Phase 2 of high-throughput sequencing as a CCPP routine diagnostic tool for variety introduction

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Year 1 of 4 (60% Complete)

Objectives

1. Development of E-probes for 14 graft-transmissible citrus pathogens based on novel sequence data analysis and publicly available databases.
2. Initiate the discussion and present data to the regulatory agencies for approval of the developed E-probes for 14 graft-transmissible pathogens of citrus.

Problem and Significance

High-throughput sequencing (HTS) is a powerful technology that combines molecular biology and computer sciences that has various applications such as diagnostics. The consensus in the scientific and regulatory community is that HTS will soon become the new gold standard for diagnostics. HTS is capable of simultaneous detection and identification of multiple pathogens from a plant sample without the need to perform multiple tests that will allow results to be obtained sooner.

One of the major challenges of HTS is the data analysis. It requires personnel with an in-depth background in bioinformatics analysis and expensive severs to perform the analysis on. A promising tool that can manage the HTS data analysis is the E-probe Diagnostic Nucleic Acid Analysis (EDNA) technology developed by Oklahoma State University (OSU). EDNA provides a user-friendly online interface to process and analyze raw HTS data for citrus pathogens without the need for intensive bioinformatics. E-probes are designed to detect the presence or absence of pathogens and the potential for the simultaneous detection of multiple pathogens in a given sample which will reduce time and cost. Once we develop the e-probes, they will need to undergo validation to prove that EDNA will work equally well or better than current regulatory approved diagnostic assays.

Benefit to Industry

The EDNA technology can be transferable to various laboratories because of the online platform and ease of use. The Citrus Clonal Protection Program (CCPP) and other citrus variety introduction programs can utilize this technology to benefit the citrus industry by providing access to pathogen tested budwood for citrus grove establishments. In addition, this technology will reduce time for the indexing pipeline and green house footprint of the CCPP. This will allow for a more streamlined introduction and distribution of new and established citrus varieties.

Progress Summary

In year one, we developed and began validating E-probes for citrus tristeza virus, Citrus exocortis viroid, and '*Candidatus* Liberibacter asiaticus'.

We have continued the development of E-probes for graft-transmissible citrus pathogens that have publicly available complete genomes. This is performed by mining the pathogen genome sequences in databases such as GenBank. The probes are developed and validated with simulated Illumina data and subsequently with real life samples. New E-probes have been developed for

citrus tatter leaf virus (CTLV), citrus vein enation virus (CVEV), *Xanthomonas citri* (X. citri), and *Xylella fastidiosa* (X. fastidiosa). These e-probes will be validated using our standardized pipeline.

For pathogens with fewer genome resources, we sequenced and assembled genomes of different isolates. We worked on *Spiroplasma citri* and related species such as *S. kunkelii*, *S. floricola*, *S. phoenicium*, and *S. melliferum* and we sequenced various viruses from the CCPP disease collection namely citrus psorosis virus, *Citrus variegation virus* and Citrus concave gum associated virus. With these newly assembled genomes, we will develop their respective E-probes and populate the diagnostic library of citrus graft transmissible pathogens.

The HTS/E-probe technology will require regulatory approval for use in citrus programs such as CCPP. We began the discussion for the regulatory approval during the “WERA 20: Virus and Virus-Like Diseases of Fruit Trees, Small Fruit, and Grapevines” conference on May 20-22. This was originally scheduled as an in person workshop with Unites States Department of Agriculture (USDA) - Animal and Plant Health Inspection Service (APHIS) regulators but due to COVID-19 restrictions we had to adjust to an online discussion with the regulatory agencies and a number of other germplasm programs for specialty crops.

During the WERA 20 meeting the implementation of HTS for routine diagnostics was extensively discussed but more importantly, we were informed that two USDA-APHIS departments; Plant Protection and Quarantine (APHIS-PPQ)-Science and Technology (S&T) as well as USDA-APHIS Germplasm Field Operations have been highly receptive in using the HTS diagnostic technology and have actually created two (2) USDA-APHIS HTS labs dedicated to using and validating this technology for routine plant diagnostics. Since the WERA 20 meeting we have initiated direct discussions with key regulatory personnel and began presenting our results on HTS/E-probe citrus diagnostics.

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Publications and Presentations

H.Wang, J. Habiger, A. Espindola, K. Cardwell, T. Dang, G. Vidalakis, A. Roy. Determining Limit of Detection of High Throughput Sequencing Diagnostics with MiFi® American Phytopathological Society online conference. 2020. Poster.

G. Vidalakis. WERA 20 UC Riverside Report, HLB status in California & CCPP activities. WERA – 20. Virus And Virus-Like Diseases of Fruit Trees, Small Fruits, And Grapevines. Annual Meeting - May 20-22, 2020 - Hawaii via Zoom.