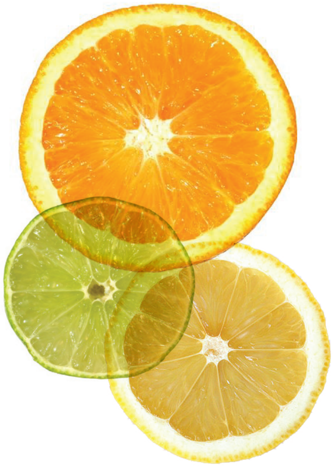

Science for Citrus Health

What makes lemons, oranges and limes look and taste different?

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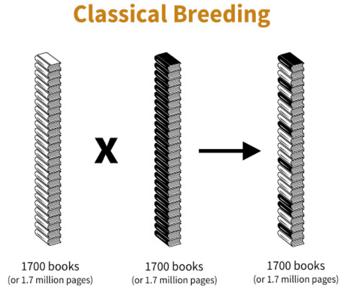
Living organisms, including plants, have many cells. Each cell has the genetic information to dictate the way the plant looks, tastes and feels. It also determines whether plants can be infected by pathogens that cause diseases, like Huanglongbing (HLB) also known as citrus greening.

The genetic information is contained in long strings of chemicals, called DNA. The DNA is organized into individual units, called genes that specify traits, like a lemon's tart taste or a tangerine's orange color. The genetic information, or genome, could be thought of as a collection of books with information on many different topics. Each organism has its own set of books and pages. While some information is similar among different organisms, some is altered - making lemons look and taste different from oranges, limes and mandarins.

If alphabetic letters were used to represent each unit in the long string of chemicals making up the genetic information of citrus, it would require about 35 books, each of 1000 pages, to contain all of the information in the cell of a citrus tree. Identity of the chemical units in sweet orange has been determined, showing that there are nearly 25,000 genes specifying its traits.

How is classical breeding used to make new citrus varieties?

Most plants consist of roots, stems and leaves joined together from the time they germinate from the seed. But citrus, and many other tree crops, are made of two parts that are grafted together to form the mature tree. A rootstock forms the part of the tree that is mostly below ground, and the scion is responsible for the upper part of the tree: trunk, branches, leaves and, most importantly, fruit.



One way breeders create better citrus trees involves selecting for different kinds of improvements in the rootstock and the scion portions of the tree. In rootstock varieties, they look for tree size, yield, disease and insect tolerance, soil adaptation, freeze tolerance, fruit quality and compatibility with the scion. In the scion varieties they look for fruit size and flavor, rind and flesh color, rind thickness, levels of acids and sugars and numbers of seeds.

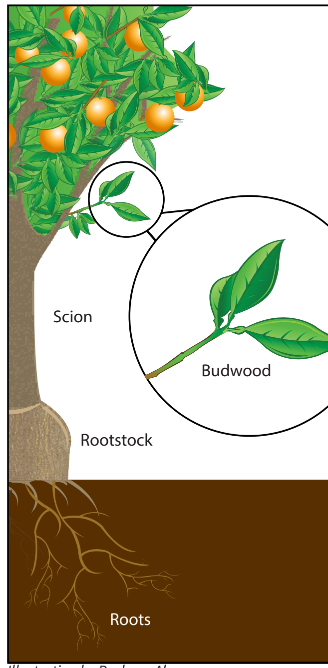


Illustration by Barbara Alonso

What if a breeder wants to create a better rootstock? One way is for the breeder to cross two citrus trees to create a new tree with improved traits. But what happens to the 35 books from each parent? Does the new tree have 70 books of information? No. Genetic rules dictate that the resulting tree can only have about 35 books. This results from randomly keeping about half of the information from one parent and half from the other. Each new tree has unique combinations of genes and traits.

Crosses are made by taking pollen (male cells), from one tree and delivering them via a pollen tube to the egg (female cell) of another compatible tree. The male and female cells come together and ultimately become seeds that create the next generation of trees. Breeders observe the resulting trees, choosing those with desired traits. Seeds resulting from classical breeding yield trees with modified genetic information containing new mixes of genes and traits. Examples in citrus from such breeding efforts are Minneola tangelo and Gold Nugget and Pixie mandarins. It is now known that many common citrus varietal types, such as oranges, lemons, and limes, originated in ancient times by such crosses.

Other methods for creating new citrus varieties

While crossing two citrus varieties can give rise to new trees, there are other ways that can be used to change traits. Sometimes changes in the order or nature of the chemical units occur naturally from the effects of sunlight. This can result in a mutation, or genetic change, that people might notice in a part of the tree. An example is the navel orange, Cara Cara. Its pink flesh resulted from a mutation in a Washington navel orange tree. Irradiating citrus budwood can also induce these genetic changes. The irradiated budwood is grafted to a rootstock and grown for a period of time and if a new trait is observed, budwood from that tree can become a new variety of citrus. Examples of varieties created by irradiating buds are the low-seeded mandarins Tango, Daisy SL, Fairchild LS and Kinnow LS.

More recently breeders have used a process, called marker-assisted selection (MAS). This approach involves creation of a "table of contents" that identifies locations for genes specifying certain traits. MAS is like using the "find" command in a word processing system to identify particular sentences in a book. By knowing phrases in a sentence, text can be found easily in the book. In plant breeding, breeders use the naturally occurring chemical tags in DNA to identify specific genes. If chemical tags are lacking, the plant does not have a particular trait and breeders don't have to wait for the plant to mature to eliminate it from the selection process. In citrus, MAS is used on a very limited basis – more for rootstocks than scions – but, as more information on tags that predict traits becomes available, it will be more widely used.

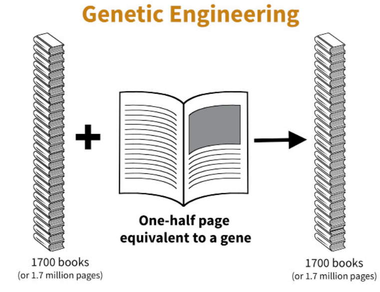
Creation of a new citrus variety by crossing two varieties, whether MAS is used or not, or by the existence or creation of mutations, whether natural or induced by radiation, results in a plant and a fruit with modified genetic information. Exactly the same process happens when two humans have a child that then has genetic information that is different from each parent.

What is a genetically engineered organism or GMO?

What if another citrus variety or another vegetable, like spinach, had a trait that would protect the citrus tree from diseases, like HLB? It turns out that the DNA "language" in in all organisms is the same, so genetic information from any organism, such as spinach, can be transferred to citrus and be used to make a new trait.

How is this done? Once certain information is known about the trait, for example a gene in spinach protects citrus against HLB, that genetic information can be introduced into the tree using a process somewhat like that in a word processing system. Once found, chemical scissors are used to remove the information, and chemical paste is used to place the information into the genetic information of another plant. The new information, equal to a half-page, will be passed on to the next generation.

Genetic Engineering



This process, termed genetic engineering, gives rise to plants with modified genetic information – termed GMO's (genetically modified organisms) by the popular press.

In citrus, the process of genetic engineering has been used experimentally to protect against citrus tristeza virus (CTV) and canker diseases, provide resistance to certain insects, create dwarf varieties and afford drought and salt tolerance. Some of these efforts use genetic information from non-citrus organisms. More recently, efforts have focused on using genes from other citrus varieties to lower acidity, create blood orange color, increase disease resistance and lower levels of the chemical in grapefruit that interferes with statin drugs.

Are genetically engineered (GMO) crops and foods on the market?

While genetic engineering has only been used experimentally in citrus, there are six major GE crop plants in the U.S. that are being grown commercially: alfalfa, canola, corn, cotton, soybean and sugar beet. Most U.S. acreage of these crops is planted in engineered varieties.

Two traits are commonly introduced into GE crops. First, insect tolerance is achieved by introducing a gene from a naturally occurring bacterium, *Bacillus thuringiensis*. This gene codes for a toxin that binds to and destroys specific cells in the guts of particular pest insects. Second, herbicide tolerance, allows engineered plants to survive when that herbicide is sprayed, while weeds are killed.

There is a common misperception among consumers that most foods are genetically engineered. This is because numerous minor ingredients from corn, canola and soybean, like cornstarch, canola oil and soy lecithin, are present in processed foods. But only three engineered whole fruits or vegetables are in the commercial market today, papaya, certain kinds of summer squash and sweet corn. Papaya and squash were engineered to resist destructive viruses. In 2015, FDA approved engineered potatoes with reduced bruising and lower starch and GE apples with reduced browning. These products may be on the market in the future.

Have genetically engineered insects been released?

The same process used to engineer plants can be used to engineer insects, like the Asian Citrus Psyllid (ACP) that carries the bacterium that causes HLB to kill citrus trees. Is it possible to engineer ACP so that it is no longer 'hospitable' to the HLB bacterium? Researchers with the USDA-sponsored NuPsyllid project are trying several ways to engineer the ACP insect so it can not host the bacterium and so no longer transmits the disease. It will take a number of years to see if such strategies are successful in curbing the spread of HLB.

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