Novel Strategies for Huanglongbing Resistance or Tolerance in Citrus by Gene Editing

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Effort Statement: Studies are still ongoing, but these methods will generate two crucial sources of plant material for growers.

Summary: Plant immune regulators' expression in response to biotic stresses like huanglongbing (HLB) determines whether the interaction leads to resistance/tolerance or disease. Thus, our research is focused on negative immune regulators of citrus to produce HLB-resistant trees. Using comparative genomics, we have identified a set of negative immune regulators. The most promising targets were identified by transiently downregulating these genes using citrus tristeza virus (CTV) RNA interference and NPR1 protein level as a molecular marker. The identified targets are being edited using three different strategies. The first strategy is CRISPR/Cas9 edit to knock out these genes. The second and third strategies manipulate the expression level of these genes by engineering intragenic microRNA (miRNA) and small interfering (siRNA) to downregulate these genes. These interfering RNA strategies create HLB-tolerant trees that produce non-genetically modified organism (non-GMO) products. This strategy complements gene editing and uses a citrus DNA-based (intragenic) vector to create RNAs in the rootstock. Because RNAs can make a root-to-shoot long-distance movement, making more of these RNAs in the rootstock (for example, in Swingle or US-942) can silence target genes and induce HLB tolerance in the scion (for example, in 'Valencia' and 'Hamlin'). As rootstocks can be used for different scions, intragenic rootstocks are particularly valuable in California and Florida, where many scion cultivars are used. This project will deliver two essential products: (1) non-GMO trees resistant to HLB directly to commercial production (see figure) and (2) intragenic rootstocks that can readily be grafted with various scions.

Take Home Message:

- This project will utilize three different gene-editing strategies in citrus to combat HLB susceptibility.
- Through our strategies to silence specific negative immune response regulators of citrus HLB, we can increase disease tolerance in both scion and rootstock citrus varieties.

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