

Using genome editing to develop HLB-resistant or -tolerant citrus

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What is the technique?

Scientists are studying the interaction at the molecular level between citrus trees and the bacterium *Candidatus Liberibacter asiaticus* (CLas) associated with huanglongbing (HLB). In particular, they are investigating how CLas interacts with citrus proteins to cause disease, hoping to disrupt this interaction and ultimately develop new resistant or tolerant varieties using a genome-editing technology called CRISPR/Cas.

What knowledge about HLB is needed to succeed in this strategy?

The first step undertaken by this team of scientists was to identify bacterial genes necessary for infection. Genes are sequences of deoxyribonucleic acid (DNA) that contain messages that code for the synthesis of proteins, which are molecules required for life processes in all organisms. Bacterial pathogens such as CLas secrete virulence proteins, called effectors, which enhance disease development by overcoming citrus defenses. Gitta Coaker's group has been sequencing the genomes (the entire genetic information in an organism) of different strains of CLas to identify DNA sequences that could code for effectors. Effectors that are common to all strains of CLas found in different geographic areas are likely to be essential for the infection process, and therefore good targets for intervention. Wenbo Ma's group is identifying the citrus proteins and biological processes that are targeted by these CLas effectors. In a complementary approach, Nian Wang has been using a genome-editing technology, the CRISPR/Cas system, to delete and modify citrus DNA sequences in order to change the citrus proteins that are targeted by the bacterial effectors, so they are no longer be susceptible to the effectors. Finally, Lisa House's group is conducting customer and grower surveys to gauge acceptance of these edited citrus varieties. Veronica Ancona and Georgios Vidalakis have also been involved in this part of the project and also coordinate extension and outreach activities in Texas and California respectively, to transfer information and the outcomes of the project to growers and stakeholders.

How does the CRISPR/Cas system work?

The CRISPR/Cas system allows scientists to precisely alter DNA sequences in order to modify gene function, a process called genome editing. This process is performed by causing a break in the DNA and using the cell's natural DNA repair machinery to introduce changes. As the DNA sequence in a gene codes for a particular protein, changing the DNA sequence causes a change in the protein that is made. Import-

tantly, in the editing process foreign genes are not introduced; rather existing genetic information is rearranged. Since the newly edited variety does not contain any DNA from other organisms, it is not regulated by the USDA as a genetically modified organism (GMO). Genome editing overcomes some problems associated with traditional breeding, such as

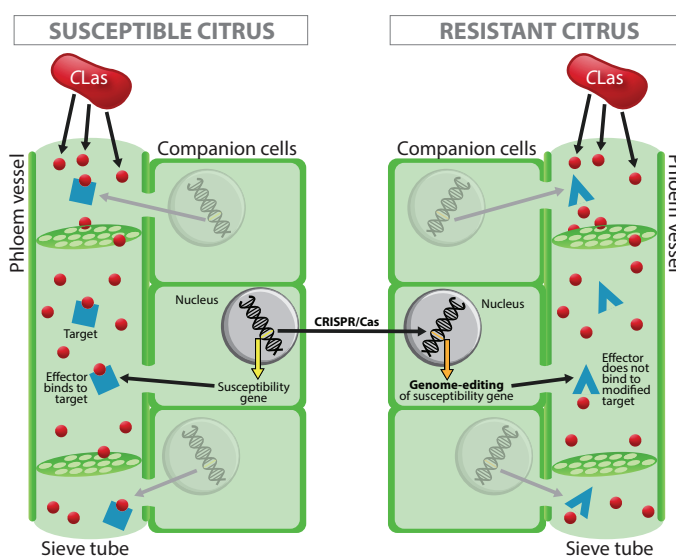


Figure drawn by Barbara Alonso

the long time to complete crosses, slow plant growth, pollen incompatibility, and other constraints particularly severe in citrus.

In this project, the CRISPR/Cas system is being used to modify citrus genes targeted by CLas effectors, attempting to make citrus proteins less susceptible to bacterial effectors, and consequently making citrus more tolerant or resistant to HLB.

Who is working on the project?

This project, funded by the USDA-NIFA Specialty Crop Research Initiative, is a collaborative effort between Wenbo Ma and Georgios Vidalakis from the University of California, Riverside; Gitta Coaker from the University of California, Davis; Nian Wang and Lisa House from the University of Florida; and Veronica Ancona from Texas A&M University-Kingsville. Some of the preliminary studies were supported by the Citrus Research Board.

What are the challenges and opportunities?

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Development of resistant or tolerant varieties is considered to be the most sustainable strategy to control plant diseases. However, no naturally occurring resistance against HLB has been found in cultivated citrus varieties. The CRISPR/Cas system is a powerful new tool to generate the much-needed resistance by identifying and modifying specific citrus genes targeted by CLas effectors, so CLas can no longer infect and/or cause damage to citrus. However, the success of this strategy depends on accurate characterization of the molecular interactions between CLas and citrus, so that appropriate genes to be modified in citrus can be identified. For this newly developed resistance to be durable, it is necessary to predict ways in which the bacterium might overcome the resistance during the co-evolutionary “arms race” with plant hosts, and design strategies to prevent this. Finally, although citrus varieties obtained through genome editing are not considered GMOs from a regulatory standpoint, consumers need to understand the CRISPR/Cas technology to help increase their acceptance of edited citrus varieties.

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