



# New acid citrus selections for Florida

Lemon and lime-like selections with niche market potential are being developed with biotechnology at the University of Florida.

By Jude Grosser, Zenaida Vilorio and Manjul Dutt

**A**re you ready for a purple margarita? Would you like a fragrant, juicy lemon for your iced tea with no seeds to clog your straw or dishwasher drain? How about some seedless lime trees that are cold-hardy enough for Central Florida? These and many more interesting acid-citrus selections are now on the horizon, including some with good ornamental potential. This article will focus on progress in using emerging biotechnologies to develop interesting new citrus cultivars in the lemon and lime group. Cultivars include some that will not have regulatory constraints, and also a genetically modified organism (GMO)-derived purple Key lime as a teaser for the future.

## LEARNING FROM THE BANANA

Have you ever wondered why you never find seeds in your bananas? Did you know that there are wild-type bananas growing in some jungles around the world that are full of seeds? The reason commercial banana cultivars do not have seeds is because

they are triploids. People and most citrus trees are diploid, meaning there are two sets of chromosomes in each cell. Triploid bananas have three sets of chromosomes per cell. This disrupts the chromosome pairing mechanism that occurs during meiosis (cell division), resulting in sterility and few, if any, seeds. The Persian

lime is a naturally occurring citrus triploid, which is why it is seedless. The new seedless watermelons in the marketplace are also triploids.

Due to the fact that new and improved citrus cultivars must be seedless to compete in the national and international marketplace, the University of Florida's Citrus Research and Education Center (UF/CREC) citrus improvement team (working with Fred Gmitter) has formulated several ways to create triploids as a key method of developing seedless citrus cultivars, especially in the mandarin group. The team's primary method for producing new, seedless, triploid hybrids is a method called **interploidy hybridization**, which is simply the

conventional breeding of a diploid parent with a tetraploid parent that has four sets of chromosomes per cell.

The team has pioneered a technique called **cell fusion** to produce most of the tetraploid parents used in this approach. Tetraploid hybrids produced by this technique could be called test-tube babies (in reality, they are petri-dish babies). This technique fuses cells isolated from tissue cultures that are **embryogenic**, meaning each cell has the capacity to develop into an embryo, just like you would find in a seed, with cells isolated from leaves of the second complementary parent. (Leaf cells cannot regenerate new trees by themselves.) Use of an embryogenic parent is necessary to provide the capacity for whole-tree regeneration from any fused cells.

Before a fusion can be performed, the rigid, cellulose plant cell walls must be removed to produce naked cells (called protoplasts). These cells still have an intact membrane and internal components. A battery of enzymes under specific osmotic conditions digests away the cell walls. The resulting naked cells can then be induced to fuse, using a chemical called polyethylene glycol.

Following the fusion, cells regrow their walls and begin dividing to form colonies, some of which develop into embryos from which we can recover whole plants. A machine called a table-top flow cytometer is used to determine the ploidy (number of sets of chromosomes) level of the regenerating plants, efficiently identifying any tetraploids. Then, a genetic fingerprinting technique is used to determine if the tetraploid resulted from the fusion of two cells from the embryogenic parent (called an autotetraploid) or the targeted fusion of cells from each parent (called an allotetraploid). Allotetraploid hybrids produced by this technique are called somatic hybrids. The tetraploids are grown in the field, and when they reach the flowering stage, they can be used in

interploid crosses. The majority of the UF/CREC team's efforts using this technology target fresh citrus groups most important to Florida, i.e. mandarins, oranges and grapefruit, but some small efforts in the acid citrus category have been made with very promising results.

## NEW HYBRIDS WITH POTENTIAL FOR COMMERCIALIZATION

Overall, the citrus improvement team has produced more than 20,000 triploid citrus hybrids, including a few hundred in the acid citrus fruit group. Many of these have flowered and fruited, and so far, 10 from this group have been selected that may have commercial potential. All 10 of these have been entered into the Division of Plant Industry Parent Tree Program, and pathogen-free budwood is now available for trials.

Two of the selections have been

approved for release by the IFAS Cultivar Release Committee (Figure 1, page 32). The first selection approved for release is C4-5-27, produced from a cross of Key lime with a tetraploid lemon (we call this a "lemonlime"). It produces nearly seedless fruit that resembles a Key lime in size and flavor, and yields quite well. This selection has ornamental potential as the new growth flushes exhibit an intense red/purple color that remains until leaves are fully expanded, and the flowers are fairly large with a beautiful, dark-pink color. Fruit from this selection does not store well, making it more attractive for dooryard use.

The second selection approved for release is the Iced Tea hybrid lemon, a seedless triploid that is uniquely shaped like a tangelo. Fruit size is larger than a standard lemon. Fruits are juicy with a very pleasant lemon fragrance. This hybrid is two-thirds lemon and one-third sweet orange, so it is

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**Figure 1.** Clockwise from the top left: seedless triploid lemonlime C4-5-27 (approved for release); the Iced Tea triploid hybrid lemon (approved for release); seedless triploid lemonlime C4-9-33; seedless triploid lemonlime C4-5-20.

expected to be slightly more cold-hardy than a standard lemon.

Also featured in Figure 1 are two seedless, triploid lime hybrids — C4-5-20 and C4-9-33 — which we refer to as “lemorlimes,” since they are one-third lime, one-third lemon and one-third sweet orange. Both of these lime-like hybrids produce larger and juicier fruit than Persian lime. The C4-5-20 fruit comes off the tree looking like it has already been waxed in the packing house. The C4-9-33 selection has an attractive green interior and very pleasant limy fragrance. These two selections will be considered for release during the next year. Triploid hybrids of this type produced by interploidy hybridization are not regulated as GMOs, and thus can be readily commercialized. The most obvious downside to this approach is that many

of the more desirable triploids produce excessively thorny trees.

## ANTHOCYANINS AND PURPLE CITRUS

Anthocyanins are naturally occurring compounds in plants and are responsible for the coloration in blueberries, grapes, cherries, pomegranates and many other fruits and vegetables. In citrus, the blood orange contains high levels of anthocyanins, and the purple coloration is due to its presence. Anthocyanins have been studied extensively for their numerous pharmacological properties. Their major roles include antioxidant benefits, ability to protect against free radical scavenging, anti-cancer activity and prevention of cardiovascular disease, among others.



Commercial blood oranges are derived from the sweet orange. They arose through mutations from a sweet orange variety in the Mediterranean region. These blood oranges cannot be commercially grown in the citrus belt in Florida due to their inability to color up in the absence of appropriate cold conditions lacking in our subtropical climate.

The UF/CREC team isolated and subsequently expressed the transcription factor *VvmybA1* (induces the anthocyanin biosynthesis in grape skins) and *Ruby* (induces the anthocyanin biosynthesis in blood oranges) in Key lime using standard genetic engineering technology (Figure 2). Expression of these transcription factors enhanced the anthocyanin production in Key limes and resulted in production of anthocyanin in most plant tissues. The transcription factor was also expressed in the fruit pulp.

These plants have both ornamental as well as potential health benefits, and the fruits with elevated anthocyanin levels are being evaluated. These genes are also currently being incorporated into other selections in the citrus improvement pipeline, including commercial-quality sweet oranges, for the development of a subtropical blood orange cultivar. Having genetically modified citrus with improved nutritional value should help convince the public that new fruits produced by this technology can be beneficial to society.

## CONCLUDING REMARKS

Emerging biotechnologies are creating many new tools to facilitate traditional plant breeding. As a result, many new selections with commercial and niche market potential are being developed and released. High-value, niche-market selections should provide new opportunities for smaller, family-type citrus farmers.



**Figure 2.** Top: A young anthocyanin overexpressing Key lime plant (left) with a regular plant for comparison on the right. Bottom: Cross-section of an anthocyanin expressing Key lime fruit.

More recent work has focused on disease resistance. Disease-resistant parents have been developed and incorporated into interploid crosses. The UF/CREC team will continue to produce new and improved high-quality citrus triploids, and many of them are expected to be more tolerant to HLB, including some in the lemon and lime category. GMO approaches with tremendous potential are also being pursued. 🍊

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