CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD March 2016

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Production of Transgenic Commercial Scion Cultivars Resistant to HLB and Canker: Continued AMP Approaches and Novel Transgenic Strategies

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
4/15/2016	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.)

Citrus trees transformed with a chimera AMP (thionin-D4E1) and the thionin alone showed remarkable resistance in citrus canker compared to control. These promising transgenic lines were replicated for HLB challenge. Replicated transgenic Carrizo lines expressing thionin, chimera and control were grafted with HLB infected rough lemon buds. Las titer was checked from new flush rough lemon leaves at six month after grafting. Las titer from 18.6-36.5 was detected in 90% of transgenics expressing the chimera. Some transgenic lines expressing thonin had lower Las titer(most in 33.3-36.4 ranges). Transgenic root sample were further tested and most were detected with las titer from 30 to 35. Root samples from control plants and transgenic Carrizo expressing chimera and thionin were taken nine months after grating inoculation. Our results showed transgenic Carrizo expressing thionin significantly inhibited Las growth (0.5% of control level) compared to control and transgenic Carrizo expressing chimera. Antibody against thionin will be produced for Western detection. Two new chimeral peptides (second generation) were developed and used to produce many Carrizo plants and Hamlin shoots. Transgenic Carrizo plants carrying second generation AMPs were obtained. DNA was isolated from 46 plants and 40 of them are PCR positive.

To explore broad spectrum resistance, a flagellin receptor gene FLS2 from tobacco was used to transform citrus. The consensus FLS2 clone was obtained and used to transform Hamlin and Carrizo so that resistance transduction may be enhanced in citrus for HLB and other diseases. Reactive Oxygen Species (ROS) assay showed typical ROS reaction in transgenic Hamlin indicating nbFLS is functional in citrus PAMP-triggered immunity. Trees showed significant canker resistance to spray inoculation. Replicated Carrizo and Hamlin were challenged with ACP feeding. Leaves were taken six months after ACP feeding inoculation. DNA will be isolated and Las titer will be tested.

To disrupt HLB development by manipulating Las pathogenesis, a luxI homolog potentially producing AHLs to bind LuxR in Las was cloned into binary vector and transformed citrus. Both transformed Carrizo and Hamlin were obtained. Replicated transgenic Carrizo plants were challenged by ACP feeding. Las titer will be tested soon. Transgenic Hamlin were propagated by grafting for HLB challenge.

In collaboration with Bill Belknap two new citrus-derived promoters have been tested using a GUS reporter gene and have been shown to have extraordinarily high levels of tissue-specific expression. The phloem-specific promoter was used to create a construct for highly phloem specific expression of the chimeral peptide using citrus genes only.

A Las protein p235 with a nuclear-localization sequence has been identified and studied. Carrizo transformed with this gene displays leaf yellowing similar to that seen in HLB-affected trees. Gene expression levels, determined by RT-qPCR , correlated with HLB-like symptoms. P235 translational fusion with GFP shows the gene product targets to citrus chloroplasts. Transcription data were obtained by RNA-Seq. Data analysis and comparison are underway.

Antibodies (ScFv) to the Las invA and TolC genes, and constructs to overproduce them, were created by John Hartung under an earlier CRDF project. We have transgenic Carrizo reflecting almost 400 independent transgenic events and 17 different ScFv ready for testing.

A series of AMP transgenics scions produced in the last several years continue to move forward in the testing pipeline. Many trees are in the field and some are growing well but are not immune to HLB. A large number of ubiquitin::D4E1 and WDV::D4E1 plants and smaller numbers with other AMPs are replicated and now in the field.

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