

Using Citrus Tristeza Virus-Based Vector as a Platform for the Management of Huanglongbing

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Effort Statement: Continue to use CTV vector to identify potential therapeutics and efficiently induce early flowering in different genotypes.

Summary: Citrus is commercially propagated from elite scion lines lacking tolerance to huanglongbing (HLB) on selected rootstocks. *Candidatus Liberibacter asiaticus* (CLAs), the causative agent of HLB, is transmitted between trees by the Asian citrus psyllid (ACP) insect vector. CLAs and citrus tristeza virus (CTV) colocalize in the phloem tissue of citrus where ACP feeds. To induce resistance or tolerance to HLB, systemic but nonvirulent CTV-T36 based vectors are being used to deliver potential therapeutics to the citrus phloem tissue. First, a major therapeutic to manage HLB is antimicrobial peptides (AMPs). A second use of the CTV vector is to identify CRISPR targets by inducing RNA interference (RNAi) against

negative regulators of citrus defense and susceptibility genes. A third use of the CTV vector is to modify psyllid citrus phloem diet by RNAi and bacterial pesticidal proteins. Furthermore, CTV vectors have other uses that include promoting early flowering by overexpressing genes promoting flowering and silencing negative regulators of flowering. In addition, we are using alternate temperature therapy to generate HLB — and CTV-free growth from citrus germplasm that is showing tolerance to HLB in the field. This will enable large scale field experiments to confirm the phenotype.

Take Home Message:

- CTV T36 overexpression and RNA interference (RNAi) vectors are readily available bio-delivery tools that ectopically deliver nucleic acids for peptide expression and the down regulation of target genes into the phloem tissue of citrus.

- Using CTV vectors, we identified antimicrobial peptides that induce tolerance against HLB in the susceptible sweet orange seedlings and working on improving their efficacy via different strategies and identified CRISPR targets to induce resistance or tolerance to HLB that our collaborators are working on generating the target modifications.
- Inducing maturity (flowering) of HLB resistance CRISPR modified plants is necessary to make available for growers as modifications are produced via protoplast or juvenile tissue transformation. We are working on developing an efficient system to induce flowering using CTV vectors in different citrus genotypes via different approaches. Upon induction of flowering CTV will be eliminated via an established alternating temperature regime with nothing left in the tree.

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