Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2023), pp. 1–3 doi: 10.1111/pbi.14014

Brief Communication

A novel QTL GL12 from wild rice increases grain length and weight in cultivated rice

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Received 10 June 2022;
revised 14 December 2022;
accepted 15 January 2023.
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Keywords: wild rice, QTL, GL12, grain length, grain weight.

Grain size and shape are determinants of grain yield and quality and have been selected during early rice domestication (Li *et al.*, 2019). Most rice cultivars have larger seeds than their ancestral progenitor *Oryza rufipogon*. Previous studies have revealed that potential genes hidden in wild rice may be important for yield-related trait improvement (Huang *et al.*, 2013). However, to date, positive regulation of grain length (GL) or yield by wild rice alleles has rarely been reported.

To explore grain shape related genes in wild rice, we constructed a set of chromosome segment substitution lines using *O. rufipogon* (accession number CWR274) as the donor parent and the *indica* cultivar '9311' as the receipt parent (Qiao *et al.*, 2016). A major QTL associated with GL was identified on Chromosome 12 and further narrowed to a 15-kb genomic region using a fine mapping approach (Figure 1a and S1). Three genes were subsequently identified in this region. Only the *LOC_Os12g39640* overexpression (OE) lines showed an increased GL phenotype. Therefore, the *LOC_Os12g39640* gene was the most likely candidate gene and named *GL12*. According to the rice annotation database, *LOC_Os12g39640/GL12* encodes a MYB transcription factor. Four nonsynonymous mutations in the coding region were detected between CWR274 and 9311, including three SNPs (+529 C>A; +2145 A>G, and +2190 T>C) and one 3-bp (GGA) insertion in the fifth exon, named as M1 to M4 (Figure 1a).

We conducted a series of genetic modification verifications in the 9311 background. Compared with 9311, the near-isogenic line (NIL) of $GL12^{w}$ exhibited increases in GL (8.1%), and 1000-grain weight (TGW) (12.9%). Using CRISPR/Cas9 technology, we generated knock-out (ko) mutants of $GL12^{w}$ in the NIL background. As expected, the GL and TGW of the mutant plants decreased significantly. OE of $GL12^{w}$ increased GL and TGW by 5% and 6.8% in the 9311 genetic background, respectively. The $GL12^{9311}$-OE lines did not display obvious difference in grain traits (Figure 1b–d). Correlation analyses showed that in both the NIL and $GL12^{w}$-OE lines, GL and TGW were positively correlated (Figure 1d5).

To explore the natural variations in the coding region of *GL12*, four $GL12^{w}$-based variants (M1 to M4) were generated (Figure 1e) and independently introduced into the *japonica* cultivar Zhonghua 11 (ZH11) under the control of the 35S promoter. OE of $GL12^{w}$-M1, M2 and M3 had no effect on GL whereas OE of $GL12^{w}$-M4 increased GL (Figure 1f,g), indicating that SNP1, 2 and 3 are functional variations. We investigated the haplotypes on the three natural polymorphisms in the RFGB 3K cultivated rice accessions dataset (Wang *et al.*, 2018), only two haplotypes, C-A-T of wild rice (Hap-W) and A-G-C of 9311 (Hap-9311) were found. Almost all *japonica* cultivars contained the three functional SNPs of Hap-W. However, in an *indica* subpopulation, 13 accessions containing Hap-W had, on average, longer grains than the other 1013 accessions containing Hap-9311 (Figure 1h).

To decipher the biological function of the wild rice $GL12^{w}$ gene, we measured the rate of grain milk filing for the NIL, $GL12^{w}$-OE and 9311. Significant differences in 100-grain dry weights were detected among NIL, $GL12^{w}$-OE and 9311 at 15 days after fertilization (Figure 1i). Therefore, we investigated the spikelet hulls before fertilization using a scanning electron microscope. There were significantly fewer longitudinal cells in spikelet hulls from the NIL and $GL12^{w}$-OE than in 9311; however, the spikelet hull cells of the NIL and $GL12^{w}$-OE were significantly longer than those of 9311 (Figure 1j1-3). Thus, the longer GL of NIL and $GL12^{w}$-OE may result from increase in the longitudinal length of cells in their spikelet hulls. We confirmed that some GL-related genes were regulated by $GL12^{w}$ using Real-time PCR (Figure 1k). Notably, the expression level of *GL3.1* (Qi *et al.*, 2012) and *GS2* (Hu *et al.*, 2015) were significantly increased in NIL and $GL12^{w}$-OE (Figure 1k). From the dual-luciferase experiments, we observed that $GL12^{w}$ promoted *GS2* and *GL3.1* transcription activity to a greater extent than $GL12^{9311}$ (Figure 1l). Further studies found that GL12 did not directly interact with *GL3.1*. A yeast one-hybrid assay showed that $GL12^{w}$ interacted with the promoter region of *GS2* (Figure 1m). EMSA analysis showed that $GL12^{w}$ binding to the same region of *GS2* promoter (Figure 1o). The *GS2* expression level was significantly increased in $GL12^{w}$-M4 transgenic plants, compared with that in $GL12^{w}$-M1, M2, and M3

Please cite this article as: Wang, Y., Yang, Z., Xing, M., Huang, J., Ding, Y., Zhang, L., Li, F., Nie, Y., Wang, S., Li, Y., Zhao, M., Ge, J., Lou, D., Liu, Z., Fan, W., Guo, W., Zheng, X., Qian, Q., Yang, Q. and Qiao, W. (2023) A novel QTL GL12 from wild rice increases grain length and weight in cultivated rice. *Plant Biotechnol. J.*, https://doi.org/10.1111/pbi.14014.

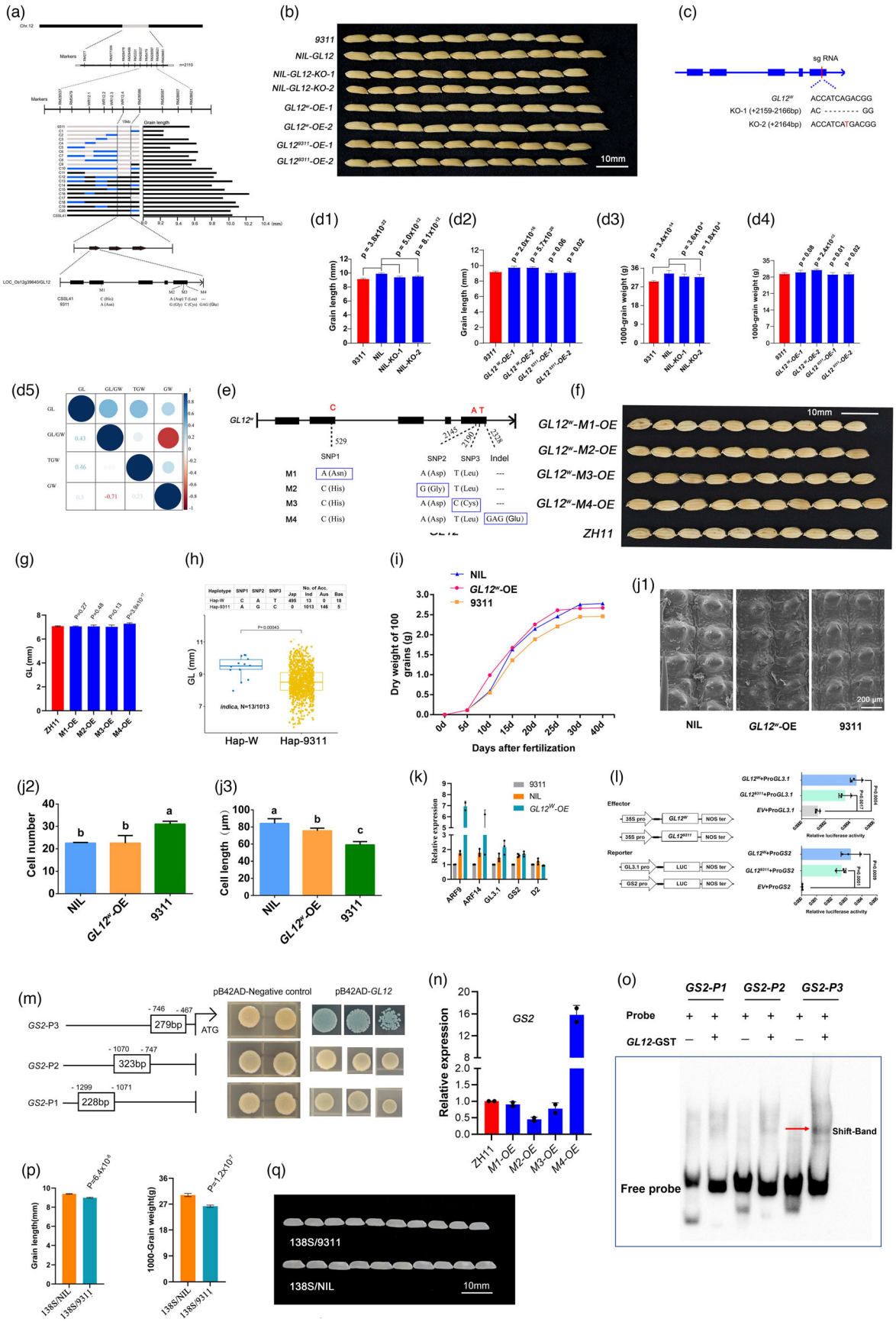

Figure 1 Wild rice *GL12* increases GL and weight in cultivated rice. (a) Fine mapping of *GL12*. CSSL41 is a CSSL line that harbours the QTL and shows increased GL compared with that of 9311. A $F_{2:3}$ Population of CSSL41/9311 was used for fine mapping. (b) Effect of *GL12* alleles on GL, in the 9311 genetic background. (c) Mutation sites in the $GL12^{W}$ gene in the NIL knockout mutants (ko1 and ko2) generated by CRISPR/Cas9 editing. (d1–d4) Statistical analysis of the GL and TGW in lines in (b). Data are shown as means ± SD ($n = 30$). Student's *t*-tests were used to generate the *P* values. (d5) Correlation analysis of GL, grain width (GW), TGW and ratio of GL to grain width (GL/GW) in the NIL and $GL12^{W}$-OE line. (e) Constructs of four variations based on the $GL12^{W}$ allele. (f) Grains of OE transgenic lines harbouring the four $GL12^{W}$-based variations and the wild type ZH11. (g) Statistical analysis of GL of OE lines harbouring the four variations. (h) Distribution of GL for the two haplotypes in *indica* rice accessions. The haplotypes and number of accessions from 1520 accessions from the RFGB 3K dataset are shown above the plot. (i) Dry weight of 100 grains for analysis of grain milk filling ($n = 5$). (j) Scanning electron microscopic analysis of the lemma (j1), and statistical analysis of longitudinal cell number (j2) and cell length (j3) in the lemmas of spikelet hulls ($n = 10$). (k) Expression levels of $GL12^{W}$-regulated genes. The expression analysis was conducted using young panicles 2–5 cm in length. Actin was used as the control and the expression levels in 9311 were set to one ($n = 3$). (l) Interaction with GL12 promotes *GS2* and *GL3.1* transcription activity. Left: Schematic diagrams of the effectors and reporters used in the dual-luciferase experiments. Right: Transient expression assays in *Nicotiana benthamiana* protoplasts. (m) Yeast one-hybrid assay showing interaction of GL12 with the *GS2* promoter region. (n) *GS2* expression level in $GL12^{W}$-M1 to M4 transgenic lines and wild type ZH11. (o) EMSA analysis of $GL12^{W}$ binding to *GS2* promoter. (p) Effect of $GL12^{W}$ on GL in hybrid rice. (q) Statistical analysis of the GL and TGW in two hybrid rice.

transgenic plants and the ZH11 wild type (Figure 1 n). These results indicated that $GL12^{W}$ might be involved in *GS2* pathway to regulate GL and weight.

Considering that 9311 is a hybrid rice restorer line, we crossed the NIL and 9311 with the photosensitive male sterile line 138S. The 138S/NIL hybrids showed considerably increased GL and TGW compared with those of 138S/9311 hybrids (Figure 1p,q). In summary, we cloned and characterized a novel GL QTL on Chromosome 12 from wild rice. Plants carrying $GL12^{W}$ had improved grain milk filing rates and increased spikelet hull cell lengths after fertilization, leading to enhanced GLs and weights. Three linked SNPs in the coding region of *GL12* were identified as functional variations. The present findings provide novel resources to increase yield in rice breeding and offer new insights into GL-related regulatory pathways in rice.

Acknowledgements

This research was supported by the National Key R&D Program of China (2021YFD1200501), and Hainan Yazhou Bay Seed Laboratory (project of B21HJ0215).

Conflict of interest

The authors declare no conflicts of interest.

Author contribution

W.Y.Y. performed most of the experiments. Y.Z.Y. and X.M. participated in the phenotyping and transgenic experiments. H.J.F. and other authors participated in field management, data analysis and logistic work. Q.Q. and Y.Q.W. supervised the study. Q.W.H. designed the study and wrote the paper.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Fine mapping of *qGL12*.

