Scientists shock plants into accepting 'naked' DNA

ITHACA, NY – With a brief, high-voltage electric shock, genetic engineers at Boyce Thompson Institute are transferring foreign DNA into plant cells and regenerating altered plants. This marks the first time the technique of electroporation has succeeded in plants.

The procedure, called electro-transformation by the BTI experimenters, is expected to allow the introduction of genetic information for improvement of the nutritional value and the synthesis of pharmaceuticals and other useful compounds in plants.

"Plant cells are totipotent; that is, an isolated, single cell can regenerate into a plant with normal roots, shoots, and leaves," explains Aladar A. Szalay, associate biochemist and director of the BTI plant genetics laboratory, where electro-transformation studies are in progress. "The introduction of foreign genetic material in the form of 'naked' or free DNA is the fundamental requirement for genetic engineering of plants."

Success of the technique, which was first used to create hormone-independent growth of gall tissue in wild carrot, was reported by William H.R. Langridge, BTI research associate, at a recent meeting of molecular biologists in Keystone, Colorado, and by Szalay at an international biotechnology symposium at Cornell University, June 23-27.

The experiments at Boyce Thompson Institute for Plant Research, an independent institution located on the campus of Cornell University, are funded by a grant from the National Science Foundation.

Electroporation refers to the opening, with high voltage electric pulses, of pores in the membranes of living cells. Electroporation DNA transfer experiments were begun at BTI in the fall of 1983 in parallel to similar experiments carried out with animal cells at Harvard Medical School.

There are three basic steps in the electro-transformation procedure, according to Langridge and Bao-Jian Li, professor of genetics at Chungshan University in the People's Republic of China, a visiting scientist in the BTI laboratory:

- foreign marker genes are constructed by recombinant DNA technology;
- the donor DNA containing the foreign genetic material with desired properties, is produced in E. coli and purified in large quantities; and
- the DNA is mixed with recipient plant cells and the mixture is subjected to a series of high voltage electrical pulses.

For their initial experiments, the BTI biologists used a foreign marker gene that would signal its presence by allowing the cultured plant cells to grow without added hormones. To obtain natrual genetic expression, they chose the Ti-plasmid DNA from ***Agrobacterium tumefaciens***, which grows in soil and causes galls in some plants.

The recipient plant cells used for the uptake of the donor DNA were obtained from ***Daucus carota*** (wild carrot). To overcome the cell wall barrier for DNA uptake, enzymes were used to remove the wall, exposing the cell membrane.

High-voltage electric pulses, a few microseconds in duration, opened pores in the carrot cell membrane, permitting introduction of the foreign DNA. Important to the process, according to the experimenters, is prevention of overheating of the plant cells by the high-voltage pulses.

After introduction of other marker genes, the carrot protoplasts were regenerated into plantlets capable of growing into mature plants. The observation of regenerability after electroporation is positive evidence of maintained viability of the plant cells, the BTI biologists stated. After 45 days, DNA-transformed embryos, known as teratomas, appeared.

"The foreign DNA was shown, by a physical method call DNA hybridization, to integrate into the plant chromosome," Langridge said. "Further, genes contained in the foreign DNA were expressed in the regenerated carrot embryos."

"Up to this time and in contrast to mammalian cells, naked DNA molecules were not successfully introduced into plant cells," Szaley said. "Electroporation seems to be a promising method for overcoming the limitations of introducing DNA into plant cells."

"The percentage of cells that take up DNA by electroporation is higher than obtained by previous methods," Langridge said. "We can now transform as many as 2 percent of the electroporated plant cells, and we are steadily improving the conditions for DNA uptake. We think the optimal conditions have not as yet been established; preliminary evidence indicates that 60 percent of the electroporated cells can be transformed."

The high level of transformation offers what the BTI researchers are calling an "exceptionally useful" tool for plant genetics. They look ahead to the application of electro-transformation to important crop plants, such as the introduction of genes carrying information for improving the nutritional value of plant proteins, and for the production of pharmaceuticals such as insulin, interferon, or peptide hormones.

In addition, the electro-transformation technique can be used by geneticists for studying the fate of engineered DNA molecules in plants, the BTI scientists believe.

"Just as the efficiency of transformation technology in bacteria and yeast has led to a tremendous explosion in knowledge of the genetics of these organisms," Szalay said, "our hope is that electroporation will lead to increased knowledge of gene expression in plants and plant organelles."