Efficient Viral-Mediated Genome Editing Technique In Tobacco noctiana and Arabidopsis thaliana Model Plants 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 and deficiencies in resources. Updated genetic engineering has been utilized to modify the DNA of various plants as one method to improve crop yields better than the previous genetic engineering methods such as ZFN and TALEN. This project aims to genetically modify crops in a shorter time without producing harmful side effects. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and associated (Cas9) systems in bacteria and Archaea use RNA guided nuclease activity to provide adaptive immunity against invading phages and plasmids. Cas9 endonuclease was over expressed using agrobacterium in in a total of 450 plants; 225 Tobacco noctiana and 225 Arabidopsis thaliana. Western blot was used to determine the existence of the Cas9 endonuclease protein inside the two model plants and Real Time Polymerase Chain Reaction to detect the presence of the Cas9’s DNA in the plant’s chromosomes. A novel technique using Tobacco Rattle Virus (TRV) was used to spread the CRISPR gRNA, based on the PDS gene that is responsible for the chlorophyll, to the overly expressed Cas9 model plants by Agro-infiltration. The model plants started bleaching in a period of 7 – 9 days after the elimination of TRV. They were successfully mutated with at least one base pair in approximately 93.33% of the samples. Targeted gene editing on a wider genetic scale to adapt plants for cultivation in extreme environments is ultimately a powerful strategy for revolutionizing plant science.

Awards Won:
First Award of $5,000
Intel ISEF Best of Category Award of $5,000