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)

Identification of virulence factors of C. Liberibactor asciaticus

Proposal Title

Characterizing the Roles of Callose and Phloem Proteins in HLB Symptom Development

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
4/18/11	Citrus Research and Development Foundation	Infection Responses

REPORT UPDATE (500 words; summarize your accomplishments)

Samples collected and fixed from HLB affected and healthy trees for a complete evaluation of phloem development and disfunction from just above the fiberous roots to one-year-old shoots will be evaluated by light and electron microscopy with revised staining technology previously described. The trees selected are 22 years-old and will allow phloem age evaluations and natural and HLB elicited changes with aging. The molecular basis of the interaction of Las with its hosts is not well understood. We hypothesized that during infection, Ca. L. asiaticus differentially expresses the genes critical for survival and pathogenicity in either host. To test this hypothesis, .quantitative reverse transcription PCR was utilized to compare the gene expression of C Las in planta and in psyllid. Overall, 362 genes were analyzed for their gene expression in planta and in psyllid. Among them, 263 genes were up-regulated in planta compared with in psyllid, 18 genes were overexpressed in the psyllid, and 81 genes showed similar levels of expression in plant and psyllid. Our study indicates that Las adjusts its expression of genes involved in transport systems, secretion system, flagella, LPS, heme biosynthesis, stress resistance, hemolysin and serralysin in a host specific manner to adapt to the distinct environment of plant and insect. To our knowledge, this is the first large-scale study to evaluate the differential expression of Las genes in a plant host and its insect vector. Two of the hypothetical genes that were overexpressed in planta were screened on Nicotiana benthamiana and showed symptoms in planta. The genes encode for unique hypothetical proteins, and the products are 80 aa and 51 aa respectively, and both have associated signal peptide and transmembrane regions. The expression of respective genes in planta was confirmed by one step Reverse-Transcriptase PCR on total RNA isolated from younger leaves. Transgenic citrus plants (Duncan) expressing these two genes were constructed at the Citrus Transformation Facility. Another potential virulence factor overexpressed in planta when compared to the psyllid was hemolysin. The first step in the characterization of hemolysin produced by C. Liberibacter asiaticus is to confirm that the protein is active or functional in the organism. For this, the gene was cloned and expressed in E. coli, and grown on sheep blood agar plates (5%) to evaluate the hemolysis on the plates. Hemolysin shows three types of lytic activity, alpha, beta and gamma. Alpha hemolysis is characterized by incomplete hemolysis, where the area surrounding the coloines is greenish /yellowish or brownish. Beta hemolysis is complete hemolysis, characterized by clearing around the colonies. There is no hemolysis associated with the Gamma hemolysis, although the plate appears brownish. In this experiment, the use of a negative control of E. coli containing an empty vector is critical, because E. coli is also known to produce hemolysin proteins. Hence, it is necessary to confirm that the hemolysis is due to the C. Liberibacter asiaticus hemolysin. Our result indicates that C. Liberibacter encodes a functional hemolysin protein which might be involved in pathogen and host interaction. Citrus plants transformed for phloem limited callose production are being introduced into a greenhouse to challenged them with HLB infected citrus psyllids.

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Total Funds (current year) $125,000.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.