QTL editing confers opposing yield performance in different rice varieties

Summary  
Grain yield is one of the most important and complex trait for genetic improvement in crops; it is known to be controlled by a number of genes known as quantitative trait loci (QTLs). In the past decade, many yield-contributing QTLs have been identified in crops. However, it remains unclear whether those QTLs confer the same yield performance in different genetic backgrounds. Here, we performed CRISPR/Cas9-mediated QTL editing in five widely-cultivated rice varieties and revealed that the same QTL can have diverse, even opposing, effects on grain yield in different genetic backgrounds.

Grain yield is a complex trait governed by many genes known as quantitative trait loci (QTLs). The most popular methodology of plant breeding in essence is generating various QTL combinations and subsequently selecting the elite ones. In the past decade, many QTLs that contribute to yield have been identified in various crops (Bai et al. 2012; Zuo and Li 2014). However, due to the vast number of QTLs in genomes, the introgression of QTLs between different varieties remains very tedious. Moreover, if the QTLs are closely linked, traditional breeding methods are nearly impossible to exploit a particular QTL of interest without introducing other QTLs into a given background. Recently, the development of the CRISPR/Cas9 system has been demonstrated to be effective for the rapid introduction of mutations at defined loci in crops (Shan et al. 2013; Wang et al. 2014). Here, we conducted CRISPR/Cas9-mediated QTL editing to explore the function of QTLs in different varieties. We found that the same QTL can have highly varied, even opposing, effects on grain yield in different backgrounds.

GRAIN SIZE3 (GS3) was the first QTL characterized to regulate grain size (Fan et al. 2006). Grain number 1a (Gn1a) was the first QTL identified to control the grain number (Ashikari et al. 2005). Loss of function of the ORF domain at the N terminus of GS3 results in long grain and enhances grain yield phenotypes (Takano-Kai et al. 2009, 2013; Mao et al. 2010). Reduction or loss of function of Gn1a increases the number of reproductive organs, resulting in enhanced grain production (Ashikari et al. 2005; Wang et al. 2015b). Both QTLs are widely used in indica varieties, but are rarely exploited in japonica varieties. We designed and constructed a CRISPR/Cas9 vector that targets the disruption of both GS3 and Gn1a (Figures 1A, S1) using a previously-described method (Shan et al. 2013; Wang et al. 2015a). Five widely-cultivated japonica varieties, namely Nanjing 9108 (N9108), Wuyunjing 27 (W27), Yangjing 4227 (Y4227), Zhejing 22 (Z22), and Zhejing 88 (Z88), were chosen for targeted mutation. In the T0 generation, we genotyped each plant and selected reading frame shift mutants of the targeted loci (Figures 1B, S2). Due to the extremely high mutation efficiency at the GS3 site, we were only able to obtain gS3 and double gS3gn1a mutants; we did not detect any gn1a single mutants. We screened the T1 generation and isolated transgenic-free homozygous mutant plants. We identified and subsequently phenotypically characterized two homozygous mutant genotypes for each of the five genetic backgrounds (gS3-N9108, gS3gn1a-N9108, gS3-W27, gS3gn1a-W27, gS3-Y4227, gS3gn1a-Y4227, gS3-Z22, gS3gn1a-Z22, gS3-Z88, and gS3gn1a-Z88) (Tables S1, S2). Confirmed unedited plants of the T1 generation were used as wild-type (WT) controls.

We quantified the effects of loss of function of GS3 and Gs1a on grain size and grain number in all of the tested japonica varieties. The grain lengths of the gS3 and gS3gn1a mutants in all of the genetic backgrounds were compared to the WT (Figures 1C–F, S4–7; Table S1). Additionally, in all of the genetic backgrounds, the gS3gn1a mutants had a larger number of grains on each of the 5 main panicles than did the gS3 mutants (Figures 1E–F, S4–7; Table S1). The results suggest that both QTLs have conserved roles between rice subspecies. We next measured the yield of each genotype. Unexpectedly, we found that 7 of the 10 novel genotypes (gS3gn1a-N9108, gS3-W27, gS3gn1a-W27, gS3-Y4227, gS3gn1a-Y4227, gS3-Z88, and gS3gn1a-Z88) had decreased grain yields (ranging from −1% to −30%) per plant as compared to the WT. Only 3 genotypes (gS3-N9108, gS3-Z22, and gS3gn1a-Z22) had higher grain yields than the WT (increases of 3–7%) (Figure 1C; Table S1).

Grain yield of rice is determined largely by four factors: the number of effective tillers or panicles per plant, the number of grains per panicle, the percentage of grains that are filled, and the grain weight (Sakamoto and Matsuoka 2008; Xing and Zhang 2010). To elucidate the underlying causes of the observed reductions in grain yield, we further analyzed the four yield-determining factors in the 7 unexpectedly low-yielding genotypes. We observed that all 7 genotypes had a reduced number of effective tillers (reduced by 1%–29%) (Figures 1H, S3A, S4–7; Table S1). Given that the rice yield of a particular plant is ultimately the summed grain weight of all of the grains from all of the tillers of that plant, we expanded our phenotypic analysis to encompass the grain number per tiller. Having expanded the scope of our analysis, we found that the grain number per tiller was reduced in gS3-W27, gS3-Y4227, gS3-Z88, and gS3gn1a-Z88 plants (Table S1). Because of the reduced number of effective tillers per plant and the decreased grain number per tiller, the total grain number of the gS3 and gS3gn1a genotypes was reduced in the W27, Y4227, and Z88 backgrounds (reduced by 9%–15%) (Figure 1I; Table S1). Accordingly, the numbers of filled grains were also reduced in these backgrounds (reduced by 6%–18%) (Figures S5B, S4–7; Table S1). These decreases in the two traits can explain the observed decrease in grain yield in these backgrounds. For the gS3gn1a-N9108 mutant, because it had a significant increase in the number of grains per effective tiller, the total grain number per plant was increased compared with that of WT. However, due to the reduced ratio of filled grains, the final grain yield of gS3gn1a-N9108 was slightly decreased compared to the WT.
Figure 1. Targeted mutation of GS3 and Gn1a led to diverse yield performance in different rice varieties
(A) Schematic diagram of the targeted sites in GS3 and Gn1a. The targeted sites are labeled in black uppercase letters. The initiation codons are underlined once. The protospacer adjacent motif (PAM) sequences are underlined twice. (B) Parts of mutations at the GS3 and Gn1a loci in T0 generation plants. The targeted sequence is highlighted in blue and the PAM sequence in red. Mutations with 1bp insertions are in red lowercase letters. The deleted sequences are shown by black hyphens.

(C) Grain shape of the N9108, gs3-N9108, and gs3gn1a-N9108 genotypes. (D) Comparison of grain lengths among plants of the N9108, gs3-N9108, and gs3gn1a-N9108 genotypes. (E) The morphology of the main panicles of plants of the N9108, gs3-N9108, and gs3gn1a-N9108 genotypes. (F) Comparison of the grain number per 5-main panicle per plant for the N9108, gs3-N9108, and gs3gn1a-N9108 genotypes. (G) Comparison of yield per plant among the WT, gs3, and gs3gn1a genotypes in the five genetic backgrounds. (H) Comparison of tiller number per plant among the WT, gs3, and gs3gn1a genotypes in the five genetic backgrounds. Values in (D, F–I) are means ± s.e.m. n = 200 grains and three replicates in (D), and n > 10 in (F), and n ≥ 8 in (G–I). Single and double asterisks represent significant differences as determined by Duncan’s Multiple Range Test at the P < 0.05 and P < 0.01 levels, respectively. Scale bars (C, E) 1 cm.
For the 3 mutant genotypes with enhanced yield, we found that the number of effective tillers of those genotypes was relatively stable or mildly reduced (ranging from −7% to 2%) compared to their respective WT background plants (Table S1). As the grain number of each effective tiller varied significantly in each plant, we divided tillers into two categories depending upon the grain number: major tillers (i.e., with a grain number count of more than one third of the average count for the 5-main tillers) and minor tillers (i.e., with a grain number count of fewer than one third of the average count for the 5-main tillers). We found that the number of major tillers of the three genotypes with increased yields was slightly increased compared with that of the respective WT plants (Figure 1H; Table S1). Furthermore, the grain number per tiller was increased in all 3 of these genotypes (ranging from 9% to 24%) (Table S1). The total grain number per plant of the mutants was therefore greater than for the respective WT plants (ranging from 7% to 14%) (Figure 1I; Table S1). These differences can explain the observed increased yields of these three mutant genotypes.

The analysis revealed that the change of major tiller number is responsible for the variation of grain yield per plant in all ten novel genotypes. Our phenotypic analyses also revealed that although the grain lengths of the gs3-Z22 and gs3gn1a-Z22 genotype were increased, their grain volumes and grain weights were both slightly reduced compared with the WT (Figure S6G-H; Table S1). These results suggest that the grains of these plants were not fully filled. In addition, although the grain number of the gs3gn1a-Z22 plants genotype was increased, we observed occasional florter development in some panicles of gs3gn1a-Z22 (Figure S6C), which might prevent the direct use of those two genotypes. Finally, it is well known that grain yield per plant does not necessarily accord with population yield in large-scale field trials. Thus, further development of appropriate field management, such as rational dense planting and controlling the number of minor tillers, may be required for the application of all those genotypes in the future.

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K.W., Q.Q. and C.Y. designed the experiments; L.S., C.W., Y.F., J.W. and Q.L. performed the experiments; X.Z. supplied plant materials; L.S., C.W. and K.W. wrote the manuscript.

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Figure S1. Schematic diagram illustrating the structure of CRISPR/Cas9-expressing vector targeting both GS3 and Gn1a.

Figure S2. Mutation types at the GS3 and Gn1a loci of the five varieties in T0 generation.

Figure S3. Comparison of yield-related traits among the N9108, gs3-N9108, and gs3gn1a-N9108 genotypes.

Figure S4. Comparison of yield-related traits among the W27, gs3-W27, and gs3gn1a-W27 genotypes.

Figure S5. Comparison of yield-related traits among the Y4227, gs3-Y4227, and gs3gn1a-Y4227 genotypes.

Figure S6. Comparison of yield-related traits among the Z22, gs3-Z22, and gs3gn1a-Z22 genotypes.

Figure S7. Comparison of yield-related traits among the Z88, gs3-Z88, and gs3gn1a-Z88 genotypes.

Table S1. Comparison of yield-related traits of WT, gs3, and gs3gn1a genotypes in the five genetic backgrounds.

Table S2. Comparison of yield-related traits of the WT, gs3, and gs3gn1a genotypes in the five genetic backgrounds.

Table S3. PCR primers used in this study.

Text S1. Materials and methods.