Florida Citrus Advanced Technology Program

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

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2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
◉ *Quarterly Report* ○ *Annual Report* ○ *Final*	Management	10/15/09

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Amorphous and filamentous phloem sieve element plugging materials associated with HLB identified.

TITLE and CONTACT INFORMATION

Proposal Title Characterizing the Roles of Callose and Phloem Proteins in HLB Symptom Development

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Organization UF Citrus Research & Education Center	*Total Direct Funds (current year)* $125,000

REPORT UPDATE (650 words; provide details about your headline)

A manuscript reporting the sequence of symptom development and the identification of callose and phloem protein 2 (PP2) as the amorphous and filamentous plugging materials, respectively, has been prepared and is in the final stages of review for submission to Plant Physiology. The upset of normal phloem translocation of carbohydrates to other parts of the plant and ultimately the starvation of the root system (Etxeberria et al, 2009, accepted) may be the main reason for tree decline in response to HLB infection. >Field samples were collected and prepared to determine the relative amounts of amorphous versus filamentous plugs in order to evaluate if one type is more important than the other in disrupting phloem sap flow. Samples include sweet orange and grapefruit collected in the Indian River District or southern flatwoods. Additionally, samples from sweet orange were collected near Lake Placid and Murcott in the southern region. Examination of these samples has begun. >Earlier work by a colleague has indicated that two of our common rootstocks do not express HLB symptoms when grown as seedlings in a greenhouse or growth chamber and infected with the HLB organism. These are being examined for possible suppression of the phloem plugging mechanism. More importantly, field grown seed source trees have been found with + PCR reactions for HLB. These will be tested for phloem plugging and HLB tolerance. >Work to understand the mechanism by which the bacterial infection leads to this phloem plugging is underway as insufficient bacteria are present in the phloem to directly plug the phloem. To determine how the causal bacteria elicits the over expression of phloem plugging materials, one Agilent microarray has been designed based on the genome sequence of Candidatus Liberibacter asiaticus. Bioinformatics analysis was performed to identify potential virulence factors. Six potential virulence factors were cloned into pGEMT-easy vector. The insertion was confirmed using PCR with gene specific primers, and will be sequenced for further confirmation. Those virulence factors are being expressed in tobacco and citrus to study their potential roles in virulence. >Transgenic approaches to achieve over-expression of the citrus ß-1,3-glucanase gene using different promoters, though protoplast/GFP co-transformation and Agrobacterium-mediated transformation are underway in order to minimize HLB associated callose-plugging. The citrus ß-1,3-glucanases gene from Valencia embryogenic callus and young leaves (McCollum et al., 1999) was cloned. Citrus β-1,3 glucanase cDNA (GenBank accession number AJ000081) was synthesized from Valencia leaf and embryogenic callus. A 1011 bp citrus β-1,3 glucanase gene fragment was amplified. To obtain the suitable restriction site and additional cMyc tag (to facilitate subsequent western analysis) on the cDNA, another PCR reaction was performed using a new primer set with a different restriction site including the cMyc tag. The final PCR product (1059bp, designated BG3) was purified, cloned into the pGEM T-Easy vector (Promega) and sequenced. The cloned BG3 fragment was ligated into a vector named pUCLON between the 35S promoter and 35S terminator, and was transformed into host E coli DH5α cells. Plasmid DNA was isolated using 5prime kit and checked through restriction digestion and also PCR analysis. The re-ligated plasmid was named pUBG3. The HindIII fragment from pUBG3 was excised and ligated into the HindIII site on another vector pCIT101 holding GFP/NPTII fusion gene on it. The final vector was designated as pCITBG3 and was transformed into Agrobacterium. It is now ready for co-transformation experiments. Plasmid vector pARS108 with the ER-targeted GFP gene was also used to make a new construct for efficient protoplast transformation. Another vector, pGASS, was constructed to target BG3 expression in the phloem tissues only. Two new sweet orange callus lines are available to carry out these and other transformations.