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Creating Novel Wx Alleles with Fine-tuned Amylose Levels and Improved Grain Quality in Rice by Promoter Editing using CRISPR/Cas9 System

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Abstract

In rice, the *Waxy* (*Wx*) gene encoding GBSSI (granule-bound starch synthase I) controls amylose synthesis in the endosperm and is the primary factor influencing grain eating and cooking quality (ECQ). The natural allelic variations within the *Wx* locus cause the broad diversity of amylose content (AC) and ECQ in modern rice. In this study, we report to create novel *Wx* alleles with the aim to fine-tune its expression and grain AC, thus catering for the unique preferences of populations from different cultures. Here, the core region of *Wx* promoter was selected for genome editing and six novel transgene-free *Wx* alleles were generated. The *Wx* expression was down-regulated and AC was fine-tuned in the rice. Among the novel *Wx* alleles, *Wx^{b-d8}*, with a moderate and stable decrease in rice AC under various temperature conditions, has a potential application in rice breeding. While other alleles, whose performances depended on temperature conditions, could also be applied in the improvement of rice quality based on local temperatures and consumer preferences. Therefore, this study provides new germplasm resources for rice breeding and efficient method for creating more novel *Wx* alleles with a wider range of *Wx* expression and grain AC. Moreover, it also demonstrated that targeted modification of gene core promoter is a universal and reliable approach for the fine-tuning of the expression of target genes.

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