MEETING INTERNATIONAL BOTANICAL CONGRESS

New Ways to Glean Medicines From Plants

ST. LOUIS—Among the developments featured at the XVI International Botanical Congress, held here early this month, were two new approaches for getting plants to serve as protein factories. In one, plant roots were induced to secrete the proteins, while another used tobacco mosaic virus to ferry new genes into plants.

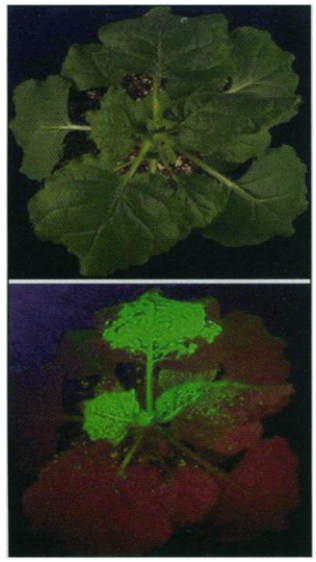
Holy Alliance?

It's hard to imagine a human disease being cured by a combination of a virus and tobacco—two notorious agents of disease. But that was the scenario described at the congress by Guy della-Cioppa of Biosource Technologies Inc., a plant biotech company located in Vacaville, California. His team devised this unlikely alliance by engineering the tobacco mosaic virus (TMV) to carry human genes and growing the virus in tobacco plants to make therapeutic products.

So far, the Biosource group has produced proteins that might be used to treat either of two diseases: Fabry's disease, a rare hereditary condition caused by lack of an enzyme called α-galactosidase, which often leads to death from kidney failure, heart attack, or stroke; and a blood cancer called non-Hodgkin's lymphoma. Because adult plants in the field or greenhouse can be easily infected by TMV, the company claims that the process is faster and cheaper than more conventional genetic engineering techniques, in which proteins are made by bacteria or in cultured mammalian cells.

"It's a good application," says Chris Somerville of the Carnegie Institution of Washington in Stanford, California. Still, he cautions that "there are limitations." The proteins might be contaminated with harmful allergens, and spraying fields with a genetically engineered virus raises environmental concerns, although the company says its early tests have allayed them.

To make their engineered TMV, company scientists link the human gene either to the regulatory sequences for the viral coat proteins, which infected cells make in large amounts, or to the coat protein genes themselves. In one field trial, begun in the early 1990s, the researchers found that plants in small outdoor plots that were infected with such a virus produced what they call "respectable" amounts of α-trichosanthin, a protein then being investigated as a possible AIDS drug. Also encouragingly, they found no detectable virus outside of plants after 2 or 3 days, an indication that it is unlikely to spread in the environment.



CREDIT: BIOSOURCE TECHNOLOGIES INC.

Lit up. The tobacco plant glows green under fluorescent light because it is infected with TMV carrying the gene for green fluorescent protein.

Since then the Biosource group has put the technique to work making α-galactosidase. The impetus came from Roscoe Brady of the National Institutes of Health (NIH) in Bethesda, Maryland, whose team wanted to see if they could help Fabry's patients by treating them with the enzyme, which would require up to a gram of protein per patient per year. Biosource's field trials, conducted this summer at the Biosource manufacturing facility in Owensboro, Kentucky, suggest that the infected tobacco plants can meet the demand, yielding "tens of grams" of the protein per acre of tobacco, says della-Cioppa. The NIH researchers are now testing the TMV-generated enzyme in mice lacking the enzyme as a prelude to human trials.

With so few Fabry's patients, Biosource CEO Robert Erwin says α-galactosidase won't be much of a moneymaker, although it gives Biosource an opportunity to demonstrate the feasibility of its technique. But a project Biosource researchers are doing with a group from Stanford could lead to a bigger market.

For over a decade, the Stanford researchers, led by immunologist Ronald Levy, have been trying to combat non-Hodgkin's lymphoma by getting the patients' own immune systems to destroy their tumor cells, which are derived from antibody-producing B cells. This involves vaccinating the patients with their own specific tumor antigens. The Levy team had been making the antigens—actually the unique antibodies displayed by the malignant B cells—by harvesting the patients' tumor cells, hybridizing them to immortal cells, and getting the resulting hybridomas, as they are called, to produce the antibodies—all of which can take from 9 to 12 months.

By contrast, with the TMV method, it took less than 30 days to get the plants producing the antigens from antibody genes cloned from the patients' tumor cells into TMV and only a day or two more to purify them. What's more, Erwin says, "we can grow a full-course vaccination from the number of plants that would fit on a desktop." He estimates that the tobacco-produced antigen could go into clinical trials in humans by next spring, provided the U.S. Food and Drug Administration gives its approval.

Some researchers, such as plant biochemist Elizabeth Hood of ProdiGene in College Station, Texas, express caution, however, raising the possibility that pathogens and allergens might slip through the purification process. But della-Cioppa says the chances of contamination are no greater than for proteins produced in bacteria or cultured mammalian cells, and that the purification procedures are largely the same in any event. If he's right, Biosource's technology may give both tobacco and viruses a chance to improve their reputations.

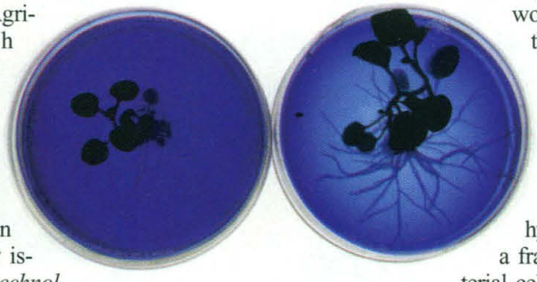
Getting to the Root of the Matter

The term "manufacturing plant" is taking on a whole new meaning as scientists shuttle human genes into plants such as tobacco or corn, hoping to turn the plants into medical protein factories. But although the gene transfers usually succeed, extracting the pure proteins from the plant material, which may contain pathogens or allergens, can be costly. Now, one group of researchers proposes solving this problem by going back to the plants' roots.

At the botanical congress, Ilya Raskin of Rutgers University in New Brunswick, New Jersey, described how his team has induced tobacco plants to secrete three different foreign proteins from their roots. If the plants are grown hydroponically in nutrient-laden liquids, the proteins can be extracted from the liquids, thus minimizing the number and cost of purification procedures. And the plants don't have to be harvested to get at the protein but can keep producing as long as they live. More recently, the team has extended the work, prodding plant roots to secrete their own defense chemicals, some of

which may have potential as antimicrobial or anticancer drugs.

"It's a nice piece of work with unique approaches and unique applications," says plant geneticist John Finer of the Ohio State Agricultural Research and Development Center in Wooster, who wrote an editorial that accompanied the Raskin team's paper on the protein results in the May issue of *Nature Biotechnology*. Still, Finer cautions, "a lot of work will be needed to show that it can be useful on a commercial scale."



Fade-out. The enzyme xylanase secreted by the roots of the transgenic plant *(right)* digests the blue-colored xylan, forming a clear zone.

In the first phase of the work, Raskin and his colleagues simply demonstrated that plants can secrete a foreign protein from their roots. The researchers tested seeds from tobacco plants into which Uwe Sonnewald's team at the Institute for Plant Genetics in Gatersleben, Germany, had introduced a bacterial gene encoding an enzyme called xylanase, which can digest a blue-colored form of the sugar xylan, turning it clear. When the seeds sprouted on petri dishes coated with nutrient agar containing the blue-colored sugar, the transgenic plants produced "clear zones" around their roots, showing that they were secreting the enzyme.

The researchers then went on to create two transgenic tobacco strains of their own. They introduced the gene for green fluorescent protein (GFP) into one and a human gene encoding an enzyme called secreted alkaline phosphatase (SEAP) into the other, along with regulatory sequences that would allow these genes to be expressed in plants. As with the xylanase gene, the group coupled GFP to a short stretch of DNA encoding "signal sequences," which tell the root cells to secrete the proteins. The *SEAP* gene already had its own secretion signal.

Grown hydroponically, the plants carrying the *GFP* gene exuded the protein from their roots, turning the hydroponic fluids a bright fluorescent green. The SEAP transgenic plants produced even more compelling results, churning out an average of 5.8 micrograms of enzyme per gram of dry root per day—a figure that went up to 20 micrograms when the researchers used a different gene construct with a stronger promoter. For comparison, Raskin points out that maize engineered to produce the protein avidin makes about 230 micrograms of the protein per gram of dry seed weight. He estimates that at the higher production rate, the tobacco roots could surpass the productivity of the transgenic corn over the life of the tobacco plants.

Raskin's team has since branched out in a new direction: coaxing plant roots to churn out higher levels of their own defense chemicals. In unpublished work presented at the meeting, Raskin showed that this can be done, for example, by exposing the roots of plants grown hydroponically to a fragment of a bacterial cell wall or a toxin from a fungus. "Basically, we press different biochemical triggers and see what comes out," he says.

The Raskin team has so far collected about 5000 samples of materials exuded by the roots of 700 different plant species. Some have been screened by the National Cancer Institute's Natural Products Branch, which found that several of these crude extracts killed various cancer cells in lab culture.

Whether the strategy will lead to any new anticancer agents or other drugs remains to be seen. But even if it does, some plant researchers caution that it may not be possible to produce either natural compounds or engineered proteins on an economically feasible scale. Elizabeth Hood, a plant researcher at ProdiGene in College Station, Texas, who has helped pioneer the production of avidin in maize, describes Raskin's technique as "a neat idea." But she adds, production "depends on greenhouse space and hydroponics, and those are very expensive."

Raskin counters that much supermarket produce, including tomatoes and cucumbers, is grown hydroponically, and consumers don't seem to object to the price. And he points out that making therapeutic proteins in mammalian or bacterial cells requires even more costly incubation techniques and sterile conditions. "Rhizosecretion is not going to work for all proteins," he maintains, "but it will work for some." **–TRISHA GURA**

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CREDITS: (TOP TO BOTTOM) RASKIN *ET AL.*; NATIONAL OBSERVATORIES OF JAPAN

ASTRONOMY

Subaru Sees an Unruly Pair

A pair of embryonic stars, swaddled in gas and dust, emit parallel jets in this image from Japan's new 8.3-meter Subaru Telescope on Mauna Kea in Hawaii. Material is still collapsing to form these protostars, a system called L1551IRS5, about 450 light-years from Earth. Theorists believe that it spirals inward to form a disk around each newborn star's equator. Some of the material acquires so much momentum, however, that it is thrown out in jets emanating from the poles.

These jets, which stretch 1500 times the distance from Earth to the sun, are pointed toward Earth and are visible partly because a stellar wind from the protostars has swept away material along the line of sight. A second set of parallel jets likely points in the opposite direction but is hidden by intervening dust and gas.

The Hubble Space Telescope discovered the jets, but Subaru is the first Earth-based telescope to see them. Its analysis has revealed the temperature of the jets—several thousand degrees—and their composition, showing that the hot gas is rich in ionized iron. Resolving the jets in such detail from the ground "is a pretty good trick," says Alan Boss, an astrophysicist at the Carnegie Institution of Washington. "It's really a tribute to this great telescope," he says, adding that Subaru's analysis could also sharpen astronomers' understanding of star formation. **–DENNIS NORMILE**