

Applications in Biotechnology: Field Testing Genetically Engineered Plants and Microorganisms

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ABSTRACT

Genetic manipulation of plants and microorganisms through molecular biology procedures has proceeded rapidly, resulting in expanding numbers of field tests and the initiation of numerous commercial development ventures. Currently, ninety-one field tests of genetically engineered plants (GEPs) and eleven of genetically engineered microorganisms (GEMs) have been conducted for at least one year, and the number of applications for additional tests is increasing. A regulatory framework has been developed between the U.S. Department of Agriculture (for GEPs) and the Environmental Protection Agency (for GEMs); this framework has functioned effectively in the application and approval process for each test. The lesser concern over field tests of GEPs (because of ease of containment) has resulted in greater numbers of approved tests than has been the case with GEMs. This paper summarizes the current status of each approved field test--geographical location, plant species and/or microorganisms involved, and the engineered genetic trait.

INTRODUCTION

The potential agricultural benefits that may result from biotechnology have been widely debated by the scientific community, public interest groups and federal regulatory agencies. Most predictions point to agriculture as one industry that will reap many of the greatest rewards from new developments in biotechnology, and more efficient and environmentally safe food and fiber production is a major potential benefit (Dines, 1985). During the 1980's, concern over widespread use of pesticidal chemicals in the environment was a major factor in promoting molecular research to develop plants and microbes that would either require fewer treatments or serve as alternatives to chemical applications.

Genetic manipulation through recombinant DNA procedures has the potential to increase the effectiveness of engineered plants and microbes and to expand the range of soils and environmental conditions into which the plants and microbes could be released (Betz, 1988). There are now several examples of successes in engineering plants for enhanced resistance to specific pathogens, insect pests, or herbicides which are currently being field tested (Cramer and Radin, 1990). The development of engineered microbes that will contribute to plant productivity by eliciting more rapid growth or protecting plants from pathogens or pests has also proceeded rapidly (Vaughan, 1988). While concerns have arisen that engineered plants and microbes could have the potential to cause environmental problems

after field release (Curtis, 1988), the evidence obtained from the detailed review process for approval of such releases indicates that foreseeable problems can be avoided (Milewski, 1990). This paper summarizes the field tests of engineered plants and microbes that have been conducted to date.

I. GENETICALLY ENGINEERED PLANTS (GEPs)

The development of genetically engineered plants (GEPs) offers potential commercial opportunities in a wide range of applications (Cramer and Radin, 1990). The use of GEPs also generates difficult questions for regulatory agencies as well as the public and the scientific community regarding the safety of field testing on a broad agricultural scale. The review and approval process must contend with the twin objectives of allowing society to benefit from new products and minimizing risks to public health and the environment (Curtis, 1988; Parry and Miksche, 1988).

Compared with conventional processes such as plant breeding and field-based germplasm screening, genetic engineering techniques have contributed, for several crops, faster and more accurate means of developing new plant lines and identifying important plant genes (An et al, 1986). The ability to screen plant material for the specific genes of interest can dramatically shorten the time required to produce new crop varieties. Most strikingly, it is now possible with genetic engineering to transfer genes between very different kinds of organisms--something not previously achievable (Vaecck et al., 1988). For example, the use of transposons to both remove and insert specific genes has permitted the transfer and expression of genes between unrelated plants and between plants and microorganisms.

The U. S. Department of Agriculture (USDA) has instituted a rigorous evaluation process under the Federal Plant Pest Act that reviews each request to field test a GEP (Miksche and Chandra, 1988). If the GEP contains genetic material from a microorganism, then the U.S. Environmental Protection Agency (EPA) also participates and conducts a joint review with USDA (Betz, 1988). The appropriate state Department of Agriculture is also contacted by USDA and may elect to hold a separate review (Milewski, 1990). To date, 91 GEP tests have been approved and initiated; others are now in the final review process (Table 1).

Herbicide Tolerance: To date, 36 field tests of GEPs have been conducted with five crops in 12 states; the first tests were initiated in 1987. The source of the resistance genes is usually a plant, such as tobacco or petunia, that is naturally tolerant to the herbicides. Since many bacteria are tolerant to herbicides, some GEPs have received their resistance genes from bacteria (Cramer and Radin, 1990). Genetic tolerance has been successfully transferred for several classes of herbicides including bromoxynil (Buctril, by Hoechst AG), glufosinate (Basta, by Rhone-Poulenc), glyphosate (Roundup, by Monsanto), and sulfonylurea (Classic and Express, by Dupont).

Insect/Pest Resistance: At present, 45 field tests of GEPs have been conducted with eight crops in 20 states, and the first tests were initiated in 1988. The goal of genetically engineering crops for insect/pest resistance is to allow more uniform control of insects and pests and to incorporate control technologies that are not dependent on a chemical application. The source of the resistant genes is usually microorganisms (bacteria and viruses) that are natural pathogens of insects (Bishop et al., 1988). The GEP is then able to use the microbial genes to produce

TABLE 1. GEP Field Tests, Target Crops, and Locations

Engineered Trait	Number of Tests	Target Crops	Test Locations
Herbicide Tolerance	36	Alfalfa, Cotton, Soybean, Tobacco, Tomato	AL,AR,AZ,CA,DE FL, HI, IA, IL, IN, KY,MN,MO,MS,NC, TN,VA
Insect/Pest Resistance	45	Alfalfa, Cantalope, Cotton, Cucumber, Potato, Squash, Tobacco, Tomato	AL,AZ,CA,DE,FL, GA,HI,IA,ID,IL, LA,MD,MN,MS,TX, NE,NC,NY,WA,WI
Wound Induced Enzymes	5	Tobacco, Poplar	DE,IA,IA
Delayed Fruit Ripening	5	Tomato	CA, FL, HI

a toxin that kills feeding insects. The discovery of important viral resistance mechanisms in bacteria and the ability to locate the responsible genes has been the basis for conferring viral resistance in many GEPs.

Genetic resistance has been successfully transferred in plants for several classes of beetles (Coleoptera) and caterpillars (Lepidoptera) and plant viruses (leaf roll and mosaic viruses)(Vaeck et al., 1988).

Wound Induced Enzymes: To date, five field tests of GEPs have been conducted with two crops in three states; they were initiated in 1988. The goal of genetically engineering crops for the production of wound induced enzymes is to enable the plant to repair cellular damage caused by pests, weather (hail), or mechanical injury. The source of the enzyme production genes is potatoes, and the genes have been successfully transferred (with bacterial transposons) to tobacco and poplar. This research is proprietary to Iowa State University, and little information is available to date.

Delayed Fruit Ripening: At present, five field tests of GEPs have been conducted with one crop (tomatoes) in three states; these were initiated in 1989. The goal of genetically engineering crops for delayed fruit ripening is to allow most of the crop to ripen over a short time span, and to permit a faster and more efficient harvest. The source of the genes that delay fruit ripening is from a non-commercial tomato variety, and the genes were transferred with bacterial transposons (which makes the recipient tomato variety a GEP).

GEP Summary: Generally, the results of the field tests shown in Table 1 have been positive and have demonstrated that GEPs can be effective under field conditions. Several herbicide tolerance studies will be in their fifth year this season, and some of the GEPs in those tests have already been entered into the commercial development process. The number of approved GEP field tests is increasing each

season, and tests with genetically engineered corn and wheat are anticipated in the near future. The first test of a GEP in Virginia occurred during the 1990 field season. The GEP was a soybean resistant to Roundup (Monsanto), and the test was conducted on a farm near Emporia, in Greensville County. The test was reviewed and approved by USDA and the Virginia Department of Agriculture and Consumer Services.

II. GENETICALLY ENGINEERED MICROORGANISMS (GEMs)

In the future, much of the leading biotechnological research that applies to agriculture will involve the development of genetically engineered microorganisms (GEMs) that are designed to perform specific functions. Compared with conventional procedures for manipulating microorganisms, genetic engineering techniques have made it possible to transfer genes between unrelated bacteria and to provide recipient bacteria with characteristics that are not naturally found (Joos et al., 1988). As with GEPs, the use of transposons have been the basis for the creation of most GEMs, and transposons have provided the means for identifying important microbial genes and then quickly developing GEMs with novel properties.

Some of the proposed functions of GEMs include enhancement of nitrogen fixation (Gerhold and Stacey, 1990), destruction of weeds, repression of fungal pathogens (Howell, 1990), control of insect pests (Bishop et al., 1988), or biodegradation of pesticide residues (Brosten, 1987; GAO, 1988). Concerns over the difficulty of containing GEMs in the field has resulted in smaller numbers of approved tests than has been the case with GEPs (Curtiss, 1988). Although no products presently sold for field use contain GEMs, several field studies with GEMs are currently in progress (Table 2).

Reduced Frost Damage: Two field trials in California involve the same organism: a strain of the bacterium *Pseudomonas syringae*. This bacterium resides on plant leaf surfaces and promotes the formation of ice crystals at temperatures near freezing. It is this formation of ice crystals that causes damage to plant cells from frost injury. Using recombinant-DNA techniques, a mutant strain that lacked the ability to enhance ice formation was developed. This mutant strain is the "ice-minus bacterium" that has received such wide coverage by the press. These mutant GEMs, when applied to plants, reduce ice formation at near-freezing temperatures and protect plants from frost injury. If successful, this product will protect sensitive crops from early frosts and will extend the growing season (Lindow, 1990).

The first test of the product was conducted in April 1987 at Brentwood, CA by Advanced Genetic Sciences, Inc. A suspension of "ice-minus" GEMs was sprayed on strawberry plants. A second test was conducted on potatoes in May of that year near Tuelle Lake, CA by scientists from the University of California. These two experiments were the first GEM field test to be conducted in the United States (Lindow and Panopoulos, 1988). Both tests were vandalized by groups claiming to represent environmental concerns. Each test was replanted, the evaluations were continued, and the tests are now in their fourth year of field trials. To date, the only environmental damage that has been documented as occurring in association with these field tests has been the damage sustained from the concentrations of herbicides dumped on the potato trial by vandals.

TABLE 2. GEM Field Tests, the Engineered Organism, Target Crops, and Test Locations.

Engineered Trait	Engineered Organism	Target Crops	Test Locations
Reduced Frost Damage	<i>Pseudomonas syringae</i>	Potatoes, Strawberries	CA
Biological Insecticides	<i>Clavibacter xyli</i>	Corn, Rice	MD, MN, NE NY, IL
Enhanced Nitrogen Fixation	<i>Bradyrhizobium japonicum</i> and <i>Rhizobium meliloti</i>	Alfalfa Soybeans	IA, WI, LA
Environmental Monitoring	<i>Pseudomonas aureofaciens</i>	None	SC, WA

Biological Insecticides: Crop Genetics International, Inc. has been conducting GEM field tests in five states. The organism *Clavibacter xyli*, a bacterium that resides in the water-conducting vessels of grass family plants, has been "designed" by recombinant DNA to control the European Corn Borer in corn. This GEM was "created" by inserting genes from another bacterium, *Bacillus thuringiensis*, that produce a toxin lethal to the European Corn Borer. The *Bacillus thuringiensis* bacterium has been known and used to control lepidopterous insects for many years; however, the bacterium will not survive long on plant surfaces (Carlton, 1988). By the incorporation of the toxin genes from *B. thuringiensis* into *Clavibacter xyli*, an endophytic bacterium of corn, it is hoped that season-long control of European Corn Borer can be obtained.

Enhanced Nitrogen Fixation: In 1988, a test of a GEM was established by Biotechnica International in Peppin County, WI. The organism is a strain of *Rhizobium meliloti* that has been genetically altered to fix greater amounts of nitrogen than strains that are currently available. Members of the genus *Rhizobium* are responsible for nodule formation on the roots of legume plants (e.g., soybeans, alfalfa, peanuts, clover, peas, and vetch). It is within these nodules that the bacteria "fix" atmospheric nitrogen into an organic form that the plant can use for growth (Gerhold and Stacey, 1990). This GEM (*Rhizobium* mutant) carries extra copies of the genes that direct the nitrogen fixation process, and in greenhouse tests, yields of alfalfa hay were enhanced. Field testing of this GEM on alfalfa is scheduled to last three years. Biotechnica International has also developed similar GEMs of *Bradyrhizobium japonicum*, the nitrogen fixing strain for soybeans, and has received approval for three additional field tests on soybeans in Iowa, Louisiana, and Wisconsin. These tests were approved and initiated in the summer of 1990.

Environmental Monitoring: In 1987, a field test of a GEM was established near Blackville, SC, in a cooperative effort between Clemson University and the Monsanto Corporation. The GEM tested is a strain *Pseudomonas aureofaciens* that contains genes obtained from another bacterium, *Escherichia coli*. These genes were transferred by recombinant DNA techniques into the *Pseudomonas* strain, and the function of the genes conferred novel properties to the *Pseudomonas* that allowed it to be "tracked" in the soil environment (Drahos et al., 1988). This field trial is scheduled to be conducted for four years in a wheat-corn-soybean rotation. The GEM being tested was not designed for a specific commercial purpose, but rather as a root colonizer to evaluate techniques that are used to "track," recover, and identify a GEM from the environment. An additional test evaluating this GEM as a biological control agent to protect wheat roots from diseases was approved in 1990 and initiated in the state of Washington.

GEM Summary: Each of the GEM tests described above has received vigorous review and evaluation by EPA, the USDA Animal and Plant Health Inspection Service (APHIS), and the state Department of Agriculture in each state where a test has been requested (Betz, 1988; Miksche and Chandra, 1988; Milewski, 1990). In addition, panels of university experts were formed to review each test application (GAO, 1988; Office of Science and Technology Policy, 1986; US-EPA, 1986).

The number of requests to conduct GEM field tests is increasing now that a regulatory framework is in place to evaluate and approve such requests. Activity in the development and testing of GEMs in the field is bringing the day closer when commercial products containing GEMs will be available to the farmer. Although no GEM field tests have yet been conducted in Virginia, the time is not far off when such tests will also be conducted in the Commonwealth, and GEM-derived products will be available to our growers. While it is unlikely that biotechnology products (GEMs and GEPs) will revolutionize agricultural practices, the materials developed through biotechnology will expand production options available to farmers, furthering their capacity to remain profitable and competitive in international markets while reducing contamination of food products and the environment by pesticide residues.

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