CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

SELECT PERIOD March 2016

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Control citrus canker by manipulating the EBE (effector binding element) of CsLOB1 which is the citrus susceptibility gene for citrus canker disease

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
4/12/2016	Citrus Research and Development Foundation	Transgenic/Metagenomic Mediation of Resistance

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

The goal of this project is to find non-copper treatment options to control citrus canker, caused by Xanthomonas citri ssp. citri (Xcc). The hypothesis of the proposed research is that we can control citrus canker by manipulating the effector binding element (EBE) of citrus susceptibility gene CsLOB1, which is indispensable for citrus canker development upon Xcc infection. We have previously identified that CsLOB1 is the citrus susceptibility gene to Xcc. The dominant pathogenicity gene pthA4 of Xcc encodes a transcription activator-like (TAL) effector which recognizes the EBE in the promoter of CsLOB1 gene, induces gene expression of CsLOB1 and causes citrus canker symptoms.

To test whether we can successfully modify the EBE in the promoter region of CsLOB1 gene, we first used Xcc-facilitated agroinfiltration to modify the PthA4-binding site in CsLOB1 promoter via Cas9/sgRNA system. Positive results have been obtained from the Cas9/sgRNA construct, which was introduced into Duncan grapefruit. We analyzed the Cas9/sgRNA-transformed Duncan grapefruit. The PthA4-binding site in CsLOB1 promoter was modified as expected. Currently we are using both Cas9/sgRNA and TALEN methods to modify EBE in sweet orange using transgenic approach. Transgenic Duncan and Valencia transformed by Cas9/sgRNA has been established. Totally four transgenic Duncan grapefruit lines have been acquired and confirmed. Mutation rate for the type I CsLOB1 promoter is up to 82%. GUS reporter assay indicated mutation of the EBE of type I CsLOB1 promoter reduces its induction by Xac. The transgenic lines are being grafted to be used for test against citrus canker.

In the presence of wild type Xcc, transgenic Duncan grapefruit developed canker symptoms 5 days post inoculation similarly as wild type. An artificially designed dTALE dCsLOB1.3, which specifically recognizes Type I CsLOBP, but not mutated Type I CsLOBP and Type II CsLOBP, was developed to evaluate whether canker symptoms, elicited by XccΔpthA4:dCsLOB1.3, could be alleviated on Duncan transformants. Both #D18 and #D22 could resist against XccΔpthA4:dCsLOB1.3, but not wild type Xcc. Our data suggest that activation of a single allele of susceptibility gene CsLOB1 by Xcc-derived PthA4 is enough to induce citrus canker disease and mutation of both alleles of CsLOB1, given that they could not be recognized by PthA4, is required to generate citrus canker resistant plants. The data has been published by Plant Biotechnology Journal

Transgenic Valencia transformed by Cas9/sgRNA has been established in our lab. Three transformants have been verified by PCR. The PthA4-binding site in CsLOB1 promoter was modified as expected, only one transgenic line seems to be bi-allelic mutant. The EBE modifed transgenic line is being evaluated for resistance against Xac.

One Cas9/sgRNA binary vector, which is designed to target CsLOB1 open reading frame, designated as GFP-Cas9/sgRNA:cslob1, was used to transform Duncan grapefruit epicotyls by Agrobacterium-mediated method. Several transgenic citrus lines were created, verified by PCR analysis and GFP detection. Cas9/sgRNA:cslob1-directed modification was verified on the targeted site, based on the direct sequencing of PCR products and the chromatograms of individual colony. Upon Xcc infection, some transgenic lines showed delayed canker symptom development. We are currently analyzing the genome modified plants using transgenic approaches including off-targets.

To generate non-transgenic DNA free canker resistant citrus, Cas9 containing nucleus localization signal was overexpressed and purified. The purified Cas9 showed activity in cutting target sequence and will be used to generate canker resistant plants.

PI First Name Nian

PI Last Name Wang

Email nianwang@ufl.edu

Phone 863-956-8828

Organization University of Florida

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Form PR-12 Quarterly Reports **SUBMIT REPORT**

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