Florida Citrus Advanced Technology Program

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

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	Management	8/26/10

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Nine polyclonal antibodies were produced and its antigenicity tested.

TITLE and CONTACT INFORMATION

Proposal Title Diagnosis of Candidatus Liberibacter asiaticus in plant and vector based on molecular and serological approaches

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Organization Centro de Citricultura - Fundag	*Total Direct Funds (current year)* $32500

REPORT UPDATE (650 words; provide details about your headline)

Concerning the Nested PCR one step we are working on improvement of sensitive of reactions but with few progress in the sensibility of NPCR one step comparing against double step nested PCR, as discussed in the annual report. On the other hand, we did great progress on development of antibodies against Ca. Liberibacter asiaticus. All the 9 peptides mentioned on the third report were injected in rabbits for the polyclonal antiserum (PA) production. Female New Zealand white rabbits weighing 2 kg were endovenously inoculated with 200 ug of each antigenic peptides. Afterwards, this procedure was repeated two times at 14 days intervals, using 300 ug and 400 ug of antigenic peptides, respectively. Seven days after the last injection, the blood was collected through cardiac puncture and the serum titer and specificity were determined by indirect enzyme-linked immunosorbent assay (ELISA) according to Clark et al. (Clark, M.F., R.M. Lister & M. Bar-Joseph. 1986. ELISA techinques, p.742-766. In H. Weissbach & A. Weissbach (eds.), Methods in enzymology, v.118. New York, Academic Press, 820p). Preimmune serum was used as a negative control. Antibody titres of nine PAs were checked by an enzyme- linked colorimetric assay (ELISA) using the Anti-Peptide Antibodies methodology (Table 1). Just one (T3699-3) of the nine PA resulted in low reactivity against the antigen (10µg/mL). For the nest steps we will test the eight PA against the Liberibacter asiatic infected and or symptomatic plants.

Table 1. ELISA assays of Polyclonal antibodies against the antigens.

Peptides name	T3699-1	T3699-2	T3699-3	T3699-4	T3699-5	T3699-6	T3699-7	T3699-8	T3699-9
Dilution	Antibody O.D. (405 nm)								
1:500	2.100	1.936	0.875	1.455	2.032	2.117	2.153	1.819	2.042
1:1.000	2.037	1.695	0.500	1.311	2.001	2.026	2.132	1.548	1.928
1:2.000	1.904	1.286	0.264	1.129	1.917	1.793	1.993	1.332	1.625
1:4.000	1.601	1.408	0.126	0.758	1.884	1.569	1.825	1.109	1.560
1:8.000	1.336	1.077	0.038	0.573	1.706	1.297	1.584	0.750	1.346
1:16.000	1.110	0.761	0.000	0.284	1.495	1.112	1.388	0.509	1.220
1:32.000	0.832	0.550	0.000	0.164	1.252	0.998	1.060	0.287	1.013