

# Multiple Non-GMO Genome-Edited Sweet Orange Lines Generated by the CRISPR Technology will be Tested for Huanglongbing Resistance in Field Trials Soon

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**Effort Statement:** Multiple non-GMO genome-edited lines for HLB target genes were generated.

**Summary:** CRISPR gene editing has been used to generate multiple canker resistant citrus genome-edited Sweet orange cv. 'Hamlin' via editing the elements in the promoter region and coding region of the canker susceptibility gene CsLOB1, demonstrating its power in generating disease-resistant citrus. The transgene-free canker-resistant 'Hamlin' plants have been approved by USDA-APHIS and are in the process of further evaluation and commercialization. Importantly, we have developed multiple transgene-free genome editing toolkits using the CRISPR technology. Among them,

one is based on the ribonucleoprotein, and another is based on a co-editing strategy. Importantly, the co-editing strategy can be used to generate non-GMO genome-edited citrus by transformation of mature tissues, thus bypassing the juvenility and speeding up the release. Our previous studies also showed that the huanglongbing (HLB) pathogen stimulates a systemic and chronic immune response in citrus phloem including reactive oxygen species (ROS) production and callose deposition, which causes systemic phloem cell death and subsequent HLB disease symptoms. We have been conducting non-transgenic citrus genome editing to reduce the systemic and chronic immune response, and to increase

antioxidant enzyme activities. We have been focusing on 40 putative targets genome editing to improve citrus resistance against HLB. We have already generated non-GMO lines for two target genes which will be tested for HLB resistance, and other horticultural traits.

## Take Home Message:

- We have developed multiple highly efficient non-GMO citrus CRISPR genome editing toolkits.
- The co-editing strategy can be used to generate non-GMO genome-edited citrus by transformation of mature tissues, thus bypassing the juvenility and speeding up the release.
- We have generated multiple non-GMO genome-edited lines.

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