CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD September 2014

○ *Quarterly Report* ● *Final*

Proposal Title

Manipulating defense signaling networks to stimulate broad-spectrum resistance to HLB and other diseases in citrus

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/30/14	Citrus Research and Development Foundation	Transgenic/Metagenomic Mediation of Resistance

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

We aim in this project to genetically manipulate defense signaling networks to produce citrus cultivars with enhanced disease resistance. Defense signaling networks have been well elucidated in the model plant Arabidopsis but not yet in citrus. Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are key hubs on the defense networks that are important for broad-spectrum disease resistance.

With a previous CRDF support, the PI's laboratory has identified ten citrus genes with potential roles as positive SA regulators. Characterization of these genes indicate that Arabidopsis can be used not only as an excellent reference to guide the discovery of citrus defense genes and but also as a powerful tool to test function of citrus genes.

This new project significantly expanded the scope of defense genes to be studied by examining the roles of negative SA regulators and genes affecting JA and ET-mediated pathways in citrus defense control. We originally proposed a three-year research with three specific objectives: 1) identify SA negative regulators and genes affecting JA- and ET-mediated defense in citrus; 2) test function of citrus genes for their disease resistance by overexpression in Arabidopsis; and 3) produce and evaluate transgenic citrus with altered expression of defense genes for resistance to HLB and other diseases. However only one-year funding was provided to support our research. With this support, our effort has been focusing on Aim 1. We have cloned 15 citrus defense genes affecting SA, ET, and/or JA pathways from citrus cDNA libraries and made overexpression constructs with the full-length cDNA clones of these genes in the binary vector pBINplusARS. While our focus is on gene cloning with the one-year support, we also initiated Arabidopsis transformation and testing disease resistance of the transgenic plants for these cloned genes (Aim 2). So far we obtained T1 seeds for 10 gene overexpression constructs of the cloned citrus genes and preliminary testing of transgenic plants overexpressing CsJAR1 or CsACD1 revealed promising results for future studies. In addition, citrus transformation with 10 citrus gene overexpression constructs has been initiated (Aim 3).

In addition, we continue to characterize transgenic citrus plants expressing the SA positive regulators, as proposed in the previous project (#129), although the support of the project was already terminated. Our recent analysis of transgenic citrus overexpressing CsNDR1 (five out of ten transgenic plants) showed that these plants had lower rates of HLB positive three months after inoculation psyllids carrying Liberibacter asiaticus, suggesting that CsNDR1 overexpression confers enhanced HLB resistance. This result is consistent with CsNDR1 overexpression in conferring enhanced disease resistance in Arabidopsis (Lu et al., 2013). We will follow up with these plants for a further test of HLB resistance in the next few months.

Lu, H., Zhang, C., Albrecht, U., Shimizu, R., Wang, G., and Bowman, K.D. (2013). Overexpression of a citrus NDR1 orthodox increases disease resistance in Arabidopsis. Front Plant Sci 4, 157.

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Project Duration (years) 1 *Year of Project* 1

Total Funds (current year) $100,000.00

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.