RNA-Guided Genome Editing in Arabidopsis thaliana Using CRISPR/CAS9

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The world today faces an increasing shortage of food production due to the exponential growth in the human population. The root of this predicament lies in limitations to further improve capacities of food production in conjunction with the deficiencies in resources and water. Previous attempts include chemical mutagenesis; which causes random genetic mutations, and Zinc Fingers Nucleases (ZFNs). However, previous testing concluded that ZFNs hold a substantial probability for errors in off-target activities and binding locations. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas9) systems in bacteria and Archaea use RNA-guided nuclease activity to provide adaptive immunity against invading phages and plasmids. In this novel project, CRISPR/CAS9 is modified to contain the Cas9 endonuclease and the targeting sequence containing the gene of interest, with the gRNA sequence downstream of it. The edited system's specificity and efficacy are tested by the modification of the Arabidopsis thaliana plant with the insertion of the Multiple Antibiotic Resistance (MAR1) gene. The recombinant plasmids engineered have been successfully transfected into the genome of approximately 20% of the Arabidopsis thaliana seeds sampled. 99.2% of the third generation (T3) seeds contained the constructed recombinant plasmid and displayed the genotype and phenotype of the desired genetic modification. The genomic modification of the plant samples was confirmed molecularly by screening for every component of the CRISPR/CAS9 system. Targeted gene editing this way on a wider genetic scale to adapt plants for cultivation in extreme environments is ultimately a powerful strategy for editing and revolutionizing plant science.

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