CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

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Project Number

S1610

Project Title

Quantifying CRISPR-Cas9 as a Method for Preventing Geminivirus Replication through Virus Replication Site Mutation

Abstract

Objectives/Goals

This experiment aims to assess the feasibility of using the CRISPR-Cas9 system to generate Geminivirus resistance in plants by targeting a site in the viral genome that is essential for viral rolling circle replication. If the viral replication is inhibited, the virus will be unable to spread throughout the plant, rendering the plant free of disease.

Methods/Materials

Agrobacterium cells were transformed with plasmids containing the gRNA and the virus. Nicotiana benthamiana plants were grown and agroinfiltrated with a derivative of the Bean Yellow Dwarf Virus and a CRISPR-Cas9 cassette targeting the Ori of said virus. DNA was extracted from plant tissue samples after agroinfiltration of both the cassette and the virus using Qiagen's Plant DNeasy Mini Kit. The efficiency at which replication was inhibited was qualified by quantitative, real-time PCR. For qPCR, a ten-fold dilution series was permformed to determine the needed concentration for flourescent detection. A ribosomal DNA standard was used for comparison with the samples.

Results

I did not find any significant difference between the viral DNA levels in between the control and gRNA containing agroinfiltrated samples in the two and three days post infiltration samples. I currently believe that the reason CRISPR-Cas9 did not work is due to too little time between agroinfiltration and DNA extraction. More samples are being run as of now with hopes that viral titer is inhibited.

Conclusions/Discussion

Repeated qPCR runs of the two and three days post infiltration samples show no statistical difference between the viral titer with presence of the gRNA and without the presence of the gRNA. As of now, it is concluded that CRISPR-Cas9 was not able to effectively cleave the virus replication site.

Summary Statement

I tested the efficiency of CRISPR-Cas9 to attenuate the spread of Geminiviruses through targeted mutation of the viral origin of replication.

Help Received

Dr. Steve Jacobsen provided access to lab facilities. Dr. Basudev Ghoshal taught me certain protocols such as Agroinfiltration, DNA extraction, and qPCR operation. Dr. Bob Sandrock assisted me with the technical aspects of the project, including the design of the gRNA.