CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Bs3 Superpromoter is triggered by multiple citrus TALEs

Proposal Title

Genetic Resistance to Citrus Canker conferred by the Pepper Bs3 Gene

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/15/11	Citrus Research and Development Foundation	Transgenic/Metagenomic Mediation of Resistance

REPORT UPDATE (500 words; summarize your accomplishments)

Over the past quarter, we have made progress in the following areas:

1. We have analyzed the contributions of individual PthA proteins by knocking out specific pthA genes from X. citri strain 306 from Brazil and testing them with GUS reporter gene fused to our super promoter Bs3 construct containing binding sites for 17 X. citri TAL effectors. Strains in which the genes pthA 1 and 2 were disrupted activated the reporter gene at levels nearly comparable to the wild-type strain. These results suggest that there is little significant contribution of these effectors to gene regulation. Strains disrupted for (i) pthA 2 and 3 showed a 25% reduction in GUS activity. (ii) pthA 1 and 3 showed a 50% reduction, pthA 1,2 and 3 showed a 60% reduction, and deletion of all four pthA genes showed a 98% reduction in GUS activity, similar to a type three secretion deficient strain, 306ΔhrpG. This results suggest that pthA4 is the principle effector in the activation of gene expression, with additional smaller contributions from the other three TALEs. These results confirm that combinations of UPT boxes allows triggering of engineered resistance promoters by more than one TALE which should reduce pressure on individual TALEs to evolve to evade detection.

2. A robust transformation system using either epicotyls or cotyledons has produced a large pipeline of transformed plant material. More than 800 Duncan grapefruit lines have been transformed, made shoots, and roots, and been transferred to soil, with six different promoter-gene constructs. 153 Ruby Red grapefruit transformants and 63 pineapple sweet orange transformants are now in soil. It is slow work, and further attrition occurs following molecular characterization to identify the desired lines.

3. Once plants are transferred to 4 inch pots and reach adequate size, the final line selection process can take place by pathogen testing. We have now begun analyzing new stably transformed Duncan plants containing the super promoter: resistance construct using the avrGf1 gene. Two candidate lines showed reduced pustule formation by pin prick inoculation, relative to a non-transformed control line, and a hypersensitive reaction to infiltration inoculation, compared to a water-soaked lesion in the susceptible control. These reactions suggest that the test construct is successfully conferring canker resistance in these stable lines. We are continuing to study these plant lines and additional stable lines that are reaching adequate size for pathogen testing. Lines identified from this analysis will be candidates for grafting and filed testing.

These results support our hypothesis that a resistance construct based on a promoter containing multiple citrus TALE binding sites can confer transcriptional activation and disease resistance to canker strains.

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Contract Number 102

Project Duration (years) 3 *Year of Project* 3

Total Funds (current year) $184,833.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.