Name(s)
Alina V. Pollner

Project Number
S1915

Project Title
Novel Strategy to Increase Fruit Production via CRISPR-Cas9 Genome Editing

Abstract

Objectives/Goals
Towards enhancing fruit production, the purpose of this experiment was to eliminate Mini Zinc Fingers 1 and 2 (MZF1/2) from Arabidopsis thaliana using the genome editing method CRISPR-Cas9. An additional purpose of this experiment was to locate the expression of MZF1 by discovering where its promoter is active.

Methods/Materials
The pJJJ2 plasmid was created to be a T-DNA vector that allows plant transformation. This vector contained a multitude of important regions, including antibiotic resistance genes, a UBQ Constitutive Promoter, Cas9, and guide RNAs. These plasmids were then transformed into multiple bacterias and ultimately transferred into the wild type model organism Arabidopsis thaliana. Later, seeds were harvested and grown. Three plants that had received the plasmid were transplanted into soil until samples were taken for genotyping.

MZF1 promoter was fused to the GUS gene reporter present in the pJJGUS T-DNA binary vector. The promoter of MZF1 was amplified by PCR and cloned into pJJGUS and transformed into plants. Transgenic plants were selected for on Hygromycin MS plates, and plants were grown for six to eight weeks until samples were taken.

Results
The gene expression studies indicated that MZF genes are active in fruit, primarily in early growth stages. The CRISPR-Cas9 mutant had an altered genome, with two early stop codons produced, due to a G insertion in MZF1 and a two nucleotide deletion in MZF2. Interestingly, this led to a chimera stem that had a 333+ % increase in fruit, a novel result that is a positive indication of future value of this work.

Conclusions/Discussion
This study uncovered a previously poorly-understood role for MZF genes as crucial components for regulating and modulating fruit development and growth. These CRISPR-Cas9 mutants could therefore produce significantly more fruit compared to wild types, and could also increase food production when applied to other organisms (such as wheat, tomatoes, etc.). This new gene editing strategy for plants is not limited to MZF genes but also applicable to investigate functions of other genes critical for plant growth, differentiation and additional development programs.

Summary Statement
Through CRISPR-Cas9 genome editing, this project demonstrated a 333% increase in fruit production, in addition to elucidating the expression pattern of Mini Zinc Finger 1.

Help Received
All work shown in this project was completed entirely by the student. I received guidance with the design of the guide RNAs. I worked at the Yanofsky Lab at the University of California at San Diego with Dr. Juan-Jose Ripoll and Prof. Martin Yanofsky.