Targeted genome editing in crop plants using a CRISPR/Cas system

Recent advances in genome engineering provide plant biologists with an important tool for understanding gene function and developing new traits. Although genome editing technologies using ZFNs (zinc finger nucleases), TALENs (transcription activator-like effector nucleases) and engineered homing endonucleases can generate genome modifications, new technologies that are robust, affordable and easy to engineer are needed. Recent studies in adaptive immune system involving type II prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR) provide an alternative genome editing strategy. The CRISPR/Cas system has been used as an RNA-guided endonuclease to perform sequence-specific genome editing in bacteria, human cells, zebrafish and mice. Funded by the NSFC, MOA and CAS, Prof. Gao Caixia and her group at the Institute of Genetics and Developmental Biology, CAS, have demonstrated that the CRISPR/Cas system can be used for rice and wheat genome modification. The work has recently been published in *Nature Biotechnology* (**2013**, 31(8): 686—688).

The gRNA guides Cas9 to recognize and cleave target DNA. Cas9 has HNH and RuvC-like domains; each cleaves one strand of a double-stranded DNA (Figure **A**). To disrupt endogenous genes in rice and wheat, four rice genes and one wheat gene, including *OsPDS*, *OsBADH2*, *Os02g23823*, *OsMPK2* and *TaMLO* were designed. Frequencies of sgRNA:Cas9 induced mutations were 14.5% to 38.0% in protoplasts (Figure **B**), and 4.0% to 9.4% in transgenic plants (Figure **C**). Biallelic mutants of *pds* were obtained in T0 generation with efficiency about 3.1%, and all the biallelic mutants show albino and dwarf phenotype (Figure **D**). Furthermore, homology-directed targeted genome modification in rice using single-stranded DNA (ssDNA) oligonucleotide as a donor template was demonstrated. The studies prove the versatility of the CRISPR/Cas system to modify the genomes of crop plants and establish a new strategy for plant genome modification.

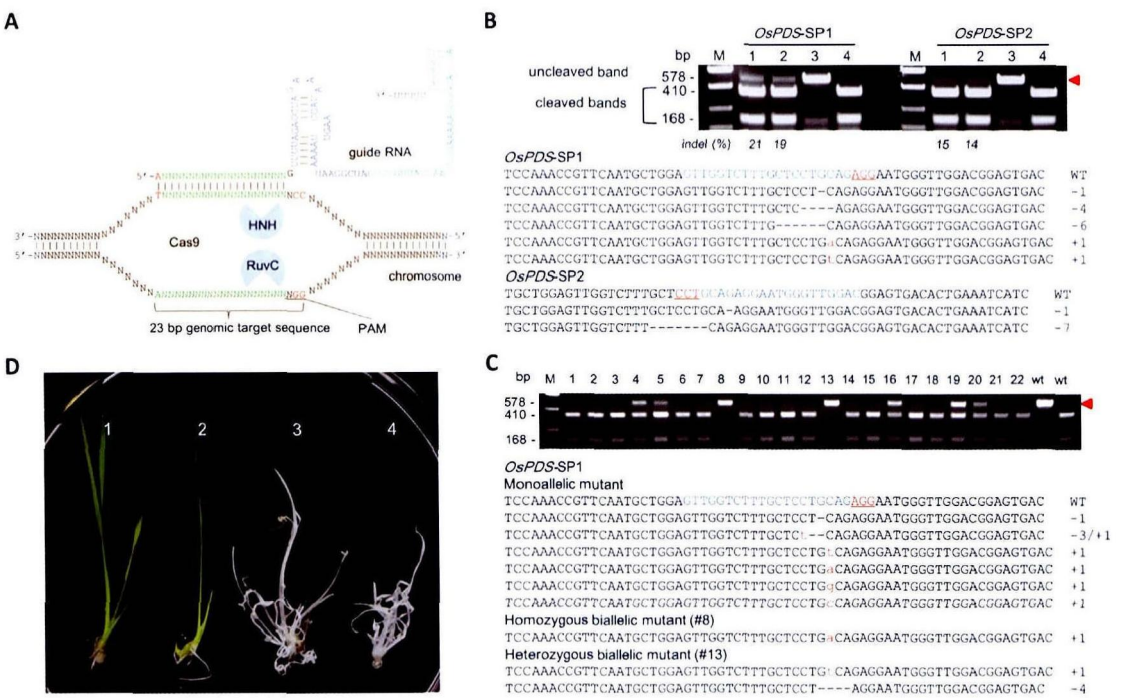

Figure **A** Schematic illustrating the engineered type II CRISPR/Cas system. **B** PCR/RE assay to detect gRNA:Cas9-induced mutations in protoplasts. **C** gRNA:Cas9-induced mutations in transgenic rice plants. **D** Phenotypes of the *pds* mutants.