CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

SELECT PERIOD December 2014

(●) *Quarterly Report* ( ) *Final*

Proposal Title

HLB resistance through transgenic expression of short chain fragment variable antibodies against key Liberibacter epitopes

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
1/14/15	Citrus Research and Development Foundation	Culture, Molecular Biology, Koch's Postulates

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

This project is a continuation of a previous project #95 "PREPARATION OF ANTIBODIES AGAINST CANDIDATUS LIBERIBACTER ASIATICUS". Progress reports for the previous project are on file. The reimbursable agreement with CRDF was established on September 5, 2012.

We have obtained the vector, pUSHRL-26, to be used for plant transformation of the scFv constructs from Ed Stover at Fort Pierce and the plasmid has been purified. We have purchased the restriction enzymes and designed primers to be used for PCR to amplify the cloned scFv encoding inserts from vector pKM19. The cloned inserts will be sequenced to confirm that they are correct and then cloned into the transformation vector.

The scFv have been modified by the addition of a four amino acid leader sequence (KDEL) and both Sma I and Spe I cloning sites. The KDEL sequence is expected to stabilize the concentration of scFv in phloem cells by facilitating proper folding of the protein in the microtubules and thereby protecting the ScFv from proteolytic digestion. Eleven scFv inserts have been sequenced to be sure that the expected sequences are correct, and five ScFv sequences have been successfully cloned into the recombinant vector pUSHRL-26 for transformation of citrus rootstocks. These inserts include three different scFv that bind to the protein InvA and two that bind to the protein TolC. The protein InvA is produced by CaLas and secreted into the host to prevent the infected host cells from entering into apoptosis, and the protein TolC targeted by the scFv, is in the external membrane and is essential for the removal of antimicrobial substances produced by the plant. The vector is designed to direct expression of the scFv into the phloem cells of citrus, where CaLas grows, and the vector encoding the scFv genes is being introduced into rootstock varieties by Agrobacterium mediated transformation. To date we have purified 19 scFv genes, and cloned them into the plant transformation vector pUSHRL-26 developed by Ed Stover at Fort Pierce. This includes 9 antibodies directed at InvA and 10 directed at the external opening of the TolC protein. Sequencing confirmed the clones were correct. The Stover laboratory has transformed the constructs into Agrobacterium and the Agrobacterium has been used to transform Carrizo seedlings. Nineteen transgenic lines have been established or are in development with between 150-400 epicotyl explants for each line. These explants include scFv for both TolC and InvA, and are being grown at Fort Pierce for subsequent evaluation by inoculation with psyllids infected with CaLas. There are hundreds of independent transformants containing these scFv constructs at Ft. Pierce (Stover). These transformants are being grafted on to high titer root stocks and will be incorporated into the separate NuPsyllid project to determine if they have adverse effect on acquisition or transmission of CaLas by the psyllid.

We are making a second recombinant antibody library using ribosomal display technology. This technology is not expected to be encumbered by patent restrictions. Mice have been obtained and have been injected 3 times with psyllid extracts, following the previously successful immunization protocol used to make the scFc library.

PI First Name John

PI Last Name Hartung

Email john.hartung@ars.usda.gov

Phone 301-504-6374

Organization USDA ARS

Project Number 5552

Project Duration (years) 3 *Year of Project* 3

Total Funds (current year) $155,680.00

Form PR-12 Quarterly Reports **SUBMIT REPORT**

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.