Innovations

Turning over a New Leaf

Not all tobacco has to go up in smoke. Anticipating a diminishing market for cigarettes and other such tobacco products in the future, researchers around the United States are studying alternative uses for tobacco plants. If the research comes to fruition, perhaps tobacco farmers may not have to change their occupation, just their customers.

Currently, it looks as though biotechnology companies could be future customers of tobacco farmers. The most promising field of research for alternative uses of tobacco involves the genetic engineering of tobacco plants to produce various substances, such as industrial chemicals, pharmaceuticals, and consumer product ingredients.

The Chosen Crop

Besides the fact that health awareness and legislation are dictating that the time is right to explore tobacco alternatives, tobacco just happens to be an ideal plant to genetically manipulate. Many researchers have called tobacco the "white rat" and the "fruit fly" of the plant kingdom, comparing it to traditional laboratory animals used for genetic bioengineering.

"Tobacco has been chosen for good reason," says Carole Cramer, a professor of plant pathology and physiology at Virginia Polytechnic Institute and State University in Blacksburg.
In order to genetically engineer a plant, Cramer says the desired genes must first be introduced into the plant’s cells. Then the cells must be enticed to reproduce a whole plant. Researchers have found that tobacco is the easiest plant to successfully genetically engineer.

In addition, Cramer says, “Tobacco is an amazing biomass producer. A seed smaller than the head of a pin can produce plants that are four to six feet tall. Tobacco has also been optimized for leaf growth.” Furthermore, tobacco has a high yield of seeds per plant: a single plant can yield up to one million seeds. Cramer says that genetically engineered seeds could produce many acres from the first generation. All these factors suggest that genetically engineered tobacco crops would be relatively easy to maintain, and that they would flourish in a short amount of time, Cramer says.

Acres of Possibilities
There are countless possibilities for tobacco, but currently the most promising research involves using the plant to produce vaccines, human enzymes, and polymers. While a majority of the research has yet to be published and is guarded under intellectual property rights, researchers are talking in general terms about their work on the potential applications for transgenic tobacco products.

Tobacco and Health Research Institute. A good example of the current wave of new applications research can be found at the Tobacco and Health Research Institute (THRI), located at the University of Kentucky in Lexington. The institute was established in 1970 by the Kentucky state legislature and is funded by a $.005 tax on each pack of cigarettes sold in Kentucky.

After more than two decades of tobacco-related medical research, the institute has refocused its mission to emphasize the development of new products based on transgenic tobacco, creating new opportunities for farmers and seeking industrial partners to collaborate with the institute. Maelor Davies, director of the THRI, says the institute is helping to develop new markets as well as new crops. THRI researchers are looking at a variety of uses for tobacco while continuing to investigate the basic science of plant bioengineering.

Deane Falcone, an assistant professor of metabolic engineering in the department of agronomy and a faculty associate at the THRI, is investigating tobacco’s genetic regulation to determine how the plant can express its less abundant compounds in greater amounts. He explains that specially modified bacteria are commonly used for introducing genetic material into tobacco. Any harmful genetic material is removed from the bacterium and the desired gene or genes are introduced into it. The researchers isolate the tobacco’s leaf cells and then cultivate the genetically engineered bacterium together with the leaf cells. The bacterium invades and infects the cells, thereby inserting the new genes into the plant’s cells. Falcone and others are working to develop promoters, or sections of DNA that must be coupled with a gene in order for the gene to be expressed in the plant tissue.

The THRI is collaborating with several companies, including InterLink Associates of Princeton, New Jersey. InterLink has developed peptides for resistance to plant diseases such as tobacco blue mold, and researchers at the THRI are attempting to produce the peptides in tobacco on a large scale. In 1997, blue mold caused an estimated $165 million loss to farmers in Kentucky alone. Davies says the peptides could be used in agriculture as environmentally compatible antibacterials, pesticides, and antifungal sprays for fruits and vegetables. He says the researchers are making good progress, and that they are close to submitting results for publication.

North Carolina State University. Raymond Long, a professor of crop science at North Carolina State University in Raleigh, and colleagues have used tobacco to express a gene protein in high concentrations and demonstrate its efficacy. In studies, the researchers sought to express bovine lysozyme, an enzyme with antibacterial properties that is well-suited for use as a preservative in foods, cosmetics, and agriculture, in transgenic tobacco plants. The results, published in the July 1997 issue of the Journal of Agricultural and Food Chemistry, showed that Long’s group was successful in expressing, isolating, and purifying bovine lysozyme at high levels in tobacco. “Certainly a wide range of compounds can be expressed in tobacco, but...
not too many at high levels," Long says, "This was a good case model."

Long's group is currently investigating the efficacy of a papilloma virus vaccine that is produced by the introduction of a gene into tobacco plants. Papilloma strikes humans and a wide range of animals. The human papilloma virus causes a variety of warts on the hands, feet, and genitalia, and 90% of cervical cancers are thought to have their origins in the virus, Long says. The researchers are first testing a vaccine for canine oral papilloma virus in dogs. If it is successful, they plan to begin testing a human papilloma virus vaccine to combat genital warts in humans.

**CropTech Development Corporation.** Promising research by Cramer and colleagues led to the creation of a company, CropTech Development Corporation, also located in Blacksburg. CropTech is working to develop genetically altered plants to make products such as drugs and vaccines. "It's all preclinical research and development at this point," says Cramer. She says three applications of transgenic tobacco look especially promising at this time.

First, the company is using tobacco plants to produce a human enzyme that could be used in the treatment of Gaucher's disease, a rare genetic disorder that affects about 2,000 people in the United States each year, causing brittle bones, distended abdomen, and in severe cases, death. There is a treatment currently available, but Cramer says its cost is often prohibitively expensive.

Another promising application is the development of a cell cycle inhibitor, which blocks cell growth. Cramer says this product may be able to help prevent cancer. Third, Cramer and colleagues are investigating a potential clot buster, or serum protein with anticoagulant properties, that she says tobacco produces in a fully active form.

While much of the research is currently confidential, Cramer explains that CropTech offers a unique approach to synthesizing pharmaceutical compounds in transgenic tobacco. She says the gene inserted in the plants is not actually expressed until after the plant is harvested. "We can separate normal plant growth from the development of the product," says Cramer. Postharvest gene expression allows the researchers to synthesize products, such as cell cycle inhibitors, that would be detrimental to the plant during growth.

Furthermore, Cramer says, this process prevents degradation that can occur when the protein is exposed to temperature changes. "As opposed to sitting out in a field for weeks exposed to the sun," she says, this process offers a "freshly synthesized" compound.

CropTech researchers are also working with the Department of Defense to produce vaccine antigens against possible biological warfare agents, Cramer says. Eventually, she says, they will target edible vaccine strategies.

**University of Central Florida and Bioelastics Research.** Henry Daniell, a molecular geneticist at the University of Central Florida in Orlando, and colleagues are investigating the potential for the use of tobacco to produce protein-based polymers in collaboration with the Birmingham, Alabama-based Bioelastics Research. Many plastics are currently made from petroleum-based polymers, the production of which requires the use of hazardous and toxic chemicals, including organic solvents such as hexane. Many of these organic solvents are carcinogenic.

Genetically engineering tobacco and other plants to generate protein-based polymers, however, is more environmentally friendly because the production process does not require hazardous chemicals. Furthermore, scientists can design the polymers to be biodegradable by "installing" chemical clocks to determine the polymers' half-lives.

Daniell's group has expressed polymers derived from human elastin genes into tobacco plants. Scientists at Bioelastics have found that these compounds can be absorbed by the skin and are biocompatible, which demonstrates their potential for use in medical applications such as the prevention of postsurgical adhesions (fibrous tissue formed in response to surgical manipulation of internal organs), as well as in tissue reconstruction and programmed drug delivery. Bioelastics hopes to soon begin conducting clinical trials on the use of a polymer produced in *Escherichia coli* and tobacco for the prevention of postsurgical adhesions.

Daniell believes the most financially promising use of tobacco, however, is the production of insulin and vaccines. Currently, insulin is produced in *E. coli*.
and then must be purified, a process that is lengthy and that recovers small amounts, making the end product very expensive, Daniell says. Daniell and colleagues are developing a one-step process in which insulin is purified in tobacco. He says the potential exists for tobacco to produce large amounts of insulin. The researchers are currently in the process of expressing insulin in tobacco plant genes, and Daniell says the insulin could be ready for clinical trials next year. Daniell's lab is also using tobacco to develop vaccines, including one for cholera, a disease that can cause diarrhea, dehydration, and death.

Implementation

There is concern about the introduction of genetically manipulated plants into nature because of the potential for foreign genes to escape through pollen dispersal, but Daniell says that tobacco is safe. Tobacco plants mainly self-pollinate and there is no evidence of any hybrids in nature. Therefore, he says, there is little risk of transfer of genetic material to other plants.

While tobacco does have advantages over other crops in the manufacture of genetically engineered products, some adjustments need to be made in order for the plant to be competitive with other crops. "We're trying to work out the kinks of tobacco production in order for it to be the plant of choice," says Davies. "Certainly the potential is there."

One issue is that the production costs of tobacco are much higher than the production costs of other crops such as corn and soybeans. The high costs are due to tobacco's unusual production style, which involves manual harvesting. The high price that tobacco currently commands allows farmers to support this high-cost production style.

Davies says that to achieve lower production costs, the development of new varieties of tobacco will be pursued. "The tobacco of the future will probably be quite a different-looking crop than [that of] today," he says. For example, THRI researchers are working to extend the life of tobacco leaves, thereby increasing the production amount of genetically engineered compounds.

Most important, however, Long says more money is needed for further research. "The failing is less in the science and technology, and more in funding," agrees Lou Tornatzky, a senior fellow with the Southern Technology Council, an organization based in Research Triangle Park, North Carolina, that works to strengthen the regional economy through the more effective development, commercialization, and deployment of technology. In March 1996, the group published a market analysis, Prospects for Plant-Based Biotechnology Products, that examined the potential alternative applications for tobacco crops that have surfaced from research by biotechnology companies. The report found that, while the economic potential of such applications is considerable, investments in research and development must be made for them to succeed.

Davies says the THRI continues to emphasize partnerships with industry because "the one limiting factor to getting molecular farming to take off is the end-user interest." He says the work is beginning to pay off, as more companies are calling and showing interest in the THRI. "The results are more encouraging all the time, and interest is picking up," he says.

If the research demonstrates that transgenic tobacco products are effective and that their production can be profitable, researchers and biotechnology companies will next have to persuade tobacco farmers to grow the modified plants. "It's a matter of the tobacco farmers understanding that they can make more production medical- use- ful compounds than medically harmful compounds," says Daniell.

Many tobacco farmers, unsure of their fate, seem open to suggestions and new uses for their crops. "We favor the use of tobacco wherever it can be best used," says J. T. Bunn, executive vice president of the Leaf Tobacco Exporters Association, which represents members who purchase, process, and deliver leaf tobacco to manufacturers. "If it can be used for [the development of] pharmaceuticals—great. It's a genetic guinea pig right now, and the more research we have on tobacco, the better off we all are." Asked if his organization's members will be open to new uses for tobacco plants, Bunn says, "Given the opportunity to produce tobacco, yes, they will. It's just that simple."

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Suggested Reading

Daniell H, Chitribabu G, McPherson DT, Zhang X, Xu J, Urry D. Hyperexpression of a synthetic protein-based polymer gene. Methods Mol Biol 63:359–371 (1997).

Daniell H, Chitribabu G. Biopolymer production in microorganisms and plants. Chem Ind 14:555–560 (1997).

Kutchan TM. Alkaloid biosynthesis—the basis for metabolic engineering of medicinal plants. Plant Cell 7:1059–1070 (1995).

Owen MRL, Pen J, eds. Transgenic plants: a production system for industrial and pharmaceutical proteins. New York: John Wiley & Sons, 1996.