CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD December 2013

(●) *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)
1/6/14	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 continue to study the literature to identify vectors to use for a future scFv library made as part of this project. The goal is to find a suitable vector that is not encumbered by intellectual property and patent issues. I have written twice to a laboratory in Germany which has published results with a suitable vector but have had no reply. We are also optimizing the cloning strategies that will be used to move already selected scFv into transgenic plants.

A visiting scientist arrived and began work 6-15-2013. 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.

Inserts from the phage vector that encode the single chain antibodies have been amplified and recloned and some have been submitted for sequencing to confirm the expected sequences. The scFv selected for this purpose recognize a TolC-like epitope of CaLas and an InvA-like epitope of CaLas. Both of these protein targets are expected to be essential for the interaction between the pathogen and host and thus are vulnerable targets for impairment by scFv. These scFv sequences have been modified by the addition of a KDEL leader sequence. The KDEL leader sequence has been reported to stabilize expression of proteins in transgenic plants. The scFv sequences have also been modified to contain SmaI and and SpeI cloning sites to enable cloning into the pUSHL26 vector to be used for transformation of plants.

Related research with the existing scFv is underway on project 551.

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Organization USDA ARS

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.