Finding New Ways To Protect and Preserve Plant Genetic Resources

"To protect and to serve" is the motto for the Los Angeles Police Department. The motto for the National Center for Genetic Resources Preservation in Fort Collins, Colorado, could be "to protect and to preserve."

The ARS center stores more than 450,000 samples of plant and animal germplasm, such as seed and semen. The Plant Germplasm Preservation Research Unit (PGPRU) at the center researches ways to manage the collection more efficiently and to get germplasm to survive longer.

Making a collection of germplasm is called genebanking—and it's not as simple as just filing seeds away. Some germplasm does not store well, so scientists must discover ways to extend shelf life. To reduce the cost of genebanking, scientists must ensure that the collection doesn't include unnecessary germplasm but is still large enough to include valuable genes. The PGPRU researchers are using genetic tools and mathematical models to help "right-size" the collection so that it includes much of what researchers and plant breeders need but is not too unwieldy.

The following examples show how PGPRU scientists solve genebanking problems to provide breeders, researchers, and growers the genetic resources needed for healthy agricultural systems at minimum cost to taxpayers.

STEPHEN AUSMUS (D050-6)

These wild-rice plants are being grown in the greenhouse in a study of how their seeds develop. Plant physiologist Christina Walters (left) and technician Lisa Hill collect the seeds and label flowers.

Preserving the Unpreservable

Consumers love wild rice. But breeders have had their work cut out for them to bring this crop into mainstream cultivation, because the seeds don't survive long.

"The seeds are called 'recalcitrant' because they can't be preserved easily. Breeders are losing valuable genetic stocks every year," according to plant physiologist Christina Walters, research leader of the unit.

Usually wild-rice breeders store seeds in a conventional refrigerator. Unfortunately, seeds stored this way may not survive even a full year and thus need to be grown out every spring.

Walters and her staff are trying to find better methods. They have found that the water content of seeds can be optimized—

making them neither too wet nor too dry. Some drying slows down seed aging and germination but does not hurt the seed. Drying also means less chance that lethal freezing will occur, so the seeds can survive at lower temperatures for longer times. Walters's group has shown that wild-rice seeds can be stored for at least 3 years at –5°C.

Storing wild-rice seeds longer is possible, but it is labor intensive, because the embryo must be dissected from the seed and then cryopreserved, that is, rapidly cooled in liquid nitrogen.

Which Garlic Is Which?

When germplasm like wild-rice seed is labor-intensive to preserve, it makes sense to store only what is needed and not waste efforts by inadvertently storing the same thing over and over again. But for some plants, like garlic, preventing duplication is a challenge.

Because garlic does not reproduce by seeds, varieties are kept as clones—that is, individuals with exactly the same genes (like identical twins). This means that bulbs from California Early garlic, for example, are clones of the same individual, whether they're purchased in Washington State or Washington, D.C.

Different varieties of garlic arose from mutations that occurred over the years. Growers gave names to the new garlic types, but over time the names changed or similar names were given to different garlics. It is now impossible to distinguish among garlics by variety name. It is also difficult to identify garlics by appearance, since cloves from the same bulb grow differently in different locations.

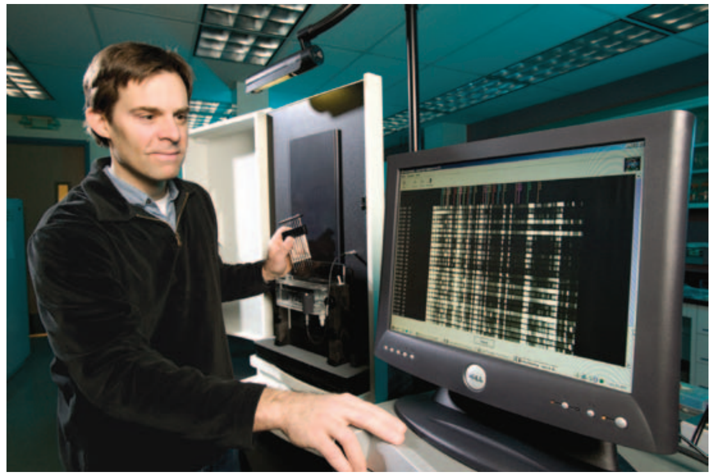
To reconcile the problem, a team led by plant physiologist Gayle M. Volk used DNA markers to examine groups of garlic varieties and determine how much diversity exists among them. The team conducted a genetic analysis of 211 garlic accessions using what's known as "amplified fragment length polymorphisms." They found that many accessions bearing different names were in fact virtually indistinguishable. But there were also plenty of unique accessions. The garlics can now be tracked by genetic identity rather than name, helping genebankers to preserve the most diverse garlics first.

Preserving Pines

Bristlecone pines could be considered Earth's oldest living inhabitants, since some of them may be 5,000 years old. Unfortunately, the bristlecone pine is being threatened by white pine blister rust, which has devastated populations of white pines in the Northwest and is rapidly moving through the Rockies. Currently, germplasm of this legacy species is not preserved in any genebank.

Geneticist Christopher M. Richards, in collaboration with U.S. Forest Service scientist Anna Schoettle, is trying to assess diversity of bristlecone pines and to identify, for collection, germplasm that represents what is in the wild.

STEPHEN AUSMUS (D053-11)



In studies of genetic diversity of bristlecone pines, molecular markers allow unique specimens or populations of trees to be identified. Here, geneticist Chris Richards loads an automated DNA sequencer for genetic analysis.

STEPHEN AUSMUS (D056-58)

These garlic shoot tips were immersed in droplets of cryoprotecting solution, then plunged into liquid nitrogen to preserve genetic diversity of the vegetatively propagated germplasm.

STEPHEN AUSMUS (D054-6)

Using a cryomicroscope, plant physiologist Gayle Volk (left) and technician Ann Caspersen examine changes that occur in cell structure during cryoprotection.

“We have to identify individual trees that have important genes, such as resistance to blister rust,” explains Richards. “Pollen from this species travels large distances, so there’s a lot of genetic mixing. We need to look at genetic diversity tree by tree.”

In his work detecting genetic diversity, Richards is using DNA-based markers called “retrotransposons,” or “jumping genes.” Sequences of these genes help uncover hidden variation within species.

Retrotransposons are genetic elements in the genomes of many living organisms, including humans, that are replicated in response to some unknown environmental cue. After several replications, there is an increasingly long trail of DNA insertions, which can be used as a DNA fingerprint. This new DNA sequencing tool is helping to distinguish tree families and identify individual trees with important genetic differences.

Guaranteeing Grapes

Just like bristlecone pines, grapes are threatened by a pathogen, in this case *Xylella fastidiosa*. This bacterium, which causes deadly Pierce’s disease, is spread by the glassy-winged sharpshooter, an insect that has invaded grape-growing regions around Davis, California, and elsewhere. Davis is also home to one of two ARS regional genebanks that specialize in preserving grapes. (The Fort Collins center acts as a backup to all the regional genebanks.)

Says plant physiologist Leigh E. Towill, “There are more than 3,300 lines of grapes preserved by ARS, and all are field-maintained,” making them vulnerable to disease or other natural disasters. Towill’s main focus is cryopreserving plant cuttings from the field collections.

“Cryopreserving these lines is an effective backup strategy that will save money and ensure the germplasm is safe.”

To buy time while other scientists battle Pierce’s disease, Towill has developed a method to store grape scions for 18 months at –3˚C. That way, if something were to happen to the Davis field collection, the grape lines could be quickly restored by simply rooting vines that were in storage.—By **David Elstein,** ARS.

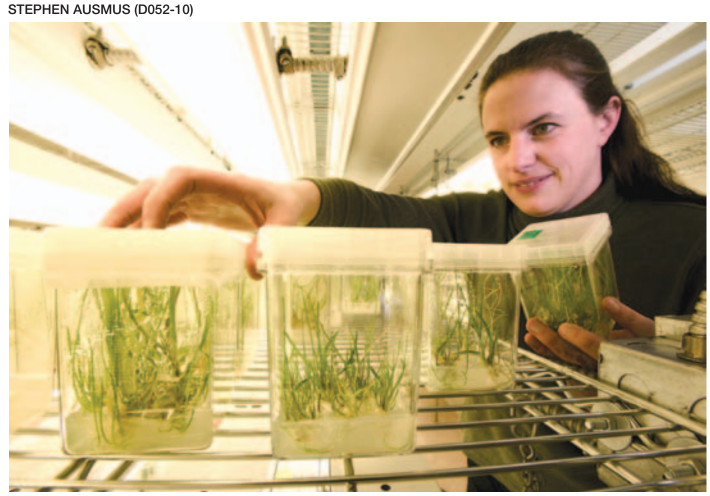
This research is part of Plant, Microbial, and Insect Genetic Resources, Genomics, and Genetic Improvement, an ARS National Program (#301) described on the World Wide Web at www.nps.ars.usda.gov

Christina Walters, Gayle M. Volk, Christopher M. Richards, and Leigh E. Towill are in the USDA-ARS Plant Germplasm Preservation Research Unit, National Center for Genetic Resources Preservation, 1111 South Mason St., Fort Collins, CO 80521-4500; phone (970) 495-3200, fax (970) 221-1427, e-mail chrisv@lamar.colostate.edu, gvolk@lamar.colostate.edu, crichard@lamar.colostate.edu, ltowill@lamar.colostate.edu. ✷

STEPHEN AUSMUS (D055-16)

Technician Remi Bonnart (foreground) flash-freezes plant shoot tips while Christina Walters places cryopreserved materials into cryovats for long-term storage. Droplets of cryoprotecting solution hold shoot tips onto foil strips. The strips are plunged into liquid nitrogen slush that is formed by placing liquid nitrogen in a vacuum.

STEPHEN AUSMUS (D052-10)



Plant physiologist Gayle Volk inspects garlic plants growing in culture. Shoot tips of garlics were cryopreserved because these plants represent the greatest genetic diversity in the collection.