Agricultural Applications of Genetic Engineering

The "green revolution" of 30 years ago depended mainly on the plant breeder's art to develop high-yielding crop plants, such as rice and corn. More recently, agricultural scientists have acquired new tools—those of recombinant DNA technology and genetic engineering—with which to tackle crop improvement and other agricultural problems. This research was the focus of a symposium, "Genetic Engineering: Applications to Agriculture," which was held on 16 to 19 May at the Agricultural Research Center of the U.S. Department of Agriculture (USDA) in Beltsville, Maryland.

Signs of an incipient "genetic revolution" were apparent at the symposium. The first stage, likely to be achieved in the next few years, will see the modification of bacteria for the production of agriculturally useful substances, including vitamins and amino acids for feed supplements, animal growth hormones, and vaccines. As Richard Flavell of the Plant Breeding Institute in Cambridge, England, points out, "The bacterial systems have been worked on for 40 years. They are extremely well understood."

Foreign genes can be readily introduced into bacterial cells and their products made there. For example, researchers at the USDA's Animal Research Center in Plum Island, New York, introduced a gene that codes for one of the coat proteins of foot-and-mouth disease virus into cells of the bacterium *Escherichia coli*. The coat protein can be harvested from the cells and used to vaccinate animals, obviating the need to use the dangerous virus. In an analogous fashion, animal growth hormones can be produced in *E. coli*, according to J. Leslie Glick of the Genex Corporation in Rockville, Maryland, although he said that *Bacillus subtilis* would be a better host for producing commercial quantities.

In addition, Robert Owens, Dean Cress, and Theodore Diener, all of the USDA's Agricultural Research Service, have used cloned DNA to identify potatoes infected with potato spindle tuber disease (PSTD), which is caused by a viroid, a small piece of naked RNA. The investigators first copied the RNA into DNA, cloned the copy, and then used the cloned material to detect the viroid in potatoes. Once PSTD is established in the field, it is hard to eliminate. But if infected stock is identified before planting, potato yields may be improved, especially in the tropics where the disease flourishes.

Genetic engineering of improved crop plants will be much more difficult, however. Flavell explains, "You are only putting a single gene into bacteria. But many genes are involved in determining plant characters. . . . The time scale for genetically engineering plant improvements is not a few years, but longer."

There may be some exceptions to this, if the trait to be engineered is specified by only a single gene. Transferring a gene that confers herbicide resistance into plants is one such possibility.

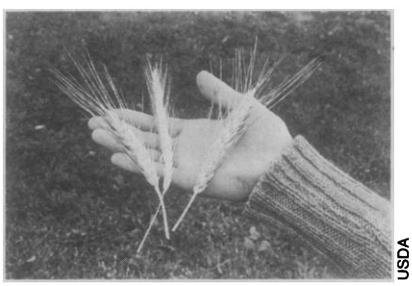
But the genetics and biochemistry of other traits are much more complex. Genetic engineering of cereal plants to make their own nitrogen fertilizer is often cited as one of the potential payoffs of recombinant DNA technology. This will not happen soon, and for several reasons it may not happen at all. Not the least of the problems is the complexity of the nitrogen-fixing (*nif*) gene complex of bacteria, which contains some 17 genes. Moreover, no one knows yet how their expression is regulated.

Even less is known about the genetic basis of other desired traits, including resistance to drought, heat, cold, salty soils, and other plant stresses. Researchers will have to identify the appropriate genes and determine how they work and are controlled before plants with improved stress resistance can be genetically engineered. More conventional approaches, including standard plant breeding methods, or use of culture techniques to select cells with the desired characteristics, may succeed first, although the culture methods require that whole plants be regenerated from single cells. This is not yet possible for most important crop plants. Jeff Schell of the Max Planck Institute for Plant Breeding in Cologne, West Germany, says of the current situation regarding genetic engineering of plants, "Right now the basic questions and the applied questions are the same."

Interface Between Molecular Biology and Plant Breeding

Plant breeders know from experience that attempts to produce new hybrids by crossing different species often fail. The reason why they fail may lie in disparities in the organization of the DNA of the different species' chromosomes, according to data presented by Richard Flavell at the Beltsville symposium.

Flavell and his colleagues at the Plant Breeding Institute in Cambridge, England, have been studying the distribution of repeated DNA sequences in the chromosomes of such economically important cereals as wheat, barley, and rye. Most of the chromosomal DNA of these and other plants consists of families of repeated sequences, which have no known function, but which are often present in large number—up to several million copies of each per genome.



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Triticale (right) with its parents, wheat (left) and rye (center)

The Cambridge workers found that the distribution of the repeated sequences may vary widely in different species, even in closely related ones. The finding has implications for the evolution of chromosomal structure. "The structure of the chromosome as we see it today," Flavell says, "seems to have been created by the amplification or deletion of these short pieces of DNA, and by moving the bits around the genome. In contrast, the coding sequences stay pretty much the same across species."

As a consequence of the structural differences, the chromosomes of a hybrid plant, which were contributed by parents of two different species, may be unable to pair up in the normal manner during meiosis. "If you do not get regular pairing at meiosis," Flavell explains, "the progeny don't inherit a balanced chromosome set. They are

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sterile. And that is hopeless from a breeder's point of view."

Inability of the chromosomes from different species to line up properly during meiosis may also handicap plant breeders in their attempts to achieve another goal, namely, the incorporation into one plant species of a small number of genes from another. For example, they would like to breed hardier strains of wheat that carry genes from rye, a species with superior stress resistance. This would require that homologous chromosomes from the two parent species recombine, that is, exchange genetic material during meiosis when the chromosomes are paired up.

Flavell says, however, "Wheat and rye chromosomes almost never recombine. The different organization of the repeated sequences in the parental chromosomes is the best explanation we now have for why recombination between homologous chromosomes fails in hybrids."

In addition, the chromosomal disparities may lead to developmental abnormalities in hybrids. Triticale is a wheat-rye hybrid that has double sets of both wheat and rye chromosomes. As a result, the wheat chromosomes can pair up with each other, as can the rye chromosomes, and triticale can produce fertile progeny. Nevertheless, the seeds are usually shriveled because the endosperm, the part of the seed that stores food reserves for the embryo, does not develop normally.

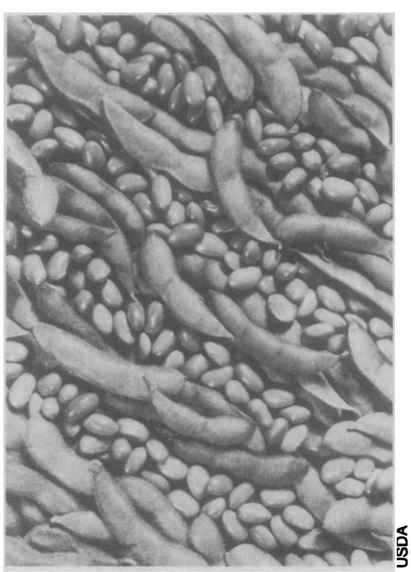
Studies by Michael Bennett of the Plant Breeding Institute and Perry Gustafson of the University of Missouri at Columbia have shown that the nuclei of badly shriveled triticale grains are abnormal. One of the ways in which the chromosomes of the two parent species of the hybrid differ is in the presence of "heterochromatic" regions, consisting of repeated DNA segments, on the ends of rye chromosomes but not on those of wheat. In lines of triticale in which some of the rye chromosomes have lost the heterochromatic regions, the endosperm nuclei appear more normal and the grains themselves are less shriveled.

Flavell cites this as a good example of how basic and applied research are intertwined. As he puts it, "The plant breeder needs the molecular biologist and the molecular biologist needs the plant breeder."

Regulation of Genes Coding for Soybean Proteins

Developing a vector, such as the Ti plasmid, to transfer genes into plant cells is just one of the problems researchers have to solve if they are to successfully engineer new, improved plants. If the transferred genes are to work in a normal fashion, they will have to be properly regulated so that the products are made at the right time and in the right amounts.

Currently there is little information about how plant genes are controlled, probably even less than about the regulation of animal genes, and the need to garner such information was a major theme of many of the participants in the Beltsville meeting. "We need to understand the regulatory processes to genetically engineer," said Robert Goldberg of the University of California at Los Angeles (UCLA).



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Soybeans with pods

Goldberg and his colleagues have been studying the organization and developmental regulation of the genes coding for the major proteins of the soybean seed. Together the four proteins in question—two storage proteins, a lectin, and the Kunitz trypsin inhibitor—constitute about 80 percent of the total protein of the seed. As much as 50 percent of the total messenger RNA content of the developing seed consists of messengers corresponding to the four genes.

The UCLA workers find the expression of the genes to be closely regulated. "The seed protein genes are activated early in embryogenesis," Goldberg says, "and repressed later. They are not expressed at all in the cells of the mature plant." The results suggest that the genes are turned on and off at the level of transcription into RNA.

When Goldberg and his colleagues examined the structures of the genes for clues as to how the genes are regulated, they found that all four are members of small multigene families. There was no evidence for gene rearrangements or amplification during embyrogenesis.

The soybean protein genes turned out to have few intervening sequences or none at all, in contrast to animal egg proteins such as ovalbumin and vitellogenin, which have several. "The main point is that these genes have relatively simple structures compared to the structures of analogous animal genes," Goldberg explains. Whether this says anything about control mechanisms is not clear. Other soybean genes, which are not expressed in seeds and the messenger RNA's of which are made in small quantities, have several introns. These genes may be more typical of the genes of plants, according to Goldberg.

The Goldberg group also looked for clues to the control puzzle in mutant soybean cells that had lost the ability to make the lectin. The mutant gene turned out to have an interesting feature. "It has an insertion element that separates the gene into two halves. This is an example of a Mendelian inherited defect that is not a point mutation," Goldberg remarks. The inserted sequence apparently prevents either transcription of the lectin gene or processing of its product without affecting expression of the genes on either side.

The UCLA workers found other copies of the inserted sequence throughout the genomes of both the mutant and normal soybean cells. It may be a representative of the movable elements that are now coming to be accepted as a common feature of eukaryotic cells.

Nevertheless, as of now the big question remains unanswered. "What are the DNA sequences required for the developmental regulation of the genes?" Goldberg asks. "That is the $64,000 question."

Jean L. Marx