PLANT BIOLOGY

# Transferred Gene Helps Plants Weather Cold Snaps

Last month, a cold front swept across the United States, leaving in its wake damaged peach trees, strawberries, blueberries, and other crops. The losses, combined with crop damage from local flooding, added up to $200 million in Georgia alone. But if work by plant molecular geneticist Michael Thomashow and his colleagues pans out, farmers may one day be able to rest easy when a sudden freeze sets in.

On page 104, Thomashow's team, located at Michigan State University in East Lansing, reports that it has created a new, cold-hardy strain of the small plant *Arabidopsis*. The researchers did this by genetically engineering *Arabidopsis*—the plant scientist's version of the lab rat—to overproduce a protein that activates at least four other genes that help the plant withstand the damaging effects of freezing temperatures. Usually, those genes come on gradually when the plant is exposed to slowly declining temperatures. But in the new strain, they are active all the time, enabling it to survive sudden temperature drops to as low as –8 degrees Celsius—4° colder than the normal killing temperature for *Arabidopsis*. "The dream ... has been to take a gene and put it into plants to make them hardy," comments Tony Chen, a plant physiologist at Oregon State University in Corvallis. "This is the first time that has been successful."

Plant physiologists caution that a relatively simple genetic manipulation like the one used on *Arabidopsis* may not be enough to generate crop plants that can endure a sudden freeze, because not all plants may have the same repertoire of cold-tolerant genes. Still, they say that making crops such as corn or soybeans even a little hardier could make a big difference in helping them weather the sudden cold snaps, early or late in the growing season, that often cause the biggest crop losses. "Even though the [Thomashow team's] work was done in *Arabidopsis*," says Charles Guy, a plant physiologist at the University of Florida in Gainesville, "it has profound implications for agriculture."

Researchers started coming across cold-tolerance genes almost 30 years ago. At first, however, they didn't know how the genes worked or whether they could protect plants against freezing as well as against cold. Indeed, 2 years ago, when Thomashow's group engineered *Arabidopsis* with an active form of one of that plant's cold-regulated (*COR*) genes, the researchers found no such protection for the whole plant—although isolated chloroplasts did fare a little better. That might have been at least partially explained by the fact that it takes a large number of the genes to really do the trick. Both *Arabidopsis* and wheat contain at least 25 cold-tolerance genes, for example. But if so, that would present an obstacle to genetic engineering of cold-tolerant plants because achieving stable transfer of that many genes is not feasible.

Plants coordinate the expression of their cold-regulated genes, however, turning them all on together as temperatures drop. And

MARY ANN CHASTAIN/AP

**Chilled out.** In the southern United States, a late winter freeze killed strawberry blossoms.

in 1994, three research teams, including Thomashow's, made a discovery that suggested it might be possible to take advantage of that synchrony. Genes are activated by proteins called transcription factors, which bind to regulatory sequences, and the teams found that the known cold-regulated genes carry the same regulatory sequences—an indication that they are all turned on by the same transcription factor.

At that time, the factor had not been identified, but Thomashow, working with Michigan State plant geneticist Eric Stockinger and plant molecular biologist Sarah Gilmour, found the gene for the transcription factor in late 1995. Thomashow then realized, he recalls, that the discovery of the gene "opened the door to using this transcriptional activator to enhance the freeze tolerance of crops" by turning on the whole battery of *COR* genes at once.

In the current work, his group tried to achieve that by attaching the transcription factor gene, called *CBF1*, to a regulatory sequence that ensures it will be active all the time. They then inserted the modified gene back into *Arabidopsis* plants. As the researchers expected, these transgenic plants began producing the COR proteins even under normal conditions. When they then froze and thawed leaves from the modified plants, they found "a dramatic enhancement in freezing tolerance," says Thomashow.

To see how well the COR proteins were protecting the cell membranes, the researchers measured the ability of the membranes in the thawed leaves to regulate the flow of ions in and out of the cell. They found that the leaves from the transgenic plants did as well as leaves from plants that had had the chance to acclimate to cold weather. In addition, the team reports, whole plants that had been frozen and then thawed survived, as did plants that were first allowed to acclimate to dropping temperatures. The work "is really a tour de force for the field," notes geneticist Gary Warren of Imperial College, London. "[Before], we didn't know for sure that cold-regulated genes were even necessary for freezing tolerance, and now he's gone and shown they are sufficient."

Recent research is also clarifying how the genes protect against freezing. At least some code for proteins that seem to protect plant cell membranes against the disruption that results when freezing or other dehydrating conditions deplete the water molecules normally found in the space between the membrane and the tough cell wall. As molecular biologist Fathey Sarhan of the University of Quebec in Montreal reports in April's *The Plant Cell*, cold-related proteins in wheat build up in this gap and seem to help keep the membrane intact.

What's more, cold-tolerance genes in important crops may be amenable to similar genetic manipulations. Sarhan says his group has data from wheat "that support the [Thomashow] result that most cold-regulated genes could be controlled by a master switch." They haven't found the switch yet but think that it could reside in one or more genes located in a small region of the wheat chromosome 5.

These demonstrations of the importance of cold-tolerance genes have spurred Thomashow's university, Michigan State, to file for a patent on the *CBF1* gene and its use in generating hardier plants. His group is now seeking commercial support for pursuing these efforts in crops. It's not a sure thing, however. Warren points out that in crops that lack freezing tolerance, such as citrus, adding an activated *CBF1* gene may not have the same effect it does in *Arabidopsis*, because they may have lost all or part of their repertoire of cold tolerance genes. But if the approach does work, perhaps farmers will no longer have to watch their strawberries be nipped in the bud by a late freeze.

**–Elizabeth Pennisi**