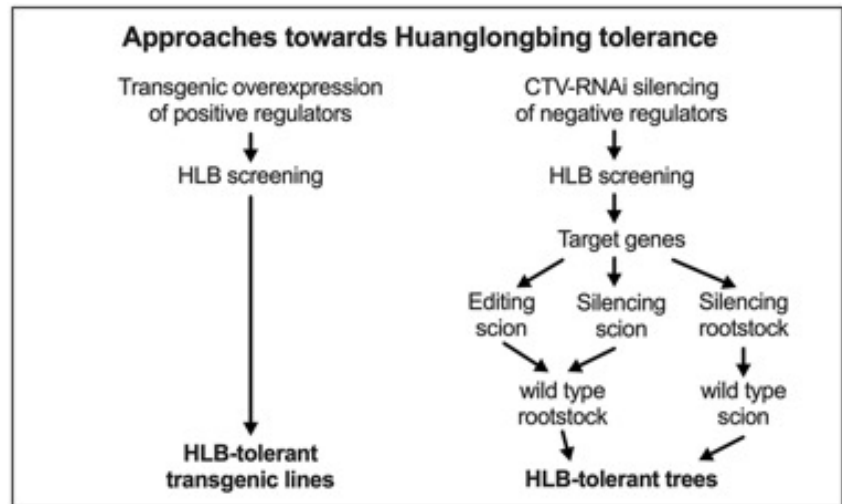


# Approaches Toward Huanglongbing Tolerance

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## Take Home Message:

- HLB tolerance can be created in citrus cultivars through genetic approaches.
- Several target genes for silencing and editing have been identified.
- Multiple approaches are being pursued to create HLB tolerance.

**Effort Statement:** We included in our approaches the generation of transgenic and/or intragenic rootstocks expressing microRNA or hairpin RNA that can silence the target genes in the scion to create HLB tolerance.

**Summary:** It is well known that the plant immune system is balanced by positive and negative regulators and the immune balance can be boosted by adding positive regulators or removing negative regulators. We

use this strategy in citrus to create long-term solutions to the devastating disease huanglongbing (HLB). Thus far, we have transgenically over-expressed 20 positive regulators in sweet orange and grapefruit and found that over-expression of certain positive regulator(s) led to robust tolerance to HLB. We also used CTV-delivered RNA interference (CTV-RNAi) technique to silence a group of 44 negative regulators to identify targets for gene editing. Two CTV-RNAi constructs that induce strong HLB tolerance have been identified and more HLB tolerance-inducing constructs are expected to come soon. Our results demonstrate that robust HLB tolerance can be achieved by modifying the citrus immune system. We are editing the target genes identified in the

CTV-RNAi screening to produce transgene-free HLB-tolerant citrus trees. Furthermore, we have built highly efficient intragenic and microRNA vectors using citrus DNA sequences, which allows production of intragenic plants. We are using these vectors to modify the citrus immune system, producing intragenic trees that are tolerant to HLB. We are also generating transgenic and/or intragenic rootstocks expressing microRNA or hairpin RNA that can silence the target genes in the scion. Finally, we continue to educate the citrus communities about the CTV-RNAi, gene editing, intragenesis, and microRNA technologies and their benefits to the citrus industry and consumers to help establish market acceptance for modern biotechnology-based citrus products.

## Funding:

 National Institute of Food and Agriculture  
U.S. DEPARTMENT OF AGRICULTURE

 Citrus Research and Development Foundation, Inc.