Dissection and validation of minor quantitative trait loci (QTLs) conferring grain size and weight in rice

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Abstract

Grain size and weight contribute greatly to the grain yield of rice. In order to identify minor QTLs conferring grain size and weight, an F2 population derived from a cross between two indica rice lines showing small difference on grain size, Guangzhan 63-4S (GZ63-4S) and Dodda, and its derived F2:3 population were developed and used for QTL analysis. Totally, 36 QTLs for grain size and weight were detected, and 7 were repeatedly detected, of which the number of beneficial alleles was contributed roughly equally by the two parents. In order to further validate effects of QTLs detected, a BC1F2 population derived from a backcross of a mixture of F2 lines with GZ63-4S was developed and subjected to QTL selection. Heterozygous regions of 3 QTLs, qGS3, qTGW6.2 and qGT7 were identified, and corresponding near-isogenic lines (NILs) of each QTL were constructed with three rounds of self-crosses. In the background of NILs, qGS3 was responsible for GL, LWR, GT and TGW, qTGW6.2 was for GL and TGW, and qGT7 was for GT and TGW. These results have laid the foundation of further fine mapping and cloning of underlying genes, and could be of great use in breeding and improvement of rice lines with desirable size and yield.

Keywords: grain size, grain weight, minor QTL, validation, NIL, rice
Introduction

Rice is one of the staple crops worldwide, and feeds more than half of the world’s population. In the face of continuously increasing population and reduced arable land, how to further improve the grain yield of rice is a major concern of scientists and breeders. Grain size, characterized by four factors viz., grain length (GL), grain width (GW), length-to-width ratio (LWR) and grain thickness (GT), contributes greatly to grain weight, which is a key determinant of grain yield [1]. Therefore, dissection of the genetic basis that underlies grain size and weight would be of great use in developing rice lines with high grain yield.

Considerable efforts have been made to investigate the genetic basis of grain size and weight in the past two decades, and results showed that the four factors of grain size, GL, GW, LWR and GT, and thousand-grain weight (TGW) are quantitative traits, and subjected to control of many genes [2, 3]. Up to now, large numbers of quantitative trait loci (QTLs) have been identified, however, only a small proportion of QTLs displaying large effect have been cloned, such as GS3 [4, 5], OsMADS1 [6, 7], GL3.3/TGW3 [8-10], GW5/GSE5 [11, 12], GS5 [13], GW8 [14], GS2/GL2 [15-17], GL7/GW7 [18, 19], etc. Although the knowledge of molecular regulation of grain size and weight has greatly increased, the mining and cloning of more QTLs, especially minor QTLs, is still of great importance to have a better understanding of underlying mechanisms and provide breeding programs with valuable gene resources.

Rice lines displaying large difference on grain size and weight were always selected to develop segregating populations for QTL analysis, which resulted in the repeated
detection of several major QTLs/genes. For example, two major genes for grain size, 
GW2 and GL3.1 were identified and cloned from genetic populations derived from 
FAZ1 and WY3, of which the TGW values differ by 23.12g [20, 21]. The two genes 
above, together with another two major genes, GS3 and GW5/GSE5, contributed to 
the huge variation of grain size and weight between N411 and N643, of which the 
TGW values differ by 54.33g [22]. The existence of major genes is likely to interfere 
the mapping and validation of minor QTLs, exemplified by the fine mapping of GS5 
[13]. Therefore, in order to identify minor QTLs for grain size and weight, rice lines 
displaying small difference should be preferred.

Quantitative traits are easily affected by environment, which leads to the instability of 
QTL detection. Therefore, genetic validation of QTLs is of great necessity in further 
breeding utilization or cloning. The most frequently used method is evaluation the 
effect of a QTL using near-isogenic lines (NILs), which are lines that carry 
segregating regions at target QTL but homozygous regions in the rest of genome [23]. 
NILs for a QTL are always developed by backcrossing lines carrying the QTL region 
from donor to the receipt several times until the non-target QTL regions were 
completely from the receipt, which could achieve the simultaneous improvement of 
target traits of recipient [24, 25]. Another simple method is to select lines carrying 
segregating target QTL regions from inbred populations that have undertaken several 
rounds of self-crosses, also known as residual heterozygous lines (RHLs) [26, 27]. 
This method is sometimes utilized for absence of laborious hybridization work. The 
NIL of Ghd8, a major QTL with pleiotropic effects on grain yield, heading date and
plant height, was constructed by screening lines carrying segregation target regions from a RIL population of the F_2 generation [28, 29].

In this study, in order to identify minor QTLs for grain size, two *indica* rice lines displaying small difference, Guangzhan 63-4S (GZ63-4S) and Dodda were selected to develop the F_2 and derived F_2:3 populations, and QTL analysis of grain size and weight were performed. In order to validate QTL detected, lines carrying heterozygous QTL regions were screened from a BC_1F_2 population derived from a backcross of a mixture of F_2 lines with GZ63-4S. NILs of three QTLs were developed by a series of self-crosses of screened BC_1F_2 lines, and further used for evaluation their genetic effect on grain size and TGW.

**Materials and methods**

Population development and cultivation

Guangzhan 63-4S (GZ63-4S) is a leading *indica* two-line male sterile line developed by the China North Japonica Hybrid Rice Research Center and Hefei Fengle Seed Company, and has been mated with many restorer lines to produce promising hybrid combinations in recent years [30]. Dodda is an *indica* cultivar with unknown origin, belonging to the core germplasm collections of our lab. The TGW values of GZ63-4S and Dodda differ by less than 10 g (data not shown).

As displayed in Fig.1, 1000 F_2 lines were produced from a cross between GZ63-4S and Dodda, and were subjected to selection of the *TMS5* locus conditioning thermo-sensitive genic male sterility with a closely linked marker [31]. 214 lines
carrying homozygous TMS5 regions were selected to make up the F2 mapping population, which was further self-crossed to produce the F2:3 population. Both the F2 and F2:3 population was exploited to map QTLs for grain size and TGW. In addition, 1200 BC1F2 lines were produced by backcrossing a mixture of F2 lines to GZ63-4S, followed by a self-cross. These lines were subjected to TMS5 selection, and 250 lines carrying homozygous TMS5 regions were selected to perform heterozygous QTL regions screening with flanking makers in the mapping process (Table 2). BC1F2 lines carrying heterozygous QTL regions were further self-crossed three times to produce the BC1F5 populations, which were utilized to validate the effect of QTLs.

The F2, F2:3 and BC1F5 populations were planted in year 2014, 2015 and 2018, respectively, during the normal rice growing seasons at the Experimental Farm of Huangzhong Agricultural University in Wuhan, China. Each F3 line consisted of 12 plants, and each BC1F5 population consisted of 100 plants.

Trait evaluation

GL, GW, LWR and TGW were measured with more than 200 grains per line or plant using the yield traits scorer [32]. GT was determined for each grain individually using an electronic digital caliper (Guanglu Measuring Instrument Co. Ltd., China), and thirty grain values were averaged for each line or plant. For the F2:3 population, the phenotypic value of each line was the average value of 12 plants.

Genetic map construction
A total of more than 1000 simple sequence repeat markers or insert/deletion markers were employed to screen for polymorphic markers between GZ63-4S and Dodda, and 143 markers were identified. Among that, 111 markers were selected to perform genotyping of the F₂ population with 4% polyacrylamide gels migration and silver staining [33]. A genetic linkage map was constructed using MapMaker/Exp3.0 with the Kosambi mapping function [34].

Data analysis

Correlation analysis was performed using the data analysis module in Microsoft Office Excel 2016. QTL analysis was performed by composite interval mapping using the software package QTLCartographer V2.5 with a logarithm of odds (LOD) threshold of 3.0 [35]. ANNOVA analysis was performed using the IBM SPSS Statistics 22.

Results

Phenotypic variation and correlation of the F₂ and F₂:₃ populations

GZ63-4S is a typical photoperiod- and thermo-sensitive genic male sterile line, and shows male sterility in the normal growing seasons in Wuhan. Therefore, the seeds could not be harvested, which abolished comparison of grain size and TGW between the two parents. All the five traits of the F₂ and F₂:₃ populations showed continuous variation and followed normal distribution in year 2014 and 2015, respectively (Fig. 2).
All the four grain size factors were significantly positively correlated with TWG in both years, except for LWR (Table 1). GL was significantly positively correlated with LWR and GT in both years, while GW was only significantly negatively correlated with LWR in both years. The three highest correlation coefficients were observed between GW and TGW in year 2014, GL in two years, and GW and LWR in year 2014, with values of 0.739, 0.731 and 0.728, respectively.

QTLs detected in the F$_2$ and F$_{2:3}$ populations

GL

Ten QTLs for GL were detected in two populations and distributed on seven chromosomes, with phenotypic variation explained by each QTL ranging from 2.58% to 25.39% (Table 2, Fig.3). Among those, the beneficial alleles of qGL3, qGL4.1 and qGL4.2 were from GZ63-4S, while that of others were from Dodda. Two QTLs, qGL3 and qGL6 were repeatedly detected, and explained 2.58% and 13.71% of the variation in the F$_2$ population, and 8.12% and 7.41% of the variation in the F$_{2:3}$ population, respectively. The remaining QTLs were detected only in the F$_{2:3}$ population, excluding qGL4.1.

GW

Five QTLs were detected for GW in the F$_{2:3}$ population, while none in the F$_2$ population (Table 2, Fig.3). Among those, the beneficial alleles of two were from GZ63-4S, while that of the other three were from Dodda.

LWR
Five QTLs for LWR were identified in the two populations, and distributed on four chromosomes (Table 2, Fig.3). Among those, two QTLs, \( qLWR3 \) and \( qLWR9 \), were repeatedly detected, and displayed nearly the same values of additive effect in opposite direction. The remaining were minor QTLs accounting for less than 6% of the variation and were detected only in one population.

GT

Seven QTLs were identified for GT in the two populations and were distributed on chromosome 2, 3, 4, 7 and 11 (Table 2, Fig.3). The beneficial allele of all eight QTLs were from GZ63-4S, except for that of \( qGT11 \). The three QTLs, \( qGT3 \), \( qGT7 \) and \( qGT11 \) were stably detected, and explained 15.16%, 6.60% and 5.83% of the variation in the \( F_2 \) population, and 20.98%, 10.25% and 6.24% of the variation in the \( F_{2:3} \) population, respectively. \( qGT2.1 \) and \( qGT2.2 \) were only detected in the \( F_{2:3} \) population, while \( qGT7 \) and \( qGT11 \) were only in the \( F_2 \) population.

TGW

Nine QTLs for TGW were detected in the two populations, which were distributed on chromosome 2, 3, 4, 6, 7 and 11 (Table 2, Fig.3). Among those, four QTLs, \( qTGW3 \), \( qTGW4 \), \( qTGW6.2 \) and \( qTGW11 \), were repeatedly detected, which accounted for 0.83%, 15.76%, 6.89% and 20.68% of the variation in the \( F_2 \) population, and 3.90%, 12.03%, 5.11% and 10.98% of the variation in the \( F_{2:3} \) population, respectively. The beneficial alleles of \( qTGW3 \) and \( qTGW4 \) were from GZ63-4S, while that of \( qTGW6.2 \) and \( qTGW11 \) were from Dodda. The remaining QTLs were all only detected in the \( F_{2:3} \) population.
The region flanked by marker LRJ99 and RM232 on chromosome 3 and consisting of four QTLs, was responsible for GL, LWR, GT and TGW in both the $F_2$ and $F_{2:3}$ population, and was termed $qGS3$, hereafter.

Validation of QTL effects using NILs

In the $BC_1F_2$ population derived from backcrossing some $F_2$ lines with GZ63-4S, heterozygous regions were screened for QTLs repeatedly detected in both the $F_2$ and $F_{2:3}$ populations or QTLs accounting for more than 10% of variation in one population using flanked markers (Fig.2, Table 1). Lines carrying heterozygous regions of three QTLs, $qGS3$, $qTGW6.2$ and $qGT7$, were identified respectively, and were self-crossed three times to produce NIL populations for each QTL.

In the NIL population of $qGS3$, significant differences were observed in the average values of GL, GT and TGW among the three genotypes, $qGS3^{Dodda}$, $qGS3^H$ and $qGS3^{GZ63-4S}$ (Table 3). Compared to NIL ($qGS3^{Dodda}$), NIL ($qGS3^{GZ63-4S}$) showed increased values by 0.21 mm in GL, 0.10 in LWR, 0.07 mm in GT and 1.47 g in TGW.

For $qTGW6.2$, significant differences were observed in the average values of GL and TGW between $qTGW6.2^{Dodda}$ and $qTGW6.2^{GZ63-4S}$, while no difference between $qTGW6.2^H$ and $qTGW6.2^{GZ63-4S}$ in the NIL population (Table 4). Compared to NIL ($qTGW6.2^{Dodda}$), NIL ($qTGW6.2^{GZ63-4S}$) showed decreased values by 0.14 mm in GL and 1.24 g in TGW.

For $qGT7$, significant differences were observed in the average values of GT among the three genotypes, $qGT7^{Dodda}$, $qGT7^H$ and $qGT7^{GZ63-4S}$, and in that of TGW between
Discussion

Evaluation of grain size and weight

Genotyping and phenotyping are two key processes in genetic analysis. With the completion of high-quality genome sequences of several rice cultivars and development of sequencing techniques, genotyping a population is becoming increasingly simple and cheap [36, 37]. Therefore, high-throughput and time-saving methods of phenotyping are in urgent need. In previous studies, grain size was always evaluated using electronic digital-display vernier caliper, and about 30 randomly chosen filled grains was used for each line, which is both pains taking and time consuming [19, 38]. SmartGrain is a phenotyping software developed for measuring grain size through image analysis, which improved greatly the throughput, but is still time consuming for the separation of adjacent seeds in the scanning process [39]. In this study, evaluation of GL, GW, LWR, and TGW was performed using the yield traits scorer (YTS) that could fulfil the measurement of a rice line represented by about 500 seeds within one minute [28]. Therefore, the YTS dramatically increases the amount of seeds evaluated and reduces the time of phenotyping, demonstrating its great power in phenotype evaluation.
In this study, a total of 37 QTLs were identified for GL, GW, LWR, GT and TGW in the F$_2$ and F$_{2:3}$ populations, and 7 QTL regions were repeatedly detected, of which the additive effects were far less than that of cloned major genes for grain size, such as $GS3$, $GL3.1/qGL3$, $GW5/GSE5$ and $GW2$ [4, 5, 11, 12, 20-22], demonstrating minor QTLs for grain size and weight. Moreover, the number of beneficial alleles was contributed roughly equally by the two parents, indicating that novel minor QTLs could be detected from rice lines that differ little in grain size.

Among QTLs detected, $qGS3$, the pleiotropic QTL for GL, LWR, GT and TGW on chromosome 3, is co-located with $OsMADS1$, of which a natural allele was reported responsible for GL, GT and TGW in two separate studies [6, 7]. However, the difference of GL between the two NILs in Yu et al. (2018) was almost twice that between our NILs, while the difference of GT was the same [7]. An appropriate explanation is that another gene for GL in the Nipponbare background may interact with $OsMADS1$ to amplify the difference in the NILs, as reported by Xia et al. (2018) [9]. Therefore, $qGS3$ is likely to be $OsMADS1$. In addition, the region of $qTGW6.2$ overlaps with that of two QTLs for TGW in the chromosomal segment substitution lines (CSSLs) population derived from Yamadanishiki or Takanari in the background of Koshihikari, respectively [40, 41]. $qGT7$, the QTL for GT on chromosome 7, is co-located with a region for GL, GW, LWR and GT reported by Liu et al (2015), which contains $GL7/GW7$, a major gene influencing GL and GW simultaneously [18, 19, 42]. As $qGT7$ has no effect on GL and GW in the NIL background, it is a novel
gene different from GL7/GW7. The region of qLWR9 was also detected for LWR only by Yin et al. (2015) [43]. The remaining QTLs are seldom reported, or maybe novel.

Validation of minor QTLs using NILs

QTLs detected in primary populations are sometimes unstable, and thus should be further validated, especially for minor QTLs. The best way to validate QTLs is the use of NILs. In this study, lines carrying heterozygous QTL regions were screened from the BC1F2 population, in case of the loss of target regions in subsequent self-crosses. Then, selected lines were subjected to three rounds of self-crosses, in order to reduce the heterozygosity of non-target regions. The method we preferred ensures that NILs for QTLs of interest are constructed, and eliminates laborious hybridization work.

In this study, the NILs of three QTLs, qGS3, qTGW6.2 and qGT7, were constructed, and effects on grain size and TGW were evaluated. The beneficial alleles of qGS3 from GZ63-4S could increase the value by 0.21mm in GL, 0.07mm in GT, and 1.47g in TGW in homozygous NILs, which was consistent with the values of additive effect in the F2 and F2:3 population on the whole (Table 2, Table 3), suggesting that qGS3 is a stable and pleiotropic QTL for GL, GT and TGW. qTGW6.2 was initially detected as a QTL for TGW, but was validated to have effect on both GL and TGW in the NIL population and act in a dominant manner (Table 4). The failure in detection of qTGW6.2 on GL in F2 and F2:3 population may be attributed to the complexity of genome background and the low variation explained, which further supported the necessity of validation of QTLs using NILs. qGT7 was repeatedly confirmed as a
QTL for GT, and had no effect on GL and GW in the F$_2$, F$_{2:3}$, and NIL populations (Table 2, Table 5). Being one of the four factors of grain size, GT has received less attention, and several cloned genes conditioning GT are responsible for GL and/or GW at the same time, such as GS2, GW8 [17, 44]. Therefore, qGT7 is a good candidate for further research of the molecular mechanism underlying GT.

Improvement of grain size, quality and yield in rice

Grain size contributes to not only grain yield, but also grain quality, especially appearance quality and milling quality [45–46]. Abundant variation of grain size is observed in rice germplasm cultivated worldwide, thus providing valuable resources for breeding or improvement of rice grain with desirable size and yield. QTLs or genes for grain size mined from germplasm resources have been or are being exploited in rice breeding programs. The gs3 allele and GW5 allele from 93-11, together with beneficial alleles controlling fine eating and cooking quality from Nipponbare, were introduced into the Teqing background, and the resulting lines displayed dramatic improvement in grain size and quality [47]. The gs3 allele and the GW7 allele from TFA were pyramided into the background of HJX74, resulting in simultaneously improvement of grain yield and quality [18]. In this study, seven QTL regions were repeated detected in both the F$_2$ and F$_{2:3}$ populations, and three of them were further validated in the NIL background, demonstrating the reality and stability these QTLs. qGL6 and qTGW6.2, the two QTLs for GL located on chromosome 6, could be used in improvement of GL and KGW of GZ63-4S with the beneficial
alleles from Dodda, and further in improvement of the yield and quality performance of hybrid combinations using GZ63-4S as the maternal parent. In addition, the QTLs detected in this study, together with QTLs or genes in other studies, could be combined in the breeding and improvement of rice grains with desirable size, quality and yield.

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Competing interests

The authors declare that they have no competing financial interests.

Author contributions

Ping Sun and Yuanyuan Zheng performed most of experiments and analyzed the date. Pingbo Li wrote the paper. Hong Ye and Hao Zhou constructed the F₂ mapping population. Guanjun Gao participated in the field management. Yuqing He designed and supervised the study.

Reference
1. Xing YZ, Zhang QF (2010) Genetic and molecular bases of rice yield. Annu Rev Plant Biol 61:421-442

2. Huang RY, Jiang LR, Zheng JS et al (2013) Genetic bases of rice grain shape: so many genes, so little known. Trends Plant Sci 18:218-226

3. Zuo JR, Li JY (2014) Molecular genetic dissection of quantitative trait loci regulating rice grain size. Annu Rev Genet 48:99-118

4. Fan CH, Xing YZ, Mao HL et al (2006) GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112:1164-1171

5. Takano-Kai N, Jiang H, Kubo Y et al (2009) Evolutionary history of GS3, a gene conferring grain length in rice. Genetics 182:1323-1334

6. Liu Q, Han RX, Wu K et al. (2018) G-protein βγ subunits determine grain size through interaction with MADS-domain transcription factors in rice. Nat Commun 9: 852

7. Yu JP, Miao JL, Zhang ZY et al (2018) Alternative splicing of OsLG3b controls grain length and yield in japonica rice. Plant Biotechnol J 16:1667-1678

8. Hu ZJ, Lu SJ, Wang MJ et al (2018) A novel QTL qTGW3 encodes the GSK3/SHAGGY-like kinase OsGSK5/OsSK41 that interacts with OsARF4 to negatively regulate grain size and weight in rice. Mol Plant 11:736-749

9. Xia D, Zhou H, Liu RJ et al (2018) GL3.3, a novel QTL encoding a GSK3/SHAGGY-like kinase, epistatically interacts with GS3 to produce extra-long grains in rice. Mol Plant 11:754-756
10. Ying JZ, Ma M, Bai C et al (2018) *TGW3*, a major QTL that negatively modulates grain length and weight in rice. Mol Plant 11:750-753

11. Duan PG, Xu JS, Zeng DL et al (2017) Natural variation in the promoter of *GSE5* contributes to grain size diversity in rice. Mol Plant 10:685-694

12. Liu JF, Chen J, Zheng XM et al (2017) *GW5* acts in the brassinosteroid signalling pathway to regulate grain width and weight in rice. Nat Plants 3:17043

13. Li YB, Fan CC, Xing YZ et al (2011) Natural variation in *GS5* plays an important role in regulating grain size and yield in rice. Nat Genet 43:1266-1269

14. Wang SK, Wu K, Yuan QB et al (2012) Control of grain size, shape and quality by *OsSPL16* in rice. Nat Genet 44:950-954

15. Che RH, Tong HN, Shi BH et al (2015) Control of grain size and rice yield by GL2-mediated brassinosteroid responses. Nat Plants 2:15195

16. Duan PG, Ni S, Wang JM et al (2015) Regulation of *OsGRF4* by OsmiR396 controls grain size and yield in rice. Nat Plants 2:15203

17. Hu J, Wang YX, Fang YX et al (2015) A rare allele of *GS2* enhances grain size and grain yield in rice. Mol Plant 8:1455-1465

18. Wang SK, Li S, Liu Q et al (2015) The *OsSPL16-GW7* regulatory module determines grain shape and simultaneously improves rice yield and grain quality. Nat Genet 47:949-954

19. Wang YX, Xiong GS, Hu J et al (2015) Copy number variation at the *GL7* locus contributes to grain size diversity in rice. Nat Genet 47:944-948

20. 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 39:623-630

21. Qi P, Lin YS, Song XJ et al (2012) The novel quantitative trait locus GL3.1 controls rice grain size and yield by regulating Cyclin-T1;3. Cell Res 22:1666-1680

22. Zhang XJ, Wang JF, Huang J et al (2012) Rare allele of OsPPKL1 associated with grain length causes extra-large grain and a significant yield increase in rice. P Natl Acad Sci USA 109:21534-21539

23. Yano M, Sasaki T (1997) Genetic and molecular dissection of quantitative traits in rice. Plant Mol Biol 35:145-153

24. Xiao C, Hu J, Ao YT et al (2016) Development and evaluation of near-isogenic lines for brown planthopper resistance in rice cv. 9311. Sci Rep 6: 38159

25. Zhou RH, Zhu ZD, Kong XY et al (2005) Development of wheat near-isogenic lines for powdery mildew resistance. Theor Appl Genet 110:640-648

26. Darvasi A, Soller M (1995) Advanced intercross lines, an experimental population for fine genetic mapping. Genetics 141:1199-1207

27. Tuinstra MR, Ejeta G, Goldsborough PB (1997) Heterogeneous inbred family (HIF) analysis: a method for developing near-isogenic lines that differ at quantitative trait loci. Theor Appl Genet 95:1005-1011

28. Yan WH, Wang P, Chen HX et al (2011) A major QTL, Ghd8, plays pleiotropic roles in regulating grain oroductivity, plant height, and heading date in rice. Mol Plant 4:319-330
29. Zhang YS, Luo LJ, Xu CG, Zhang QF, Xing YZ (2006) Quantitative trait loci for panicle size, heading date and plant height co-segregating in trait-performance derived near-isogenic lines of rice (Oryza sativa). Theor Appl Genet 113:361-368

30. Shen QW, Jiang H (2014) Performance of male sterile line Guangzhan 63-4S on breeding and its enlightenment of Hubei middle rice. Hubei Agricultural Sciences 53: 2490-2492 (Chinese with English abstract)

31. Zhou H, Zhou M, Yang YZ et al (2014) RNase ZS1 processes UbL40 mRNAs and controls thermosensitive genic male sterility in rice. Nat Commun 5: 4884

32. Yang WN, Guo ZL, Huang CL et al (2014) Combining high-throughput phenotyping and genome-wide association studies to reveal natural genetic variation in rice. Nat Commun 5: 5087

33. Panaud O, Chen X, McCouch SR (1996) Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (Oryza sativa L.). Mol Gen Genet 252:597-607

34. Lincoln SE, Daly MJ and Lander ES (1992) Constructing genetic maps with MAPMAKER/EXP3.0. Whitehead Institute Technical Report, Whitehead Institute, Cambridge

35. Wang SC, Christopher JB and Zeng ZB (2012) Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC

36. Huang XH, Lu TT, Han B (2013) Resequencing rice genomes: an emerging new era of rice genomics. Trends Genet 29:225-232

37. Xie WB, Feng Q, Yu HH et al (2010) Parent-independent genotyping for
constructing an ultrahigh-density linkage map based on population sequencing. P
Natl Acad Sci USA 107:10578-10583

38. Sun L, Ma DP, Yu HH et al (2013) Identification of quantitative trait loci for grain
size and the contributions of major grain-size QTLs to grain weight in rice. Mol
Breeding 31:451-461

39. Tanabata T, Shibaya T, Hori K, Ebana K, Yano M (2012) SmartGrain:
High-throughput phenotyping software for measuring seed shape through image
analysis. Plant Physiol 160:1871-1880

40. Okadao S, Onogi A, Iijima K et al (2018) Identification of QTLs for rice grain
size using a novel set of chromosomal segment substitution lines derived from
Yamadanishiki in the genetic background of Koshihikari. Breeding Sci
68:210-218

41. Takai T, Ikka T, Kondo K et al (2014) Genetic mechanisms underlying yield
potential in the rice high-yielding cultivar Takanari, based on reciprocal
chromosome segment substitution lines. BMC Plant Biol 14:295

42. Liu DL, Kang MH, Wang F et al (2015) Mapping of the genetic determinant for
grain size in rice using a recombinant inbred line (RIL) population generated from
two elite *indica* parents. Euphytica 206:159-173

43. Yin CB, Li HH, Li SS, Xu LD, Zhao ZG, Wang JK (2015) Genetic dissection on
rice grain shape by the two-dimensional image analysis in one *japonica x indica*
population consisting of recombinant inbred lines. Theor Appl Genet
128:1969-1986
44. Gao FY, Luo ZL, Ren JS et al (2015) Fine mapping and candidate gene analysis of \(qGT8\), a major QTL for grain thickness in rice. Sci Agric Sin 48:4859-4871

45. Fitzgerald MA, McCouch SR, Hall RD (2009) Not just a grain of rice: the quest for quality. Trends Plant Sci 14:133-139

46. Zheng TQ, Xu JL, Li ZK, Zhai HQ, Wan JM (2007) Genomic regions associated with milling quality and grain shape identified in a set of random introgression lines of rice (\textit{Oryza sativa} L.). Plant Breeding 126:158-163

47. Zeng DL, Tian ZX, Rao YC et al (2017) Rational design of high-yield and superior-quality rice. Nat Plants 3: 17031

Table 1 Correlations of GL, GW, LWR, GT and TGW of the \(F_2\) and \(F_{2:3}\) populations in year 2014 and 2015

|       | GL14 | GL15 | GW14 | GW15 | LWR14 | LWR15 | GT14 | GT15 | TGW14 | TGW15 |
|-------|------|------|------|------|-------|-------|------|------|-------|-------|
| GL14  |      |      |      |      |       |       |      |      |       |       |
| GL15  | 0.731** |      |      |      |       |       |      |      |       |       |
| GW14  | 0.157 | 0.055 |      |      |       |       |      |      |       |       |
| GW15  | 0.046 | 0.139 | 0.489** |      |       |       |      |      |       |       |
| LWR14 | 0.525** | 0.453** | -0.728** | -0.424** |       |       |      |      |       |       |
| LWR15 | 0.477** | 0.585** | -0.348** | -0.692** | 0.657** |       |      |      |       |       |
| GT14  | 0.387** | 0.381** | 0.126 | 0.269** | 0.144 | 0.053 |      |      |       |       |
| GT15  | 0.225* | 0.452** | 0.109 | 0.235* | 0.057 | 0.118 | 0.677** |      |      |       |       |
| TGW14 | 0.656** | 0.558** | 0.378** | 0.415** | 0.129 | 0.046 | 0.739** | 0.495** |      |       |       |
| TGW15 | 0.484** | 0.712** | 0.261** | 0.450** | 0.093 | 0.132 | 0.584** | 0.707** | 0.700** |       |

GL14, GL in 2014; GL15, GL in 2015; GW14, GW in 2014; GW15, GW in 2015; LWR14, LWR in 2014; LWR15, LWR in 2015; GT14, GT in 2014; GT15, GT in 2015; TGW14, TGW in 2014; TGW15, TGW in 2015.

*, ** Significantly at \(P<0.05\) and \(P<0.01\), respectively.

Table 2 QTLs detected for GL, GW, LWR, GT and TGW in the \(F_2\) and \(F_{2:3}\) populations.
| Trait | QTL | Chr | Interval         | 2014 F₂ | 2015 F₂⁻³ | 2014 F₂ | 2015 F₂⁻³ |
|-------|-----|-----|------------------|---------|-----------|---------|-----------|
|       |     |     |                  | LOD     | Add(¹)    | Dom(²)  | R²(⁻³)    |
|       |     |     |                  | LOD     | Add       | Dom     | R²        |
| GL    | qGL1.1 | 1   | RM283-RM259      | 3.68    | 0.09      | -0.02   | 6.14      |
|       | qGL1.2 | 1   | RM582-RM562      | 3.04    | 0.07      | -0.04   | 5.80      |
|       | qGL3  | 3   | LRJ99-RM232      | 4.52    | -0.09     | -0.07   | 2.58      |
|       | qGL4.1 | 4   | RM252-RM470      | 3.51    | -0.10     | -0.01   | 5.36      |
|       | qGL4.2 | 4   | RM470-RM349      | 16.71   | -0.20     | -0.02   | 25.39     |
|       | qGL5  | 5   | RM516-RM3381     | 3.34    | 0.12      | 0.03    | 7.15      |
|       | qGL6  | 6   | RM402-RM5963     | 5.29    | 0.10      | -0.08   | 13.71     |
|       | qGL9  | 9   | RM566-YH16.8     | 5.95    | 0.13      | -0.03   | 12.69     |
|       | qGL11 | 11  | RM286-RM26085    | 4.86    | 0.09      | -0.02   | 7.86      |
| GW    | qGW4  | 4   | RM349-RM567      | 8.21    | -0.05     | -0.01   | 13.97     |
|       | qGW8  | 8   | RM126-RM515      | 3.35    | -0.03     | 0.00    | 6.96      |
|       | qGW11 | 11  | RM27181-RM224    | 4.19    | 0.03      | 0.01    | 4.58      |
|       | qGW12.1| 12  | RM511-RM313     | 4.15    | 0.03      | 0.01    | 5.11      |
|       | qGW12.2| 12  | RM309-RM3726    | 4.67    | 0.03      | 0.01    | 5.09      |
| LWR   | qLWR2.1| 2   | RM7252-RM233    | 3.95    | 0.06      | 0.02    | 4.07      |
|       | qLWR2.2| 2   | RM279-RM555     | 3.23    | 0.08      | 0.02    | 5.96      |
|       | qLWR3 | 3   | LRJ99-RM232     | 7.03    | -0.08     | -0.06   | 3.96      |
|       | qLWR9 | 9   | RM566-YH16.8    | 5.13    | 0.08      | 0.03    | 8.02      |
|       | qLWR11| 11  | RM27181-RM224   | 5.23    | -0.06     | -0.05   | 1.46      |
| GT    | qGT2.1| 2   | RM7252-RM233    | 6.84    | -0.02     | 0.02    | 11.73     |
|       | qGT2.2| 2   | RM327-RM263     | 6.00    | -0.03     | -0.01   | 11.16     |
|       | qGT3  | 3   | LRJ99-RM232     | 16.94   | -0.04     | -0.02   | 15.16     |
|       | qGT4.1| 4   | RM6659-RM16616  | 4.31    | -0.02     | 0.01    | 7.99      |
|       | qGT4.2| 4   | RM16653-RM16820 | 4.52    | -0.02     | 0.01    | 10.05     |
|       | qGT7  | 7   | RM560-RM234     | 4.95    | -0.02     | -0.00   | 6.60      |
|       | qGT11 | 11  | RM286-RM26085   | 3.00    | 0.01      | -0.01   | 5.83      |
| TGW   | qTGW2.1| 2   | RM7252-RM233    | 3.01    | 0.00      | 0.63    | 3.01      |
|       | qTGW2.2| 2   | RM327-RM263     | 5.08    | -0.68     | -0.13   | 8.28      |
|       | qTGW3 | 3   | LRJ99-RM232     | 3.54    | -0.49     | -0.54   | 0.83      |
|       | qTGW4 | 4   | RM470-RM349     | 5.08    | -0.83     | 0.46    | 15.76     |
|       | qTGW6.1| 6   | RM402-RM5963    | 4.47    | 0.60      | -0.01   | 7.25      |
|       | qTGW6.2| 6   | RM3183-RM20048  | 3.09    | 0.55      | -0.25   | 6.89      |
|       | qTGW7.1| 7   | RM21242-RM542   | 3.16    | -0.40     | 0.29    | 5.11      |
|       | qTGW7.2| 7   | RM455-RM234     | 5.75    | -0.62     | 0.50    | 12.83     |
Table 3 Genetic effect of \( q_{GS3} \) in the NIL population

| Number of lines | GL (mm)     | GW (mm)     | LWR      | GT (mm)     | TGW (g)     |
|-----------------|-------------|-------------|----------|-------------|-------------|
| NIL(\( q_{GS3}^{Dodda} \)) | 21 | 9.06±0.14 a | 2.77±0.05 | 3.31±0.08 a | 1.97±0.02 a | 24.10±0.96 a |
| NIL(\( q_{GS3}^H \)) | 40 | 9.15±0.14 b | 2.77±0.06 | 3.33±0.07 a | 2.00±0.02 b | 24.88±1.10 b |
| NIL(\( q_{GS3}^{GZ63-4S} \)) | 19 | 9.27±0.11 c | 2.75±0.01 | 3.41±0.05 b | 2.04±0.03 c | 25.57±1.08 c |

Lower-case letters indicate statistically significant \((P<0.05)\) differences between the mean values within each column (Duncan test).

NIL(\( q_{GS3}^{Dodda} \)) and NIL(\( q_{GS3}^{GZ63-4S} \)) are the lines carrying homozygous \( q_{GS3} \) regions from Dodda and GZ63-4S in the NIL population, respectively, while NIL(\( q_{GS3}^H \)) is the line carrying heterozygous \( q_{GS3} \) regions.

Table 4 Genetic effect of \( q_{TGW6.2} \) in the NIL population

| Number of lines | GL (mm)     | GW (mm)     | LWR      | GT (mm)     | TGW (g)     |
|-----------------|-------------|-------------|----------|-------------|-------------|
| NIL(\( q_{TGW6.2}^{Dodda} \)) | 19 | 9.64±0.11 b | 2.64±0.05 b | 3.70±0.06 | 2.15±0.03 | 26.86±1.25 b |
| NIL(\( q_{TGW6.2}^H \)) | 34 | 9.55±0.11 a | 2.61±0.06 a | 3.71±0.08 | 2.14±0.03 | 25.96±1.10 a |
| NIL(\( q_{TGW6.2}^{GZ63-4S} \)) | 18 | 9.50±0.11 a | 2.61±0.04 ab | 3.69±0.07 | 2.14±0.03 | 25.62±1.08 a |

Lower-case letters indicate statistically significant \((P<0.05)\) differences between the mean values within each column (Duncan test).

NIL(\( q_{TGW6.2}^{Dodda} \)) and NIL(\( q_{TGW6.2}^{GZ63-4S} \)) are the lines carrying homozygous \( q_{TGW6.2} \) regions from Dodda and GZ63-4S in the NIL population, respectively, while NIL(\( q_{TGW6.2}^H \)) is the line carrying heterozygous \( q_{TGW6.2} \) regions.
| NIL      | Number of lines | Mean±SE GL (mm) | Mean±SE GW (mm) | Mean±SE LWR | Mean±SE GT (mm) | Mean±SE TGW (g) |
|----------|----------------|-----------------|-----------------|-------------|----------------|-----------------|
| NIL(qGT7Dodda) | 14           | 9.66±0.12       | 2.57±0.06      | 3.81±0.10 b | 1.96±0.03 a    | 21.84±1.20 a    |
| NIL(qGT7H)    | 21           | 9.62±0.14       | 2.61±0.08      | 3.74±0.08 a | 1.99±0.03 b    | 22.81±1.19 b    |
| NIL(qGT7GZ63-4S) | 13          | 9.60±0.13       | 2.60±0.06      | 3.76±0.07 ab| 2.03±0.03 c    | 23.17±1.05 b    |

Lower-case letters indicate statistically significant (P<0.05) differences between the mean values within each column (Duncan test).

NIL(qGT7Dodda) and NIL(qGT7GZ63-4S) are the lines carrying homozygous qGT7 regions from Dodda and GZ63-4S in the NIL population, respectively, while NIL(qGT7H) is the line carrying heterozygous qGT7 regions.

**Figure legends**

Fig. 1 Schematic representation of the experimental design.

Fig. 2 Frequency distribution of the F₂ and F₂:3 populations for GL, GW, LWR, GT and TGW in year 2014 and 2015.

Arrow indicates the value of Dodda.

Fig. 3 Distribution of putative QTLs for GL, GW, LWR, GT and TGW identified in the F₂ and F₂:3 populations on the linkage map.

GL14, QTLs for GL detected in the F₂ population in year 2014; GL15, QTLs for GL detected in the F₂:3 population in year 2014. The QTLs for GW, LWR, GT and TGW are represented as the same manner as that for GL.
Figure 1

GZ63-4S × Dodda

F₁

F₂

TMS5 Selection

GZ63-4S × F₂

BC₁F₁

BC₁F₂

TMS5 Selection

QTL Screening

BC₁F₂

BC₁F₅

QTL Validation
Figure 3