Brief Communication

Natural variation in OsGASR7 regulates grain length in rice

Zhengbin Tang1, Xiuying Gao1, Xiangyun Zhan1, Nengyan Fang1,2, Ruqin Wang1, Chengfang Zhan1, Jiachi Zhang1, Guang Cai1, Jiping Cheng1, Yongmei Bao1, Hongsheng Zhang1 and Ji Huang1,2

1State Key Laboratory of Crop Genetics and Germplasm Enhancement, College of Agriculture, Nanjing Agricultural University, Nanjing, China
2Institute of Crop Sciences, Fujian Academy of Agricultural Sciences, Fuzhou, China

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*Correspondence (Tel +86 25 84399532; fax +86 25 84396075; email huang@njau.edu.cn)

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Identification of quantitative trait loci (QTLs) for grain length (GL) is important to rice breeding for increasing grain yield and appearance quality. Recent studies have identified a number of QTLs genes as key grain length regulators by linkage mapping or genome-wide association study (GWAS). These regulators are involved in G protein signalling, phytohormone signalling or transcriptional regulation, etc (Li et al., 2018). However, our current knowledge on GL is still fragmented in molecular mechanism and breeding utilization in rice. Here, we detected QTLs for GL by GWAS using 210 rice accessions from rice diversity panel 1 (RDP1) (McCouch et al., 2016) and linkage mapping using a recombinant inbred line (RIL) population with 116 lines derived from geng/japonica rice Suyunuo (long grain) and Bodoa (short grain) (Figure 1a). Interestingly, one co-localized locus was identified and LOC_Os06g15620 encoding a gibberellin acid-stimulated regulator (GASR) protein included in this region was confirmed to control GL.

A GWAS performed for GL using the efficient mixed-model association eXpedited (EMMAX) algorithm approach identified an associated locus over the threshold on chromosome 6 (−log10(1/401 085) ≈ 5.6) (Figure 1b1). This locus was further narrowed down to a 62-Kb region by linkage mapping (Figure 1b2). According to the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/), 10 annotated genes were located within the above-described locus (Figure 1b3). Among them, a candidate gene OsGASR7 was also previously identified in a GWAS analysis for GL (Huang et al., 2012) and was found to be responsive to gibberellins and brassinosteroids in rice (Wang et al., 2009). Moreover, the wheat orthologous counterpart of OsGASR7, TaGASR7-A1, was found to affect GL in common wheat under multiple cultivation conditions (Dong et al., 2014), and its CRISPR/Cas9-induced aabbdd mutant significantly elevated thousand kernel weight (Zhang et al., 2016). Recently, OsGASR7/GW6 was found to regulate grain width and weight (Shi et al., 2020). Thus, OsGASR7 is likely a candidate for controlling GL. OsGASR7 contains a 437-bp open reading frame (ORF) with two exons and one intron, and the sequence comparison of exons between Bodoa and Suyunuo revealed three SNPs and one Indel (Suyunuo to Bodoa: SNP1, 58G → A; SNP2, 70G → T; SNP3, 196A → G; Indel1, +AGCAGC between 209C and 210G). These variations lead to three amino acid substitutions and additional two serines (Figure 1b4).

To confirm the effect of OsGASR7 on grain length, the functional complementation test of OsGASR7 was performed. We introduced the entire genomic region of OsGASR7 from Suyunuo (SYN) into Bodoa (BD) and Nipponbare (Nip) which carries OsGASR7RD, respectively, and generated two types of transgenic complemental rice, BD-pOsGASR7SYN::OsGASR7SYN (B_CP) and Nip-pOsGASR7SYN::OsGASR7SYN (N_CP). As expected, both BD-pOsGASR7SYN::OsGASR7SYN and Nip-pOsGASR7SYN::OsGASR7SYN exhibited increases in GL, confirming the role of OsGASR7 in controlling GL (Figure 1c1–c4). As grain elongation is related to cell division and/or cell expansion, the outer lemma surfaces of mature seeds were examined by scanning electron microscopy (SEM). The results showed that there was no significant difference in cell length between Bodoa and BD-pOsGASR7SYN::OsGASR7SYN (Figure 1c5, c6), indicating the involvement of OsGASR7 in cell division during spikelet head development to control GL.

In order to further explore the variation in OsGASR7 in controlling GL, we conducted OsGASR7-based candidate gene association analysis using 2004 accessions from RFGB (Wang et al., 2019) by general linear model (GLM) algorithm. The association analysis showed that the Indel1 of 6-bp insertion (AGCAGC) was the most significantly associated with GL (Figure 1d1). Interestingly, Indel1 locates in the variable region containing a polyglycine tract of OsGASR7, which is specific in cereal species including common wheat (Dong et al., 2014). It indicates that Indel1 is critical for grain length regulation, and we thus divided OsGASR7 into six major genotypes based on the variable region where Indel1 locates (OsGASR7RD, OsGASR7SYN, OsGASR7II, OsGASR7II, OsGASR7II and OsGASR7RE, respectively, and OsGASR7SYN has the strongest effect on GL no matter in xianindica (Xi), geng/japonica (GJ) or total population (Figure 1e1). Especially in GJ, the grains of rice with OsGASR7SYN are significantly longer than that without OsGASR7SYN (Figure 1e1), and this is consistent with the results of complementation test. Although containing the Indel1, rice accessions with OsGASR7RD and OsGASR7II exhibit short grains. However, these rare alleles also show variations in flanking sequence of Indel1 compared to OsGASR7SYN, indicating that the flanking sequence or the position of Indel1 may also affect the grain length regulation. To find out whether OsGASR7SYN has been utilized in
OsGASR7 regulates grain length in rice. (a) Grain morphology of Bodao (BD), Suyunuo (SYN) and some representative lines/accessions from RILs/RDP1. Scale bar, 5 mm. (b) Identification and positional cloning of OsGASR7 combining GWAS and linkage mapping. (b1) The genome-wide association signals for grain length are shown in the region at about 8.5–10.0 Mb on chromosome 6 (x-axis). Negative log10-transformed P-values from the EMMAX algorithm are plotted on the y-axis. The position of the peak SNP is indicated by the red dot. The horizontal red line indicates the threshold (–log10(0.05) = 5.6). (b2) The QTL for grain length is shown in the region at 8.848–8.91 Mb on chromosome 6 (x-axis). LOD values from ICIM-ADD algorithm are plotted on the y-axis. The horizontal red line is the LOD threshold (2.82). (b3) The physical position of the predicted ORFs (filled arrows). (b4) Non-synonymous variants of OsGASR7 between Bodao and Suyunuo. Black lines represent introns, and colour bars represent exons. (c) OsGASR7 controls grain length. (c1, c2) Grain morphology of wild-type Bodao (BD), Nipponbare (Nip) and transgenic complemental rice BD-pOsGASR7SYN::OsGASR7SYN (B_CP), Nip-pOsGASR7SYN::OsGASR7SYN (N_CP). Scale bar, 5 mm. (c3, c4) Statistical analysis of (brown) grain length of wild-type plants and transgenic plants. The dashed lines and error lines represent means ± SD (n ≥ 4 plants, shown by dots). (c5) SEM observation of mature seeds. Scale bar, 1 mm for seed and 100 μm for lemma. (c6) Statistical analysis of cell length of the glume outer surfaces. Data are presented as means ± SD (n = 9). The P-values are calculated by Student’s t-test. (d) InDel1 is significantly associated with grain length. (d1) The x-axis indicates position of each variation in the ORF of OsGASR7 and the y-axis is negative log10-transformed P-values from GLM algorithm. (d2) Six major genotypes of OsGASR7. (e) Genotype analysis of OsGASR7 for subpopulation distribution and grain length in 3K accessions (e1) and cultivars (e2). GI: geng/japonica; XI: xian/indica; CA: centrum-Aus; CB: centrum-Basmati; BD: OsGASR7BD; SYN: OsGASR7SYN; I: OsGASR7I; II: OsGASR7II; III: OsGASR7III; IV: OsGASR7IV; nonSYN: cultivars without OsGASR7SYN; number in brackets: number of statistical samples. Significance analysis is performed by Student’s t-test. (f) The subcellular localization of OsGASR7BD-GFP and OsGASR7SYN-GFP. Scale bar, 5 μm.
breeding, we analysed OsGASR7 sequences of 42 cultivars. Compared with the distribution of OsGASR7SYN in XI of 2004 accessions, that in XI cultivars has increased (57% versus 74%) (Figure 1e). What is more, the grains of cultivars with OsGASR7SYN are still longer than those without OsGASR7SYN (non-OsGASR7SYN) (Figure 1e). These results show that OsGASR7SYN has been likely selected in many XI cultivars.

At last, we examined the subcellular localization of OsGASR7-SYN and OsGASR7BD protein carrying green fluorescent protein (GFP) in rice protoplast expressing a nuclear marker OsD53-mCherry. Both fluorescence of OsGASR7SYN-GFP and OsGASR7BD-GFP were mainly observed in cytoplasm (Figure 1f). Through in silico gene expression analysis, it was found that OsGASR7 was regulated by brassinolide treatment and accumulated in panicles, suggesting that OsGASR7 may involve brassinosteroid (BR) pathway to regulate grain length in rice.

Functions of some GASR genes have been studied in rice. OsGASR1 controls seedling growth and amylase production (Lee et al., 2017), and OsGASR9 plays a positive role in the response to GA and grain development (Li et al., 2019). In this work, we confirmed that a GASR protein OsGASR7 is responsible for grain length regulation in rice by GWAS, linkage analysis and transgenic complementary study. More importantly, a natural variation of 6-bp insertion (AGCAGC) in OsGASR7 was found to be significantly associated with GL and could be potentially used as a molecular marker for rice breeding. The allele variations in different rice cultivars may lead to the functional diversity of OsGASR7 observed in the studies of GW6 (Shi et al., 2020) and this work. Further studies will be conducted to clarify how OsGASR7 modulates grain length and the role of additions of two serines in OsGASR7 function and regulation.

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Conflicts of interest

The authors declare no conflict of interest.

Author contributions

J.H. and H.Z. contributed to the original concept of the project. Z.T., N.F., R.W., C.Z. and J.Z. performed the experiments and analysed the data. X.Z. and G.C. were responsible for material planting in field. J.C., X.G. and Y.B. participated in the design and coordination of the study. Z.T. and J.H. wrote the manuscript. All authors read and approved the final version of the manuscript.

References

Dong, L.L., Wang, F.M., Liu, T., Dong, Z.Y., Li, A.L., Jing, R.L., Mao, L. et al. (2014) Natural variation of TaGASR7-A1 affects grain length in common wheat under multiple cultivation conditions. Mol. Breed. 34, 937–947.

Huang, X.H., Zhao, Y., Wei, X.H., Li, C.Y., Wang, A., Zhao, Q., Li, W.J. et al. (2012) Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. Nature Genet. 44, 32–39.

Lee, S.C., Kim, S.J., Han, S.K., An, G. and Kim, S.R. (2017) A gibberellin-stimulated transcript, OsGASR7, controls seedling growth and alpha-amylase expression in rice. J. Plant Physiol. 214, 116–122.

Li, N., Xu, R., Duan, P. and Li, Y. (2018) Control of grain size in rice. Plant Reprod. 31, 237–251.

Li, S., Tao, Q., Tao, Y., Mao, J., Peng, X., Li, C., Yang, Z. et al. (2019) OsGASR9 positively regulates grain size and yield in rice (Oryza sativa). Plant Sci. 286, 17–27.

McCouch, S.R., Wright, M.H., Tung, C.-W., Maron, L.G., McNally, K.L., Fitzgerald, M., Singh, N. et al. (2016) Coriendum: Open access resources for genome-wide association mapping in rice. Nat. Commun. 7, 11346.

Shi, C.L., Dong, N.Q., Guo, T., Ye, W.W., Shan, J.X. and Lin, H.X. (2020) A quantitative trait locus GW6 controls rice grain size and yield through the gibberellin pathway. Plant J. https://doi.org/10.1111/tpj.14793.

Wang, L., Wang, Z., Xu, Y.Y., Joo, S.H., Kim, S.K., Xue, Z., Xu, Z.H. et al. (2009) OsGASR7 is involved in crosstalk between gibberellins and brassinosteroids in rice. Plant J. 57, 498–510.

Wang, C.C., Yu, H., Huang, J., Wang, W.S., Faruque, M., Zhang, F., Zhao, X.Q. et al. (2019) Towards a deeper haplotype mining of complex traits in rice with RFGB v2.0. Plant Biotechnol. J. 18, 14–16.

Zhang, Y., Liang, Z., Zong, Y., Wang, Y.P., Liu, J.X., Chen, K.L., Qiu, J.L. et al. (2016) Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. Nat. Commun. 7, 12617.