PLANT BIOTECHNOLOGY

High-Tech Plants Promise a Bumper Crop of New Products

Agriculture is one of the all too rare U.S. industries that can hold their own in the toughest international competition. Indeed, if anything, it's been too successful: Year after year it yields huge surpluses of crops that end up in storage—at great cost to the public, which ultimately has to pay both for the storage and for the government price supports that maintain farmers' incomes in the face of the production glut. Might plant biotechnology come to the rescue?

Within the past 2 to 3 years, researchers in several labs around the world have genetically engineered plants, including such common crop plants as potato and tobacco, to manufacture a wide variety of materials, including human proteins such as albumin and interferon, alpha-amylase, a bacterial enzyme that is widely used in the food processing industry, and natural polymers, including a type of polyester. The work may make it possible to divert agricultural production from surplus crops, such as feed corn, and from those of questionable social value, such as smoking tobacco, to more useful commodities, says Charles Hess, former assistant secretary for science and education at the U.S. Department of Agriculture (USDA) and now a faculty member at the University of California, Davis. "Plants have a great deal of flexibility and the potential for vast productivity," adds plant biochemist Chris Somerville of Michigan State University in East Lansing.

Using plants to manufacture new products could have "multiple societal benefits," Hess predicts. Besides reducing the need for agricultural subsidies and allowing the farmers of the southeastern United States to wean themselves from their dependence on the cigarette industry without disrupting local economies, farmlands might become renewable production factories for oils and other products that now come from a nonrenewable resource, petroleum. And while it may be a decade or more before large-scale manufacture of drugs and other products in plants becomes a reality, the idea is being taken sufficiently seriously that in March of this year USDA Secretary Edward Madigan appointed a commission, known as the "Alternative Agriculture Research and Commercialization Board," to explore novel industrial uses for farm and forest products.

Much of the current work depends on gene transfer technology developed for plants about 10 years ago by Jozef Schell, Marc Van Montagu, and their colleagues at the Max Planck Institute for Plant Breeding in Cologne, Germany, and the State University of Ghent, Belgium, as well as by Rob Schilperoot at the State University of Leiden in the Netherlands, and researchers at Monsanto Corp. in St. Louis. These groups showed that a modified form of the "Ti plasmid," a piece of DNA from the bacterium *Agrobacterium tumefaciens* that naturally infects plant cells, is an efficient vector for carrying new genes into plants.

The new wave. Since then, plant genetic engineers have used the Ti plasmid to create many strains of "transgenic" plants bearing new genes. But it wasn't until early 1989 that efforts to develop plants as factories for biological and chemical products—described by Rob Fraley of Monsanto as the "third wave" in plant biotechnology—got under way. "The first area of application for genetically engineered plants was the delivery to established crops of new, improved agronomic traits, such as disease resistance. Then plants were altered with improved traits that affect food processing, such as by slowing down ripening in tomatoes," he explains. "Now, plants are being used to produce specialty chemicals and novel biopolymers."

Although many of the same products can be produced in either normal or genetically engineered bacteria, plants may have some advantages over the bacteria. While fermentation vats of the microbes successfully produce gram quantities of the product, farms of genetically engineered plants might yield tens or hundreds of pounds of extracted protein per acre, says Laurence Grill of Biosource Genetics Corp. in Vacaville, California, one of the companies doing the work. That would be a big plus, helping to hold down costs for those pharmaceuticals, industrial enzymes, and other chemicals that are needed by the ton.

In addition, mammalian proteins generally undergo sugar additions and other modifications that can affect their activity. The proteins made by bacteria lack these sugar side chains, whereas plants can carry out the modifications much more normally.

Researchers have shown, for example, that plants are very good at making several human and mouse proteins that have potential application in therapeutic and diagnostic medicine.

MOGEN INTERNATIONAL

Instant packaging. Genetically engineered tobacco seeds can be used as a source of bacterial alpha-amylase.

In two cases, the discoveries came serendipitously in the course of experiments designed to produce plants with improved disease or pest resistance. In late 1988, when Gus de Zoeten, now at Michigan State University, and Thomas Hohn, then at the Friederich Miescher Institute in Basel, Switzerland, introduced the gene for human interferon, a protein with natural antiviral activity, into turnip plants, their goal was to see whether the interferon would make the plants more resistant to viral infections. Although the experiment failed to achieve its goal—turnips making the human interferon were just as susceptible to infection by turnip yellow mosaic virus as the control plants—the Swiss workers noted that their transgenic plants made large amounts of the interferon. And just as important, the protein was biologically active in animals.

At about the same time, Andy Hiatt's group at the Scripps Research Institute in La Jolla, California, began experiments aimed at improving the resistance of tobacco plants to tobacco mosaic virus (TMV) and also to certain nematodes that are serious plant pests. The Scripps workers' strategy was to genetically engineer the plants to produce antibodies against the pests. The researchers haven't yet completed those experiments, making it difficult to determine if the antibodies make the plants more resistant.

But they have shown that tobacco plants can synthesize large quantities of mouse antibodies and that the plant-made antibodies—the researchers call them "plantibodies"—behave just like normal mouse antibodies, at least in test-tube studies. "There is nothing in the alien environment of the plant that compromised the antigenic recognition of the antibodies," Hiatt says. And that opens up other potential applications for the plant-made antibodies. They may be useful, for example, as supplements for infant formulas.

Meanwhile, other researchers deliberately set out to see whether plants could be used as bioreactors for manufacturing human biologicals. Also in 1989, for example, a group including researchers at the biotech company, Plant Genetic Systems, N.V., and the Rijks University, both in Ghent, genetically engineered two different species, the oilseed rape plant (*Brassica napus*) and *Arabidopsis thaliana*, a small plant that has become a favorite for many different kinds of experiments, to produce the peptide leu-enkephalin, one of the brain's own opiates. And still another group, this one at the agricultural biotechnology company Mogen International, N.V., in Leiden in the Netherlands, found that genetically transformed potato and tobacco plants could produce human serum albumin that is indistinguishable from the genuine human protein. Human albumin solutions are widely used in medicine for fluid replacement in burn patients and others.

But while these results point up the potential importance of plants as factories for human biologicals, the first marketable chemicals from transgenic plants will probably not be drugs, which will have to undergo rigorous testing for safety and efficacy. Instead, they are likely to be enzymes used for food processing and other industrial applications. Take, for example, the starch-digesting enzyme alpha-amylase, which is used to make foods including bread and low-calorie beer, as well as to clarify wines and juices.

Promising yield. Currently this enzyme is produced by bacterial fermentation. But both Mogen and the U.S. biotech firm Biosource Genetics have shown that tobacco plants can make the protein. The big question now is whether the plants will make the protein in sufficiently high concentrations to make the plant product economically competitive with the bacterial enzyme. And there, results look promising, says Peter van den Elzen, Mogen's research director.

The Mogen group, with researchers from Gist-brocades, N.V., in Delft, found that alpha-amylase constituted about 0.5% of the soluble protein in their transgenic tobacco plants. Tobacco plants making this much alpha-amylase would produce about a pound per acre. What's more, the transgenic plants grew just as vigorously as control plants, and the plant enzyme was just as effective in breaking down starch as the bacterial enzyme. Indeed, the researchers didn't even have to purify the enzyme. They simply milled seeds from the transgenic plants. "The best surprise of the study was the stability of the enzyme in the seeds," van den Elzen says. This means that seeds may offer a natural, long-lasting packaging for the amylase enzyme, and perhaps other enzymes as well.

All this early work involved a relatively simple genetic manipulation: the introduction of a new single gene specifying a particular protein or peptide into plants. But within the past month, plant biotechnology research has taken a new twist that should open the door to using plants to make a much wider array of products. Three independent research teams have shown that it's possible to use gene transfer technology to redirect the innate biosynthetic pathways of plants. With such genetic manipulations, plants can be coaxed into producing more of the natural products that they normally make, such as edible oils, waxes, lipids, and starches, as well as things not normally in their biochemical repertoire, such as industrial oils and biopolymers, including polyesters.

One such demonstration comes from Michigan State's Somerville, his postdoc Yves Poirier, and their colleagues. In the 24 April issue of *Science*, they reported genetically engineering *Arabidopsis* to produce granules of polyhydroxybutyrate (PHB), a polyester used for plastic containers that is normally obtained from the bacterium *Alcaligenes eutrophus*. They did this by transferring two of the genes the bacterium uses to make PHB into the plants. As a result, the flow of carbon through the plants' metabolic cycles was directed away from a pathway that leads to production of plant hormones into PHB synthesis. The plant PHB was stored in granules, much as it is in bacteria.

Somerville says he was surprised by how well the experiment went: "A lot of things could have gone wrong. The new enzymes could have acted on other cell constituents and killed the plant, or the stored granules of PHB might have killed the plant." Still, he adds, there are problems that will have to be solved before plants can be routinely used as production factories for PHB. For one thing, the plants that made the most PHB showed poor growth and seed production, and the PHB granules were spread throughout the cell. It would be better, Somerville says, if they were concentrated in the small, intracellular structures known as plastids.

Redirected pathways. At last month's Keystone Symposium in Colorado on "Crop Improvement via Biotechnology," two other groups offered proof that plants' biosynthetic pathways can be redirected. Vic Knauf reported, for example, that scientists at his company, Calgene Inc. of Davis, California, got canola plants to produce either of two different oils with unique fatty acid compositions that are of value to the food and detergent industries. Moreover, in another project that is not as far along, Calgene researchers are introducing two genes into canola that should allow the plant to express an enzyme that catalyzes the production of jojoba oil, a valuable, multipurpose oil used in cosmetics, as well as in transmission fluids and other lubricants.

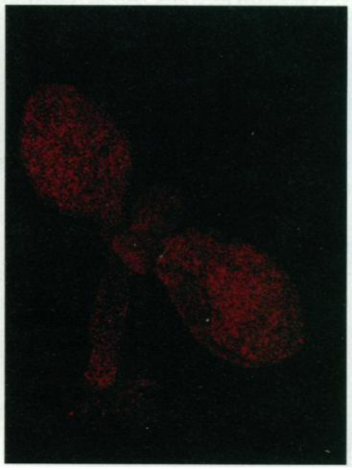
And Monsanto's Ganesh Kishore described a method for getting potatoes to synthesize more starch, which is in high demand for making high-fructose syrup and bioethanol. Potatoes with a high content of starch solids are also cheaper and easier to process for french fries and potato chips.

Normally, the amount of starch that is made is limited by the availability of one of the enzymes (called adenosine diphosphate (ADP)-glucose pyrophosphorylase) in the synthetic pathway. The Monsanto workers modified the gene sequences that control the synthesis of the enzyme so that more of it would be made, and then introduced the modified gene into potato plants. The result: The plants made increased amounts of starch, although the original experiments were not total successes as the generalized overproduction of starch impaired plant growth. But when researchers inserted an additional gene that limited starch production to the potato tuber, those problems disappeared.

The researchers doing this work point out that it is just beginning, and a key obstacle to further development of the productive potential of plants is ignorance of the details of plant metabolism. Nevertheless, enough scientific obstacles have already been toppled to give real hope that plants may soon be grown to provide much more than the basics of food and fiber. So, instead of producing surpluses of a few foodstuffs, U.S. agriculture might be redirected to yield useful quantities of a broader variety of goods.

–**Anne Simon Moffat**

MONSANTO CORP.



POIRIER *ET AL.*

Putting plants to work. The dark staining shows increased starch production in the genetically engineered potato *(above)*. At right is a modified *Arabidopsis* with polyester (PHB) granules.