



# CALIFORNIA SCIENCE & ENGINEERING FAIR 2019 PROJECT SUMMARY

<b>Name(s)</b>  <b>Alina Pollner</b>	<b>Project Number</b>  <b>S1810</b>
<b>Project Title</b>  <b>Bioengineering Plant Genomes to Increase Crop Production via CRISPR-Cas9</b>	
<p style="text-align: center;"><b>Abstract</b></p> <p><b>Objectives</b> Since plants are the major source of food for our planet, a comprehensive understanding of factors affecting their growth and development is critical. This project employed classical molecular approaches together with new genome editing tools based on CRISPR-Cas9 technologies to investigate the role of a subset of transcriptional regulatory genes, Mini Zinc Fingers (MZF), in the model system <i>Arabidopsis thaliana</i>. In the hope of finding keys to increased fruit production, MZF1 and MZF2 genes were targeted using a CRISPR-Cas9 strategy to generate loss-of-function mutants, and demonstrate the first steps of a Super-Mendelian inheritance model that will allow efficient propagation of beneficial mutations.</p> <p><b>Methods</b> Bacterial transformations through <i>Escherichia coli</i> and <i>Agrobacterium tumefaciens</i> allowed the creation of two gRNA CRISPR-Cas9 constructs, which targeted MZF1 and, separately, MZF2. These constructs were then inserted into the <i>A. thaliana</i> genome, plants were grown, and transgenics identified. The mutant plants were genotyped and phenotypic observations were drawn.</p> <p><b>Results</b> Plants with knocked-out MZF1 expression alone produced an average of 87% more fruit than wild type and had 78% longer stem length (<math>p &lt; 0.001</math> for both). MZF2 knockout plants did not have statistically significantly more fruit per stem or longer stem length. A double knockout of MZF1 and MZF2 produced plants with an average 294% more fruit compared to wild type (<math>p &lt; 0.001</math>). The single knockouts are the first steps in a new Super-Mendelian inheritance model mediated by Cas9, ensuring that when engineered plants breed with wild type plants, both copies of mutant alleles are inherited by future progeny.</p> <p><b>Conclusions</b> Plants with both MZF1 and MZF2 knocked out produce the greatest increase in fruit production as compared to wild type. Results also confirm that MZF1 plays a greater role than MZF2 in the inhibition of Zinc Finger transcription factors. This project provides the framework for an implementation of a Super-Mendelian inheritance model, ensuring propagation of the beneficial mutation, thereby potentially allowing increased crop production in the field. The use of these beneficial mutations in the agricultural field could potentially have a global impact on increasing overall food production.</p>	
<b>Summary Statement</b>  Editing the genome of <i>A. thaliana</i> via CRISPR-Cas9 led to a significant increase in fruit production with potential applications toward solving world hunger.	
<b>Help Received</b>  I am very grateful to the Yanofsky Lab at UCSD under the mentorship of Prof. Yanofsky and Dr. Juan-Jose Ripoll. All work shown was done by the student (designing of gRNAs, PCRs, bacterial transformation, phenotypic conclusions, etc.).	