Florida Citrus Advanced Technology Program

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

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	Genetics	1/15/10

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Creation of citrus canker resistance by the NPR1 genes

TITLE and CONTACT INFORMATION

Proposal Title Engineering Resistance Against Citrus Canker and Greening Using Candidate Genes

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Organization UF-Department of Plant Pathology	*Total Direct Funds (current year)* 84,313.00

REPORT UPDATE (650 words; provide details about your headline)

The goal of this project is to transform the citrus and Arabidopsis NPR1 genes (CtNPR1 and AtNPR1), and the rice XIN31 gene into citrus, and to evaluate their resistance to both citrus canker (caused by Xanthomonas axonopodis pv. citri (Xac)) and greening diseases. The first year objectives include: (1) Molecular characterization of the transgenic plants; (2) Inoculation of the transgenic plants with Xac. (3) Inoculation of the transgenic plants with the HLB pathogen and monitoring of the bacterium in planta with quantitative PCR; (4) Transformation of SUC2::NPR1 into citrus; (5) Plant maintenance.

During the first nine months of studies, we have identified three transgenic lines overexpressing CtNPR1 and AtNPR1, respectively, by using Northern blot analysis. These NPR1 overexpression lines were inoculated with 105 cfu/ml of Xac306 and the results showed high levels of resistance from the NPR1 overexpression lines, but not from the control plants, suggesting that both CtNPR1 and AtNPR1 are functional in citrus resistance to canker disease. Establishment of resistance to Xac by CtNPR1 is particularly significant for engineering resistance in citrus in the future. Preparation of a manuscript describing these findings is in progress.

We have also inoculated the transgenic plants expressing a truncated XIN31 and the preliminary data showed resistance to Xac306. We will further characterize these plants in year 2 of this project.

To prepare for greening inoculation, we have grafted the six NPR1 (three each of CtNPR1 and AtNPR1) overexpression lines and the control onto more root stocks to propagate the transgenic population. A total of 7-15 individuals have been produced for each of the transgenic lines. All these plants are currently maintained in green-houses located at the Citrus Research and Education Center in Lake Alfred, and will be used for greening inoculations in year 2 of this project.

Finally, we have finished the SUC2::CtNPR1 construct, in which CtNPR1 is driven by a phloem-specific promoter from the Arabidopsis SUC2 gene. This construct may increase the expression of CtNPR1 in citrus phloem thereby maximizing the opportunity for resistance to greening. Citrus transformation of this construct will be started soon.

In summary, we have achieved most of the goals for year 1, which establishes a firm foundation for the research in next year. The delay for objective 3 in year 1 is largely due to the fact that greening inoculation requires the transgenic plants growing to relative bigger sizes. To continue our research, we request funds for the second year to achieve the following goals as proposed originally: (1) Inoculation of the characterized NPR1 transgenic plants with the HLB pathogen and monitoring of the bacterium in planta with quantitative PCR; (2) Characterization of transgenic plants expressing the truncated XIN31; (3) Transformation of SUC2::CtNPR1 into citrus; (4) Microarray analysis of the CtNPR1 plants in response to Xac or greening inoculations; (5) Examination of changes in hormone (abscisic acid, auxin, jasmonic acids and salicylic acids) levels in the CtNPR1 plants infected with Xac or HLB; (6) Plant maintenance.

Accomplishment of these goals will very likely generate transgenic citrus plants with resistance to the HLB pathogen and advance our understanding of how citrus responds to these two diseases and could lead to new tools and strategies for the control of these two important diseases in Florida.