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*	Management	4/5/10

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

Analyze defense phenotypes of Arabidopsis expressing citrus SA regulators

TITLE and CONTACT INFORMATION

Proposal Title Manipulating SA-mediated defense signaling to stimulate broad-spectrum resistance to HLB and other diseases in citrus

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Organization University of Maryland Baltimore County	*Total Direct Funds (current year)* 160897.00

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

In the fourth quarter, we have primarily focused on selecting T1 Arabidopsis transformants expressing citrus SA homologs and testing some T2 transgenic plants in disease resistance. So far, preliminary data obtained from Arabidopsis expressing ctNDR1 look promising. The Arabidopsis ndr1-1 mutant was shown previously to lack a hypersensitive response (HR) when challenged with the Pseudomonas syringae avrRpt2 strain. We transformed ndr1-1 with ctNDR1 and obtained T2 seeds. The T2 plants were infected with the P. syringae avrRpt2 strain at OD=0.1 for a HR test. We found that T2 plants from 10 independently transformed lines were segregated into HR+ and HR-, consistent with the fact that the T2 transgenic plants are heterozygous in the transgene. In the control experiments, all T2 plants showed no HR when challenged with a virulent isogenic P. syringae strain. In addition, when we infected the T2 ndr1-1 plants expressing ctNDR1 with P. syringae avrRpt2 strain at OD=0.0001 for a disease resistance test, we observed a segregation of resistance and susceptibility among the T2 lines. As controls, WT plants were resistance and ndr1-1 plants were more susceptible to this strain. These data suggest that the ndr1-1 mutant is likely complemented by the ctNDR gene. We will isolate homozygotes of the transgenic plants and test them again for defense response in the next generation. In the meantime, the ctNDR1/pBINplusARS construct will be placed in the pipeline for citrus transformation.

Summary for additional transgenic plants are in the following:
1. CtNPR1/pBINplusARS to Col or the npr1-1 mutant: we infected T2 transgenic npr1-1 plants expressing ctNPR1. So far we did not observe a drastic change of disease resistance in the T2 plants. We will isolate homozygous lines and plant them side by side with WT and the npr1-1 mutant for a disease resistance test in the next generation.
2. CtPAD4/pBINplusARS to Col or the pad4-1 mutant: We obtained T2 seeds from Col and the pad4-1 mutant expressing ctPAD4 and will infect the T2 plants soon with P. syringae.
3. CtEDS5/pBINplusARS to Col or the eds5-1 mutant: We screen T0 seeds for transgenic plants expressing ctEDS5 but were not able to obtain any lines. This is possibly due to the toxicity of overexpressing ctEDS5 and/or the failure of transformation. We are now repeating the transformation process. In the meantime, we will transiently express ctEDS5 in tobacco to see if overexpression of this gene could cause cell death or other damages to tobacco cells.

In addition, we continue to clone additional SA genes from citrus. We are currently working on cloning several additional SA homologs from citrus, including ctEDS1, ctSID2, ctALD1, and ctWIN3. All these genes were previously shown to be involved in either SA biosynthesis or regulating SA levels in Arabidopsis. We already obtained 3' end of ctEDS1 and in the process of cloning other genes.