BIOTECHNOLOGY

Genetic Engineering Yields First Pest-Resistant Seeds

Over the past decade, plant genetic engineers have racked up impressive successes in designing crop plants with improved resistance to insects and viruses. So far, that research has focused on endowing plants with genes that give them a survival edge in the field. But for many important crops, including cereal grains, beans, and peas, the danger from pests doesn't end with the harvest. Weevils and other insects may cause losses just as great—or greater—during storage. The problem is particularly acute in developing countries, where farmers can rarely afford protective chemical fumigants. Brazilian farmers, for instance, lose a staggering 20% to 40% of their beans to storage pests, leading to periodic food shortages, says plant molecular biologist Roger Beachy of the Scripps Research Institute in La Jolla, California.

Now, a team of U.S. and Australian researchers has shown how biotechnology can combat pests in the storage bin as well as in the field. In the August issue of *Bio/Technology*, the team, led by molecular biologist Maarten Chrispeels of the University of California, San Diego (UCSD), plant biologist T. J. Higgins of CSIRO's Division of Plant Industry in Canberra, Australia, and entomologist Larry Murdock of Purdue University in West Lafayette, Indiana, reports having created a strain of garden pea that resists attack by two weevil species that damage stored crops. The genetic stratagem the researchers used involves inserting a foreign gene into the pea plants that triggers production of a protein that inhibits feeding by weevil larvae; the protein is expressed only in the pea seeds.

This achievement, which marks the first time that seeds have been genetically engineered for pest resistance, is expected to pave the way for new varieties of weevil-protected legumes, including some that are important sources of protein for people around the world, such as chickpeas, cowpeas (black-eyed peas), mung beans, and kidney and pinto beans. "This kind of biotechnology, in which you get post-harvest protection of the seed, is going to be extremely useful in the developing countries," predicts Indra Vasil, a plant molecular biologist at the University of Florida in Gainesville and chair of UNESCO's Biotechnology Action Council.

Chrispeels, Higgins, and Murdock's interest in genetically engineering desirable new traits into legume seeds merged about 4 years ago. Several groups had already provided one important piece of information: In the early 1980s they identified a DNA sequence that acts as a switch for turning genes on in seeds. Chrispeels knew just which gene he wanted to get this switch to turn on: the gene for a seed protein called α-amylase inhibitor, which had just been cloned in his lab. Several researchers, including Murdock working with Richard Shade of Purdue, had shown that the inhibitor protein protects seeds of the common bean (*Phaseolus vulgaris*) from two storage pests, the cowpea weevil (*Callosobruchus maculatus*) and the Azuki-bean weevil (*C. chinensis*).

Protected. The pea seeds on the left have been genetically engineered to make them resistant to weevil larvae.

The inhibitor apparently exerts its protective effect by blocking the action of the starch-digesting enzyme α-amylase, thus preventing weevil larvae from digesting the starches in the seeds. Transferring this pest-resistance trait to seeds of other legumes seemed like a very attractive possibility, says Murdock, because moving a gene from one food plant to another raises fewer safety concerns than, say, putting a gene from a virus or bacterium into a plant. But he and his colleagues weren't sure which bean or pea species should be the recipient, since genetic engineers hadn't had much luck working with leguminous plants.

The catch came in the second part of the two-step process whereby foreign genes are introduced into plant cells. A gene is put into cells of the target plant in culture, after which whole plants must be regenerated from those cultured cells. Plant regeneration, however, is as much an art as a science, and researchers have had trouble finding the right conditions for growing legume plants from cultured tissue. Says Vasil: "There's this impression that these crops are very difficult, so many people have shied away from working on them."

Defying this perception, Higgins and Hartmut Schroeder dove into the problem and by last year had reported a reliable protocol for introducing foreign genes into the garden pea (*Pisum sativum L.*), a legume Murdock and Shade had found to be vulnerable to the cowpea and Azuki-bean weevils. The key to their success, says Higgins, was the discovery that they could achieve reliable regeneration only by using embryonic pea axis tissue (the part that develops into the plant stem); this tissue is available for just a few days during development.

While this protocol was being worked out, Chrispeels' lab constructed a hybrid gene containing the seed-specific switch attached to the α-amylase inhibitor sequence. The CSIRO and UCSD researchers used this hybrid gene to make their transgenic pea plants and then sent the resulting seeds to Purdue for testing against weevils.

The results were striking. Not only did pea seeds from the transgenic plants make the α-amylase inhibitor, producing up to 1.2% by weight of the inhibitor protein, but "lo and behold," says Murdock, "the peas that were the high expressers were the ones most resistant to the weevils." Nearly every Azuki-bean weevil that fed on peas with a detectable amount of the inhibitor died, and those that survived developed more slowly than normal. The effects on cowpea weevils were less dramatic, although their development was also inhibited.

"This opens the way for putting all kinds of interesting genes into peas and into lots of other legumes as well," says Higgins, who envisions using the technique to raise the protein content of legume seeds. There's also potential for using other insecticidal genes from plants, although Chrispeels cautions that much remains to be learned about which plant genes encode chemical defense proteins and which pests they deter.

While the hunt for additional genes to insert into peas goes on, researchers are working out the protocols for gene transfer in other legumes. As Vasil notes, now that this has been done for the garden pea, "it's really just a matter of time and effort" before it will be accomplished for other legumes. And when that happens, the seeds of a better fed future for the developing countries will have been well and truly planted.

–Karen Schmidt

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