Plant Genetic Engineering: Progress and Promise[1]

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TOOLS OF GENETIC ENGINEERING

Plant genetic engineering technology has evolved as a spinoff of fundamental studies of crown gall disease, a plant cancer that is caused by *Agrobacterium tumefaciens* (Smith and Townsend, 1907). This soil bacterium causes galls on dicotyledonous plants by transferring genes into the chromosome of a cell at a wound site (Bevan and Chilton, 1982). The genes cause the cell to produce the plant growth hormones (auxin and cytokinin) that cause growth of the gall (Nester and Kosuge, 1981). One gene causes synthesis of a new metabolite (octopine or nopaline, for example) that serves as a specific nutrient for the inciting *Agrobacterium* strain. The gall can be viewed as a factory for production of specific food for the bacterium. The bacterium brings this about by a process of genetically engineering host plant cells.

Over the past 10 years, means have been developed to exploit the gene-transfer mechanism used by *Agrobacterium* for introduction of desirable genes into crop plants. The transferred genes are on a large circular plasmid called the Ti (tumor-inducing) plasmid. We now know how to introduce genes of our own choosing onto a "disarmed" Ti plasmid and allow *Agrobacterium* to transfer these to plant cells (Fraley et al., 1985; Matzke and Chilton, 1981; Zambryski et al., 1983). With certain kinds of plant cells, especially tobacco, it is possible to regenerate complete plants from such cells. The genetically engineered plant contains the new genes in all its cells and transfers them as a Mendelian trait to its progeny (Barton et al., 1983).

The first genetically engineered tobacco plant was described in a publication in April 1983, less than 5 years ago (Barton et al., 1983). We now know that totally alien genes, such as bacterial or yeast genes, do not function in the plant cell. However, it is possible to make them intelligible to the plant by splicing the coding region of the alien gene to "promoter" and "trailer" elements from genes that do express in plants—for example, the nopaline and octopine synthase genes. The resulting chimeric genes now function in the plant. For example, a bacterial kanamycin or chloramphenicol resistance gene, spliced to nopaline or octopine synthase promoter and trailer elements, functions to make a plant resistant to kanamycin or chloramphenicol (Bevan et al., 1983; Herrera-Estrella et al., 1983). The promoters of light-regulated plant genes, together with foreign coding regions, have been spliced together to make chimeric genes that when introduced into the original plant are light regulated (Facciotti et al., 1985). Other chimeric gene forms can direct the protein to chloroplast or mitochondrial target sites in the plant cell (Boutry et al., 1987; della-Cioppe et al., 1987). This approach can presumably be used to make chimeric genes that function at the desired level, time, and place in plant development.

HERBICIDE-RESISTANT PLANTS

Herbicides are chemicals that interfere with plant-specific processes, preferably processes not shared with other organisms. For example, inhibitors of photosynthetic processes or of essential amino acid biosynthetic pathways make potentially desirable herbicides, in that they are unlikely to be toxic to animals. Herbicide resistance can be conferred on a crop plant by two strategies: addition of a gene that allows the plant to detoxify the herbicide or addition of a gene that makes the target protein either in large amounts or in a resistant form. Both of these strategies have been used in projects developed in industry and academia over the last 3 years.

Plant Genetic Systems has produced tobacco, potato, and tomato plants that are resistant to the herbicide Basta. They used a gene from *Streptomyces hygroscopicus* that acetylates the herbicide to form an inactive product (Newmark, 1987).

CIBA-Geigy has engineered tobacco plants tolerant to atrazine by introduction of a gene encoding a glutathione-*S*-transferase that detoxifies the herbicide. Atrazine is a corn herbicide, and corn uses this detoxification pathway to make itself tolerant to atrazine. Soybeans grown in rotation with corn in certain soil types may occasionally be damaged by carryover. Thus, tolerance to atrazine would be a desirable trait to introduce into soybeans. CIBA-Geigy was given permission to field-test candidate atrazine-tolerant tobacco lines in North Carolina last summer. For the purpose of containment, the plants were prevented from outcrossing with native plants by removal of immature flowers continuously through the growing season.

Du Pont scientists (Chaleff and Mauvais, 1984; Yadav et al., 1986) and Sommerville's group at Michigan State University (Haughn et al., 1987) have studied resistance to sulfonylurea herbicides. Sommerville isolated a mutant weed (*Arabidopsis thaliana*) that was resistant to the herbicide because of a resistant form of the target enzyme, acetohydroxyacid synthase (AHAS). Sommerville and collaborators cloned the gene for the resistant enzyme, transferred it to sensitive tobacco plants, and found them to be resistant to the herbicide (Haughn et al., 1987).

Glyphosate resistance has been studied by Monsanto scientists and, more recently, by Calgene researchers. This herbicide has as its target EPSP synthase, an enzyme in the aromatic amino acid biosynthetic pathway. Monsanto scientists cloned the gene encoding this enzyme, made a chimeric form of the gene that increased the amount of enzyme produced, and reintroduced the high-level gene into petunia plants, which became fairly resistant to the herbicide (Shah et al., 1986). Calgene scientists isolated a mutant bacterium with a form of the enzyme that is resistant to glyphosate. A chimeric form of the corresponding gene, when introduced into tobacco plants, made them somewhat resistant to the herbicide (Comai et al., 1985). The bacterial enzyme lacks a "signal peptide" found in the plant enzyme that directs the enzyme to the chloroplast. A signal peptide can be added by more gene-splicing work, and presumably a chimeric construct encoding resistant EPSP synthase (della-Cioppa et al., 1987) will be more effective when restructured in this manner.

INSECT-RESISTANT PLANTS

Scientists at Plant Genetic Systems have engineered a toxin gene into tobacco plants to confer toxicity to lepidopteran larvae (Vaeck et al., 1987). The toxin gene originates from *Bacillus thuringiensis*, a bacterium currently used as a microbial insecticide. The bacterium forms a

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crystalline protein toxin of extremely high specificity. The toxin is the product of a single gene, which has been cloned and used to produce a chimeric form that expresses in plants. The engineered plant survives an onslaught of tobacco hornworms that is sufficient to strip the control plant down the midribs. Monsanto researchers have had equal success with introduction of a similar gene into tomato plants (Fischhoff et al., 1987).

Introduction of a gene encoding a protease inhibitor into tobacco plants also confers insect resistance (Hilder et al., 1987). Dr. Don Boulter at the University of Durham found that a trypsin inhibitor gene from an insect resistant strain of cowpea, when transplanted to tobacco, confers protection against budworm.

VIRUS-RESISTANT PLANTS

Dr. Roger Beachy and collaborators at Washington University and at Monsanto have engineered TMV resistance into tobacco and tomato plants by a process that may mimic classical cross-protection. The coat protein gene from the virus, when expressed in the host plant at a high level, confers protection from incoming virus (Abel et al., 1986). This discovery could provide an effective means of control of many viruses.

EMERGING TECHNOLOGIES

A. tumefaciens can transfer genes to many dicots, and recent evidence shows that certain monocots are also in its host range. Tumors have been incited on yam corms (Schaefer et al., 1987). Gene transfer from *Agrobacterium* to corn seedlings has been demonstrated by Dr. Barbara Hohn and her collaborators (Grimsley et al., 1987), who inserted tandem copies of the maize streak virus into a Ti plasmid vector. Maize plants inoculated with the engineered bacteria came down with maize streak virus infection. While this is not a genetically engineered maize plant, symbolically it shows that the approach has promise. Graves and Goldman (1986) have presented evidence for transfer of the octopine and nopaline synthase genes of T-DNA to maize shoots by inoculating seedlings with *Agrobacterium*. Despite these promising leads, no one has thus far reported producing a genetically engineered corn plant by using *Agrobacterium* gene vectors.

Protoplasts of many kinds of plant cells can be transformed without benefit of *Agrobacterium* or Ti plasmid vectors: naked DNA can enter protoplasts, aided by poly(ethylene glycol) or calcium phosphate, facilitated by electroporation to make the membrane permeable. This technology has been perfected by Dr. Ingo Potrykus and his collaborators (Shillito et al., 1985). DNA can also be microinjected into plant cell protoplasts, an approach taken by Calgene scientists and by Dr. Brian Miki of Agriculture Canada (Reich et al., 1986). By all of the above means, plant cells can be transformed, and in some cases complete plants can be regenerated. The principal problem for application to cereal crops is that, in general, cereal protoplasts do not regenerate to plants. However, a recent report (Abdullah et al., 1986) described regeneration of rice plants from protoplasts, giving hope that other cereals may be manipulated by similar procedures.

ENVIRONMENTAL ISSUES

In approving genetically engineered plants for field test, regulatory agencies will face a variety of potential concerns. Questions that such agencies must consider include the following:

1. Will the genetically engineered crop plant become a noxious weed?

Clearly, if this were a serious possibility, field testing should not be approved. However, several lines of argument can be marshaled to support the view that a genetically engineered crop plant is still going to be the same crop plant, not a weed:

A. Weediness is a consequence of a combination of a large number of traits. Introduction of one or two well-characterized traits could never bring about significant weediness in a crop plant.

B. Wide crosses of crop plants with weedy relatives are commonly done by plant breeders to introduce disease resistance into crop plants. The resulting progeny are regarded as safe for unregulated field testing. Genetically engineered crop plants have a far lower potential for weediness than wide cross progeny.

2. Will the gene we have engineered into the crop plant "escape" to weedy relatives through sexual crossing?

This is a serious concern and should be considered case by case. For wind-pollinated crops grown in the same geographical region as weedy relatives, escape of herbicide resistance could be a problem, e.g. out of sorghum into shattercane or Johnson grass.

3. Will the gene "escape" from the engineered crop plant into weeds by other means?

No mechanism of "horizontal" (asexual) gene transfer from plant to plant is known. If such a process did occur in nature, herbicide resistance (a selectable trait) would teach us of its existence. Natural herbicide resistance has not revealed any such mechanism. For example, the corn plant's GST detoxification system for atrazine has almost never appeared in atrazine-resistant weeds: they are nearly always resistant by an entirely different mechanism.

A potential concern would be a genetically engineered plant that still contains some of the vector used to engineer it. *Agrobacterium* or DNA vector systems are long gone by the time the plant is regenerated. Thus, for current technology, there is no known cause for concern. Virus vector systems, if any are ever developed, might pose some concern in the future.

4. Will the crop plant be toxic to man or beast?

This is a significant concern, but one that can and should be solved by suitable testing. It should only be a problem for a crop plant that is eaten, not for pine trees or cotton plants, for example. Even an old benign toxin such as BT toxin needs to be reexamined when introduced into a plant, to be sure that it produces no novel breakdown products with new toxicity spectra.

CONCLUDING REMARKS

The patentability of plants in the United States should put this country at the leading edge as a potential market for genetically engineered crop plants in years to come. This promising technology may produce disease and insect resistance traits that will be extremely difficult for these pests to overcome because of their novel mechanisms. Crop plants may be engineered to produce seed storage proteins of more nutritious amino acid composition than their natural counterparts. Undesired biochemical pathways may be diminished or eliminated from certain plants. This could reduce the need for chemical processing and refinement steps. In the far distant future, this technology may give us crop plants of novel morphology and architecture. These are challenging goals indeed, but the rapid rate of progress in this science over the past 5 years augurs well that its future will be bright.

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