GW10, a member of P450 subfamily regulates grain size and grain number in rice

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Abstract

Key message A quantitative trait locus GW10 is located on Chromosome 10 by map-based cloning, which encodes a P450 Subfamily protein. The GW10 regulates grain size and grain number in rice involved in the BR pathway.

Abstract Grain size and grain number play extremely important roles in rice grain yield. Here, we identify GW10, which encodes a P450 subfamily protein and controls grain size and grain number by using Lemont (tropical japonica) as donor parent and HJX74 (indica) as recipient parent. The GW10 locus was mapped into a 14.6 kb region in HJX74 genomic on the long arm of chromosome 10. Lower expression of the gw10 in panicle is contributed to the shorter and narrower rice grain, and the increased number of grains per panicle. In contrast, overexpression of GW10 is contributed to longer and wider rice grain. Furthermore, the higher expression levels of some of the brassinosteroid (BR) biosynthesis and response genes are associated with the NIL-GW10. The sensitivity of the leaf angle to exogenous BR in NIL-GW10 is lower than that in NIL-gw10 and in the KO-GW10, which implied that the GW10 should involve in the brassinosteroid-mediated regulation of rice grain size and grain number.

Abbreviations
cM Centimorgan
QTL Quantitative trait loci
SSR Simple sequence repeats
SSSL Single segment substitution line

Introduction

Rice (Oryza sativa L.) is one of the most crucial cereal foods in the world, which provides over 21% calorie for the world population and over 76% calorie for the Asians, and it has been deeply ingrained in their daily lives (Fitzgerald et al. 2009). Grain yield controlling factors include grain weight, grain number per panicle and panicle number per plant (Zuo et al. 2014). Grain size plays a key role in grain weight, and it is one of the most frequently selected traits during domestication and breeding (Meyer et al. 2013).

Grain weight and grain size are controlled mainly by the grain length, grain width and grain thickness. A number of major quantitative trait loci (QTLs) controlling grain size have been successfully cloned and described. Grain size could be controlled by transcriptional regulators including GW8 (Wang et al. 2012), GLW7 (Si et al. 2016), GS2 (Hu et al. 2015), GL4 (Wu et al. 2017), MAD51 (Yu et al. 2018), GS9 (Zhao et al. 2018) and GW6a (Song et al. 2015; Li et al. 2019). Previously, some major QTLs such as TGW3 (Hu et al. 2018a, b), GS5 (Li et al. 2011), GW5 (Liu et al. 2017), TGW6 (Ishimaru et al. 2013), GW6 (Shi et al. 2020) and GS9 (Zhao et al. 2018) were proved to change grain size by phytohormone signals. In the auxin signaling pathway, TGW6 encodes an indole-3-acetic acid (IAA)-glucose hydrolase activity protein and negatively regulates grain size. TGW3 protein interacts with OsARF4 (Auxin response factor 4) repressing downstream auxin response genes’ expression (Ishimaru et al. 2013; Hu et al. 2018a, b). GW6 encodes a GA-regulated GAST family protein and positively regulates grain width (Shi et al. 2020). The other three major QTLs of grain size, GS3, GW2 and GL3.1, are involved in...
G-protein signaling pathway, ubiquitin–proteasome pathway and cell cycle regulation, respectively (Song et al. 2007; Mao et al. 2010; Qi et al. 2012). In the BR signaling pathway, GS5 binds to OsBAK1—7 affecting BR signaling and grain size (Li et al. 2011). GW5 protein represses GSK2 activity resulting in the altered expression of BR-response genes (Liu et al. 2017). GSK2 influences BR signaling pathway by the phosphatases OsBZR1 and DLT (Tong et al. 2012). GS9 interacts with OsOPP14 and OsOPP8, whereas OsGSK2 interacts and phosphatases OsOPP8 affecting the BR signaling pathway (Zhao et al. 2018).

The cytochrome P450 family is one of the biggest protein families in plants. In several sequenced angiosperms, the CYP genes constitute up to 1% of the total gene annotations of each plant species (Nelson et al. 2004). Several P450 genes have been studied to participate in biosynthesis and catabolism of phytohormones (Mizutani and Ohta 2010). ELL1 encodes a cytochrome P450 monooxygenase and influences chlorophyll contents and ROS accumulation (Cui et al. 2021). CYP701A and CYP88A regulate gibberellins (GAs) biosynthesis to influence seed germination and shoot growth in Arabidopsis (Helliwell et al. 1998, 2001). Some P450 genes regulate brassinosteroids (BRs) biosynthesis to influence plant growth development and regulate strigolactones (SLs) biosynthesis to influence seed germination of root parasitic plants (Mizutani 2012). In rice, DWARF11 (D11)/CPB11/GNS4, encoding cytochrome P450 proteins are involved in grain size control and brassinosteroid (BR) biosynthesis pathway (Tanabe et al. 2005; Wu et al. 2016; Zhou et al. 2017a, b).

In this study, we identified a P450 subfamily gene, named as GW10 (Grain Width gene on chromosome 10), which controlled grain size and grain number. The near-isogenic line gw10 (NIL-gw10) produces more grain number per panicle than that of the NIL-GW10 line, while the grain size of the NIL-gw10 is smaller than that of the NIL-GW10. The expression levels of the BR biosynthesis and response genes, such as D2, DWARF, CPD, BZR1 and DLT, are higher in the NIL-GW10 than those in the NIL-gw10, which suggests that GW10 might be involved in the BR signaling pathway to regulate grain size and grain number in rice. The GW10 might be a potential target for rice breeding by design.

Genetic analysis and fine mapping of the GW10

We developed F2 population from the cross between NIL-GW10 (HJX74) and NIL-gw10. We selected polymorphic simple sequence repeat (SSR) markers between these two NILs according to the rice linkage map (http://www.grame ne.org). A series of Indel and SNP polymorphic markers which were designed according to the sequence variations between the japonica cultivar Nipponbare (http://rapdb. dna.affrc.go.jp) and HJX74 (http://192.168.87.153/) were used for GW10 fine mapping (Li et al. 2021) (Table S2). The linkage analysis between the markers and the grain width locus for the F2 population mapped the GW10 gene to a chromosome 10 region flanked by polymorphic markers.

Genomic DNA extraction and PCR analysis

The fresh young leaves of individual rice were collected into 2 ml centrifuge tube and then were grounded in liquid nitrogen. The solution used in the experiment has been reported previously (Chen et al. 2015). The PCR program for the initial denaturing step was at 94 °C for 5 min, followed by 38 cycles for 30 s at 94 °C, 30 s at 55 °C, 45 s at 72 °C, with a final extension at 72 °C for 5 min. The 6% nondenaturing polyacrylamide gel was used for separating PCR products, and the genomic DNA polymorphism analysis was carried out by the silver staining method.

RNA extraction and quantitative real-time PCR

Total RNA was isolated from young panicles of NIL-GW10 and NIL-gw10 by using TRIZOL reagent (Invitrogen) following the manufacturer’s instruction. First-strand cDNA was reverse transcribed from 5 × All-In-One RT MasterMix (with AccuRT Genomic DNA Removal Kit), which was implemented for detected gene semi-quantitative/quantitative analysis with specific primers. Gene transcript levels were measured by qRT-PCR using the ABI 7500 real-time
PCR system, while actin gene was used as internal control. Each qRT-PCR program was performed in total volume of 20 µl schema containing 1×SYBR Green Master Mix. Each experiment was repeated three times, and the relative quantitative method was used to evaluate quantitative variation. The qRT-PCR reaction program was directed at 94 °C for 10 min, followed by 40 cycles at 94 °C for 30 s, 60 °C for 1 min. Primers for the experiment were referred to Table S3.

Gene cloning and sequence analysis

Candidate genes were cloned by the gene specific primers, and the primers were produced by using HJX74 genome sequence as reference. The KOD FX DNA polymerase (TOYOB, Japan) was employed in the PCR assay. The information of ORFs (Open Reading Frames) and its corresponding predicted protein sequence was obtained from NCBI (https://www.ncbi.nlm.nih.gov/). The multiple DNA and protein sequences of candidate genes in NIL-GW10 and NIL-gw10 were aligned by software DNAman. The phylogenetic analysis was presented by MAGA6 based on the neighbor-joining (NJ) model, and the bootstrap values were estimated with 1000 replicates.

Vector construction and rice transformation

To generate the Cas9 vector for GW10, two targets were designed in the ORF of candidate gene. The intermediate SK-gRNA vector was constructed using the isocaudomer ligation method. The SK-gRNA-target1 and SK-gRNA-target2 were digested with Kpn1/NheI and Xba1/BglII, respectively. The digested products were gel purified and ligated into pC1300-Cas9 binary vector (digested with KpnI/BamHI). To construct the overexpression vector, the full ORF of GW10 was amplified from the NIL-GW10 cDNA and cloned into pXQ35S vector. The vectors were transformed into Agrobacterium tumefaciens EHA105 cells, and resulting strains were implemented to transform the callus of HJX74 and NIL-gw10, respectively. (Hu et al. 2018a, b). Primers for these experiments were referred to Table S4.

Agronomic traits evaluation and statistical analysis

Agronomic traits were evaluated in the different period of plant growth. Plant height and number of effective tillers were measured at the maturity stage. Heading date was calculated at the time when the first panicle sprouting. Meanwhile, panicle length, primary branch, secondary branch, 1000-grain weight, grain length and grain width were investigated after the rice harvest at the maturity stage. Grain length and grain width were measured by Microtek Scan-Wizard EZ scanner V-2.140 and Wan Shen grain analyzer software. Each set of groups was recorded about ten plants, and more than 100 grains were measured per plant. The effect of BR on the lamina inclination was examined by epiBL treatment assay according to Li (2017). And the lamina inclination angles were measured by using the ImageJ software. All the data were analyzed using IBM SPASS statistic 20. The significance was accepted at $P \leq 0.05$ and $P \leq 0.01$.

Results

Comparison of grain and panicle traits of NIL-GW10 and NIL-gw10

Grain size and grain number per panicle play extremely important roles in rice grain yield (Li et al. 2019). We constructed a set of SSSLs by crossing Lemont (donor parent) and HJX74 (recipient parent). Each of the SSSLs carries only one substitution segment from the Lemont on the genetics background of HJX74. Then, QTL analysis showed the presence of a minor grain size locus $qGW10^{GW10}$ on the long arm of chromosome 10 (Table S1). The near-isogenic line gw10 which was derived from Lemont produced significantly shorter grain length than that of NIL-GW10 (namely HJX74) (Fig. 1c, d). The grain width of NIL-gw10 was also markedly narrower than that of NIL-GW10, and the 1000-grain weight decreased accordingly in NIL-gw10 (Fig. 1e, f). In addition, the plant of NIL-gw10 is slightly higher than that of the NIL-GW10, and the difference in plant height between the NIL-gw10 and NIL-GW10 was statistically significant (Figs. 1a and 2b). The panicle length of NIL-gw10 is markedly longer than that of NIL-GW10 (Fig. 2d). There were no differences between the two NILs in the heading date (Fig. 2a) and number of tillers (Fig. 2c). Moreover, the secondary branch per panicle of NIL-gw10 was much more than that of NIL-GW10, which resulted in an increased number of grains per plant (Fig. 2e, f). These results indicated that the introgressed substitution segment from Lemont in HJX74 contributed to the decrease in grain length and grain width, meanwhile, to the increase in the grain number per panicle.

Genetic analysis and mapping of GW10

To study the genetic factor for $qGW10^{GW10}$, we crossed NIL-GW10 with NIL-gw10 to generate F2 population. The grain width was used as a target trait. The genotype and phenotype of F2 population conformed to a segregation ratio 1:2:1 ($\chi^2 = 0.11 < \chi^2_{0.01,2} = 9.21$). The inheritance patterns of the F2 plants indicated that a semidominant $qGW10^{GW10}$ allele from HJX74 controlled grain size (Fig. 1b). A 3200 F2 segregant was bred from the cross between the NIL-gw10 and HJX74. A subsequent high-resolution
map was constructed on the basis of the $F_2$ population. The region of $qGW10_{Lemont}$ was narrowed down to a 14.6 kb flanked by marker Z4 and Z5 on chromosome 10 (Fig. 3a, b). The segment contains only one predicted open reading frame ($ORF1$) and is very close to the start codon of $ORF2$ according to the HJX74 genome (Fig. 3c). The $ORF1$ and $ORF2$ correspond to $Os10g0515400$ and $Os10g0515900$ in Nipponbare, respectively. The expression profiles of $ORF2$ in various tissues of NIL-$GW10$ and NIL-$gw10$ were tested by RT-PCR analysis. No expression of $ORF2$ was detected at all the tissues (Fig. S1b), while the qPCR analysis indicated that $ORF1$ was expressed in root, stem, leaf and developing panicles (Fig. S1a). The expression was significantly reduced in the young panicle of the NIL-$gw10$ compared to that of the NIL-$GW10$ especially in the panicles of 6 cm in length (Fig. 3d). It is strongly suggested that $ORF1$ is the candidate gene for $GW10$. The $ORF1(Os10g0515400)$ encodes a P450 subfamily protein CYP89A2 (Fig. S2). The sequence comparison of $GW10$ in Lemont and HJX74 revealed four polymorphisms in the promoter region and two polymorphisms in the coding sequence. There is one synonymous polymorphism (C 792 G) and an in frame 3 bp Indel in the coding sequence. A 3326 bp Indel in the upstream of the coding region of $Os10g0515400$ gene locus was probably
associated with the down regulation of the gene in NIL-
gw10 (Fig. 3c and Table S5). The role of Os10g0515400 in rice development is not clear. Phylogenetic analysis of GW10 protein indicates that it is ubiquitous in the poaceae (Fig. S3) and suggests that GW10 might play a vital role in the poaceae development.

GW10 controls grain size and grain number

We generated the GW10 knockout transgenic plants by the CRISP/cas9 genome editing system in HJX74 and obtained homozygous transgenic T2 plants (Figs. S4 and S5). The grain length and grain width were examined in the NIL-GW10, NIL-gw10 and KO-GW10 lines at the mature stage. The NIL-GW10 line produced grains with length of 8.40 mm and width of 2.82 mm, while the NIL-
gw10 line produced grains with length of 7.93 mm and width of 2.72 mm. The KO-GW10 line had a grain length of 7.71 mm and a grain width of 2.71 mm (Fig. 4a, c, d). These results indicate that the grain size of KO-GW10 line was significantly smaller than that of NIL-GW10 and NIL-gw10. The 1000-grain weight of KO-GW10 line decreased 5.5% and 14.6% compared to that of NIL-gw10 and NIL-GW10, respectively (Fig. 4e). Interestingly, the number of grains per plant was obviously different in the three lines. The KO-GW10 line had much more grain number compared to the NIL-GW10 and NIL-gw10 line (Fig. 4b, f). We generated OE-GW10 transgenic plant line in NIL-gw10 background, the grain width was wider than that of NIL-gw10 and so did the grain length (Fig. S6a–c). Moreover, the number of grains per plant decreased in OE-GW10 plants (Fig. S6d). The plant height and panicle length were different between the line of NIL-GW10 and NIL-gw10 (Fig. 1a and Fig. 2b, d). But the panicle length of KO-GW10 had no difference compared to that of NIL-GW10 (Fig. S5a, e), and the plant height had no significant difference between KO-GW10 and NIL-GW10 (Fig. S5a, c). These results indicate that the GW10 contributed to the different rice grain size and grain number. In the meantime, there should be some loci that contribute to the plant height and panicle length in the same substitution segment on chromosome 10.
GW10 involved in the BR signaling pathway

The GW10 encodes a cytochrome P450 subfamily 89A2 homology protein. In the previous reports, a new allele of DWARF2 (D2), smg11, encoding a cytochrome P450 protein controls grain size and grain number by involving in brassinosteroid (BR) biosynthesis pathway (Fang et al. 2016). Our materials have similar phenotypes to those of the smg11. Therefore, the GW10 might participate in the BR biosynthesis pathway. We examined the expression levels of BR biosynthetic and BR signaling genes in the 0.2 cm young panicle. The expression levels of D2, DWARF, CPD, BZR1 and DLT of NIL-GW10 were markedly higher than those of NIL-gw10, while the expression levels of BU1 and GSK2 were significantly lower in NIL-GW10 than those in NIL-gw10. The BRI1 expression level was not altered in the NIL-gw10 and NIL-GW10 lines (Fig. 5a). The expression levels of GS2 and GS9 had no significant differences in the two NILs, but the GW5 and DEP2 expressions were significantly different with \( P \leq 0.01 \) and \( P \leq 0.05 \) levels, respectively (Fig. 5b). The epiBL treatment assay showed that the NIL-gw10 and KO-GW10 were more sensitive to exogenously applied BLs compared to the NIL-GW10. And the more BLs, the bigger lamena inclination angles (Fig. S7a, b). These results suggest that the GW10 involves in the BR signaling pathway in the regulation of grain size.
Discussion

It was difficult to determine the precise location of QTLs in rice genome by using the $F_2$ plants, Recombinant Inbred Lines (RILs), Backcross Inbred Lines (BILs) and Doubled Haploid Lines (DHLs) (Ashikari et al. 2006). Consequently, the development of Nearly Isogenic Lines (NILs), Chromosome Fragment Substitution Line (CSSL) and Single Segment Substitution Line (SSSL) was necessary for QTL fine mapping and gene cloning (Ashikari et al. 2006; Guo et al. 2016; Zhou et al. 2017a, b; Wang et al. 2018; Yang et al. 2018; Luan et al. 2019; Tan et al. 2020; Tan et al. 2021), especially for the minor genes. The additive effect value was $GW10$ lower than some major genes for grain size, such as $gs3$, $GW5$ and $GW7$ (Wang et al. 2015). The SSLSs were excellent material for the cloning and functional analysis of the $GW10$. Many QTLs for grain size have been identified and distributed across nearly all the 12 chromosomes in rice. However, only a few QTLs for grain size were reported on chromosome 10 (Li et al. 2019). The $GW10$ was located on the long arm of chromosome 10 and was incomplete dominant inheritance (Fig. 1b). The qPCR was performed to

![Image](image_url)
examine the expression level of GW10 in various rice tissues, including roots, stems, leaf and panicles in different growth stages. The GW10 is constitutively transcribed in all tested tissues (Fig. 3d and Fig S1a). The GW10 encodes a P450 Subfamily 89A2 homology protein. The cytochrome P450 family is one of the biggest protein families in plants, and Subfamily 89A2 homology protein. The cytochrome P450 superfamily protein affected grain size and other agronomic traits in rice. The OE-CPB1 transgenic plants showed increasing grain length, while the RNAi-CPB1 transgenic plants showed smaller grain size and semidwarf in stature (Wu et al. 2016). A small grain (sng11) mutant in rice exhibited more secondary panicle branch, small grains and increased number of grains per panicle (Fang et al. 2016). The NBG4, also reported as Small grain 4 (Shi et al. 2015)/ Dwarf 11 (Tanabe et al. 2005)/Clustered spokelets 4 (Guo et al. 2014)/ GNS4 (Zhou et al. 2017a, b), encodes a cytochrome P450 (CYP724B1). A 10-bp deletion in the CDS resulted in the lower expression of nb4g than that of the NBG4 in ZH11, and the deletion was contributed to the grain shape (Tong et al. 2018). In this study, one of synonymous polymorphism (C 792 G) and a 3 bp deletion in the ORF1 coding sequence, and three single base variations and a 3326 bp deletion in the promoter between NIL-GW10 and NIL-gw10 (Fig. 3c, Table S5). It was found that the grain size could be influenced by the expression level of grain size genes such as the GW8, GW5 and GW6 and there were Indel variations in the promoter of the GW8, GW5 and GW6 (Wang et al. 2012; Liu et al. 2017; Shi et al. 2020). The lower expression level of gw10 in young panicle was associated with the smaller grain size and the more grain number in NIL-gw10 (Fig. 1 and Fig. 2f). Additionally, the grain size of the KO-GW10 line was smaller than that of the NIL-gw10 and of the NIL-GW10 (Fig. 4a), while OE-GW10 lines revealed significantly increased grain length and grain width than that of the NIL-gw10 (Fig. 6a–c). It implied that the 3326 bp deletion in the promoter would be the key potential cis-regulators for the smaller grain size in NIL-gw10. In addition, the epiBL treatment assay implied that the mutations in the CDS of GW10 might contribute to the different phenotype between NIL-GW10 and NIL-gw10 (Fig. S7).

It was reported that the CYP724B1 could control grain size by affecting BR-related genes (Shi et al. 2015; Zhou et al. 2017a, b). BRs regulate various aspects of plant development, including root development, anther and pollen development, stem elongation and cellulose biosynthesis in plants (Yang et al. 2011). GSK2 is one of the orthologs of BIN2 which plays crucial roles in the BR signaling pathway, and GSK2 could inhibit the activities of OsBZR1 and DLT by phosphorylating the two proteins (Tong et al. 2012). BUI protein acts as a positive regulator of the BR pathway and participates in BR signaling pathways through OsBRI1 and RGA1 (Tanaka et al. 2009). GW5 could physically interact with GSK2 and inhibit its kinase activities toward BZR1 and DLT (Liu et al. 2017). In NIL-gw10, the expression of GW5 was significantly higher than that of NIL-GW10. Beside, the expression levels of a series of GSK2-regulated genes, including BZR1, DLT, D2, Dwarf and CPD, were significantly downregulated (Fig. 5a), which implies that GW10 reduces the GSK2 expression by influencing GW5. DEP2 mainly affects the rapid elongation of rachis and primary and secondary branch (Li et al. 2010). The expression level of DEP2 is higher in NIL-gw10 than in NIL-GW10, which suggests that GW10 should involve in the regulatory of DEP2 for grain size and secondary branch (Fig. 5b). Furthermore, the NIL-gw10 and KO-GW10 showed bigger leaf angles than that of NIL-GW10 after treated by the exogenous BR (Fig. S7). As shown above, BR signaling was involved in the GW10-mediated regulation of grain size. However, the detailed mechanism of GW10 in rice grain size regulation still needs further clarification.

The rice grain size is regulated by a complicated network. Although recent studies have identified some key grain size genes and molecular pathways, the complete regulatory network for grain size is poorly understood (Li et al. 2019). Our
work provides a piece of this complex puzzle and makes a contribution to the enrichment of breeding germplasm resources bases.

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**Author contribution statement** SW and GZ designed and supervised works. PZ performed most of the experiments, analyzed experimental data and prepared the draft of manuscript. XW, XW, ZX, SM, SL and FL conducted a part of experiments. SB, ZL, HZ and GL developed the materials. SW wrote the paper. All authors read and approved the final manuscript.

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**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

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Ashikari M, Matsuoka M (2006) Identification, isolation and pyramid-interest. The authors declare that they have no conflict of interest. The online version contains supplementary resources bases.
**DWARF**1 allele that affects brassinosteroid biosynthesis in rice. Sci Bull 60:905–915

Si L, Chen J, Huang X, Gong H, Luo J, Hou Q, Zhou T, Lu T, Zhu J, Shangguan Y, Chen E, Gong C, Zhao Q, Jing Y, Zhao Y, Li Y, Cui L, Fan D, Lu Y, Weng Q, Wang Y, Zhan Q, Liu K, Wei X, An K, An G, Han B (2016) *OsSPL13* controls grain size in cultivated rice. Nat Genet 48:447–456

Song X, Huang W, Shi M, Zhu M, Lin H (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet 39:623–630

Song XJ, Kuroha T, Ayano M, Furuta T, Nagai K, Komeda N, Segami T, Li X, Chen J, Huang X, Gong H, Luo J, Hou Q, Zhou T, Lu T, Zhu J, Wang S, Li S, Liu Q, Wu K, Zhang J, Wang S, Wang Y, Chen X, Zhang G, Liu G (2018) QTL epistatic regression rice grain yield, and plant biomass in rice. Proc Natl Acad Sci 115:76–81

Tan Q, Wang C, Luan X, Zheng L, Ni Y, Yang W, Yang Z, Zhu H, Zeng R, Liu G, Wang S, Zhang G (2021) Dissection of closely linked QTLs controlling stigma exsertion rate in rice by substitution mapping. Theor Appl Genet 134:1253–1262

Tan Q, Zou T, Zheng M, Ni Y, Luan X, Li X, Yang W, Yang Z, Zhu H, Zeng R, Liu G, Wang S, Fu X, Zhan G (2020) Substitution mapping of the major quantitative trait loci controlling stigma exsertion rate from *Oryza glumaepatula*. Rice 13:37

Tanaka A, Nakagawa H, Tomita C, Shimatani Z, Ohtake M, Nomura T, Watanabe A, Nakashima H, Tomita C, Mori M (2009) A novel cytochrome p450 is implicated in brassinosteroid biosynthesis via the characterization of a rice dwarf mutant, *dwarf11*, with reduced seed length. Plant Cell 17:776–790

Takahashi H, Kuroha T, Ayano M, Furuta T, Nagai K, Komeda N, Segami T, Li X, Chen J, Huang X, Gong H, Luo J, Hou Q, Zhou T, Lu T, Zhu J, Wang S, Li S, Liu Q, Wu K, Zhang J, Wang S, Wang Y, Chen X, Zhang G, Liu G (2018) QTL epistatic regression rice grain yield, and plant biomass in rice. Proc Natl Acad Sci 115:76–81

Tanaka A, Nakagawa H, Tomita C, Shimatani Z, Ohtake M, Nomura T, Jiang C, Dubouzet JG, Kikuchi S, Sekimoto H, Yokota T, Asami T, Kamakura T, Mori M (2009) BRASSINOSTEROID UPREGULATED1, encoding a Helix-Loop-Helix protein, is a novel gene involved in brassinosteroid signaling and controls bending of the lamina joint in rice. Plant Physiol 151:669–680

Tang H, Liu L, Jin Y, Du L, Yin Y, Qian Q, Zhu L, Chu C (2012) DWARF and LOW-TILLERING acts as a direct downstream target of a GSK3/SHAGGY-Like kinase to mediate brassinosteroid responses in rice. Plant Cell 24:2562–2577

Tong X, Wang Y, Sun A, Bello B, Ni S, Zhang J (2018) *Notched belly grain 4*, a novel allele of *dwarf 11*, regulates grain shape and seed germination in rice (*Oryza sativa*). Int J of Mol Sci 19:4009

Wang X, Jin L, Zhu H, Wang S, Zhang G, Liu G (2018) QTL epistatic analysis for yield components with single-segment substitution lines in rice. Plant Breed 137:346–354

Wang S, Li S, Liu Q, Wu K, Zhang J, Wang S, Wang Y, Chen X, Zhang Y, Gao C, Wang F, Huang H, Fu X (2015) The *OsSPL16-GW7* regulatory module determines grain shape and simultaneously improves rice yield and grain quality. Nat Genet 47:949–954

Wang S, Wu K, Yuan Q, Liu X, Liu Z, Lin X, Zeng R, Zhu H, Dong G, Qian Q, Zhang G, Fu X (2012) Control of grain size, shape and quality by *OsSPL16* in rice. Nat Genet 44:950–954

Wu Y, Fu Y, Zhao S, Gu P, Zhu Z, Sun C, Tan L (2016) CLUSTERED PRIMARY BRANCH 1, a new allele of *DWARF*11, controls panicle architecture and seed size in rice. Plant Biotechnol J 14:377–386

Wu W, Liu X, Wang M, Meyer RS, Luo X, Ndjiondjop M, Tan L, Zhang J, Wu J, Cai H, Sun C, Wang X, Wing RA, Zhu Z (2017) A single-nucleotide polymorphism causes smaller grain size and loss of seed shattering during African rice domestication. Nat Plants 3:17064

Yang Z, Jin L, Zhu H, Wang S, Zhang G, Liu G (2018) Analysis of epistasis among QTLs on heading date based on single segment substitution lines in rice. Sci Rep 8:3059

Yang C, Zhang C, Lu Y, Jin J, Wang X (2011) The Mechanisms of brassinosteroids’ action: from signal transduction to plant development. Mol Plant 4:588–600

Yu J, Miao J, Zhang Z, Xiong H, Zhu X, Sun X, Pan Y, Liang Y, Zhang Q, Abdul Rehman RM, Li J, Zhang H, Li Z (2018) Alternative splicing of *OsLG3b* controls grain length and yield in *japonica* rice. Plant Biotechnol J 16:1667–1678

Zhang GQ (2019) The platform of breeding by design based on the SSSL library in rice. Hereditas (beijing) 41:754–760

Zhu Z, Li Q, Zhang C, Zhang C, Yang Q, Pan L, Ren X, Lu J, Gu M, Liu Q (2018) *GS9* acts as a transcriptional activator to regulate rice grain shape and appearance quality. Nat Commun 9:1240

Zhou Y, Tao Y, Zhu J, Miao J, Liu J, Liu Y, Yi C, Yang Z, Geng Z, Liang G (2017a) *GNS4*, a novel allele of *DWARF*11, regulates grain number and grain size in a high-yield rice cultivar. Rice 10:34

Zhou Y, Xie Y, Cai J, Liu C, Zhu H, Jiang R, Zhong Y, Zhang G, Tan B, Liu G, Fu X, Liu Z, Wang S, Zhang G, Zeng R (2017b) Substitution mapping of QTLs controlling seed dormancy using single segment substitution lines derived from multiple cultivated rice donors in seven cropping seasons. Theor Appl Genet 130:1191–1205

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

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