Biotechnology

Dawn D. Bennett and Julie Ann Miller report from the USDA symposium in Beltsville, Md., on Biotechnology for Solving Agricultural Problems

Bold approach to gene engineering

Although scientists are making rapid progress in the transfer of single genes into plant cells, most traits important to agriculture depend on the coordinated activity of a large set of genes, often with more than 100 members, says Robert J. Griesbach of USDA's Agricultural Research Service (ARS) in Beltsville, Md. Such traits include the ability to withstand high salt levels or drought periods or to coexist with nitrogen-fixing bacteria. Sometimes the genes behind a trait are scattered throughout the plant's genetic material, but often they appear to be clustered on a single chromosome. Griesbach has developed a technique to transfer a single chromosome, or a piece of a chromosome, into a foreign plant cell. He now reports the first evidence of expression of the genes transferred.

Griesbach uses a fine glass needle to inject a chromosome into a plant cell. Although scientists have been successfully injecting chromosomes into animal cells for five years, major obstacles have slowed the work on plants. First, the rigid cell wall has to be removed with enzymes, leaving a cell called a protoplast. Second, a method had to be developed to avoid puncturing the large structure, called the central vacuole, which contains toxic materials that can kill the cell.

Griesbach removes the vacuole by spinning the protoplast in a centrifuge. The vacuole contains oily chemicals, so it rises against the centrifugal force in the spinning protoplast and eventually is pinched off. When Griesbach, working with petunias, injects a chromosome into the remaining protoplast, about 25 percent of the protoplasts are capable of regenerating into full plants. In about a third of these cells the new chromosome is stably inherited when the cells divide, and Griesbach has evidence from 2-dimensional gel electrophoresis that the foreign genes direct production of proteins.

Jumping genes in soybean?

Pieces of DNA that move from one position to another within chromosomes, turning off or on the more sedentary genes they invade, have been well studied in maize, but few have been described in other plants. Now Lila O. Vodkin, Patsy R. Rhodes and their colleagues at ARS report the first evidence for such a mobile element, often called a transposon, in soybean plants. They believe transposons will be useful for identifying and isolating plant genes of agricultural interest, and transposons may eventually serve as a genetic engineering tool for carrying genes from plant to plant.

The ARS scientists discovered the soybean transposon during their genetic analysis of lectin, a major protein of the soy seed. A few varieties of soybean produce no lectin at all. Vodkin and her colleagues found that the lectin gene in a lectin-less variety is interrupted near the middle by a large piece of DNA, which prevents the gene's function. They next observed junctions between the lectin gene and the insert that are characteristic of mobile elements. Vodkin has named the soybean element Tgm1 — indicating it is the first transposon of soybean, which has the species name *Glycine max*.

The newly discovered soybean transposon has surprising similarities to elements of other plant species, Vodkin says. It resembles transposons recently identified in snapdragons and in corn. These soybean, snapdragon and corn transposons all have unusual structures containing many repeats of short DNA sequences that shape the molecule into a series of linked "hairpin" structures.

So far Vodkin has no direct evidence that Tgm1, identified as a transposon on the basis of its structure, moves in and out of the soybean gene. She is searching for the Tgm1 in other soybean genes, especially those that act like corn genes known to harbor transposons and create a variegated pattern — for instance, dappled green and yellow coloration in the leaves.

Poultry coccidiosis vaccine on horizon

Poultry farmers and chicken lovers take heart. Scientists are one step closer to developing a vaccine against coccidiosis, a parasitic disease that costs the U.S. poultry industry $300 million a year. The disease also causes chickens to have a skin color paler than the yellow color desired by consumers.

With monoclonal antibodies produced by the ARS, Russell McCandliss and his colleagues at Genex Corp. of Rockville, Md., used genetic engineering to produce a characteristic protein of one major species of coccidia. When injected into chickens, this experimental antigen stimulates production of antibodies, McCandliss says.

Coccidial protozoans attack birds' intestinal tracts, killing them or weakening them by interfering with efficient feed conversion. Antigens of some coccidial species induce an immune response that keeps the parasites from penetrating intestinal cells, while others cause the immune system to block the parasites' development once they are inside cells. The ARS and Genex researchers do not yet know how the bioengineered antigen they are using works.

ARS microbiologists Harry Danforth and Patricia C. Augustine used standard hybridoma (monoclonal antibody) technology to develop antibodies that were then used by Genex to isolate coccidial antigens. The ARS researchers took spleen cells from mice that had been injected with coccidia and were producing antibodies against the parasites. They then fused the spleen cells with mouse cancer cells growing in cultures. The cancer cells reproduce rapidly, producing large amounts of the antibodies.

Danforth cautions that the genetically engineered antigen as it exists now provides only partial protection. He hopes further research can alter it to provide more complete protection.

Other possibilities for introducing the coccidial antigen into birds, Danforth says, include isolating the gene coding for the antigen and inserting it into vaccinia viruses or putting the antigen into birds' feed or water. Direct insertion of the antigen gene coding for coccidial resistance in some chicken species is possible, but a long way off, he says. "We haven't considered cloning the gene and putting it into the chicken line yet, but that would be a very interesting prospect."

Pesticide-eating bacteria march on

Bacteria that degrade toxic substances are nothing new, but microbiologist Jeffrey Karns of the ARS has just added two important examples to the list. Karns recently described how enzymes produced by *Flavobacterium* degrade coumaphos, a pesticide used to kill insect pests of livestock, and how *Achromobacter* enzymes degrade carbofuran, a pesticide used to control corn rootworm and other crop insects.

Coumaphos is a "recalcitrant" molecule that stays in the soil for a long time before being broken down. Although coumaphos is water insoluble and thus doesn't pollute groundwater, it can be toxic to living things while it remains in the soil.

Previous attempts to degrade coumaphos waste have focused on ozonation, exposure of the pesticide to ultraviolet light. This did not work, Karns says, because coumaphos is a turbid solution and could not be destroyed by the light. But he and his colleagues found that incubating the waste with *Flavobacterium* beforehand degrades it to chlorferon, a clearer solution that can then be further degraded by ozonation.

Carbofuran can be degraded by *Achromobacter,* a bacterial species that uses the pesticide as its only source of nitrogen, Karns says. These bacteria also degrade several other N-methyl carbamate insecticides, he says.

Karns is working on cloning the genes coding for the degradative enzymes of *Flavobacterium* and *Achromobacter.* "If this can be done," he says, "pesticide degradation will be more efficient because fewer [bacterial] cells will have to be used."