CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD September 2015

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Control citrus HLB by blocking the functions of two critical effectors encoded by 'Candidatus Liberibacter asiaticus'

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/16/15	Citrus Research and Development Foundation	Unclassified

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

The objectives of this project are to characterize the molecular interactions between the effectors and the host mitochondrial proteins; to screen for molecules that inhibit the effector functions; and to control HLB using the inhibitor(s) and/or other related molecules.

Transgenic Arabidopsis plants expressing lasAI or lasAII showed a different degree of impaired growth. In particular, the LasAI contains domains responsible for abnormal growth of the root and/or meristem. Trangenic citrus plants expressing Las AI also display growth retardation. Meanwhile, to further study the function of LasAI in citrus, transgenic citrus were generated to express LasAI, LasAI N-terminal, LasAI C-terminal, LasAI repeat region, LasAII and GFP control, respectively. We have obtained transgenic citrus plants transformed with different domains of LasAI, Interestingly, transgenic plants show different degree of growth retardation, in particular the full length LasA1 and LasA1 C-terminal shows slower growth compared to the other constructs. Using RNAseq and RT-qPCR, we were able to identify the up- and down- regulations of some important genes involved in host-pathogen interactions and biosynthesis of secondary metabolites in these transgenic plants.

Transient expression of LasAI and three different LasAI domains, LasAI-N-terminal, LasAI-repeat, LasAI-C-terminal allowed us to visualize the sub-cellular localizations of different domains. Because of high level expression of these effector proteins, we developed a novel in vitro screening system that can evaluate small molecules against these Las effectors. The library consists of more than 30 million compounds obtained from the small molecule libraries of the TPIMS (Torrey Pines Institute for Molecular Studies). Interestingly, a few groups of compounds showed interference activity against the mitochondrial localization of LasAI. Meanwhile, to concert this screening, we developed another in vitro screening system in conjunction with the culture screening using Liberibacter cresence (Lcr). From these screening of 65 scaffold chemicals, we identified a number of chemical groups that disrupted the interaction between LasA1 and mitochondria and inhibit both Las and Lcr growth. We are narrowing down to individual compounds that inhibit the function of the Las AI effector or kill Las bacteria via other pathways, and measured the dosage effect of these potential candidate.

In addition, another hypothetical protein has been expressed in planta via transient and stable transformation, and founded to affect host resistance to a bacterial pathogen. The antibody against this protein was able to detect this antigen both in the transgenic plants and in the Las-infected plants. Meanwhile, the Western blot results revealed unique formation of this protein in E. coli and plants. Citrus plants with high level expression of this transgene displayed HLB-like symptoms, yellow shoot and impaired growth. Further characterization of this effector revealed its unique sub-cellular localizations. Understanding the molecular mechanism of how the effector induces HLB-like symptom is underway.

We also analyzed the expression of LasA1 in Las-infected citrus plants. RT-PCR results indicate that LasA1 expression is correlated with severity of HLB symptoms. In particular, LasA1 was expressed more in the yellow leaves or the yellow spots than green spots of the symptomatic leaves with blotchy mottle.

We successfully optimized the production of LasA1 polyclonal antibody. The new antibody increased the detection sensitivity and specificity. The new LasA1 antibody can detect LasA1 protein in Las infected periwinkle plants using western blot. Moreover, we detected LasA1 in Las infected citrus leaves in histological localization experiments. From the tissue print results, we identified the presence of a strong diffuse signal in the vascular bundle, indicating that LasA1 is abundant in las infected tissue and that diffuses in the phloem, which indicates that LasA1 was secreted outside of Las bacteria and move in the phloem system.

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.