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)

Speeding up the delivery of transgenic solutions to HLB

Proposal Title

Accelerating the Commercialization of Transformed Juvenile Citrus

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
2/2/12	Citrus Research and Development Foundation	Transgenic/Metagenomic Mediation of Resistance

REPORT UPDATE (500 words; summarize your accomplishments)

Continued efforts to improve transformation efficiency:
• Evaluation of transgene expression of transgenic citrus plants with different phloem specific promoters. Several transgenic 'Mexican lime' lines containing the d35s promoter and the 4 phloem specific promoters were evaluated for transgene activity. Transgene analysis was carried out using PCR, RT-PCR, q-PCR and Southern Blot analyses. Publicataion: Dutt M., Ananthakrishnan G, Jaromin MK, Brlansky RH, & Grosser JW (2012) Evaluation of four phloem-specific promoters in vegetative tissues of transgenic citrus plants. Tree Physiology 32(1):83-93.

• q-PCR approach to evaluate copy number and gene expression levels. Copy number of several transgenic lines has being evaluated using gene specific TaQMAN probes. Most transgenic lines had 1 – 4 copies of the transgene stably incorporated into the genome. In addition a qPR-PCR approach is being used to evaluate gene expression levels in all transgenic lines.

Horticultural manipulations to reduce juvenility in commercial citrus:
• Continued to grow selected precocious rootstock seedlings for subsequent budding with transgenic precocious sweet oranges (Vernia and OLL series). Several transgenic lines of our precious sweet orange and mandarin transgenic lines (B4-79, W. Murcott, B10-68 and OLL8) have been grafted onto precocious rootstock including Amblycarpa + Benton and Changsha + Benton. Grafted trees have been transplanted into airpots in a heated greenhouse for evaluation (Fig 1.), with plans to grow the trees in a RES (Rapid Evaluation System horticultural manipulation) type system in the greenhouse. We are now planning to establish a transgenic site on CREC property, and hope to be able to include a small structure to apply the RES technology (horticultural manipulation to reduce the time of juvenility) to actual transgenic plants.

Transformation with early-flowering genes:
• We have regenerated many transgenic plants with the poplar FT behind either the 35S or heat shock promoter. Some of them are quite large now and the ones with HS promoter were maintained in a growth chamber at high temperature for several months, but none have bloomed yet. We are growing T1 tobacco with all 3 citrus FTs in order to determine phenotypes. We developed a co-transformation strategy to transform Carrizo citrange with two cassettes, one containing 35S-cft1 and the other containing AtSUC2 – gus. We generated 122 transgenic Carrizo plants using the two vectors. PCR analysis revealed that 16 lines contained both cassettes. Plants have not flowered 12 months after transformation. Plants are currently being evaluated in an unheated greenhouse for cold stress in order to initiate flowering in spring 2012. Numerous transgenic plantlets of Hamlin and Carrizo were regenerated containing P27, P28, P29, PATFT and pPTFT.

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Organization Citrus REC

Contract Number 00077976

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

Total Funds (current year) $178,000.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.