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

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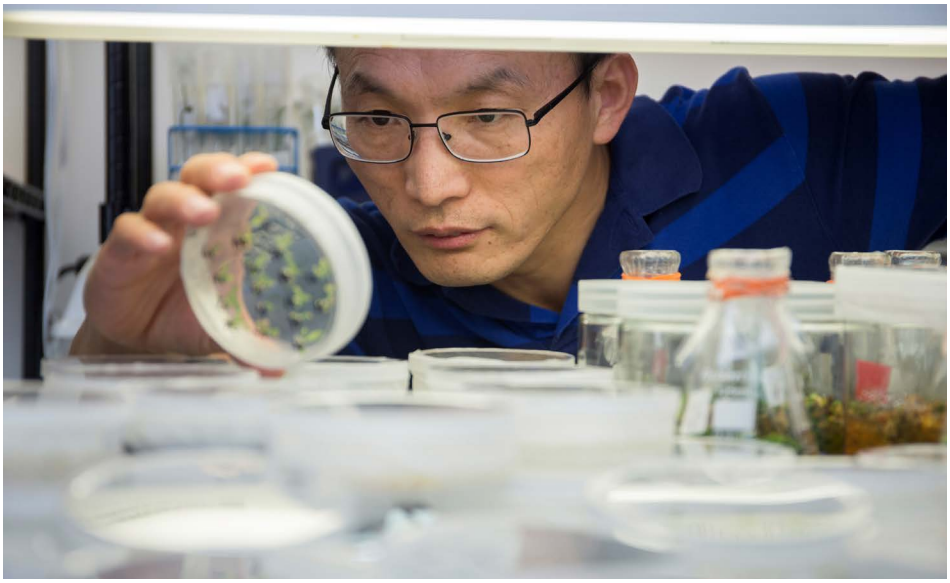

CRISPR gene editing has been used to generate multiple canker resistant citrus gene-edited lines via editing the elements in the promoter region and coding region of the canker susceptibility gene *CsLOB1*, demonstrating its power in generating disease-resistant citrus. We have previously identified six critical citrus genes (such as CsACD2) that *C*Las targets to suppress the plant's natural defense and promote the disease's spread in the tree. Importantly, we have identified critical residues of CsACD2 that interact with SDE15, an important effector of *C*Las. 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 the six HLB susceptibility 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. We have successfully generated one biallelic mutant line in the promoter region of CsACD2 of 'Hamlin' sweet orange. 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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