Making Plants Hormone-Deaf

Molecular biologists mute ethylene's control of aging and ripening.

Genes tell plants how and when to make flowers and seeds. They say whether corn, dandelion, or walnut seeds must separate from their parent plants. The separation has a name: abscission. Because of it, plants also drop their wilted flowers and ripened fruit and amputate their diseased stems.

Abscission is Mark Tucker's scientific obsession.

"Few labs around the world are examining abscission at the genetic level," he says.

But Tucker and colleagues have been untangling its molecular script at an Agricultural Research Service laboratory in Beltsville, Maryland.

Abscission zones are thin layers of cells. In response to the hormone ethylene, these cells churn out cellulase and other enzymes that biochemically snip off flowers, leaves, fruit, stems, or seeds. Genes send abscission orders when appropriate to the season, temperature, growth stage, disease or insect threats, or other events.

But ethylene triggers far more than abscission. This gaseous compound, first identified in the early 1900's, also induces ripening and decay as it leads plants throughout the biological journey.

Recently, researchers at Beltsville's Plant Molecular Biology Laboratory (PMBL) isolated two tomato genes that offer new approaches to genetically engineering the control of ethylene in several crops. Goals include preventing, delaying, or promoting ripening and abscission.

We would like to find ways to turn ethylene off when its activity is inconvenient," says Tucker, who is a molecular biologist. Early findings point toward strategies for engineering plants for higher yield, longer storage and shelf life of cut flowers and fresh produce, more efficient harvesting, and reduced losses in storage (for example, yellowing and aging of broccoli and other vegetables).

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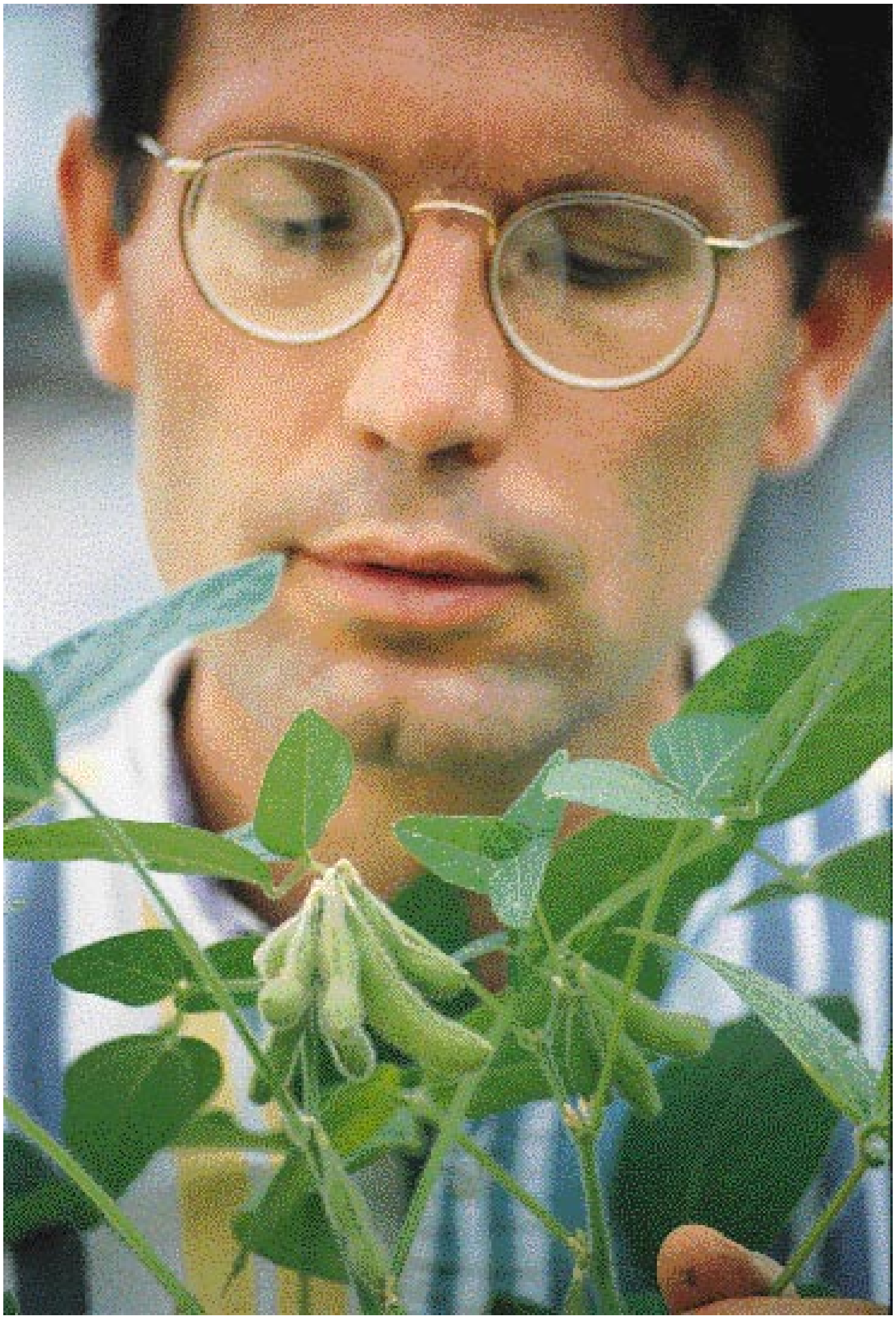

In studying ways to increase yield by reducing the number of flowers and pods that prematurely drop, or abscise, plant molecular biologist Mark Tucker examines the pod set of a healthy soybean plant. (K7331-10)

The ARS researchers seek to build genes that will shut off production of the very first protein that can "hear" ethylene's biochemical message.

Scientists call this ethylene receptor protein ETR1.

"You might not want to disable an *ETR1* gene permanently," Tucker says. "That could make the crop unmarketable. Instead, you may want to turn ethylene on—or off—depending on what's convenient."

Farmers, for example, would generally prefer a whole crop to start and finish ripening on the same time schedule. This usually captures the highest yield. But modified *ETR1* genes might let growers avoid giving up on—or spending extra harvesting time, energy, and labor on—the portion of crop that matures early or late.

Modified *ETR1* genes might also help make crop plants cooperate better with hand or machine harvesting. For perennials like apples and nuts, growers want the trees to gently but surely give up their fruits or nuts. With annuals, the ideal is to harvest only the usable part of the crop. That means less time and labor to cull stems, stalks, and leaves that often get yanked along with bean pods, cotton bolls, or tomatoes.

ETR1 genes may also enable plants to shed fewer of their flowers and develop more of them into fruits and seeds.

"About 70 to 80 percent of soybean flowers drop prematurely because of drought, insects, or other stresses," Tucker notes. "You wouldn't want every flower to stay on, because the plant couldn't support all the resulting soybeans. But holding on to more flowers could increase the potential yield."

Autar Mattoo, who heads the PMBL, says putting a muzzle—or a leash—on ethylene may offer other tantalizing possibilities.

"Physiological evidence implicates this hormone in the infection of tomato plants by root knot nematodes," Mattoo says. Evidence also indicates ethylene increases the severity of fungal diseases on leaves or flowers of tomatoes, peppers, cucumbers, roses, carnations, and other plants.

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In response to the hormone ethylene, plant cells churn out cellulase and other enzymes that biochemically snip off flowers, leaves, fruit, stems, or seeds. (K7332-15)

Many pathogens and insects use plant volatiles, including ethylene, to recognize their targets. So engineering plants to reduce the strength of ethylene's hormonal signal could change the volatile mix and thus help shield plants from pests.

"Ethylene alters the makeup of the natural volatile chemicals that plants emit," Mattoo explains. "Many pathogens and insects use the volatiles, including ethylene, to recognize their targets. So engineering plants to be deaf to ethylene—or reducing the strength of its hormonal signal—could change the volatile mix and thus help shield plants."

ETR1 genes were first isolated in the early 1990's, when two were found in *Arabidopsis*, a mustard relative often used as a "lab rat" in plant gene studies. In 1994, Tucker, Mattoo, and molecular biologist Dingbo Zhou used molecular probes to isolate one of the first *ETR1* genes found in tomatoes. The scientists named the gene transcript *eTAE1*, for extended Tomato Abscission Ethylene library 1.

"We isolated this gene from abscission zones, but it appears to be active throughout the tomato plant," Tucker says. "Its DNA sequences are 74 percent identical—and its amino acids 81 percent identical—to those of an *ETR1* gene from *Arabidopsis*." In 1995, in tomato fruit, the researchers found a different but closely related *ETR1* gene.

Ethylene: A Gaseous Jekyll-and-Hyde

The scientists work with tomatoes partly because they are easy to bioengineer for basic genetic studies. But Tucker says bioengineering a tomato or other plant with a modified *ETR1* gene could offer an alternative to current biotech approaches to controlling the problematic influences of ethylene.

To keep consumers happy, the growers, packers, shippers, and

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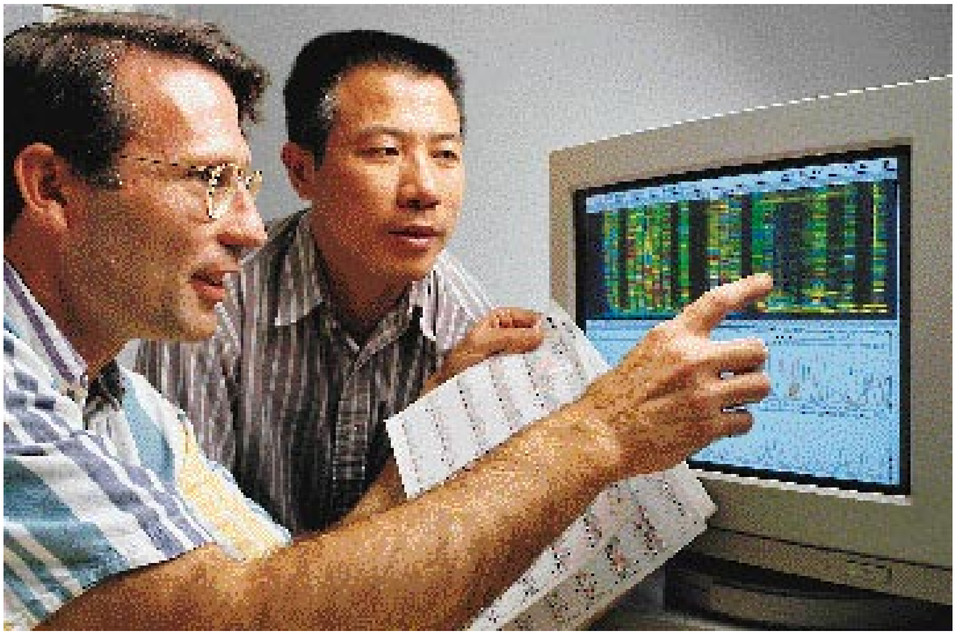

Molecular biologists Mark Tucker and Dingbo Zhou analyze electrophoretic data to confirm the DNA sequence for the ethylene receptor gene, *eTAE1*. (K7330-2)

retailers of tomatoes and other fresh produce try to sustain optimal ripeness and quality as long as possible.

But while ethylene carries fruit and other produce to the peak of ripeness, it quickly pushes them on to excess softening or decay. This Jekyll-and-Hyde trait largely explains why tomatoes grown for long-distance shipping are usually harvested hard and green.

Picked and packed before ethylene makes them soft, red, and ripe, tomatoes stand up well to the rigors of harvesting. The fruits ripen inside their shipping cartons. But their flavor can lack the full development often found in vine-ripened tomatoes from backyard gardens and local farm stands.

To try to improve flavor—and sidestep ethylene troubles—two U.S. companies recently developed commercial varieties of gene-engineered tomatoes. For different reasons, fruit of both varieties may be left on the vine longer to build flavor, but with a reduced risk of mushy tomatoes at harvest time.

One of the new varieties is the Flavr Savr of Monsanto Co. of St. Louis, Missouri. Flavr Savr tomatoes are engineered to make less of an enzyme called polygalacturonase. It and other enzymes cause cell walls to loosen; that is, the enzymes make the fruit soften as it ripens.

The second variety is the Endless Summer of DNA Plant Technology Corp. of Oakland, California. DNAP developed this variety with licensed technology invented and patented by ARS plant physiologist Athanasios Theologis and colleagues at the Plant Gene Expression Center.

The center, in Albany, California, is operated by ARS and the University of California at Berkeley. The DNAP scientists manipulated ethylene's biochemical "front end." They turned down the hormone's volume by disabling an enzyme, ACC synthase, necessary to produce it.

Endless Summer tomatoes make little ethylene on their own. They ripen only in storage—from exposure to pumped-in ethylene gas.

Tucker's approach, instead of turning down ethylene's volume, would stop the hormone by making cells in selected tissues unable to hear and respond to its tune. Plants would make and broadcast ethylene. But its cellular "listening posts," the ETR1 proteins, would be turned off.

To make this approach work, Tucker says, a modified *ETR1* gene will require two features. One is an appropriate gene switch, or promoter. "The best promoter," he says, "may be one that growers or processors can activate; for example, with storage temperature or with a safe calcium-based spray."

A second essential feature would let the modified *ETR1* gene override a plant's pre-existing ones. For this, Tucker wants to exploit a natural, well-known but rare gene mutation in tomato plants. Fruit with the *Never-ripe* mutation ripens only slightly. More intriguing for Tucker, the *Never-ripe* defect disrupts the plant's other, normal genes that might correctly sense and transmit ethylene's messages.

"By inserting a modified *ETR1* gene with *Never-ripe's* dominance feature, we may be able to engineer plants that will not recognize ethylene in selected tissues—and will prevent the plant's other *ETR1* genes from doing so," Tucker says.

The researchers continue to search for potentially useful combinations among many lab-modified *ETR1* genes and promoters.—By **Jim De Quattro,** ARS.

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