PEGGY GREB (D738-1)

A Plum That Stands Up to Pox

Combining classical and molecular techniques to protect a favorite fruit

HoneySweet plums, sweet and flavorful, are highly resistant to plum pox virus.

Every day, folks enjoy eating tasty, nutritious plums. Whether eaten as fresh or dried fruit, plums provide a wholesome dietary choice, particularly for people on the go. But a nasty virus of stone fruit trees could steal those snacks away.

Plum pox virus, or PPV, has already destroyed well over 100 million stone fruit trees throughout Europe. In 1999, PPV was first found stateside in Pennsylvania. Despite extensive eradication efforts, the virus was reported in 2006 for the first time in New York and Michigan. Fortunately, an international group of public researchers has succeeded in producing a PPV-resistant plum variety—named "HoneySweet"—as a preemptive measure to protect thriving plum industries.

Since 1990, ARS horticulturist Ralph Scorza has teamed with molecular virologist Michel Ravelonandro, of INRA, a French counterpart to ARS located in Bordeaux, and ARS geneticist Ann Callahan to develop and test the strength of HoneySweet's virus resistance. Scorza and Callahan are located at the Appalachian Fruit Research Station in Kearneysville, West Virginia.

Their cooperators have included Dennis Gonsalves, then at Cornell University in Geneva, New York, now center director for ARS at Hilo, Hawaii, and plant pathologist Vernon Damsteegt, with ARS's Foreign Disease-Weed Science Laboratory at Fort Detrick, Maryland. The team also has collaborators in Spain, Poland, Romania, Chile, and the Czech Republic.

They used a system to regenerate plum trees from embryonic cells that were genetically enhanced to resist PPV. Regeneration is a time-consuming, delicate process used to produce whole trees whose inherent DNA—and that of their progeny—contains the new trait.

How does this plum fend off the destructive virus?

"One of HoneySweet's unique genetic traits is that it prevents the virus from making the proteins it needs to spread," says Scorza.

Starting With the Best

To endow modern crop varieties with just the right mix of desirable traits, plant breeders painstakingly accept or reject dozens of genes through many generations of crosses. To produce HoneySweet, the researchers combined the benefits of age-old breeding with new molecular tools.

"We started with germplasm from Bluebyrd—a commercial variety developed at Kearneysville through more than 20 years of traditional breeding," says Scorza.

Next, they extracted tiny embryos—only a few millimeters long—from Bluebyrd seeds, sliced them, and placed them on a growth medium.

The researchers then isolated a gene from PPV that codes for a protein in the virus's outer coat. They then inserted this gene into the bacterium *Agrobacterium tumefaciens*. This bacterium acted as an effective carrier of the desired gene into Bluebyrd's embryonic tissue. Biotechnologists use *Agrobacterium* as a genetic transporter because of its ability to inject any gene of choice. The embryonic tissue that received the genes was then put into a regeneration medium containing various additives, including nutrients and growth regulators. The plants that developed contained the introduced gene.

HoneySweet uses the small pieces of RNA produced by its new coat-protein gene to recognize PPV as an invader and activates its defenses against the virus once exposed.

"HoneySweet produces no detectable coat protein—even though it contains the coat-protein gene—because of the way it was inserted into the plant," says molecular biologist Christopher Dardick, also with ARS in Kearneysville. "The mechanism, termed 'gene silencing,' has recently been shown to be a natural way plants fend off viruses. This plum has the ability to seek out and specifically degrade the genetic material of the invading virus."

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Horticulturist Ralph Scorza (right) and technician Mark Demuth inspect ripe HoneySweet plums.

Passing the Test

The PPV-resistant plum variety has passed a long line of resistance-gauging tests conducted during the past 16 years. HoneySweet was found to be extremely resistant in three European countries where the virus is well established. No trees were found to be infected after exposure to aphids, the insect that normally transmits the disease. The researchers worked with the approval of all required European regulatory agencies to test HoneySweet in isolated and protected areas under strict confinement.

"The completion of this test phase—the most comprehensive testing to date—in areas of Europe where PPV is endemic underscores HoneySweet's success as a tree that's extremely resistant to plum pox virus," says Scorza. "This success also reflects the potential value of using this innovative technology to protect other stone fruit crops vulnerable to PPV or other viral diseases."

HoneySweet may only be grown in U.S. orchards for sale or consumption after its safety has been determined by all three agencies that regulate genetic engineering of crops in the United States—the USDA Animal and Plant Health Inspection Service (APHIS), the U.S. Food and Drug Administration (FDA), and the U.S. Environmental Protection Agency (EPA). In July 2007, APHIS approved an ARS-submitted petition seeking a determination of nonregulated status for HoneySweet. With this deregulated status, HoneySweet and its progeny can be freely moved and planted without the requirement of permits or other regulatory oversight by APHIS. ARS has also submitted documentation on HoneySweet to FDA for consultative review. And an application for regulatory review and registration has been filed with EPA. More information about the HoneySweet plum tree can be found at www.ars.usda.gov/is/br/plumpox.

Once approved for safety and effectiveness, PPV-resistant HoneySweet could potentially be available for propagation by nurseries in 5 years, according to the researchers. Full-grown trees are able to provide budwood—used in grafting—to ramp up the number of trees available.

HoneySweet is a productive variety in its own right—capable of flourishing in mid-Atlantic, eastern, and northeastern U.S. regions. And because HoneySweet trees transfer their viral resistance to their seedlings, breeders can use the germplasm to create new resistant varieties once it is approved.

"If HoneySweet were to be used as a parent in breeding programs, additional varieties could emerge with ideal characteristics for other growing seasons and industry requirements," says Scorza. "For example, the dried-fruit and fresh-fruit industries have different varietal preferences. And breeders could further develop cultivars to adapt to conditions in other U.S. regions."

Once approved for planting, HoneySweet and its progeny hold promise to help protect the future supply of healthful and delicious plums.—By **Rosalie Marion Bliss,** ARS.

This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement (#301), an ARS National Program described on the World Wide Web at www.nps.ars.usda.gov.

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Postdoctoral associate Jean-Michel Hily (left) and technician Kevin Webb evaluate growth of genetically engineered plums in tissue culture. These new lines will provide additional plum-pox-virus-resistant germplasm for plum breeders and new resistant plum varieties.