Title:
Citrus tristeza virus-based RNA-interference (RNAi) vector and its potential in combating citrus Huanglongbing (HLB)

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Author:
Hajeri, Shubash, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850 USA
El-Mohtar, Choaa, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850 USA
Dawson, William O., Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850 USA
Gowda, Siddarame, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850 USA

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Abstract:
Citrus tristeza virus (CTV), a plus-sense ssRNA virus, is member of the genus Closterovirus, family Closteroviridae. RNA viruses are inducers as well as targets of gene silencing defense mechanism of host plants and this has been exploited as a tool in functional genomics. CTV was developed into virus-induced gene silencing (VIGS) or RNA-interference (RNAi) vector, which interferes with expression of endogenous genes in citrus or GFP-transgene in Nicotiana benthamiana (16c) in a sequence specific manner. Photobleaching phenotype indicative of silencing of endogenous gene, phytoene desaturase in citrus, and red color under UV indicative of silencing of transgene GFP in N. benthamiana (16c) was observed using CTV-RNAi vector. CTV-RNAi vector has great potentials in combating huanglongbing (HLB) disease through (1) enhancing basal defense of citrus by silencing of auxin signaling F-Box receptor genes while simultaneously overexpressing microRNAs; (2) down-regulation of overexpressed genes, callose synthase and phloem protein-2, responsible for phloem-plugging in citrus by HLB; (3) expressing dsRNA specific to essential genes of insect vector psyllid (Diaphorina citri) to disable transmission of 'Candidatus' Liberibacter asiaticus pathogen. Simultaneous silencing of multiple endogenous genes of a metabolic pathway is possible through tandem engineering of potential siRNA eliciting regions in CTV-RNAi vector.

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Hajeri, S., El-Mohtar, C., Dawson, W.O., and Gowda, S.

Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850 USA

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