In the bag. These cultured carrot cells are engineered to make a human drug.

Is the Drought Over for Pharming?

Despite technological, economic, and social issues, companies are plowing ahead, making drugs and other compounds in plants

MANY A CHILD HAS BEEN TOLD "CARROTS are good for you." That advice could soon take on new meaning for people with Gaucher disease, an inherited metabolic disorder that leads to liver and bone problems. Patients must now be injected every 2 weeks with a manufactured enzyme that costs on average $200,000 a year, making it one of the most expensive drugs ever. If ongoing clinical trials go well, the 5000 Gaucher patients on the therapy could soon have a second option—a cheaper version of the enzyme that stays in the bloodstream longer and can be injected less often.

If the U.S. Food and Drug Administration (FDA) approves recombinant glucocerebrosidase, it will be good news not only for medicine but also for a community far removed from the clinic: plant scientists. Protalix Biotherapeutics in Karmiel, Israel, produces this new version of the protein in giant plastic bags, not in steel vats of mammalian cells like most biologics are. The bags are filled with transgenic carrot cells that are cultured and then processed to extract the drug. "If Protalix gets regulatory approval, that would [make it] the first plant-made pharmaceutical," says plant scientist Charles Arntzen of Arizona State University in Tempe. "For people who work in this field, it will be a very exciting step forward."

Arntzen is chasing an elusive dream: using whole plants as factories to make drugs. Nearly 20 years ago, when researchers first showed that a tobacco plant could be engineered to crank out an antibody, they envisioned harvesting cheap supplies of therapeutic proteins, antibodies, and vaccines from vast fields of crops. For this approach, researchers isolate the target gene and usually insert it into a bacterium called *Agrobacterium* that readily infects the plants and passes on the gene. The gene becomes part of the plant and is passed from one generation to the next, producing foreign protein much as if it were one of the plant's own genes.

However, technological hurdles and a lack of interest from drug companies have hamstrung "pharming," as have worries that pharma crops will escape from their experimental plots and taint the food supply. As a result, many companies have abandoned this research or gone under. And no plant-made drugs for humans have made it to the pharmacy.

But academic scientists and some companies have persisted, improving yields of plant-made drugs and developing innovative ways to keep pharming inside the lab, or the greenhouse. Several plant-made pharmaceuticals (PMPs) are now in patient trials (see chart, p. 474). Moreover, the European Union, the Bill and Melinda Gates Foundation, and the U.S. Department of Defense are fertilizing the field with new funding. "We're actually not doing too bad," says Julian Ma, an immunologist at St. George's University of London in the U.K. "It's just that everyone is in a hurry."

Fields of dreams

The excitement over plant-made pharmaceuticals began with a 1989 paper in *Nature* showing that monoclonal antibodies could be produced in tobacco. The paper "really captured the imagination," says Ma. Monoclonal antibodies were being used to treat a growing number of diseases, from arthritis to cancer, but were expensive to make in mammalian cells. So-called plantibodies appeared to offer a cheaper production method—a kilogram might cost $100 rather than $3 million—and might be simpler to process because they would be free of animal pathogens.

Other discoveries followed. In 1995, for instance, Arntzen's group reported in *Science* that potatoes engineered to make a cholera protein worked as a vaccine when the spuds were fed to mice. Such "edible vaccines" could offer developing countries cheap oral vaccines that didn't require refrigeration, Arntzen suggested (*Science*, 5 May 1995, p. 658).

A company called Large Scale Biology Corp. in Vacaville, California, came up with a shortcut. It didn't bother to create a new tobacco strain when it wanted to produce an antigen for a lymphoma vaccine. It simply sprayed tobacco plants with a tobacco mosaic virus carrying the appropriate gene. The leaves produced useful amounts of the vaccine protein within 14 days. The drug worked in mice, suggesting that vaccines tailored to lymphoma patients' tumors could be made in plants in just weeks. And because the plants carried the foreign gene only until they shed their leaves, they were potentially more acceptable than permanently modified crops.

Temporary transgenic. Fluorescing protein shows tobacco leaf's pharming potential.

CREDITS (TOP TO BOTTOM): PROTALIX; BAYER AG

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Selected Plant-Made Pharmaceuticals

Company	Plant	Grown in	Drug or product	Disease	Status
Human drugs					
Protalix Biotherapeutics	carrot	cell culture	glucocerebrosidase	Gaucher disease	Phase III trial*
Biolex Therapeutics	duckweed	indoor chambers	alpha interferon	hepatitis C	Phase II trial*
SemBioSys Genetics	safflower	field	insulin	diabetes	Phase I/II trial †
Meristem Therapeutics	corn	field	lipase	cystic fibrosis	Phase III trial †
Other products					
Ventria Bioscience	rice	field	lactoferrin, lysozyme	diarrhea	Efficacy trial §
Cobento	*Arabidopsis*	greenhouse	human intrinsic factor	Vitamin B-12 deficiency	Approved ††
Planet Biotechnology	tobacco	field	secretory antibody vaccine	tooth decay	E.U. approved
Dow AgroSciences	tobacco	cell culture	poultry vaccine	Newcastle disease	USDA approved
CIGB, Cuba	tobacco	greenhouse	vaccine purification antibody	hepatitis B	On market

* Ongoing; † Projected late 2008; § Completed; †† In Ukraine.

Steps along the way. No plant-made human drug has made it through final clinical trials, but several "pharmed" proteins are close to or on the market as supplements, a vaccine reagent, and a medical device.

Scores of biotech companies sprang up to commercialize these discoveries, and some big agbiotech companies got involved as well. By the mid-1990s, more than 180 companies and organizations were working on pharming, according to the Biotechnology Industry Organization.

The companies soon ran into technological snags, however. Biotechnologists couldn't always get plants to express enough protein and had trouble purifying the protein product. Efforts to make edible vaccines stalled after researchers realized that the amount of antigen fluctuated widely from plant to plant. Arntzen thinks that oral vaccines made from dried plant material could work for developing countries, but a vaccine without a strictly controlled dose "would never be approved" in the United States, he says.

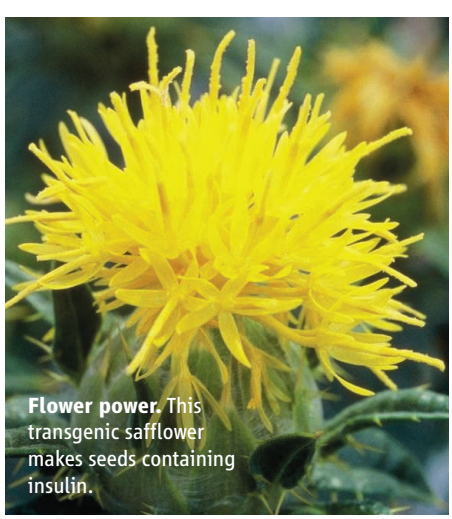
Another reality check: lukewarm interest from the big drug companies. They didn't much care that plant-made drugs would be cheaper to make because production is a small chunk of the cost of drug development; the big-ticket item is clinical trials. The companies were also leery of the regulatory hurdles, because both the drug and the new production process would have to clear FDA. "Most pharmaceutical companies aren't willing to take a chance on a drug produced in plants," says Roger Beachy, president of the Donald Danforth Plant Science Center in St. Louis, Missouri.

Also, like other genetically modified crops (see pp. 468, 472), pharma plants can be a public relations nightmare. In 2002, leftover corn plants engineered by ProdiGene Inc. to make a pig vaccine sprouted in a soybean field in Nebraska. For this and an Iowa mishap, the U.S. Department of Agriculture (USDA) fined the company $250,000 and made it pay $3 million to buy and destroy tainted soybeans. The incident stoked opposition from farmers and activists worried about "drugs in your cornflakes."

Other companies underestimated the public's concerns. A company called Ventria Bioscience that wanted to conduct field trials of rice containing two breast-milk proteins useful in combating diarrhea drew the ire of rice growers in California, then Missouri. It wound up in Kansas, where no other rice is grown.

USDA tightened its rules for field trials of pharma plants in 2003 to prevent mistakes like the ProdiGene episode. But skeptics were not assuaged. Bill Freese of the Center for Food Safety in Washington, D.C., says enforcement is "horrendous." As a result, "we don't think [drugs] should be in any food crops, indoors or outdoors," he adds. Many ecologists and some plant scientists are also leery of using food crops for pharma. "It's too dangerous," says Kenneth Palmer, former director of the vaccine program at Large Scale Biology.

These concerns drove many companies away from using food crops such as corn for pharmaceuticals. A few big companies, such as Monsanto, dropped PMP research altogether. Stung by bad press and lack of interest from drug companies, many leading plant pharma companies have folded, including ProdiGene and Large Scale Biology. As Palmer puts it, "the field imploded."



Flower power. This transgenic safflower makes seeds containing insulin.

Close to the clinic

Despite the setbacks, a handful of companies in the United States and Europe haven't given up. A few have plowed ahead with food crops, grown outdoors, for their pharma products; others have focused on other plants or on unconventional growing schemes.

Meristem Therapeutics in Clermont-Ferrand, France, plans to start final clinical trials for a corn-grown gastric lipase for cystic fibrosis patients by the end of the year. And the Canadian company SemBioSys Genetics Inc. uses transgenic safflower—"much less of a lightning rod than some other crops," says CEO Andrew Baum—to produce insulin, which should be in clinical trials this year. Companies such as Protalix and Biolex Therapeutics sidestep the growing of crops altogether: the former with its carrot-cell culture to make a Gaucher disease enzyme, and the latter by producing interferon using duckweed, tiny clonal plants grown as a layer in clear plastic bags. "We are careful *not* to be associated with whole-plant transgenic technology," says Protalix CEO David Aviezer.

New technologies are attracting attention. To boost expression, the German biotech Icon Genetics relies on bacteria to get transgene-laden viruses into tobacco plants. The company dips the plants into a solution of *Agrobacterium* that carries the DNA for a deconstructed tobacco mosaic virus, which in turn contains the gene for the desired drug. The bacterial bath, followed by a few seconds in a vacuum, gets far more of the virus into plant-leaf tissue than conventional spraying.

In a 2006 paper in the *Proceedings of the*

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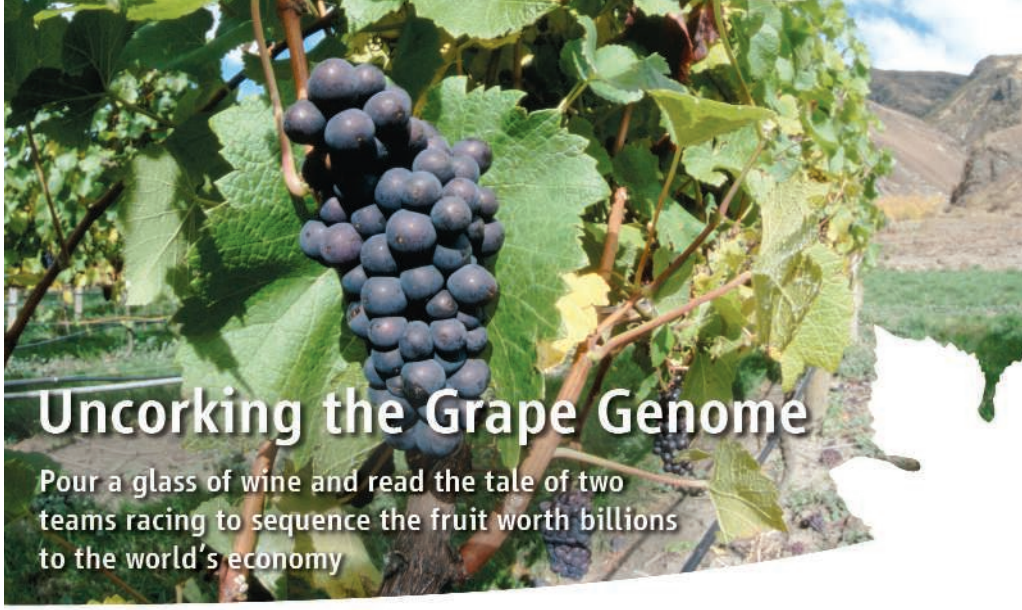
National Academy of Sciences (*PNAS*), they reported that this method, combined with other techniques, increases the amount of antibody by up to 100-fold, reducing the size of the crop needed and making it feasible to grow plants commercially indoors. Compared with making a transgenic plant, which takes a year or two to develop, this "magnifection" can go from gene to grams of protein in a couple of weeks. "It's incredibly promising technology," says Ma, who, like other academic researchers, is trying out magnifection.

With help from the drug giant Bayer, which bought the company in 2006, Icon Genetics will open a clinical-grade manufacturing plant in June. It expects to begin trials with a cancer vaccine tailored to individual patients in 2009, says CEO Yuri Gleba.

Bayer's move is a healthy sign of regrowth for the pharming field, Ma and others say. And other new sources of support are helping too. Last month, Pharma-Planta, a €12 million, 5-year, European Union–funded project co-coordinated by Ma, described in *PNAS* an anti-HIV microbicide grown in corn or tobacco that could be ready for testing next year. The Defense Department and other U.S. government agencies have provided the Fraunhofer USA Center for Molecular Biotechnology in Newark, Delaware, nearly $14 million to use a technique like magnifection to make vaccines. It has tested anthrax and plague vaccines in nonhuman primates and a pandemic flu vaccine in ferrets. "[We] can do things much faster than any other technology," says Executive Director Vidadi Yusibov, slashing in half the 6 months it now takes to make flu vaccine the traditional way, in chicken eggs. The organization also has $8 million from the Gates Foundation for plant-based vaccines for malaria, sleeping sickness, and flu.

As visions of endless fields of pharma crops have faded, so have unrealistic expectations for pharming. Scientists say they now realize that they need to be smarter about the marketability of the drugs they develop in plants. They think the best bets—Protalix aside—may be high-volume biologics, such as microbicides, monoclonal antibodies, and vaccines, particularly for use in developing countries. Getting these first low-hanging fruits through clinical trials and FDA approval should allay concerns about safety and environmental risks. Says Palmer, now at the University of Louisville in Kentucky, "Once two or three products [win approval], the field should really take off." **–JOCELYN KAISER**

CREDITS (TOP TO BOTTOM): CHARLES O'REAR/CORBIS; HOLT STUDIOS INTERNATIONAL LTD / ALAMY



Uncorking the Grape Genome

Pour a glass of wine and read the tale of two teams racing to sequence the fruit worth billions to the world's economy

AMONG WINE CONNOISSEURS, OPINIONS differ about whether 2007 will prove a good year for Pinot Noir. But among plant geneticists, it's the finest vintage ever: Last year, two European teams published high-quality drafts of two Pinot Noir–derived genomes.

Plant biologists are toasting the genomic double-header. This is the first fleshy fruit and just the fourth flowering plant to have its genome decoded. And in economic terms, grapes top the world's fruit crops: We consume them fresh or dried, crush them into juice, and use them to make wine that can sell for many thousands of dollars a bottle. "The contributions of these sequencing efforts are enormous and historical," says grape researcher Steven Lund of the University of British Columbia in Vancouver, Canada.

Wine woes. Powdery mildew (*above*) and other fungal diseases can devastate vineyards.

The story behind the grape genome is one in which a worldwide scientific community came together, then partially splintered into rival camps; money to support sequencing was hard to come by; and success has brought both new insights and delicious questions. The rivalry provided the drama of the story. For a while, a French-Italian grape genome alliance called Vigna/Vigne looked like it was going to be beaten by a disgruntled researcher who started his own genome effort. "Undoubtedly, competition was a driver here, perhaps in a microcosm of the human genome sequence drama of years past," says Lund, referring to the bitter contest between public and private programs to decipher our genetic code. Recently, however, at a workshop* in Udine, Italy, the two grape genome groups began to put aside their rivalry. "I'm hopeful there will be more collaboration now," says Vigna/Vigne member David Horner of the University of Milan in Italy. "It's cool there are two cultivars done. It allows more comparative work."

A key motivation for deciphering the grape genome is to prevent a repeat of the economic devastation that struck the European wine industry in the late 1800s. At that time, phylloxera, sap-sucking insects from North America, ravaged European grapevines. Today, winemakers and grape researchers are struggling to combat new threats, particularly downy and powdery mildew, diseases that have made their way to Europe from the United States over the past century. These fungi are an environmental as well as an economic nightmare: Although only about 5% of Europe's farmland is dedicated to wine vineyards, they account for about 70% of the region's fungicide use.

The new genome information should speed the creation of hardier vines, which has been slow going. "The target now is clearly resistance genes," says Vigna/Vigne member Michele Morgante from the Institute of Genomic Applications (IGA) in Udine. New insights into the locations of these genes can assist breeders as they try to develop better varieties, for example. And identifying genes in the few grapes that are resistant to drought

*Tuning the Taste of Wine, 7 March 2008, Udine, Italy.

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