Engineering Plants to Cope With Metals

Newfound genes and enzymes could enable crops to flourish on metal-rich soils and help other plants clean up heavy metal contamination

Along with disease, drought, and pests, metals are a key enemy of plant growth. Aluminum, for example, the most abundant metal in Earth's crust, is normally locked up in minerals. But in acid soils, like those of the southeastern United States, Central and South America, North Africa, and parts of India and China, aluminum is set free as ions that poison plant roots, probably by making the cells rigid and unable to lengthen. The result is stunted plants and poor harvests, a problem on up to 12% of soils under cultivation worldwide.

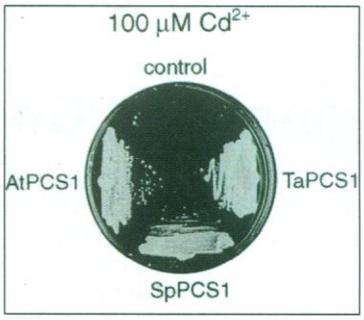
For decades, plant breeders coped with metals in soils by crossing metal-sensitive plant varieties with the few species that thrive despite their presence. But tolerant crops are few, and classical plant breeding is slow because crop genomes are large and complex. Lately, however, crop researchers have turned to genetic engineering to improve traits ranging from pest resistance to nutritional value (see Reviews beginning on p. 372)—and now they are taking the first steps toward producing metal-tolerant plants as well.

Within the last year, several research groups have identified metal-resistance genes, or their approximate locations, in mutant plants and other organisms. In some cases, they have gone on to identify the enzymes made by the genes, which help cells cope with metals by excluding them, sequestering them within the cell, or transforming them into volatile forms that can escape to the air. "This recent work is exciting," says plant biochemist Himadri Pakrasi of Washington University in St. Louis. "Now we have mechanisms" for coping with toxic metals—and the possibility of inserting them into crops to boost their growth.

The findings could also aid efforts to use other plants as cost-effective agents of environmental remediation—growing them on soils contaminated with mercury, copper, or cadmium, for example, where they would extract and store the metals. The plants could then be harvested and incinerated (*Science*, 21 July 1995, p. 302). U.S. Department of Agriculture (USDA) agronomist Rufus Chaney estimates that the cost of using plants to clean polluted soils could be "less than one-tenth the price tag for either digging up and trucking the soil to a hazardous waste landfill or making it into concrete."

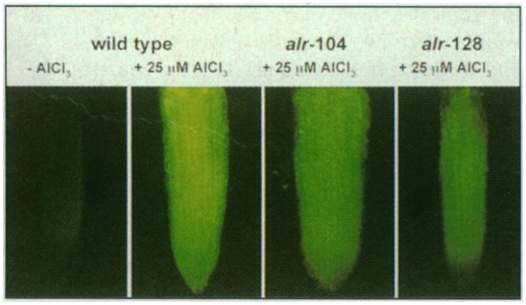
One advance came about a year ago, when Stephen H. Howell of the Boyce Thompson Institute for Plant Research in Ithaca, New York, Leon V. Kochian of the USDA's Plant, Soil and Nutrition Laboratory, also in Ithaca, and their colleagues used chemicals to create random mutations in the small experimental plant *Arabidopsis*, then screened the mutants for aluminum tolerance. They found two that could thrive in soils containing four times the level of aluminum that stunted the growth of normal plants.

One of the mutants coped with aluminum by secreting organic acids, such as citric and malic acids, which bound the metal ions outside the cell as aluminum malate and aluminum citrate before they had a chance to enter the root tip. It's not the only plant known to employ this strategy for coping with aluminum, Howell says. "Emanuel Delhaize [of the CSIRO in Canberra, Australia] and others discovered about 5 years ago that wheat resistant to aluminum released a variety of organic acids," he says. But finding this defense in *Arabidopsis* opens the way to tracking down the gene. Already, Howell and his colleagues have mapped the still-unidentified gene to chromosome 1 of the plant's four chromosomes.



Enzyme's mettle. Genes for a metal-resistance enzyme called phytochelatin synthase from wheat (right), yeast (bottom), and *Arabidopsis* (left) enable yeast cells to survive a dose of cadmium that kills control cells.

A second *Arabidopsis* mutant had a very different way of dealing with aluminum. Plants with this mutation, which mapped to chromosome 4, increased the flux of hydrogen ions into the root tip, alkalinizing the medium outside the root. The slight increase in external pH, by as little as 0.15 unit, was enough to transform Al^{+3} ions—the form in which free aluminum travels through groundwater—into aluminum hydroxides and aluminum precipitates, which don't enter and harm the root. Howell says the next phase of the research is to isolate and clone the genes—and perhaps introduce them into other plants.



Aluminum excluded. The roots of two aluminum-resistant *Arabidopsis* mutants (right panels) take up less of the metal than the normal plant.

Two other metals, cadmium and copper, build up in soils contaminated by industry or heavy fertilizer application. They harm plants by producing free oxygen radicals, which damage cells, or by displacing essential metal ions such as zinc from plant enzymes, disabling them. Many plants cope with these metals by binding them in complexes with a class of peptides called phytochelatins and sequestering the complexes inside their cells. Now three groups have isolated genes for the enzymes, called phytochelatin synthases, that make the metal-binding peptides when the cell is exposed to toxic metals. The groups—led by Christopher Cobbett of the University of Melbourne in Australia, Phil Rea of the University of Pennsylvania, Philadelphia, and Julian Schroeder of the University of California, San Diego—identified the genes in *Arabidopsis*, wheat, and yeast. After searching genome databases, they also found counterparts of the plant genes in the roundworm *Caenorhabditis elegans*.

The finding implies that animals may deal with unwanted metals in the same way as plants. "There doesn't seem to be a kingdom barrier," says Cobbett. The gene sequences also shed light on how the enzymes sense metal levels: They indicate that one end of the phytochelatin synthase molecules contains many cysteine amino acids, residues that bind heavy metals.

Looking to the future, Schroeder says that scientists would like to fine-tune the regulation of the phytochelatin synthase genes so that they are expressed at the highest levels in the shoots and leaves, rather than in the roots. The resulting plants would make better allies in environmental cleanup, because it is far easier to harvest the aboveground portions than to gather metal-laden roots.

Richard Meagher of the University of Georgia, Athens, also hopes to manipulate

CREDITS: (TOP) JULIAN SCHROEDER; (BOTTOM) STEPHEN HOWELL plants to create a green cleanup crew, in this case for mercury, a lethal waste product found at various industrial sites. Microbes in soil and aquatic sediments transform the element into methyl mercury, which is a particularly serious problem because it accumulates in the food chain and causes neurological damage in humans. A few years ago Meagher made use of a bacterial enzyme, mercuric ion reductase, that converts ionic mercury in mercuric salts to the elemental form, the least toxic form of mercury. When this gene was placed in *Arabidopsis*, canola, tobacco, and even yellow poplar, it allowed the plants to grow on mercury-laden media and release the metal into the air. By eliminating mercury from these sites, the plants block the formation of methyl mercury.

Some might cringe at the notion of plants emitting trails of mercury vapor, but Meagher argues that, compared to the global pool of mercury in the air, the amount emitted from contaminated sites would be a trace. And he has an additional strategy for cleaning up mercury contamination. Last month, he and his colleagues reported in the *Proceedings of the National Academy of Sciences* that they had endowed *Arabidopsis* with a second modified bacterial gene that enabled the plants to break down methyl mercury directly. The gene, encoding an organomercurial lyase, catalyzed the split of the carbon-mercury bond, releasing less-toxic ionic mercury. Meagher hopes that the same enzyme can be engineered into trees, shrubs, and aquatic grasses, allowing these plants to detoxify dangerous methyl mercury. "Our working hypothesis is that the appropriate transgenic plant, expressing these genes, will remove mercury from sites polluted by mining, agriculture, and bleaching, for example, and prevent methyl mercury from entering the food chain," says Meagher.

Pakrasi cautions, however, that before transgenic plants can be widely planted on contaminated soils, researchers will need to do extensive field trials. Transgenic plants adept at handling metals in laboratory and greenhouse experiments may not perform as well when the soil, moisture, and climate vary, for example. "There are a lot of unknowns," Pakrasi says.

–ANNE SIMON MOFFAT

NEWS

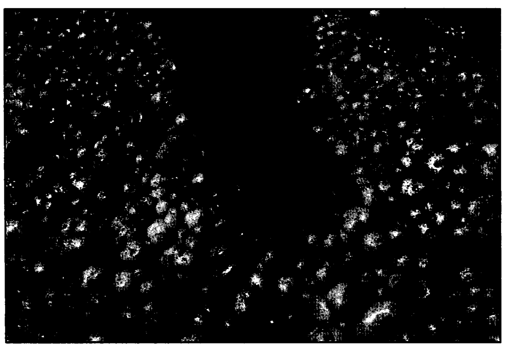
Crop Engineering Goes South

The staple crops of the developed world—wheat, corn, rice, and soybeans—get most of the attention from genetic engineers, who are endowing them with genes for resistance to disease and herbicides. Now some researchers are turning their attention to so-called nonprimary crops, often native to the subtropics or tropics, that have untapped potential for producing food, fiber, fuel, and medicines. One such crop, sweet potato, has already been genetically altered to improve its protein quality and may soon be planted commercially (*Science*, 18 December 1998, p. 2176). Enhanced versions of other crops that produce food or products for export in the developing world are in the pipeline.

Bringing the Potato Back Home

Although potatoes are everywhere in the Western diet—baked, fried, and made into chips and starch thickeners—per capita consumption is relatively low in developing countries, even in South America, where the crop originated. The problem? The huge gains in U.S. yields—a doubling over the last 50 years, achieved via classical plant breeding and irrigation, pesticides, and fertilizers—have been made with potatoes that cater to U.S. tastes and climate. But genetic engineering could allow the developing world to close the gap.

At last May's China Workshop on Plant Biotechnology in Beijing, Alejandro Mentaberry of the Research Institute of Genetic Engineering and Molecular Biology in Buenos Aires announced that researchers there had modified potato types commonly grown in Chile, Argentina, Uruguay, Cuba, and Brazil. Using *Agrobacterium* as a gene shuttle, the researchers created 16 transgenic lines, each carrying a different two-gene combination coding for resistance to various viral, fungal, and bacterial diseases. Several of the transgenic lines, which express antimicrobial proteins such as lysozyme and attacin from the giant silk moth, are resistant to the *Erwinia* bacterium, a serious potato pathogen, and are being field tested in Chile and Brazil.



Spuds of the Andes. Yellow potatoes in Ecuador.

A consortium of 13 Latin American and European laboratories now hopes to introduce an array of six genes—for resistance to various fungal, bacterial, and viral pests, for herbicide resistance, and for the natural insecticide *Bt* toxin—into a single tropical cultivar. Some researchers worry that such a heavy load of foreign genes will depress yields. Indeed, a single gene for an insecticidal toxin may have reduced yields when it was added to another strain of potatoes tested in upstate New York. But Roger Beachy, president and director of the new Donald Danforth Plant Science Center in St. Louis, who has put 14 genes into one rice strain, says: "I'm optimistic about their goals. The precedent for that sort of project is pretty good."

A Healthier Cassava

Known to Western societies as a source of tapioca, cassava is a mainstay of the developing world's diet. The leaves and starchy roots of this shrub, when powdered, boiled, fried, or fermented, make up the world's third largest source of calories, after rice and corn. About 60 years ago, British scientists working in East Africa began a program of plant breeding to increase the size and number of edible roots. Yields improved at first, but over the years the gains plateaued in the face of increasing losses to fungal, viral, and bacterial diseases. Now scientists are trying to push up yields again with the tools of biotechnology.

Rooting for sustenance. Freshly dug cassava.

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The impetus comes from three organizations: the International Laboratory for Tropical Agriculture and Biotechnology (ILTAB), originally based in La Jolla, California, but now moving to the Donald Danforth Plant Science Center in St. Louis; CIAT (the Center for International Tropical Agriculture) in Cali,