Genes, Genomes and Genetic Engineering in Citrus

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http://ucanr.edu/sites/scienceforcitrushealth/

Living organisms, like plants, have many cells – each with genetic information to specify how plants look, taste and feel. This genetic information also determines whether plants can be infected by disease-causing organisms, like the bacterium causing Huanglongbing (HLB) and the degree of damage caused by the disease.

That genetic information, containing long strings of chemicals called DNA, is organized in individual units, called genes, that specify traits, like a lemon's tartness or a tangerine's color. All of the genetic information, called a genome, is like a collection of books with information on many topics. Each organism has its own set of books – with some of the same information and some different - making lemons look and taste different from oranges and limes.

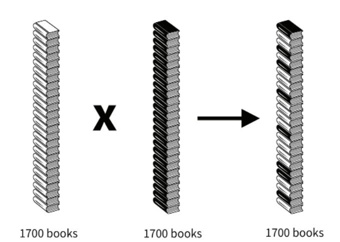
If alphabetic letters are used to represent each chemical in the long DNA string, it would require about 35 books, each of 1000 pages, to contain all information in a citrus cell. All of the chemical units in sweet orange are known, revealing nearly 25,000 genes.

How is classical breeding used to make new citrus varieties?

Most plants develop roots, stems and leaves after seeds germinate. But mature trees of citrus, and other tree crops, grow from two parts of other trees, grafted together. The rootstock is mostly below ground. The scion forms the tree's upper part, its trunk, branches, leaves and, most importantly, fruit.

One way breeders create new citrus varieties is to select rootstocks and scions that provide different improvements. In rootstocks, they look for traits for tree size, yield, disease and insect tolerance, and scion compatibility. In the scion, they look for fruit size and flavor, rind and flesh color, ratios of acid and sugar and the numbers of seeds.

Classical Breeding



To create a new rootstock, or scion, breeders cross two citrus varieties. In crosses, pollen (male, sperm cells) is taken from one tree and delivered to the egg (female cell) of another tree. Male and female cells fuse, divide and become seeds for the next generation, which has new mixes of genes and traits. Many common citrus types, like oranges, lemons, and limes, originated long ago from such crosses of citrons, mandarins and other early varieties. For example, the sweet orange came from a cross between a mandarin and a pummelo. What happens to the 35 books of genetic information from each parent? Genetic rules dictate the resulting tree can only have 35 books, half randomly received from one parent, half from the other, resulting in next generation trees with unique combinations of genes and traits.

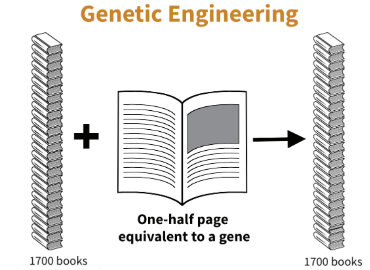
Other methods of creating new citrus varieties

Besides classical breeding, the order or nature of the chemical units can be changed the through effects of sunlight on DNA. This action results in mutations, or genetic changes. The pink-fleshed, navel orange, Cara Cara, arose from a mutation in a Washington navel orange tree. Irradiating citrus budwood can also act like sunlight and rapidly induce genetic changes, an approach that scientists used to create the low-seeded mandarins Tango, Daisy SL, Fairchild LS and Kinnow LS.

More recently breeders have used marker-assisted selection (MAS), which involves creating a "table of contents" with tags for locations of genes specifying certain traits. Breeders use the naturally occurring DNA tags to identify whether desired genes are present. In citrus, MAS has been used on a very limited basis in, for example, rootstock breeding programs to introduce citrus nematode resistance.

What is a genetically engineered (GE) organism or GMO?

Genetic Engineering



DNA in all organisms is written in the same chemical language, so genetic information can be exchanged among organisms. How could this be used to improve citrus? Once genetic information for useful traits is identified, it can be introduced into genetic information in the citrus cell, using a cut and paste process, like that in a word processing system. Once the trait's chemical text is found (equal to about a half-page of information), chemical scissors are used to remove the information, and chemical paste is used to place that information stably into the DNA of the same or another plant.

Continued on following page

This process, termed genetic engineering (GE) , gives rise to plants with modified genetic information – often termed GMO (genetically modified organism).

How can breeding and engineering methods be used to protect against HLB?

Citrus fruits and their relatives originated in South-east Asia, New Caledonia and Australia and they have been cultivated in tropical and temperate parts of the world for more than 2000 years. During that time, natural crossing and mutations have occurred but, since the late 1980's, breeders have made intentional crosses and identified natural mutations, like the pink flesh of the Cara Cara orange. But fruit tree breeding is slow, taking 10-15 years to identify parents with desirable traits, crossing them and then waiting for fruit. After that, a few more years to insure there are no unwanted characteristics and trees and fruits thrive. In order to use conventional breeding to combat HLB, there must be a source of resistance within a sexually compatible variety and to date this has not been identified within citrus.

In citrus, genetic engineering has been used in experimental approaches to protect against diseases, to provide certain insect resistance, to create dwarf varieties and to afford drought and salt tolerance. In more recent experimentS, scientists are now using genes from other citrus varieties to engineer citrus with lower acidity, unique blood orange colors, increased disease resistance and lower levels in grapefruit of the chemical that interferes with statin drugs. Further along, but still experimental, with regard to HLB, a population of genetically engineered sweet orange trees, with a piece of genetic information from watercress, was created in 2017 and is currently being monitored for HLB resistance. Several lines have remained HLB-negative for over five years at a site that has >95% HLB infection. There are other genes, like those from spinach, that are currently being assessed for protection against HLB, thus demonstrating potential benefits from genetic engineering. A new genetic tool, termed genome editing, is being explored to identify new approaches for resistance within the citrus family.

What are the challenges for breeders?

The challenges for breeders using conventional approaches are finding a citrus relative that has resistance to HLB and the long amount of time it takes to do crosses and determine if the approach has created a resistant variety. In the case of genetic engineering, researchers have to identify genes that are effective against the bacterium causing HLB and insert them into the citrus plant. Then time is required to assure that the trees are protected against the bacterium and remain protected over the long-term. Finally, even if an effective approach is found with either classical breeding or genetic engineering, it has to be introduced into all varieties of interest and then orchards have to be replanted.

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