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

Mature Citrus Transformation to Survive Citrus Greening

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/2/13	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.)

The Mature Citrus Biotechnology Facility (MCBF) continues to increase our capacity for genetic transformation of mature scion budded onto immature rootstock. To this end, we have double budded immature rootstocks with mature scion. The number of explants will be significantly increased if this double budding scheme works well. Thus far, the results are promising for grapefruit but we are still waiting for results in sweet orange. Sweet orange is not as vigorous as grapefruit. We are also increasing the number of transgenic events produced by transforming immature rootstock. Different transgenic/wild-type combinations of rootstock/scion will be tested in field studies.

A number of clients have provided genetic constructs important to imparting tolerance to citrus canker. Genetic transformations of Valencia using two constructs obtained from Dr. Wang's lab have been performed and putative transgenic shoots are regenerating which will be micro-grafted in the future. Transformations using a construct from Dr. Mou's lab (originally provided by Dr. Dong at Duke, NC) have been used with Hamlin, two batches of Pineapple and Ray Ruby grapefruit. Mature shoot explants of Valencia will be transformed in the near future as explants become available. This construct has also been used with explants of Carrizo. Swingle and Macrophylla rootstocks will be transformed in the near future. Additional genetic constructs will be obtained from citrus researchers as the capacity of our lab increases.

Molecular analyses of putative transgenics, transformed with marker genes, are underway, and these plants are expected to flower in the near future. The number of transgenes for each putative transgenic is being determined by qReal-time PCR or Southern blot. Additional laboratory equipment and consumables are being purchased and another employee hired to accomplish this objective. The temperature, photoperiod, and light source have been changed in Growth Room A to induce flowering in these transgenics. Dr. Pena indicated that thorns in mature citrus transgenics regenerated from tissue culture is normal, transitory, and a sign of vigor.

During the last quarter, additional experiments were conducted with marker genes (Table 1). The number of positive shoots recovered in some experiments is relatively low, but still acceptable for mature citrus transformation.

Table 1. Mature citrus transformation experiments showing transformation efficiencies.

Cultivar	Date	Batch	Plasmid	Explants	Positive Shoots	Transformation Efficiencies (%)
Ham 19	6/11/13	36	p2301	240	1	0.8
Ham 20	7/2/13	31	p2301	300	2	2.2
Pine 8	6/11/13	35B	p2301	240	1	0.8
Pine 9	7/9/13	40	pE121	260	3	4.5
Val 20	5/21/13	30	pE121	810	28	2.1
Ray 2	7/30/13	37X	pE121	640	7	6.7

Book chapter from previous lab manager: Orbovic, Shankar, Peeples and Hubbard (in press) Citrus Transformation using Mature Tissue. Edited by Kan Wang, IN Agrobacterium Protocols Vol 2

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