CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD July 2020

Quarterly Report Final

Proposal Title

Control citrus Huanglongbing by exploiting the interactions between Candidatus Liberibacter asiaticus and citrus

Today's Date 08/08/2020

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

Category (drop down) CLas Bacteria

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

The goal is to understand how citrus interacts with *Candidatus* Liberibacter asiaticus (Las) infection and develop improved and long term HLB management strategies.

Objective 1. Identification of the receptors for Las PAMPs in susceptible and tolerant citrus varieties

Potential PAMPs from Las (either homologous to known PAMPs or pilin genes) LasFlaA (flagellin), LasEF-Tu, LasCSP (cold shock protein), LasSSBP (single strand binding protein) and pilin assembly genes were cloned under 35S promoter and the Arabidopsis phloem specific promoter *SUC2* and introduced into Agrobacterium. We have tested their receptors in Tobacco and citrus. Specifically, we are identifying the receptors in HLB susceptible variety Valencia sweet orange and HLB resistant variety Poncirus and HLB tolerant variety Sugar Belle. We have identified multiple receptors for the aforementioned PAMPs and are in the process of confirmation using pull-down assay or co-immunoprecipitation assays.

We also hypothesized that Las outer membrane proteins might directly induce plant immune response in the phloem sieve elements because Las lives in the phloem. 21 outer membrane proteins have been cloned and the putative targets in citrus are being identified using Yeast 2 hybrid (Y2H) system and surface plasmon resonance (SPR) assay. Two outer membrane proteins showed positive interactions with citrus proteins based on Y2H assays. We are further confirming the interactions using GST pull-down assays

In addition, multiple Las PAMPs have been tested for their effects in inducing plant defense against Las in the greenhouse and at least four different Las PAMPs showed significant effect in inducing plant immunity. We are testing whether those Las PAMPs can inhibit Las titers after foliar spray in the greenhouse.

We have conducted RNA-seq analyses of Poncirus and sweet orange and we currently analyzing the data.

Objective 2. Generate transgenic/cisgenic citrus expressing PAMP receptors recognizing Las

We are transgenically expressing putative receptors or targets (identified in Poncirus) of Las PAMPs in Valencia sweet orange or Duncan grapefruit. They are driven by 35S promoter and phloem specific promoter AtSuc2. We will conduct Las inoculation via grafting or psyllid transmission once the transgenic plants are about one year old.

For those identified receptors or targets, we are sequencing the promoter regions in Valencia, Sugar Belle, and Poncirus to compare their differences. If the native promoter of Poncirus is strong enough, we will use Poncirus promoter to drive the expression of PAMP receptors or other target genes to avoid concerns about 35S promoter or AtSUC2 promoter. We are also driving the expression of one defense inducing gene using a pathogen-inducing promoter. Several plants expressing the constructs were generated.

Objective 3. Investigate the roles of effectors in HLB disease development

We have completed screening of 30 putative Las effectors and 4 of them repressed plant defense. We are

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Project Duration (years 3 *Year of Project* 2

% Completion of Objectives 71.00%

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.