CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD September 2013

(●) *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)
10/18/13	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 scions continue to advance which have been transformed with diverse constructs including AMPs, hairpins to suppress PP-2 through RNAi (to test possible reduction in vascular blockage even when CLas is present), a citrus promoter driving citrus defensins (citGRP1 and citGRP2) designed by Bill Belknap of USDA/ARS, Albany, CA), and genes which may induce deciduousness in citrus.

Putative transgenic plants of several PP-2 hairpins and of PP-2 directly are grafted in the greenhouse and growing for transgene verification, replication and testing. Over 40 putative transgenic plants with citGRP1 are growing and will soon be tested. Forty of them were test by PCR and twenty two of them are transgenic plants with citGRP1 insertion. RNA was isolated from some of them and RT-PCR showed gene expression. More than thirty kan resistant shoots were obtained from citGRP1 transformed Hamilin. About 10 transgenic Hamlin shoots with citGRP2 were rooted in the medium and nine of them were planted in soil. Over 60 transgenic Carrizo with GRP2 were transferred to soil and are ready for PCR test. Belknap reports that potatoes transformed with citGRP2 are displaying considerable resistance to Zebra Chip in Washington state. Fifteen transgenic Hamlin shoots with peach dormancy related gene MADS6 are in the rooting medium for rooting. Seven transgenic Hamlin with MADS6 were planted in soil. In addition, numerous putative transformants are present on the selective media transformed with different constructs. A chimeral construct that should enhance AMP effectiveness (designed by Goutam Gupta of Los Alamos National Lab) is being tested. Many kanamycin resistant transformants were generated on the selective media. About twenty kanamycin resistant shoots are rooted in-vitro and one Hamlin transformant is in soil.

To explore broad spectrum resistant plants, a flagellin receptor gene FLS2 from tobacco was amplified and cloned into pBinARSplus vector. Flagellins are frequently PAMPS (pathogenesis associated molecular patterns) in disease systems and CLas has a full flagellin gene despite having no flagella detected to date. The consensus FLS2 clone was obtained and used to transform Hamlin and Carrizo so that resistance transduction may be enhanced in citrus responding to HLB and other diseases. The construct pBinARSplus:nbFLS2 was used to transform Hamlin and Carrizo. Many putative transformants were generated on the selective media. About ninety transgenic shoots were rooted in rooting medium and eighty Carrizo and ten Hamlin transformants were plant in soil. Other targets identified in genomic analyses are also being pursued.

A series of transgenics scions produced in the last several years continue to move forward in the testing pipeline. Several D35S::D4E1 sweet oranges show initial growth in the field which exceeds that of controls. A large number of ubiquitin::D4E1 and WDV::D4E1 plants and smaller numbers with other AMPs are replicated and in early stages of testing.

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Organization USDA/ARS/USHRL

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.