Gene Editing in Alfalfa

Zeng-Yu Wang, Qingdao Agricultural University - Qingdao, China

Zeng-Yu Wang received his Ph.D from China Agricultural University in 1990. He completed a five-year postdoctoral appointment at Swiss Federal Institute of Technology, Zurich, Switzerland, in 1995, and then moved to Australia and served as a research scientist for three years at Agriculture Victoria, Melbourne. He joined the Noble Foundation in Oklahoma USA in 1998 and rose through the ranks from Assistant Professor to Full Professor. He retired from Noble and then joined Qingdao Agricultural University in 2019. He is currently Professor and Dean of the College of Grassland Science of Qingdao Agricultural University.

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9) system has become the most preferred genome editing tool. Alfalfa (*Medicago sativa*) is an outcrossing tetraploid legume species; the existence of gene duplication creates difficulties in the mutagenesis process, and the outcrossing nature makes it complicated to generate homozygous mutants. Our initial single gRNA-CRISPR/Cas9 system had very low mutagenesis efficiency in alfalfa with no mutant phenotype. In order to develop an optimized genome editing system in alfalfa, we constructed multiplex gRNA-CRISPR/Cas9 vectors by a polycistronic tRNA-gRNA approach targeting the stay-green gene (MsSGR) gene. The replacement of CaMV35S promoter by the Arabidopsis ubiquitin promoter (AtUBQ10) to drive Cas9 expression in the multiplex gRNA system led to a significant improvement in genome editing efficiency, whereas modification of the gRNA scaffold resulted in lower editing efficiency. The most effective multiplex system exhibited 75% genotypic mutagenesis efficiency, which is 30fold more efficient than the single gRNA vector. Importantly, phenotypic change was easily observed in the mutants, and the phenotypic mutation efficiency reached 68%. This highly efficient multiplex gRNA-CRISPR/Cas9 genome editing system allowed the generation of homozygous mutants with a complete knockout of the four allelic copies in the T0 generation. This optimized system offers an effective way of testing gene functions and overcomes a major barrier in the utilization of genome editing for alfalfa improvement.