CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Cloning citrus SA regulators and analyzing Arabidopsis expres

Proposal Title

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

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
11/10/10	Citrus Research and Development Foundation	Transgenic/Metagenomic Mediation of Resistance

REPORT UPDATE (500 words; summarize your accomplishments)

In cloning the three SA genes, EDS1, SID2, and WIN3, we currently confirmed the cloning of the full-length ctEDS1 and are in the process of moving the sequence to the binary vector for plant transformation. We showed in the last progress report that we obtained 3' end RACE sequence for ctWIN3 and 5' end RACE sequence for ctSID2. In order to amplify the other ends of the two genes, we tried to design different primers for RT-PCR. We also performed TAIL PCR, in which we used citrus genomic DNA as a template in a series of PCR in order to obtain the missing regions of the two genes. However, these attempts were unsuccessful.

With Carrizo sequence database (http://citrus.pw.usda.gov/) recently available, we have been doing bioinformatics analysis and have identified additional SA genes that have citrus homologs with available sequence. We are currently design primers to further amplify these additional SA genes.

For ctEDS5/pBINplusARS transformation, we obtained 5 Col-0 and 5 eds5-1 carrying the transgene. We are in the process of screening T0 seeds for additional independent transformants. In the meantime, we planted these 10 transgenic plants for disease resistance assay with Pseudomonas infection.

We continue to characterize the transgenic plants overexpressing ctNDR1/pBINplusARS, ctNPR1/pBINplusARS, or ctPAD4/pBINplusARS. We obtained 4 homozygous ndr1 + ctNDR1/pBINplusARS and performed disease resistance assay. The recent data confirmed our earlier report that ctNDR1 complemented Arabidopsis ndr1 mutant. Additional analysis will be conducted to verify this result and to further characterize the defense phenotypes of the transgenic plants.

For plants overexpressing ctNPR1/pBINplusARS or ctPAD4/pBINplusARS, we did not observe complementation of npr1 or pad4 mutant with transgenic plants currently obtained. We reason that overexpression of these two genes may be toxic or citrus cDNA clones may not be well expressed in Arabidopsis. We are currently trying to clone the genomic fragments of these two genes. We will repeat npr1 or pad4 complementation once we obtain the genomic clones.

PI First Name Hua

PI Last Name Lu

Email hualu@umbc.edu

Phone 410-455-5972

Organization University of Maryland Baltimore County

Contract Number 0000005072

Project Duration (years) 3 *Year of Project* 2

Total Funds (current year) $160,834.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.