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*	HLB Pathology	11/4/10

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

Genetic Analysis of "Candidatus Liberibacter asiaticus" Populations

TITLE and CONTACT INFORMATION

Proposal Title Development of SSR markers for detection, genotyping, phenotyping and genetic diversity assessment of "Candidatus Liberibacter" strains in Florida

Principal Investigator Hong Lin	*PI Last Name* Lin
Email hong.lin@ars.usda.gov	*FDACS Contract Number* 125
Phone 559-596-2933	*Project Duration (years)* 3 *Year of Project* 1
Organization USDA, ARS, Parlier, CA	*Total Direct Funds (current year)* $105,342

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

The objectives of the first year's research project are focusing on:

1. Conduct genome-wide sequence analysis to identify Simple Sequence Repeat (SSR) loci from genomic sequences of "Candidatus Liberibacter asiaticus" (Las). Design and develop PCR-based multi-locus SSR molecular diagnosis assay.
2. Analyzing Las population structures, assessing the genetic diversity of Las in Florida populations.

We assessed Florida Las population and compared it with a global genetic diversity of Las populations using a multi-locus SSR marker system. A total 293 HLB samples used for population analysis were composed of isolates obtained from the US (177 isolates), China (42 isolates), India (35 isolates), Brazil (22 isolates), Cambodia (11 isolates), Vietnam (3 isolates), Taiwan (1 isolate), Thailand (1 isolate), and Japan (1 isolate). This sample population had an abundance of sequence types, consistent with the sampling diversity and the type of marker (SSR) used for this study; a total of 147 sequence types (STs) were identified from the 293-member sample pool (Supplemental Table 1). All of the STs identified within the sample population were restricted to the boundaries of their country of origin; in other words, we found no individual ST in more than one country. Profile frequencies and allele frequencies were calculated for this sample population. Based on allele frequencies of SSR loci among these populations, a genetic similarity matrix that consisted of 71 alleles and 293 samples was generated. The genetic distance analyses were performed using Neil's coefficient with 1,000 bootstrap and 95% confidential intervals. A pair wise population genetic analysis indicated that among four Las populations, the genetic distance between Florida vs China Las populations is 0.61, while the genetic distance between Florida vs India is 1.35, Florida vs Brazil is 0.96, China vs Brazil is 0.84, India vs Brazil is 0.95 and China vs India is 1.72, respectively. The results indicated that the genetic distance between Florida and China have the closest distance (0.61) as compared with other populations. Analysis of genotypic profiles present amongst an HLB sample population allowed us to make inferences about the relationships between L. asiaticus isolates associated with HLB. From this work, we conclude that at least two major clonal complexes of L. asiaticus are associated with HLB worldwide and that the recent introduction of HLB into the southeastern US probably occurred as a result of at least two separate events. The panel of markers we introduce here will be useful for future population studies of HLB and may also aid in the identification of segments of the L. asiaticus chromosome associated with bacterial virulence. High percentage of commonly-shared allele types detected in both populations suggests that Las populations in Florida and China are more related. This leads to a hypothesis that Las populations in Florida could be possibly derived from China. It is not clear if the introduction was from single or multiple times and/or from multiple sources as well. Our results demonstrate the useful of multi-locus SSR marker system for genetic analysis of Las. Bacterium has very compact genome. Sequencing variations of SSR loci located within or near the coding and/or gene regulatory regions could have profound effect on gene expression and functions. We have mapped SSR loci genome containing genes of interest. Our next step is to examine the functionality of the candidate genes using a standard in vitro heterologous expression system. This type of study will facilitate the link of DNA-based genotyping to phenotyping of Las. We have accomplished objective 1 and 2 during the first year of the project. Research had been summarized and reported in 2009 and 2010 American Phytopathological Society meetings.