Gene Editing via CRISPR-Cas9 System for Prolamin Suppression in Rice

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Rice prolamins, which account for 20-30% of seed storage proteins (SSP), are encoded by 34 genes and are classified into 10, 13, and 16-kDa prolamins depending on their relative molecular weights. The 13-kDa prolamins are sub-grouped into pro13a and pro13b according to the number of cysteines. Prolamins have been reported to be indigestible, resulting in decreasing its nutritional value. To improve the nutritional quality, prolamin levels were reduced in seed storage proteins. Herein, to investigate the phenotypic and molecular features of prolamin deficiency transgenic rice plants, the plants suppressing multiple prolamins were generated using CRISPR/Cas9. In this study, we designed two sgRNAs targeting 13kDa prolamin genes, including sgRNA-pro13aI (targeting pro13a1 & a2 gene); sgRNA-pro13b (targeting pro13b1, b2, b3 & b8 gene) and generated T-DNA-free homozygous editing lines with reduced prolamin content compared to the wild-type. The results revealed that 13kDa prolamins were markedly suppressed but other SSPs such as 10kDa prolamin, and Glutelin B were increased at the transcription and translation levels in the seeds of the transgenic lines compared to those of the wild-type seeds. Further, the prolamin mutants exhibit a chalky grain owing to loosely packed, small, spherical starch granules in the ventral region of the endosperm. In conclusion, our results not only reveal the crucial role of 13kDa prolamin in regulating rice grain quality but also highlight the application potentials of the CRISPR/Cas9 system in improving rice grain quality by molecular breeding.

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