CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD June 2013

Quarterly Report ◉ Final

Proposal Title

Functional study of the putative effectors of 'Candidatus Liberibacter asiaticus' using Citrus tristeza virus vector

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
11/18/13	Citrus Research and Development Foundation	Pathogen/Disease Detection

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

Sequence information of Candidatus Liberibacter asiaticus (CLas) revealed number of putative effectors unique to CLas, the causal agent of citrus Huanglongbing (HLB). The effectors identified to be part of prophage related gene clusters and/or pathogenicity islands believed to contain bacterial pathogenicity, multiplication and virulence determinants. We cloned the effectors into binary vector based citrus tristeza virus (CTV) vector, between CP and CP ORFs as a separate cistron under an heterologous beet yellows virus coat protein controller element. Additionally, based on the observation that engineering the foreign genes at the 3' end of CTV vector resulted in optimal expression, the potential effectors were cloned into CTV and citrus plants were generated containing the following; CLIBASIA_05165, CLIBASIA_05605, CLIBASIA_01555, CLIBASIA_05195, CLIBASIA_05200, CLIBASIA_05620, CLIBASIA_05635, CLIBASIA_05665, CLIBASIA_05130, CLIBASIA_05155, CLIBASIA_05265, CLIBASIA_05560, CLIBASIA_05150, CLIBASIA_05180, CLIBASIA_05245, CLIBASIA_02250, CLIBASIA_03020, CLIBASIA_03025, CLIBASIA_02090 and CLIBASIA_02905. Citrus plants were tested by reverse-transcription-PCR (RT-PCR) for the presence of the effector sequence as part of CTV genome. However, the effectors did not elicit any unusual phenotype in citrus.

We also tested full-length Flagellin gene of CLas and bacterial Flagellin for possible effector function in citrus because of our earlier observation of the Pathogen-associated Molecular Patterns (PAMP) activity associated with Flagellin domain of HLB. We used a faster approach using Potato virus x (PVX) vector system to screen for virulence genes of HLB. Transient expression of prophage-related gene cluster of HLB in PVX vector did not induce obvious phenotype changes in tobacco plant. However, transient expression of HLB-flagellin, and flg22, HLB flagellin domain, induced cell death and callose deposition in N. benthamiana. Substitution experiments revealed that the 38th and 39th amino acid residues were essential for callose induction. However, the synthetic peptide (DRVSSGLRVSDAADNAAYWSIA) could not induce cell death, but retained the ability to induce callose deposition at the minimal concentration of 20 µM. These results demonstrated that Flg22Las has PAMP activity, and may play an important role in determining citrus resistance to HLB. These results have been published in the journal PLoS One.

A useful outcome of this project is the observation that CTV-vector can be used as an Virus Induced Gene Silencing (VIGS) vector. We used this property of CTV to silence citrus endogenous Phytoene desaturase (PDS). It has also been used to potentially silence endogenous genes of psyllids (vecotr of HLB). In co-operation with scientists at CREC and USDA, Fort Pierce we have been successful in silencing of psyllid endogenous genes. This line of research potentially holds great promise in mitigating the spread of HLB by psyllid vector. CTV-RNAi vector, engineered with truncated abnormal wing disc (Awd) gene of D. citri, upon replication in citrus generated RNA silencing triggers, which induced altered Awd expression when ingested by feeding D. citri nymphs. Decreased Awd in nymphs resulted in malformed wing phenotype in adults and increased adult mortality. This curtailed the ability of D. citri to fly, and potentially would limit the successful vectoring of CLas bacteria between citrus trees in the grove. CTV-RNAi vector would be relevant for fast-track screening of candidate sequences for RNAi-mediated pest control.

The work accomplished during this investigation have been presented in national and international meetings and another manuscript is submitted to Plant Pathology journal. Two post doctoral researchers, DR. Husong Zou and Dr. Subash Hajeri mailnly were involved in these investigations. Dr. Zou returned back to China to a new assignment in Fujian Agricult and Forestry University and Dr. Hajeri has since accepted a position with California Citrus Industry.

PI First Name Siddarame

PI Last Name Gowda

Email gowda@ufl.edu

Phone 8639568715

Organization IFAS-CREC

Project Number 00087697

Project Duration (years) 2 *Year of Project* 2

Total Funds (current year) $119,200.00

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