern of candidate genes did not well explain the difference of target traits, that is no dominant expression. They need further studying by genetic complementary experiment etc. In conclusion, 12 QTLs (*qPN5*, *qGPP12*, *qSPP12*, *qGL2*, *qGL3*, *qGW5*, *qRLW2*, *qRLW3*, *qRLW5–2*, *qGWT2*, *qGWT3*, *qGWT5–2*) were still unreported. These results will be important for both functional studies of target QTLs in theory and breeding novel hybrid varieties in application.

SSSLs, DSSLs, and TSSLs can improve efficiency of QTL identification and epistatic effects detection between QTLs, thus are ideal materials for favorable gene pyramiding.

Breeding by design refers to the breeding of varieties by crop design utilizing favorable alleles dispersed in different genetic resources in a genome (Peleman and van der Voort 2003). Zhang (2021) has applied a three-step strategy for research on rice breeding by design based on single-segment substitution lines (SSSLs) library of HJX 74 and argued that target chromosome segment substitution is a way to breed by design in rice. Thus, SSSLs are ideal materials to realize the strategy owing to each SSSL carrying...
only one single segment from the donor parent in the genome of the recipient. At first, SSSLs can improve the efficiency of QTL identification (Zhao et al., 2016b; Balakrishnan et al., 2019; Wang et al., 2020). In this study, we developed six SSSLs (S1~S6) from progenies of the Xihui18/Z431 F2 population. And using these SSSLs, we validated eleven QTLs (qPL3, qPN3, qGPP12, qSPP12, qGL3, qGW5, qRLW2, qRLW3, qRLW5, qGWT3, and qGWT5-2) among 13 QTLs which were detected by the Xihui18/Z431 F2 population. However, qSSR5 and qGWT5-1 could not be validated by SSSLs, these results indicated that some minor QTLs were affected easily by environmental conditions. Paterson et al. (1991) has demonstrated that QTLs for traits with low heritability appear to show a range of sensitivities to the environment, such as yield per plant and seed setting rate. Liu et al. (2008) argued that the total effect of a QTL in any specific environment includes the main effect and the Q×E interaction effect for that environment. Thus, qSSR5 and qGWT5-1 should have a Q×E interaction effect. Zhao et al. (2016b) found that different agronomic traits displayed different Q×E interactions and the Q×E interaction effect was specific to a particular environmental condition. In addition, we identified 15 QTLs (qPN5, qGL1, qGL2, qGL5, qGW1, qGW5-1, qRLW1, qRLW5-2, qGWT1, qGWT2, qYD1, qYD2, qYD3, qYD5, qYD12) by these SSSLs, which were not detected in the F2 population. These results indicated that SSSLs showed higher QTL detection efficiency. Many pieces of research also supported the conclusion (Zhao et al., 2016a, b; Balakrishnan et al., 2019; Wang et al., 2020; Wang et al. 2021a, b; Liang et al. 2021).

In addition to efficiently detecting single QTL using SSSLs, the epistatic interaction between QTLs could be studied by dual-segment substitution lines (DSSLs) combined with according SSSLs. Due to many important agronomic traits controlled by more than one QTL, thus, it is necessary to check whether these QTLs belong to independent inheritance or epistatic interaction. The epistatic effect between target QTLs is very important for design breeding. Wang et al. (2018) argued that epistasis is an important genetic component affecting the pyramid effect, especially in QTLs for complex quantitative traits. Zou et al (2020) showed that there were significant differences in epistatic effects among seven 2-QTL pyramiding lines, presenting in both directions and magnitudes. The epistatic effects between QTLs for stigma exertion rate (qSERb3-1/qSERb6-1, qSERb3-1/qSERb8-1, and qSERb3-1/qSERb12-1) in 3 pyramiding lines were all positive, which induced very larger pyramiding effects as 51.49%, 43.54%, and 55.51% (Zou et al. 2020). In this study, we analyzed epistatic effects and pyramid performance of many pair QTLs by 6 SSSLs, 3 DSSLs, and 2 TSSLs. The results showed that some QTLs belonged to independent inheritance, such as pyramiding of qGW1 and qGW5, qGWT2 and qGWT3, qGWT2 and qGWT5, qYD2 and qYD5, etc., while most QTLs or loci exhibited epistatic interactions. For example, pyramiding of qGL2 (a = −0.47) and qGL3 (a = −0.31) yielded an epistatic effect of 0.62, resulting in grain length (10.03) in D1 significantly longer than that (9.39) of S2 (qGL2). Pyramiding of qYD2 (a = −1.69), qYD3 (a = 6.72), and qYD5 (a = 7.45) produced an epistatic effect of −7.79, leading to the yield per plant (37.18 g) of T2 that was significantly higher than that (24.41) of S2 and that (27.66 g) of Xihui18. Pyramiding of qPN3 (a = 1.63) and qPN5 (a = 1.43) in D3 yielded an epistatic effect of −1.86, resulting in less number of panicles (6.75) than those (7.60 and 7.20) of S3 (qPN3) and S5 (qPN5). Zhang et al. (2020) showed that pyramiding qGL5 (a = 0.30) and qGL6 (a = 0.13) for long grain yielded positive epistatic effects of 0.31 and produced longer grain (8.67 mm) than that (7.80 and 7.46 mm) of the responding SSSLs. Wang et al. (2021a) pyramided qSSD5 (a = 14.10), qSSD8 (a = 11.38), and qSSD10 (a = 5.11) for seed setting density produced an epistatic effect of −9.36 and resulted in increasing seed setting density. In conclusion, pyramiding different QTLs yielded different genetic models. Hence, in breeding of design, we need to understand the genetic effects of all target QTLs, including the additive effect of single QTL and epistatic effects between QTLs. With the explicit genetic backgrounds of the excellent recipient and known favorable alleles, SSSLs, DSSLs, and TSSLs can be directly used in our design breeding. According to the additive effects and epistatic effects of target genes, we can predict the ideal phenotype of a needful genotype and design personalized novel varieties.

Acknowledgements Professor Shizhong Xu of the University of California, Riverside, USA, wrote the stem program for QTL mapping.
Author contribution: FM Zhao proposed the structure and content. SF Sun completed QTL mapping, cytological analysis, candidate gene analysis, and DNA sequencing and drafted the manuscript. ZB Wang completed the trait correlation analysis, verification, and pyramid of QTMs, as well as qRT-PCR analysis. FM Zhao, SF Sun, SQ Xiang, M Lv, K Zhou, J Li, and PX Liang developed and identified the chromosome segment substitution line Z431 and the development of SSSLs, DSSLs, and TSSLs. RX Li, MM Li, GH He, and YH Ling assessed the agronomic traits. All authors read and approved the final version.

Funding: The study was supported by the National Key Research Plan Project (2016YFD0101107). The study was supported by the Chongqing Science and Technology Commission Special Project (CSTC2019jscx-msxmX0392) and the Chongqing Innovation Project of returned from studying abroad.

Availability of data and material: All data generated or analyzed during this study are included in this published article.

Declarations:

Consent for publication: Informed consent for publication was obtained from all participants.

Conflict of interest: The authors declare no competing interests.

References:

Akagi H, Nakamura A, Yokozeki-Misono Y, Inagaki A, Takahashi H, 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. https://doi.org/10.1007/s00122-004-1591-2
Ashikari M, Sakakibara H, Lin SY, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka M (2005) Cytokinin oxidase regulates rice grain production. Sci 309(5735):741–745. https://doi.org/10.1126/science.1113373
Balakrishnan D, Surapaneni M, Mesapogu S, Neelamraju S (2019) Development and use of chromosome segment substitution lines as a genetic resource for crop improvement. Theor Appl Genet 132(1):1–25. https://doi.org/10.1007/s00122-018-3219-y
Cai J, Liao QP, Dai ZJ, Zhu HT, Zeng RZ, Zhang GQ (2013) Allelic differentiation and effects of the Rf3 and Rf4 genes on fertility restoration in rice with wild abortive cytoplasmic male sterility. Biol Plant 57(2):274–280. https://doi.org/10.1007/s10535-012-0294-9
Choi MS, Koh EB, Woo MO, Piao RH, Oh CS, Koh HJ (2012) Tiller formation in rice is altered by overexpression of OsIAGLU gene encoding an IAA-conjugating enzyme or exogenous treatment of free IAA. J Plant Biol 55(6):429–435. https://doi.org/10.1007/s12374-012-0238-0
Cui YR, Li RD, Li GW, Zhang F, Zhu TT, Zhang QF, Ali J, Xu SZ (2020) Hybrid breeding of rice via genomic selection. Plant Biotechnol J 18(1):57–67. https://doi.org/10.1111/pbi.13170
Dhath BK, Paul P, Sandhu J, Hussain W, Irvin L, Zhu F, Adviento-Borre MA, Lorence A, Staswick P, Yu H, Morota G, Waila H (2021) Allelic variation in rice fertilization independent endosperm 1 contributes to grain width under high night temperature stress. New Phytol 229(1):335–350. https://doi.org/10.1111/nph.16897
Etsuko I, Natsuko I, Sota F, Tomohikok K, Kinya T (2011) The fertility restorer gene, Rf2, for lead rice-type cytoplasmic male sterility of rice encodes a mitochondrial glycine-rich protein. Plant J 65:359–367. https://doi.org/10.1111/j.1365-313X.2010.04427.x
Hu ZJ, Cao LM, Sun XJ, Zhu Y, Zhang TY, Jiang L, Liu YH, Dong SQ, Sun DY, Yang JS, Luo XJ (2018) Fine mapping of a major quantitative trait locus, qggnp7(t), controlling grain number per panicle in African rice (Oryza glaberrima S.). Breeding Sci 68(5):606–613. https://doi.org/10.1270/jsbbs.18084
Kashif H, Zhang YX, Workie A, Aamir R, Adil A, M. Haseenuzzaman R, Wang H, Shen XH, Cao LY, Cheng SH (2020) Association mapping of quantitative trait loci for grain size in introgression line derived from Oryza rufipogon. Rice Sci 27(03): 246-256. https://doi.org/10.1016/j.rsci.2020.04.007
Li J, Zhou JW, Zhang Y, Yang Y, Pu QH, Tao DY (2020) New insights into the nature of interspecific hybrid sterility in rice. Front Plant Sci 11:555572. https://doi.org/10.3389/fpls.2020
Li N, Li Y (2016) Signaling pathways of seed size control in plants. Curr Opin Plant Biol 33:23–32. https://doi.org/10.1016/j.molp.2016.05.008
Li ZH, Aamir R, Zhang YX, Galal BA, Zhu AK, Cao LY, Cheng SH (2019) Quantitative trait loci mapping for rice yield-related traits using chromosomal segment substitution lines. Rice Sci 26(5):261–264. https://doi.org/10.1270/jsbbs.18047
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. https://doi.org/10.1186/s12284-021-00477-w
Liu DQ, Yan YL, Xu DH (2018) Identification and validation of QTLs for 100-seed weight using chromosome segment substitution lines in soybean. Breeding Sci 68(4):442–448. https://doi.org/10.1270/jsbsbs.17127
Liu GF, Zhang ZM, Zhu HT, Zhao FM, Ding XH, Zeng RZ, Li WT, Zhang GQ (2008) Detection of QTLs with additive effects and additive-by-environment interaction effects on panicle number in rice (Oryza sativa L.) with single-segment substitution lines. Theor Appl Genet 116(7):923–931. https://doi.org/10.1007/s00122-008-0724-4
Liu JF, Chen JH, Zhong XM, Wu FQ, Lin QB, Heng YQ, Tian P, Cheng ZJ, Yu XW, Zhou KN, Zhang X, Yu XD, Wang JL, Wan JM (2017) GW5 acts in the brassinosteroid signalling pathway to regulate grain width and weight in rice. Nat Plants 3(5):17043. https://doi.org/10.1038/nplants.2017.43
Liu LC, Tong HN, Xiao YH, Che RG, Xu F, Hu B, Liang CZ, Chu JF, Li YJ, Chu CC (2015) Activation of big grain1 significantly improves grain size by regulating auxin transport in rice. Proc Natl Acad Sci 112(35):11102–11107. https://doi.org/10.1073/pnas.1512748112

Ma FY, Du J, Wang DC, Wang H, Zhao BB, He GH, Yang ZL, Zhang T, Wu RH, Zhao FM (2020a) Identification of long-chromosome segment substitution line Z744 and QTL analysis for agronomic traits in rice. J Integr Agr 19(suppl.1):1163–1169. https://doi.org/10.1007/S11032-020-8627-5

Ma FY, 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(04):494–503. https://doi.org/10.1016/j.cj.2018.12.009

Ma XJ, Wei X, Gao GJ, Jiang HC (2020b) Development and evaluation of improved lines based on an elite rice variety 9311 for overcoming hybrid sterility in rice. Mol Breeding 40(11):102. https://doi.org/10.1007/s11032-020-01180-2

Mora-García S, Vert G, in YH, Caño-Delgado A, Cheong H, Chory J (2004) Nuclear protein phosphatases with Kelch-repeat domains modulate the response to brassinosteroids in Arabidopsis. Genes Dev 18:448–460. http://www.genesdev.org/cgi/doi/10.1101/gad.1174204

Nadir S, Khan S, Zhu Q, Henry D, Wei L, Chen LJ (2018) An overview on reproductive isolation in Oryza sativa complex. AoB Plants 10(6):ply060. https://doi.org/10.1093/aobpla/ply060

Okada S, Onogi A, Iijima K, Hori K, Iwata H, Yokoyama W, Suehiro M, Yamasaki M (2018) Identification of QTLs for rice grain size using a novel set of chromosomal segment substitution lines derived from Yamanadanishiki in the genetic background of Koshikihari. Breed Sci 68(2):210–218. https://doi.org/10.1270/jsbbs.17112

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Okada S, Miura K, Ogawa D, Kamura T, Suzuki T, Higashiyama T, Yamasaki M, Mori H, Inukai Y, Wu JZ, Kitano H, Sakakibara H, Jacobsen SE, Ashikari M (2015) Rare allele of a previously unidentified histone H4 acetyltransferase enhances grain weight, yield, and plant biomass in rice. Proc Natl Acad Sci U S A 112(1):76–81. https://doi.org/10.1073/pnas.1421271112

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. https://doi.org/10.1038/s41467-018-03141-y

Tang HW, Luo DP, Zhou DG, Zhang QY, Tian DS, Zeng XM, Liu YG (2014) The rice restorer Rhf4 for wild-abortive cytoplasmic male sterility encodes a mitochondrion-localized PPR protein that functions in reduction of WA352 transcripts. Mol Plant 7(9):1497–1500. https://doi.org/10.1093/molp/ssu047

Tao YJ, Zhu YJ, Xu JJ, Wang LJ, Gu HW, Zhou RH, Yang ZF, Liang GH (2016) Exploitation of heterosis loci for yield and yield components in rice using chromosome segment substitution lines. Sci Rep 6:36802. https://doi.org/10.1038/srep36802

Wang DC, Zhou K, Xiang SQ, Zhang QL, Li RX, Li MM, Liang PX, Forkhanda N, He GH, Ling YH, Zhao FM (2021a) Identification, pyramid and candidate genes of QTLs for associated traits based on a dense erect panicle rice CSSL-Z749 and five SDSLs, three DSSLs and one TSSL. Rice 14(1):55. https://doi.org/10.1186/s12284-021-00496-7

Wang DW, Sun WQ, Yuan ZY, Sun Q, Fan K, Yu SB (2021b) Identification of a novel QTL and candidate gene associated with grain size using chromosome segment substitution lines in rice. Sci Rep 11(1):189. https://doi.org/10.1038/s41598-020-80667-6

Wang H, Zhang JY, Naz F, 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(05):414–423. https://doi.org/10.1016/j.rsci.2020.04.008

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. https://doi.org/10.1038/ng.3352

Wang XL, Jin LL, Zhu HT, Wang SK, Zhang QG, Liu GF (2018) QTL epistatic analysis for yield components with single-segment substitution lines in rice. Plant Breed 137(3):346–354. https://doi.org/10.1111/pbr.12578

Wu GL, Deng HD, Yu MX, Cai YC, Zhou DH, Tan JG, Yu JF, Luo X, Tong S, Wang P, Zhang XY, Li CJ, Li CJ, Wang YN, Cheng Q, He HH, Bian JM (2020) Genetic analysis of rice seed recovery under low-temperature胁迫条件下的水稻育种。Front Plant Sci 11:564824. https://doi.org/10.3389/fpls.2020.564824
conditions using a new CSSL population with a high-density genetic map in rice. Mol Breeding 40(12):109. https://doi.org/10.1007/s11032-020-01189-7

Xing YZ, Zhang QF (2010) Genetic and molecular bases of rice yield. Annu Rev Plant Biol 61:421–442. https://doi.org/10.1146/annurev-arplant-042809-112209

Yang C, Ma YM, He Y, Li JX (2018) OsOFP19 modulates plant architecture by integrating the cell division pattern and brassinosteroid signaling. Plant J 93(3):489–501. https://doi.org/10.1111/tjp.13793

Zhang GQ (2021) Target chromosome-segment substitution: a way to breeding by design in rice. The Crop Journal 9(03):658–668. https://doi.org/10.1016/j.cj.2021.03.001

Zhao FM, Tan Y, Zheng LY, Zhou K, He GH, Ling YH, Zhang LH, Xu SZ (2016a) Identification of rice chromosome segment substitution line Z322-1-10 and mapping QTLs for agronomic traits from the F3 population. Cereal Res Commun 44(3):370–380. https://doi.org/10.1556/080644.2016.022

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.