CALIFORNIA CITRUS NURSERY BOARD

Progress Report for 2013
California Citrus Nursery Board
Agreement # 58-5302-3-392

Project Year: 2013

Progress Report: Year One of a Two-Year Project

Project Leader: Raymond K. Yokomi

Phone: 559.596.2990 FAX: 559.596.2992

E-Mail: ray.yokomi@ars.usda.gov

Location: USDA ARS San Joaquin Valley Agricultural Sciences Center, 9611 S. River-bend Ave. Parlier, CA 93648

Co-Project Leader: Richard Lee and Manjunath Keremane

Phone: 951-827-4399 FAX: 951-827-4398

E-Mail: Richard.Lee@ars.usda.gov and Manjunath.Keremane@ars.usda.gov

Location: USDA ARS Germplasm, 1060 Martin Luther King Blvd, Riverside, CA 92507

Project Title: Development of a TaqMan® Array Plate for Multiplex Detection of Citrus Pathogens by Real-Time PCR from a single sample

General Objectives

Develop and validate a Taqman® Array Card (TAC®) and/or plate for simultaneous detection of multiple citrus pathogens from a single sample by real-time PCR (qPCR). The sensitivity of the TAC qPCR assay was designed for use with standardized high throughput extraction robots to obtain high quality nucleic acid targets from pathogen-infected citrus tissue using magnetic beads from commercial kits.

Specific Objectives:

1. Develop singleplex qRT-PCR assay for RNA pathogens for use with TaqMan Array cards.
2. Develop singleplex qPCR assay for DNA pathogens for use with TaqMan Array cards.
3. Develop a custom Taqman Array card or plates as a standardized test for both RNA and DNA pathogens of regulatory importance for the California citrus nursery industry.

Progress Report for 2013 (Year 1):

Unique conserved sequences of key citrus RNA and DNA pathogens were selected from NCBI database and used to develop primer sets to identify pathogens by quantitative PCR (qPCR) assay. Using Primer Express Version 3.0.1 (Life Technologies Corp, Carlsbad, CA), primers and TaqMan probes were optimized to have the same annealing and melting

profiles between pathogens or strains to operate with a uniform set of PCR conditions (reagent concentrations, temperature, cycle number, annealing, melting temperature, extension, etc.). Table 1 shows a list of the pathogens included.

Table 1.

Pathogen or strain	Type	ID
CTV	RNA	CTV P25
CTV T30	RNA	CTV T30
CTV T36	RNA	CTV P27 T36
CTV T36NS	RNA	CTV P27 T36NS
CTV VT3	RNA	CTV P27 VT3
Stubborn1	DNA	spirilin
Stubborn 2	DNA	citriP58
Internal Std 1	DNA	COX
Internal Std 2	RNA	NAD
Exocortis	RNA	CEVd
Hop stunt/Cahexia	RNA	HSVd/CVd-II
HLB	DNA	HLB
Viroid generic	RNA	CVd
Citrus bent leaf	RNA	CVd-I
Citrus viroid III	RNA	CVd-III
Citrus viroid IV	RNA	CVd-IV
Citrus viroid V	RNA	CVd-V
Citrus viroid VI	RNA	CVd-VI
Citrus tatterleaf	RNA	CTLV
Leprosis	RNA	CiLV
Citrus varigated chlorosis	DNA	CVC
Witches broom	DNA	Phytopl
Citrus leaf blotch-Dweet mottle	RNA	CLBV

In Year 1, research concentrated on detection of *Citrus tristeza virus* (CTV), '*Candidatus* Liberibacter asiaticus' (*C*LAS) (Huanglongbing or HLB) and *Spiroplasma citri,* causal agent of citrus stubborn disease. High throughput extraction was employed with citrus samples from greenhouse and field infected with CTV, citrus viroids (CEVd and HSVd) and *C*LAS. The qPCR protocol successfully detected and differentiated severe CTV strains (VT and T3 genotypes) from mild CTV strains, *C*Las, CEVd or HSVd in single or mixed infections (Saponari et al. 2013). Success was also obtained to detect and differentiate Hopstunt viroid variants by real-time RT-PCR and high resolution melting temperature analysis (Loconsole et al. 2013).

A CTV RT-qPCR kit was designed to rapidly and economically differentiate between economic and non-economic CTV strains. The kit was tested and validated with 2013 field samples provided by the CCTEA and successfully detected a VT genotype of CTV in several locations in the Tulare County Pest Control District. Therefore, the procedure can expedite, supplement or replace the existing MCA13 monoclonal antibody-based ELISA assay currently used by the CCTEA.

More sensitive detection of *S. citri* was accomplished by developing and using pPCR primers based on several conserved multi-copy prophage gene sequences with SYBR® Green. *S. citri* detection was improved 2-3 log orders of magnitude compared to the single-copy house-keeping gene, spiralin (Wang et al., in preparation).

All qPCR primers that detected the pathogens described above work in the Taqman array card or plate system (Table 1). Therefore excellent progress was made to develop, validate and implement the pathogen detection tools being developed in this project.

Publications.

Loconsole, G., Onelge, N., Yokomi; R. K., Abou Kubaa, R., Savino, V., and Saponari, M. 2013. Rapid differentiation of citrus Hop stunt viroid variants by real-time RT-PCR and high resolution melting analysis. Molecular and Cellular Probes. 27: 221-229.

Saponari, M., Loconsole, G., Liao, H.-H., Jiang, B., Savino, V., and Yokomi, R. K. 2013. Validation of high-throughput real time polymerase chain reaction assays for simultaneous detection of invasive citrus pathogens. Journal of Virological Methods 193:478-486.

Wang, X., Doddapaneni, H., Chen, J., and Yokomi, R. K. (In preparation). Improved real-time PCR assay for sensitive detection of *Spiroplasma citri* by targeting multicopy phage/prophage genes. Plant Disease.