Engineering the Rice Genome via CRISPR/Cas9 to Achieve Herbicide Resistance

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Rice is a major food source worldwide with 3.5 billion people depending on it. Rice yield loss is up to 47% annually because of the use of herbicides. To address this problem, CRISPR/Cas9 systems were utilized to develop genetically modified rice. CRISPR/Cas9 was used to fuse with activation-induced cytidine deaminase (AID), and hyperactive AID (AID*) for point mutagenesis at specific genomic regions guided by single-guide RNA (sgRNA). The chimeric single-guide RNA (sgRNA) molecules will carry information to the targeted site; acetolactate synthase (ALS). ALS is inhibited by urea herbicides causing that plant to die. Genetic modification is an effective method in rice protoplasts. sgRNA molecules were utilized in this study to achieve herbicide resistance in rice. After being expressed in the rice expression vectors, both dCas9-AID and dCas9-AID* successfully edited the rice genome at the ALS gene. As was predicted, the dCas9-AID* was able to cover more of the genome and editing was distant relative to the PAM position in the protoplasts unlike dCas9-AID. With modifying the ALS gene and using specific plasmids, transformed rice calluses were able to grow and produce a new line of crops that obtained the new trait of herbicide resistance. There were a total of 60 rice seedlings tested, 45 transfected with the new gene and 15 controls, with bispyribac sodium at 0.5μM. The entire control group didn’t survive, while 9 out of the 45 (20%) transfected rice seeds survived and are being used to plant the next generation.