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Genome Editing for Rapeseed Genetic Improvement

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Pod shattering resistance is a key agronomical trait for facilitating the development of rapeseed mechanization. To identify rapeseed pod shattering resistance germplasm resources, over 200 rapeseed accessions, originated from China, Europe, Australia, Oceania, North America and India, were collected in our lab to identify the resistance level. However, only 3 rapeseed accessions exhibit pod shattering resistance. Based on genome wide association study (GWAS) coupled with fine mapping of key quantitative trait loci (QTL), a candidate gene (qSRI.A9.2) was identified. Sequence analysis results demonstrated that a 1 bp deletion and 629 bp insertion were occurred in the second extron of (Resistance material 1) R1 qSRI.A9.2 gene compared with wild type sequence. Overexpression of the wild type qSRI.A9.2 gene in R1 causes highly prone to pod shattering, which suggested that qSRI.A9.2 may acts as negative regulator in controlling rapeseed pod shattering. In order to further investigate the role of qSRI.A9.2 in rapeseed pod shattering, a high efficient CRISPR/Cas9 genome editing platform was established for specific targeting the genome region of qSRI.A9.2 gene. Mutation identification data suggested that all deigned qSRI.A9.2 genome sites can be effective mutated by this set of CRISPR/Cas9 system, which the editing frequency of CRISPR/Cas9-induced mutagenesis ranged from 96.8 to 100.0%. To further broaden the feasibility of genome editing system in rapeseed breeding application, zhongshuang 11 variety, an widely used elite breeding material for cultivating hybrid variety in China, was selected for testing the efficacy of CRISPR/Cas9 genome editing system. Our result indicated that the mutation efficiency is around 20% in zhongshuang 11 variety. Give that pod shattering resistance materials are very limited in Brassica napus natural population, several orthologs of pod shattering related genes in Arabidopsis (Arabidopsis thaliana) were selected for CRISPR/Cas9-targeted mutagenesis in rapeseed, including BnSHP1, BnSHP2, BnALC, BnIND et al. Based on PAGE-mediated mutation screening, many mutated single and multiple homoeologous gene copies lines were identified and exhibited different roles in regulating rapeseed pod shattering resistance, which will promote mechanism dissection underlying rapeseed pod shattering resistance and the later stage applications.