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	3/20/10

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

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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 have conducted genome wide sequence analysis to identify (SSR) loci in Las. This resulted in the identification of ~175 total sequencing loci in Las containing mono, di-, tri-, tetra-, penta-, hex- type of perfects, in-perfect and compound types of simple repeats. Based on the criteria for primer design and BLAST analysis against available microbe sequences in the NCBI databases, 112 SSR loci were selected for primer design. We then designed and experimentally evaluated Las SSR primers. These evaluations were based on PCR experiments using 2-3 Las isolates collected from US Florida, India, China and Brazil, respectively. These efforts led to the successful validation and development of 10 SSR primers. While we continue working on more SSR marker development, these 10 SSR markers that showed to be useful for unambiguous detection and discrimination of Las genotypes were used for Las population genetic analysis. To adapt high through-put sample analysis platform, these SSR markers were labeled with fluorescent dyes (FAM, NET, VIC). These labeled SSR markers can be multiplexed and analyzed by an automatic ABI 3130 Genetic Analyzer. This Las genetic analysis system can process 4 x 96 samples in 2.5 hours.
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 of 166 HLB isolates representing four major citrus production regions from US Florida, Brazil, India and China were analyzed using 5 SSR primers. Two of them (India and China) represent populations in Asian continent where HLB has been for centuries while another two (US Florida and Brazil) represent recently emerging populations in American continent. Based on allele frequencies of SSR loci among the four populations, a genetic similarity matrix that consisted of 71 alleles and 166 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. 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.