CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL PROGRESS REPORT FORM: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

▶ SELECT PERIOD October 2020

● Quarterly Report ○ Final

Proposal Title

Optimization of the CRISPR technology for citrus genome editing

Today's Date: 11/03/2020

Sponsoring Organization (drop-down): Citrus Research and Development Foundation

Category (drop down): Plant Improvement

ABSTRACT (750-word Executive Summary-public report-do not disclose proprietary information or intellectual property)

The purpose of this project is to optimize the CRISPR technology for citrus genome editing. This study is related to the CRDF RMC-18 Research Priorities 4AB.

Objective 1. Expanding the toolbox of citrus genome editing. In this study, we will adapt StCas9, NmCas9, AsCpf1 (from Acidaminococcus), FnCpf1 (from *Francisella novicida*) and LbCpf1 (from Lachnospiraceae) on genome modification of citrus. Lately, we have shown CRISPR-Cpf1 can be readily used as a powerful tool for citrus genome editing.

In our recent study, we employed CRISPR-LbCas12a (LbCpf1), which is derived from Lachnospiraceae bacterium ND2006, to edit a citrus genome for the first time. Our study showed that CRISPR-LbCas12a can readily be used as a powerful tool for citrus genome editing. One manuscript entitled CRISPR-LbCas12a-mediated modification of citrus has been published on Plant Biotechnol J. We are currently further optimizing LbCas12a-crRNA-mediated genome editing to make homologous biallelic mutations.

We are also testing AsCpf1 and FnCpf1 for their application in citrus genome editing and generating homologous biallelic mutations. We have successfully generated both homozygous and biallelic mutations in the EBE region of LOB1 gene in pumlo. This work has been submitted for publication. We are in the process of generating homozygous and biallelic lines of other citrus varieties.

Recently, we have developed multiplex genome editing toolkits for citrus including a PEG mediated protoplast transformation, a GFP reporter system that allows rapid assessment of the CRISPR constructs, citrus U6 promoters with improved efficacy, tRNA-mediated or Csy4-mediated multiplex genome editing. Using the toolkits, we have successfully conducted genome modification of embryogenic protoplast cells and epicotyl tissues. We have achieved a biallelic mutation rate of 44.4% and a homozygous mutation rate of 11.1%, indicating that the CRISPR-mediated citrus genome editing technology is mature and could be implemented in citrus genetic improvement as a viable approach. In addition, our study lay the foundation for non-transgenic genome editing of citrus. One manuscript entitled Development of multiplex genome editing toolkits for citrus with high efficacy in biallelic and homozygous mutations has been published on Plant Molecular Biology.

We have successfully developed base editing tools for citrus genome editing.

Objective 2. Optimization of the CRISPR-Cas mediated genome editing of citrus. In this study, we are testing different promoters including INCURVATA2 promoter, the cell division-specific YAO promoter, and the germ-line-specific SPOROCYTELESS promoter, and ubiquitin promoter in driving the expression of Cas9 and Cpf1 orthologs.

To optimize the expression of sgRNA and crRNA, we have identified multiple citrus U6 promoters and two of

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% Completion of Objectives 60.00%

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