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Redesign of starch biosynthetic pathway in rice by CRISPR/Cas9-mediated genome editing toward human diets

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CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated Cas9 endonuclease)-mediated genome editing has revolutionized biological research and crop improvement because of its specificity, simplicity, and versatility. Editing a gene by CRISPR/Cas9 only has three requirements: (1) expression of the nuclear localized Cas9 protein; (2) production of a guide RNA (gRNA) molecule, whose first 20 nucleotides are complementary to the target gene; (3) the NGG PAM site that is located immediately adjacent to the 30 end of the target sequence. The majority of the reported CRISPR/Cas9-mediated gene editing in plants belongs to this category. CRISPR/Cas9-mediated gene editing technology has the potential to greatly facilitate plant breeding. However, so far only a very few examples of improvement of agronomic important traits and creation of novel germplasm in crop plants have been reported. Here, we succeeded in constructing individual target gene editing objects for 22 genes related to starch biosynthesis in rice via CRISPR/Cas9. A total of 1685 T0 plants (60 sgRNAs per each) were analyzed by NGS for genetic modification, resulting in a mutation in the target gene of 965 individuals. From these mutants, T1 seeds were grown through single copy and homo-, hetero-, di-allelic studies and used for selection null plants. We defined the roles of some genes example SBEI and SBEIIb etc, in determining the amylose content, fine structure of amylopectin, and physiochemical properties of starch. This work enables the improvement of nutritional properties of starch in rice grain, thus potentially providing health benefits to many people.

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