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	1/14/10

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

Progress Report (October, 2009 to January, 2010)

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)

This progress report summarized research activities from October 15, 2009 to January 15, 2010. During this period, we continued working on (1) "Identification of additional Simple Sequence Repeat (SSR) loci and design and validation of new Las SSR markers" and (2), "Analyses of genetic diversity of HLB Las populations. Followings are the summary of research activities and accomplishments during this period.

Previously, we reported that we had designed and experimentally evaluated 40 new Las SSR primers. Among them, 25 primers showed clean and single amplified bands with expected amplicon sizes while the rest of 15 primers showed multiple bands when PCR evaluations were conducted. We then re-evaluated 15 primers by adjusting annealing temperature and Mg ++ concentrations. PCR experiments were conducted with annealing temperatures 55oC, 57oC and 59oC along with 1.0 mM 1.5 mM and 2.0 mM Mg++ concentrations using an ABI Veriti thermal-cycler with a temperature gradient heating block (ABI, Foster City, CA). The results showed that PCR amplification conditions with 57oC and 1.0 Mg++ improved the specificity of PCR amplification. 8 primers that showed clean and single bands under this condition were therefore selected. Those primers that passed quality test were then subjected to discrimination power test. Total 33 primers (8 primers and 25 primers) were amplified with six asiaticus-infected DNA samples, 2 from Florida, 2 from India and 2 from China, respectively. Amplified PCR products were then separated by the high resolution of 5% polyacrylamide gels. Gels were visualized by silver staining. 16 out of 33 primers showed polymorphism with alleles ranging from 150bp to 320 bp per locus. Seven of SSR primers were synthesized with 5'-labeled fluorescent dyes (FAM, NET, VIC) and assayed by an ABI 3130 Genetic Analyzer. All rest of primer evaluation will be completed in next three months.

We conducted global genetic assessment of asiaticus populations. A total of 166 HLB isolates representing four populations from US Florida, Brazil, India and China were analyzed using multilocus SSR markers. Based allele frequencies of SSR marker loci across four populations, a data matrix that consisted of 71 alleles and 166 samples was generated. The genetic distance analyses were then performed using Neil's coefficient (Neil, M.,1972) with 1,000 bootstrap and 95% confidential intervals. A pair wise population genetic analysis showed that the genetic distance of the populations between Brazil and China, or Brazil and Florida or Brazil and India were 0.8261, 0.9598 and 0.9330, respectively, while genetic distances between Florida and China populations, or Florida and India populations were 0.6016 and 1.3327, respectively. The results indicated that the genetic distances among the populations are not always reflected to their geographic relationships. For example, the genetic distance between Florida Las population and China Las population was closer (0.6016) than that of any other populations compared while the Las populations between China and India are most genetically distant (1.3327) even though both are in Asia. Since Brazil Las population has more or less equal distance to other three populations, it brings up a question of why both populations are closely related or what is the origin of Florida HLB populations. High numbers of commonly-shared alleles of Las populations in Florida and in China suggest both populations are genetically related. In contrast, the genetic distance of Las populations between India and China is 1.7212, the largest distance as compared with other populations indicating that both populations have been differentiated for certain time and there is limited gene flow between the populations.

Reference: Neil, M. 1972. Genetic distance between populations. Amer. Natur. 106:238-292.