

Figure S1. Schematic diagram for designing sgRNAs

Figure S2. Cas9 cleavage assay for checking sgRNA efficiency *in vitro*. Digesting templates were genomic DNA amplified using the specific primers for each prolamin gene subgroups (Pro13a-I and Pro13b-I/II) and the testing sgRNAs were transcribed *in vitro* (using the Guide-it Complete sgRNA Screening System). Symbol (+) and (-) indicate the presence or absence of sgRNA-Cas9 complex, respectively. S/M: size marker. Fragments of cleaved-Pro13a-I are 210bp and 190bp. Fragments of cleaved Pro13b-I/II are 250bp and 180bp.

Figure S3. Construction of a CRISPR-Cas9 binary vector for editing rice 13 kDa prolamin genes.

Figure S4. Genotype of 13 kDa prolamin-knockout lines in T₀ generation. (A) Sequence analysis of target genes in T₀ plants showing various indel mutations. Sequences of sgRNAs were marked with underlines, PAM sequence was marked in boxes; dash lines and bold letters represent deletions and insertions of nucleotides, respectively; and “*” represents nucleotides identical to those in the original WT sequences. (B) Transgenic assay. The gel image represents PCR amplification of 1839-bp *SpCas9* gene and a 615-bp *HygR* gene in the putative T₀ transgenic plants. S/M: size marker.

Figure S5. Transgenic assay of T₁ plants. The gel image represents PCR amplification of 1839-bp *SpCas9* gene and a 615-bp *HygR* gene in the putative T₁ transgenic plants. S/M: size marker

Figure S6. Regression plot showing strong positive linear relationship between RNA seq data and qRT-PCR data with an R-square > 0.8. The qRT-PCR data is normalized using 2^{-ΔΔCT} method and the RNA seq transcript is presented as log₂[foldchange] data. The plot was visualized using SRplot “Pearson spearman scatte” (https://www.bioinformatics.com.cn/plot_basic_pcc_scatter_plot_049_en).

Figure S7. Expression level of SSP genes in 13 kDa prolamin-knockout lines. (A) Heatmap displays the expression profiles of the SSP genes from RNAseq analysis. Normalized expression of each gene is represented by Z-score. (B) qRT-PCR analysis of SSP genes in 13 kDa prolamin-knockout lines. Transcripts encoding different SSPs were analyzed in the immature seeds of WT and mutant lines. Normalized expression of the target genes was calculated using the 2^{-ΔΔCT} method and is represented as log₂ value. Error bars denote ± SD of three replicates. P values were calculated using the Student’s t-test (* p < 0.1, ** p < 0.01 and *** p < 0.001).

Figure S8. Sequence alignment of all the 13 kDa prolamins genes.