CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ SELECT PERIOD June 2014

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Identification of key components in HLB using effectors as probes

Today's Date: 6/24/14

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

Category (drop down): Infection Consequences

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 understand the biology of HLB by identifying key components and processes involved in disease development. We identified four secreted proteins (also called effectors) from the causative agent, Candidatus Liberibacter asiaticus (CLas), which are consistently, and in some cases, highly expressed in infected citrus trees. We have generated antibodies against these CLas-specific proteins and developed serological detection methods for HLB. This method is currently under extensive evaluation using field and greenhouse trees. We are also using these effectors as molecular probes to identify their targets in citrus because effectors have been shown to perform key virulence functions during bacterial infection. This project will reveal important information of HLB pathogenesis, therefore providing guidance for disease management.

A major approach that we are using to find the effector targets is yeast two hybrid (Y2H) screen. In the first year of this project, we cloned the four CLas effector genes into an Y2H bait vector, transformed them into the yeast strain AH109, and confirmed that the effectors are highly expressed in the yeast without self activation activities. Therefore, these constructs are appropriate for Y2H screens.

In the past quarter (April to June, 2014), our main progress include:

1) Finished the construction of a citrus cDNA library, which is currently used for Y2H screening. We collected RNA samples from asymptomatic and symptomatic tissues of HLB-infected sweet orange leaves. These RNA samples were mixed with RNA extracted from healthy tissues to ensure that we would be able to cover as many genes as possible. A cDNA library was constructed and further normalized to reduce the level of cDNAs representing frequent transcripts using double strand specific nuclease (Evrogen). The cDNA was also size-fractionated to achieve an average insert size of >1 kb, with additional enrichment for long cDNAs that tend to represent full length transcripts. The cDNA library was transformed to a target complexity of about 3 millions of primary clones. Normalization of the library was confirmed with sequencing of 50 randomly picked colonies, which exhibited increase in complexity measured by diversity of transcripts. We are now in the process of Y2H screening using this cDNA library and the four CLas effectors as the baits.

2) Determined the subcellular localization of the effectors in plant cells. We have made gene expression constructs that produce fusion proteins with each CLas effector gene tagged to two consecutive genes encoding the yellow fluorescence protein (YFP). We used two YFP tags because these CLas effectors are small in size and may therefore diffuse to certain sub-cellular localizations non-specifically. We expressed these fusion proteins in plant cells and examined their localizations using confocal microscope. Our data showed that three of the four proteins can enter plant nucleus. This is interesting because they may manipulate plant physiology by associating with specific plant targets in the nucleus. These results will provide insight into the biological function of the effectors and also provide guidance when we interpret the Y2H data.

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Form PR-12 Quarterly Reports

SUBMIT REPORT