Founder lines used to improve HLB tolerance

DISEASE MANAGEMENT

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What is the technique?

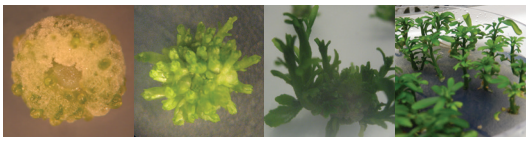
"Founder Lines" can be used to genetically engineer citrus plants with desirable traits, like tolerance to huanglongbing (HLB). But, what are Founder Lines? Founder lines are plants that have a DNA sequence, or piece of genetic material, that is used as a docking site for the insertion of one or more genes for desired traits at specific locations in the genome. Having a docking site ensures that insertions do not interrupt desirable genes, that high and consistent activity of the inserted sequence is achieved, which is more likely if only a single copy of the gene is introduced. The approach also leads to removal of marker genes for antibiotic resistance, that are needed to identify engineered tissues, and to reduce the time and cost of creating engineered varieties.

How is genetic engineering involved?

The Founder line approach uses primarily citrus genes to genetically engineer citrus varieties in a very precise way. The first step is creating a citrus line with target sites inserted in its genome, and the first founder line utilized the Carrizo variety. DNA is introduced into young stem slices and then, using appropriate media, individual cells in the stem are "coaxed" to form plantlets (see below).

DNA can be introduced into cells in the slice in several ways. First it can be done via an electrical current, which pokes holes in the cell wall, allowing DNA to enter and insert permanently into the genome. Secondly, DNA can be introduced via a natural soil-born organism, *Agrobacterium tumefaciens**, which can inject into plant cells DNA that inserts into the genome.

Once the target sites are identified and validated, they can be used for future docking efforts. This is because the docking site is recognized by special enzymes, called recombinases, which specifically identify the DNA sequence (recognition site) within the docking site and insert new genetic information at that location. The recombinase is so precise that the new information can be introduced without gaining or losing even one single chemical unit of DNA.



Stem section (left); regenerating plantlets (middle); plants (right)

The next part of the process is introducing recognition sites that are used by the recombinase to insert the new genetic material, such as HLB resistance, into the site and to remove the marker gene(s).

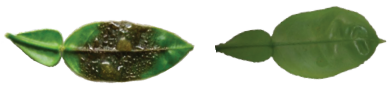
The same strategy can be used to "stack" additional traits into the same location, using the target sites and the recombinase. For example, an existing trait for HLB resistance can be stacked with another trait for resistance to the fungal pathogen *Phytophthora*.

Who is working on the Project?

This approach is being developed by James Thomson, a research scientist at the USDA-ARS in Albany CA. To date, through collaborative efforts with other scientists around the country, such as Ed Stover at USDA-ARS Ft. Pierce FL, Eliezer Louzada at Texas A&M, Gitta Coaker at UC Davis and William Belknap at USDA-ARS in Albany, 19 potential resistance genes have been identified and are being introduced into citrus as single genes and in some cases "stacked" as multigene cassettes. Once completed, the engineered plants will be given to collaborators for disease resistance evaluation.

What are the challenges and opportunities?

Although initial introductions into citrus of DNA with the target sites are complete and many different lines have been micrografted onto plants in the greenhouse, these must be characterized to select "Founder Lines" with the desired characteristic of HLB disease resistance. The team is also focusing on improving transformation/regeneration efficiency and rates of recombinase-mediated gene insertion. In other words they are trying to get more plants with DNA where they want it with less effort.



Engineered citrus leaves infected with Phytophthora (right); nonengineered (left)

One important benefit of the Founder Line technique is the fact that the time and effort for federal regulatory oversight could be reduced because introduced traits will always be inserted into the same location in the genome.

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** Agrobacterium tumefaciens is a bacterium that causes Crown Gall disease in many plants. It does this by inserting a small piece of its DNA into the plants genome. Researchers figured out how to use Agro to insert the pieces of DNA that they wanted to introduce into the plant, and genetic engineering "was born".*

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