## PLANTING NEW GENES

Bacteria aid first step toward improved crops by direct genetic manipulation

## BY JULIE ANN MILLER

Genetic engineering has been going on for ages in plants — with bacteria as the genetic engineers. Microorganisms, called agrobacteria, insert DNA into plant cells and force the plant to produce a compound on which only the agrobacteria can thrive. Human scientists interested in improving crops are now trying to take advantage of this natural system. And the most difficult problem of gene transfer, how to successfully insert and protect genes in a recipient cell, is already worked out for them by nature.

A gene chosen by scientists has for the first time been piggybacked onto the bacterial DNA carrier and moved into plant cells, Marc Van Montagu told SCIENCE NEWS. Experiments have just confirmed the presence of the new DNA in the plant. Van Montagu sees the result as a promising step toward endowing plants with desirable characteristics, such as resistance to insects and rotting or better protein production.

Agrobacteria are the microorganisms that produce crown gall tumors, a significant problem in almond and peach orchards and in rose gardens. But the tumors are interesting to scientists. Many of the tumors produce a rare amino acid, an opine, that only the bacteria can use as a carbon and nitrogen source. "It's a kind of genetic colonization of plants by bacteria," Van Montagu says. "This strange amino acid synthesis creates an ecological niche for the bacteria in the tumor."

The ability to synthesize and use the rare amino acids, as well as the ability to produce tumors, is carried on a plasmid, an independent ring of bacterial DNA. The plasmid is found in all agrobacteria in tumors, but not in bacteria living freely in the soil. The bacteria enter plants through wounds, and the plasmid passes into the plant cells. Then a segment of the plasmid snaps into the plant DNA where it is maintained during subsequent cell divisions.

Van Montagu and Jeff Schell of the State University of Gent in Belgium and Mary-Dell Chilton of the University of Washington have been leading investigations of this natural genetic engineering system. Chilton speculates that the system evolved in two steps: First, the plasmid acquired a gene for the rare amino acid production, then it developed a gene that lets the bacteria subsist on that amino acid.

Among agrobacteria, a variety of plasmids cause crown gall tumors. Chilton has found a common region of about 10,000

nucleotide pairs in all seven plasmids she has examined. The common region sits at one end of the plasmid segment maintained in the plant tumor. That region must contain any DNA coding for the tumor induction, she reasons. The more variable part of the maintained DNA may contain the directions for producing the rare amino acids, octopine in some tumors and nopaline in others.

Chilton and co-workers Martin H. Drummond, Milton P. Gordon and Eugene W. Nester have reported that the plasmid dna is active in tumor cells. They detected messenger RNA molecules transcribed on the foreign template (NATURE, Vol. 269, p. 535, 1977). But they do not know how the foreign dna brings about tumorous growth.

The agrobacterial plasmid seems an ideal vehicle for moving selected genes into plants. The Belgian workers now have slipped chosen DNA into the plasmids and allowed bacteria to carry it into plants. The foreign DNA is a mobile element, a transposon (SN: 6/17/78, p. 390), carrying a drug resistance gene. Schell and Van Montagu chose a plasmid where the transposon had snapped into the opine production gene. Plant cells incorporating that plasmid become tumorous, but do not make the rare amino acid. The investigators have now analyzed the plant tumor and they find the transposon appropriately inserted.

The next step, according to Van Montagu, is to try a similar experiment with a plant gene, instead of an antibiotic resistance gene from bacteria. He is optimistic because transposons are generally able to carry any DNA inserted in their centers.

But more work is needed before these techniques apply to agriculture. "It's unfortunate that you end up with a tumor," Chilton says. "No one wants to eat a tumor for dinner."

Under laboratory conditions, normal plants can be produced from crown gall tumors grown in culture. The plant cells have fewer copies of the plasmid DNA than do tumor cells, but they still produce the rare amino acid. So far the ability to produce a rare amino acid has not been successfully passed to a second generation through seeds. But improved plants might be cultivated through cuttings or tissue propagation.

Van Montagu is hopeful that breeding altered plants will become possible because many of the seeds are sterile. He says. "That shows the genes do go into



Crown gall tumor on leaf is agrobacteria's self-styled exclusive niche.





Chilton and Van Montagu plan to use agrobacteria to manipulate plant genes.

seeds; they behave abnormally."

Plant breeders may be able to take advantage of the agrobacterium plasmid before the details are known about how it operates, Chilton points out. But those fine points are important in another arena. The tumor cells in the plant grow uncontrollably in a manner analogous to many human tumors. Medical insights may result from learning how that small amount of foreign DNA frees plant tissue from its normal growth control.

JULY 15, 1978 45