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	10/2/09

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

Progress Report (July - September, 2009)

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	*Total Direct Funds (current year)* $105,342

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

The research activities during this period were working on objectives;

(1) Identification of Simple Sequence Repeat (SSR) loci and validating SSR markers designed from genome sequences of "Candidatus Liberibacter asiaticus" (Las).

(2) Analyses of global genetic diversity of HLB Las strains in American and Asian continents (U.S. Florida, Brazil, China and India) using new multilocus SSR markers. Followings are the summary of research activities and accomplishments during this period.

1. Extensive genome wide sequence search resulted in identifying 58 new loci containing perfect, imperfect or compound SSR repeats that can be potentially useful for designing SSR markers. Most of these loci are either located in or next to the Open Reading Frames (ORFs). Molecular Beacon software v7.0 was used to design SSR primers with the same criteria reported earlier. 40 new SSR primers were evaluated by PCR experiments. Those primers produced single clean bands with expected amplicon sizes were further evaluated their polymorphic power by using HLB asiaticus strains collected from U.S. Florida and other geographic regions. Amplified products were separated by the high resolution of 5% polyacrylamide sequencing gels. Primers that passed the test were synthesized with 5'-labeled fluorescent dyes (FAM, NET, VIC) and run by an ABI 3130 Genetic Analyzer.

2. Genetic diversity analyses were conducted for HLB Las strains collected from Florida, Brazil, China and India. These included 43 samples from 14 counties in Florida, 35 samples from 15 cities in Brazil, 30 samples from 8 areas in northern and southern India and 58 samples from five southern provinces in China. Five SSR primers were selected for this set of data, Preliminary data showed that polymorphic allele types ranged from 3 to 18 in five SSR loci among total 166 isolates with average of polymorphism 34% in Florida, 32% in Brazil, 52% in China and 57% in India, respectively. Relative lower genetic diversities detected in Florida and Brazil (34% and 32%) reflect recent introduction of HLB in south and north American continent while higher genetic diversities in India and China (57% and 52%) account for the long history of the evolutionary process resulting in differentiation of these populations. These results also demonstrated that the multi-locus SSR molecular markers developed in this project are a useful tool for detection and population genetic analysis for HLB Las.