Brief Communication

Production of novel beneficial alleles of a rice yield-related QTL by CRISPR/Cas9

Yongtao Cui1,2,†, Xingming Hu1,3,†, Guohua Liang4,†, Anhui Feng1, Fanmiao Wang3,†, Shuang Ruan1, Guojun Dong1, Lan Shen1, Bin Zhang1, Dongdong Chen1, Li Zhu1, Jiangu Hu1, Yongjun Lin1,†, Longbiao Guo1, Makoto Matsuoka3,3,† and Qian Qian1,4,†

1State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou, China
2National Key Laboratory of Crop Genetic Improvement and National Centre of Plant Gene Research, Huazhong Agricultural University, Wuhan, China
3Bioscience and Biotechnology Center, Nagoya University, Nagoya, Japan
4Jiangsu Key Laboratory of Crop Genetics and Physiology/Co-Innovation Center for Modern Production Technology of Grain Crops, Key Laboratory of Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou, China

Keywords: CRISPR-Cas9, QTL, SCM3/OsTB1/FC1, Noncoding region.

Breeding high-yield crop cultivars has improved agronomic performance for key grain crops (Hirano et al., 2017). However traditional breeding takes years. Molecular genetic studies have identified beneficial trait-associated alleles in elite cultivars providing a platform for gene-editing techniques such as the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system (Rothen et al., 2019).

Most current ‘super rice’ varieties with high yields have several beneficial agronomic traits. For example the variety Liang-You-Pei-Jiu has strong culms for lodging resistance and large panicles for high yield. Our previous study using recombinant inbred lines (RILs) from a cross between 93-11 and PA64 identified 43 quantitative trait loci (QTLs) associated with many agronomic traits including heading date spikelet number per panicle and stem area (Gao et al., 2013). However no QTL for culm strength or panic size was detected.

Here we studied culm strength by measuring stem cross-section area (SCSA) of the fourth internode (Figure 1a). By using T27 chromosome substitution segment lines (CSSLs) from a cross between 93-11 and Nipponbare (NPB) we detected three QTLs associated with SCSA (Figure 1b,c,d) (Xu et al., 2010). The position of qSCSA1-1 (Chr1: 42290679-43345963) overlapped with STRONG CULM1 (SCM1) (Mulsanti et al., 2018) and SEMI DWARF1 (SD1)-qSCSA6-1 (Chr6: 28410389-28439277) overlapped with SCM2 (Figure 1b,d). For both QTLs the alleles from NPB positively contributed to SCSA.

The region of qSCSA3-1 on the long arm of Chr3 (28406301-30831094) included SCM3/RICE TEOSINTE BRANCH1 (OsTB1)/FINE CULM1 (FC1) (Figure 1b,c,d) (Yano et al., 2015) and the 93-11 allele had a positive effect on culm strength. To test whether OsTB1 is the causal gene for qSCSA3-1 fine mapped qSCSA3-1 using near-isogenic lines (NILs). qSCSA3-1 mapped to a 51-kb region between markers CT11 and CT17 a region that includes OsTB1 (Figure 1e). Quantitative-PCR (qPCR) showed that the expression of OsTB1 in 93-11 inflorescences was higher than that of NPB and NIL-93-11 carrying the NPB allele NIL-qSCSA3-1NPB (Figure 1f).

In addition to affecting SCSA introgression of the NPB allele resulted in a decrease in bending stress area and number of spikelets per panicle and an increase in tiller number but had no effect on plant height (Figure 1f). These observations indicated that OsTB1 has pleiotropic effects on different traits. Therefore we examined the effect of OsTB1 using mutant and complemented plants (Figure 1g). The null mutant of OsTB1 fc1-2 showed lower bending stress smaller panicles and increased tiller number compared with rescued plants expressing OsTB1 from 93-11 or Kasalath (Figure 1h). These observations confirmed that qSCSA3-1 is allelic to SCM3/OsTB1 and has pleiotropic effects.

We compared the sequence of OsTB1 among rice cultivars Nipponica (Koshihikari (nipponica) and four varieties with large SCSA: 93-11 (indica) Kasalath (indica) Zhongcha123 (japonica) and Chugoku117 (indica) and found polymorphisms in the 5'-flanking coding sequence (CDS) (Figure 1i,j). Interestingly the four varieties with large SCSA contained a TGTG insertion at + 219 in the 5'-noncoding region suggesting that this insertion might be important for OsTB1 expression and consequently affect SCSA.

Based on this prediction we introduced mutations in the promoter and 5'-noncoding regions of SCM3/OsTB1 in NPB using CRISPR-Cas9. We designed six single-guide RNAs (sgRNAs) four targeting the promoter sequence and two sgRNAs and 6 targeting the region proximal to the TGTG insertion in the 5'-flanking CDS. These six sgRNAs were integrated into one plasmid and transformed into NPB using Agrobacterium. We obtained 23 plants carrying 9 different mutations (Figure 1j). Relative to wild-type (WT) NPB Type 1 had more tillers and smaller culms and panicles; Type 2 had similar phenotypes to WT; Type 3 had fewer tillers and larger culms and panicles (Figure 1k). The Type 1 plants 67-1-1 63-1-6 71-3-2 and 66-2-3 were similar to the null mutant of OsTB1 fc1-2 (Minakuchi et al., 2010). Indeed 71-3-2 and 66-2-3 contained deletions in the 5'-noncoding region of OsTB1. OsSPL14/IPA1 positively regulates OsTB1 expression through binding to a GTAC motif (Lu et al., 2013) and this motif was deleted in these plants likely resulting in the null phenotypes.

The Type 3 plants 83-4-1 and 76-1-2 mimicked the phenotypes of NIL-qSCSA6 (Yano et al., 2015) and 93-11 suggesting that mutations in these plants enhance OsTB1 expression. Indeed these plants showed higher OsTB1 expression than the control whereas the Type 1 and Type 2 plants showed reduced and unchanged expression levels respectively (Figure 1j). 76-1-2 had a
TGTG insertion which also occurs in varieties with large SCSA (Figure 1i) confirming that the TGTG insertion enhances OsTB1 expression. Although 83-4-1 had mutations at six sites in the 5'-flanking CDS region it showed a gain-of-function phenotype (Figure 1k). Thus different sequence(s) in the 5'-flanking CDS region in WT may repress or enhance transcription. In 84-5-1 a 36-bp deletion around the TGTG insertion did not increase OsTB1 expression suggesting that the TGTG insertion did not disrupt a repressive site. Instead the TGTG insertion may create a binding site for transcription activator(s) hypotheses that will require further validation.

For Type 2 plants which showed similar phenotypes to the control plants (Figure 1k) OsTB1 expression did not differ from the control although they had various deletions and single-nucleotide polymorphisms upstream of the gene (Figure 1l). These results demonstrated that targeted editing of OsTB1 cis-regulatory elements could produce alleles having different expression levels and phenotypes (Figure 1m).

Figure 1 CRISPR-Cas9 alleles alter OsTB1 transcript levels and plant phenotypes. (a) Global features and cross-section of the 4th and 5th internodes (the 2nd and most basal internodes) of 93-11 (indica), NPB (japonica) and NIL-qSCSA3-1NPB. Bar: 20 cm. (b) QTLs for SCSA. (c) NIL-qSCSA3-1NPB carrying the NPB allele. (d) Three QTLs associated with SCSA. qSCSA1-1, qSCSA3-1 and qSCSA6-1 overlapped with SCM1/SD1, SCM3/OsTB1/FC1 and SCM2/APO1, respectively. (e) Mapping of qSCSA3-1. Its candidate region included OsTB1. (f) OsTB1 expression and agronomic traits in 93-11 (indica), NPB (japonica), and NIL-qSCSA3-1NPB. (g) Appearance of plants transformed with the empty vector (left) and its whole genome sequence of 93-11 (middle) or Kasalath (right). Bar: 20 cm. (h) OsTB1 expression and agronomic traits in the transgenic plants. (i) The 4th internode phenotype of NPB, 93-11, 93-11/NPB(F1), Kasalath, Zhongchao 123, fc1. Upper panel: 4th internode of NPB, 93-11, 93-11/NPB(F1), Kasalath, Zhongchao 123, fc1 (from left to right) Bar = 1 cm. Bottom panel: Cross-sections of the 4th internode NPB, 93-11, 93-11/NPB(F1), Kasalath, Zhongchao 123, fc1 (from left to right) Bar = 5 mm. (j) OsTB1 genomic region with DNA polymorphisms in four varieties with large SCSA, (9311, Kasalath, Zhongchao123, and Chugoku117), positions of sgRNAs and mutated sequences in the nine genome-edited plants. IPA1 target site (GTAC + 78) is indicated. (k) Phenotypes of the edited plants. NPB (leftmost) was used as a control. Top to bottom, plant appearance, cross-section of the 4th internode and panicle structure. Scale bars, 20 cm, 7 mm and 7 cm, respectively. (l) OsTB1 expression in edited plants. (m) Agronomic traits in these plants. Error bars represent mean ± SD (n = 3). The different letters indicate statistical differences by Duncan’s multiple range test (p < 0.05). All RNAs were isolated from 2-cm inflorescences.
genes associated with agronomic traits of interest may accelerate molecular design of desirable traits.

Acknowledgements

This research was financially supported by the Natural Science Foundation of China (Grant No: 31461143014).

Authors’ contributions

Xingming Hu, Qian Qian and Makoto Matsuoka conceived the project and designed the research strategies. Yongtao Cui, Xingming Hu, Lan Shen, Fanmiao Wang, Shuang Ruan and Anhui Feng cloned the CRISPR-Cas9 plasmid, identified mutants and performed qRT-PCR. Guohuang Liang, Bin Zhang, Yongjun Lin, Dongdong Chen, Li Zhu and Longbiao Guo performed the SASA QTL analysis and mapping. Qian Qian, Xingming Hu, Guojun Dong and Jiang Hu performed phenotype observations and genetic tests. Xingming Hu, Makoto Matsuoka and Qian Qian wrote the article.

Competing interests

The authors declare no competing interests.

References

Gao, Z.Y., Zhao, S.C., He, W.M., Guo, L.B., Peng, Y.L., Wang, J.J., Guo, X.S. et al. (2013) Dissecting yield-associated loci in super hybrid rice by resequencing recombinant inbred lines and improving parental genome sequences. Proc Natl Acad Sci USA 110, 14492–14497.

Hirano, K., Ordonio, R.L. and Matsuoka, M. (2017) Engineering the lodging resistance mechanism of post-Green Revolution rice to meet future demands. Proc Jpn Acad Ser B Phys Biol Sci 93, 220–233.

Huo, X., Wu, S., Zhu, Z., Liu, F., Fu, Y., Cai, H., Sun, X. et al. (2017) NOG1 increases grain production in rice. Nat Commun 8, 1497.

Lu, Z., Yu, H., Xiong, G., Wang, J., Jiao, Y., Liu, G., Jing, Y. et al. (2013) Genome-wide binding analysis of the transcription activator ideal plant architecture1 reveals a complex network regulating rice plant architecture. The Plant cell 25, 3743–3759.

Minakuchi, K., Kameoka, H., Yasuno, N., Urushihara, M., Luo, L., Kobayashi, K., Hanada, A. et al. (2010) FINE CULM1 (FC1) works downstream of strigolactones to inhibit the outgrowth of axillary buds in rice. Plant Cell Physiol 51, 1127–1135.

Mulani, I.W., Yamamoto, T., Ueda, T., Samadi, A.F., Kamahora, E., Runanti, I.A., Thanh, V.C. et al. (2018) Finding the superior allele of japonica-type for increasing stem lodging resistance in indica rice varieties using chromosome segment substitution lines. Rice 11, 25.

Rodriguez-Leal, D., Lemmon, Z.H., Man, J., Bartlett, M.E. and Lippman, Z.B. (2017) Engineering quantitative trait variation for crop improvement by genome editing. Cell 171, 470–480.e8.

Rothan, C., Diouf, I. and Causse, M. (2019) Trait discovery and editing in tomato. Plant J 97, 73–90.

Xu, J., Zhao, Q., Du, P., Xu, C., Wang, B., Peng, Q., Liu, Q. et al. (2010) Developing high throughput genotyped chromosome segment substitution lines based on population whole-genome re-sequencing in rice (Oryza sativa L.). BMC Genom 11, 656.

Yano, K., Okawa, T., Aya, K., Ochiai, Y., Hirasawa, T., Ebisui, T., Takarada, T. et al. (2015) Isolation of a novel lodging resistance QTL gene involved in strigolactone signaling and its pyramiding with a QTL gene involved in another mechanism. Mol Plant 8, 303–314.

Supporting information

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

Figure S1 Mapping of SCSA (Stem Cross-Section Area) QTLs CSSLs.

Figure S2 Identification of hygromycin in different edited plants.

Table S1 Primer sequences used in this study.

Data S1 Materials and Methods.