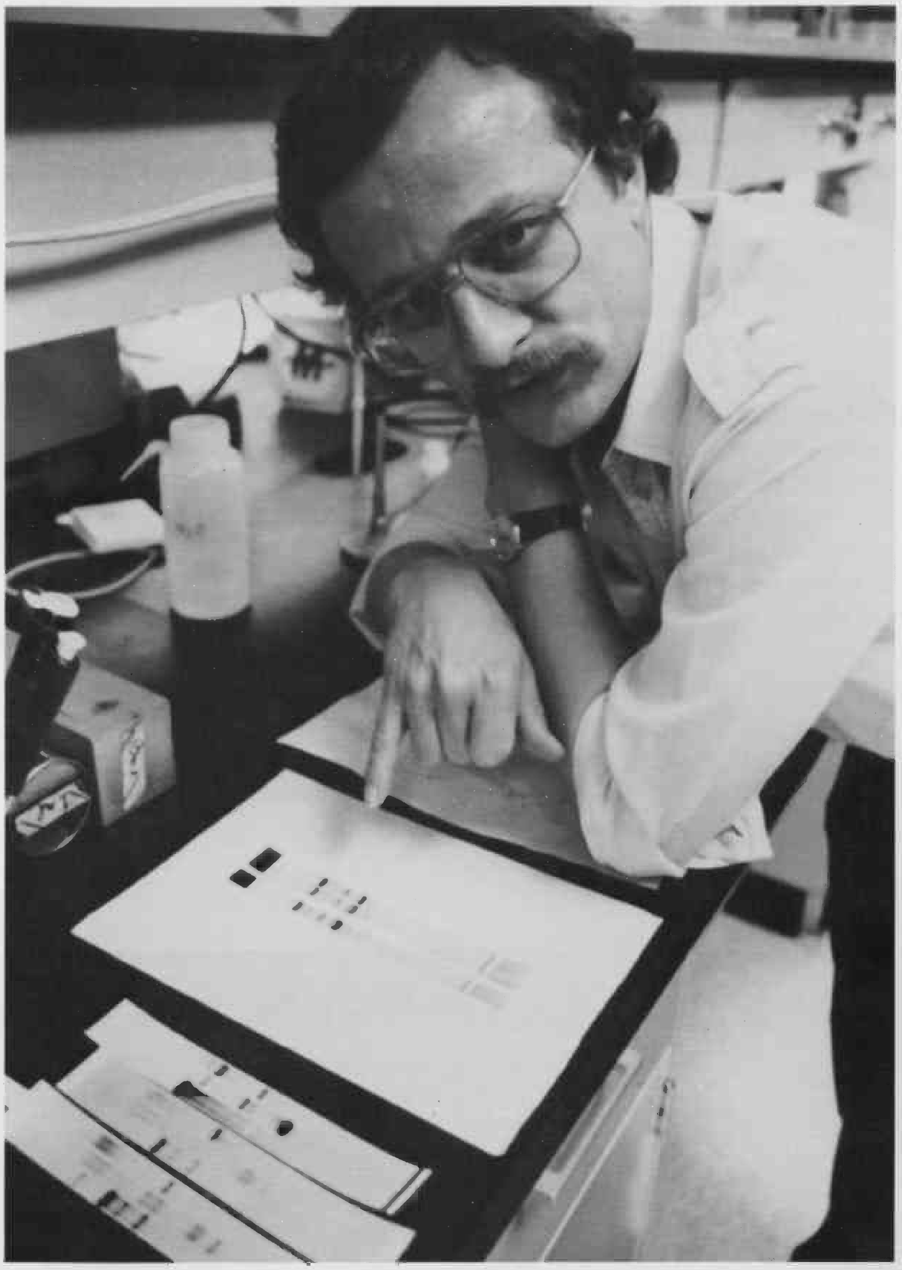

Bacteria Smuggle Genes Into Tobacco Plants

In a University of Arizona laboratory, specially altered bacteria are smuggling selected genes into plants from other types of plants and from bacteria.

UA biochemist Dr. Hans J. Bohnert, in charge of the project, predicted that the ability to move selected genes into crop plants "will have a wider impact than any other application of genetic engineering." However, he said, few researchers work on plant gene engineering compared with the number in animal projects such as moving genes from rats to mice or getting modified animal cells to churn out useful biochemicals.

Bohnert has already used the smuggler bacteria, which he helped develop, to put genes from peas and bacteria into tobacco plants.

He and colleagues are working to select genes for improving plants' resistance to salt and drought and for loosening an apparent bottleneck in plants' conversion of sunlight into food.

Tobacco plants have given Bohnert some advantages in the initial development of techniques. He is also working on cotton plants and intends to apply the gene additions to food plants.

Dr. Hans Bohnert describes a technique for identifying specific enzymes, which are coded by specific genes. (Photos by Ted Bundy.)

The process for adding genes works now, he said, "but the problem with adding genes to plants is that it's hard to know what will be a useful gene."

Seeking Genes for Salt Tolerance

The genes he has transferred so far do not carry traits with a foreseeable agricultural advantage. One makes the plant resistant to an antibiotic named kanamycin. Another makes an enzyme that changes one amino acid in the plant, arginine, into a related compound, nopaline, which the smuggler bacteria like to eat. He has also transferred important genetic stop-and-go signals needed for the plant to interpret its new genes.

Salt resistance is one trait with great potential for agricultural benefits. Salt in water or soil limits crop choices and productivity for farmers in many areas worldwide. In Arizona, salt problems affect some areas near Safford, Buckeye, Yuma and elsewhere.

In search of a gene that adds salt resistance, Bohnert's group of researchers is using iceplant. It is a succulent plant that changes some of its enzymes according to whether it is grown in salty or unsalty conditions.

"We have identified some enzymes that are made only under high-salt conditions," said Bohnert. "Finding the genes for them is the next step." The enzymes allow the plant to store up carbon dioxide from the atmosphere at night. Photosynthesis in daylight turns the carbon dioxide into food. Without these enzymes, the plant would need to keep more pores open in daytime to take in carbon dioxide. Keeping pores closed reduces evaporation of water from the plant.

Plant Cells Begin the Same:

Frank Katterman

The cells in a leaf are genetically the same as the cells in the same plant's root, so how do roots and leaves become different?

Dr. Frank R. H. Katterman, a University of Arizona plant scientist, studies what makes cells that start out the same divide into cells that begin to differ from each other. He has developed a system in which he can spot the exact stage when differentiation starts, so he can check various biochemical changes that may trigger the differentiation.

An improved ability to regenerate plants from test-tube cultures of cells would widen the variety of agricultural benefits likely from the genetic-engineering breakthroughs of the past decade, Katterman said. However, for many types of plants, including some important crops, getting generalized cell cultures to start differentiating into functioning plants is difficult.

"For example, you can grow soybean cells in cultures for 10 years, but you can't get a root to start from them," said Katterman.

He and his graduate student John Thomas are studying how differentiation starts. Their system uses undifferentiated cells from carrots, growing in test-tube cultures, rather than normally developing plant embryos in seeds.

Dr. Hans Bohnert and several other scientists worldwide are altering plants with new techniques of genetic engineering. Possible crop benefits include reduced needs for water or fertilizer, and improved characteristics in the harvested product. Selected genes can be smuggled into plants' chromosomes, or two types of plants that are mismatched for normal interbreeding can be genetically combined through a technique called proto-

Drought Tolerance and Photosynthesis

"Salt tolerance and drought tolerance are closely related," said Bohnert. Less evaporation from the plant means less salt stress because evaporation concentrates the salts left behind. The enzymes that his group has isolated from iceplant are also found in several other salt-tolerant and drought-tolerant plants.

The researchers plan to use the smuggler bacteria to put various ice-plant genes, including unidentified ones, into millions of other plant cells, then test those by large-scale screening tests to identify the ones with the right genes.

Bohnert helped engineer the smuggler bacterium at Germany's Max Plank Institute. He moved from there to the University of Arizona in 1983.

"It's called agrobacterium," he said. "Normally it infects plants and makes tumors grow on them, but we have modified it so that after the infection, the result is not a tumor, but another plant."

The process for putting kanamycin resistance into tobacco plants not only shows that this method for genetic engineering can work on plants, it also gives a tool for finding plant genes that might be worth moving from one plant to another. Where the inserted genes cut into the tobacco plant's lineup of genes, they make it easier to identify adjacent genes, said Bohnert.

In another possible application, Bohnert is working with a UA bio-chemist Dr. Richard C. Jensen, an expert on photosynthesis. They will check ways that genetic tinkering might speed up this basic step in all food production.

The clumped growth of cells on this plant is caused by a bacteria that can slip new genes into plant chromosomes.

Divide Into Roots and Leaves

plast fusion. Both methods depend on manipulations at the cell-culture level. Katterman said, "If you get a beneficial change, but can't regenerate the cells into a plant, it does no good."

Genetic engineering with plants has concentrated on the few types easiest to clone, such as tobacco. On the other hand, some cereal grains and other crops have not been successfully regenerated from cell cultures. Success has been spotty with other crops. For example, varieties of corn and cotton have been regenerated using formulas found through lengthy trial and error, "but then the same recipes usually won't work for closely related varieties," said Katterman. "We want to understand more of the general principles that apply to all plants. What makes plant cells differentiate in the first place?"

In his carrot cell cultures, differentiation shows up when individual cells take on characteristic shapes, well ahead of large-scale changes such as the formation of a visible root structure. By manipulating these cultures with plant hormones and with a chemical that is like a faulty letter in the DNA code, Katterman and Thomas have probed how closely differentiation is tied the replication of DNA.

Katterman said, "There apparently is a relationship between DNA replication and cell differentiation. The next step will be to determine which genes of the replicating DNA are directly responsible for the onset of differentiation. This is where the tools of genetic engineering will be extremely helpful."