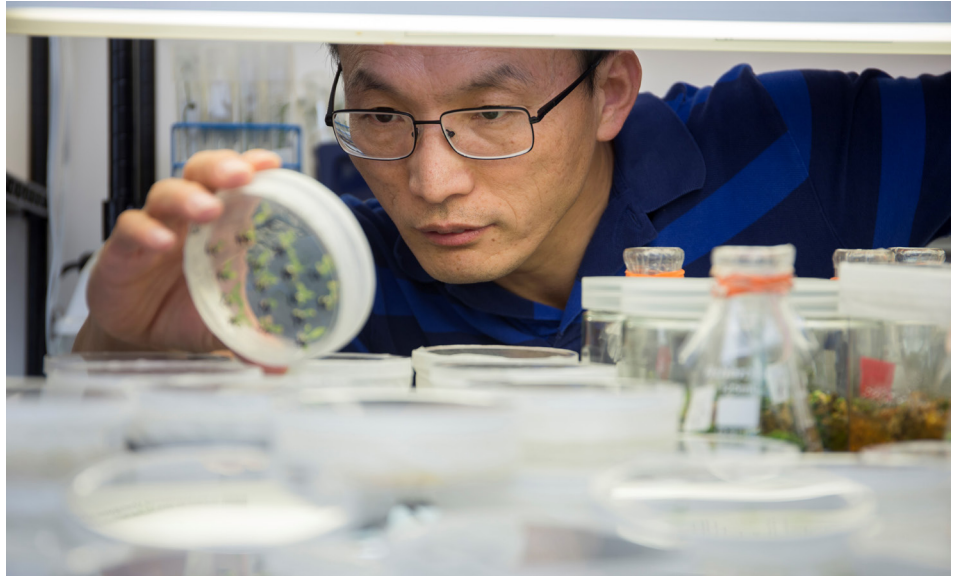


Non-Transgenic CRISPR Gene Editing is Ready to Join the Force to Fight HLB

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CRISPR gene editing has been used to generate multiple canker resistant citrus gene-edited lines 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. We have shown that genome editing of key genes controlling CLAs-triggered reactive oxygen species (ROS) production might render citrus HLB resistant/tolerant. Our data suggest that respiratory burst oxidative homolog D (RBOHD)

is likely responsible for CLAs triggered overproduction of ROS. In addition, key genes required for RBOHD activation are being identified. We are actively editing RBOHD genes and related genes. The method for non-transgenic gene editing of embryogenic sweet orange protoplast cells has been successfully developed and is mature and can be used for citrus gene editing to generate disease-resistant citrus varieties by editing the promoter or coding region of RBOHD genes and related genes. Base editors have been

successfully used to precisely edit specific residues of citrus genes and transient expression of base editors in epicotyl tissues successfully generated transgene-free citrus plants, thus providing an alternative approach for non-transgenic gene-editing of citrus. Non-transgenic gene-editing technology for sweet orange is mature now and non-transgenic HLB-resistant lines have a much simpler and easier path for regulatory approval, thus accelerating their potential commercialization.

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