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Genetically Engineering Plants for Crop Improvement

CHARLES S. GASSER AND ROBERT T. FRALEY

Dramatic progress has been made in the development of gene transfer systems for higher plants. The ability to introduce foreign genes into plant cells and tissues and to regenerate viable, fertile plants has allowed for explosive expansion of our understanding of plant biology and has provided an unparalleled opportunity to modify and improve crop plants. Genetic engineering of plants offers significant potential for seed, agrichemical, food processing, specialty chemical, and pharmaceutical industries to develop new products and manufacturing processes. The extent to which genetically engineered plants will have an impact on key industries will be determined both by continued technical progress and by issues such as regulatory approval, proprietary protection, and public perception.

THE STABLE INTRODUCTION OF FOREIGN GENES INTO plants represents one of the most significant developments in a continuum of advances in agricultural technology that includes modern plant breeding, hybrid seed production, farm mechanization, and the use of agrichemicals to provide nutrients and control pests. The first-generation applications of genetic engineering to crop agriculture are targeted at issues that are currently being addressed by traditional breeding and agrichemical discovery efforts: (i) improved production efficiency, (ii) increased market focus, and (iii) enhanced environmental conservation. Genetic engineering methods complement plant breeding efforts by increasing the diversity of genes and germplasm available for incorporation into crops and by shortening the time required for the production of new varieties and hybrids. Genetic engineering of plants also offers exciting opportunities for the agrichemical, food processing, specialty chemical, and pharmaceutical industries to develop new products and manufacturing processes.

The first transgenic plants expressing engineered foreign genes were tobacco plants produced by the use of *Agrobacterium tumefaciens* vectors (1). Transformation was confirmed by the presence of foreign DNA sequences in both primary transformants and their progeny and by an antibiotic resistance phenotype conferred by a chimeric neomycin phosphotransferase gene. These early transformation experiments often utilized plant protoplasts as the recipient cells; the subsequent development of transformation methods based on regenerable explants (2) such as leaves, stems, and roots contrib-

uted significantly to the facile and routine transformation methods that are used today for many dicotyledonous plant species. A variety of free DNA delivery methods, including microinjection, electroporation, and particle gun technology are being developed for the transformation of monocotyledonous plants such as corn, wheat, and rice. In view of the rapid progress that is being made, it is likely that all major dicotyledonous and monocotyledonous crop species will be amenable to improvement by genetic engineering within the next few years.

In this article, we describe transformation methods that have been developed for plants and discuss some of the applications of genetically engineered plants in agriculture. We also address some of the critical issues that will influence the commercialization of genetically engineered crops.

Methods for Introducing Genes into Plants

Agrobacterium tumefaciens-mediated gene transfer. Derivatives of the plant pathogen *Agrobacterium tumefaciens* have proved to be efficient, highly versatile vehicles for the introduction of genes into plants and plant cells. Most transgenic plants produced to date were created through the use of the *Agrobacterium* system. *Agrobacterium tumefaciens* is the etiological agent of crown gall disease and produces tumorous crown galls on infected species. The utility of this bacterium as a gene transfer system was first recognized when it was demonstrated that the crown galls were actually produced as a result of the transfer and integration of genes from the bacterium into the genome of the plant cells (3). Virulent strains of *Agrobacterium* contain large Ti (for tumor inducing) plasmids, which are responsible for the DNA transfer and subsequent disease symptoms. Genetic and molecular analyses showed that Ti plasmids contain two sets of sequences necessary for gene transfer to plants; one or more T-DNA (transferred DNA) regions that are transferred to the plant, and the *Vir* (virulence) genes which are not, themselves, transferred during infection. The T-DNA regions are flanked by border sequences that were shown to be responsible for the definition of the region that is to be transferred to the infected plant cell. The T-DNA contains 8 to 13 genes (4), including a set for production of phytohormones, which are responsible for formation of the characteristic tumors when transferred to infected plants. Several excellent reviews on the biology of this and other pathogenic species of *Agrobacterium* have been published for those who desire more detailed information (4).

Early experiments demonstrated that heterologous DNA inserted into the T-DNA could be transferred to plants along with the existing T-DNA genes (5). Efficient plant transformation systems were constructed by removing the phytohormone biosynthetic

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genes from the T-DNA region, thereby eliminating the ability of the bacteria to induce aberrant cell proliferation (6). Modern plant transformation vectors are capable of replication in *Escherichia coli* as well as *Agrobacterium*, allowing for convenient manipulations (7). The general features of these vectors and the process of transfer to plant cells are outlined in Fig. 1. Recent technological advances in vectors for *Agrobacterium*-mediated gene transfer have involved improvements in the arrangements of genes and restriction sites in the plasmids that facilitate construction of new expression vectors. Vectors in current use have convenient multilinker regions, which may be flanked by a promoter and a polyadenylate addition site for direct expression of inserted coding sequences (8).

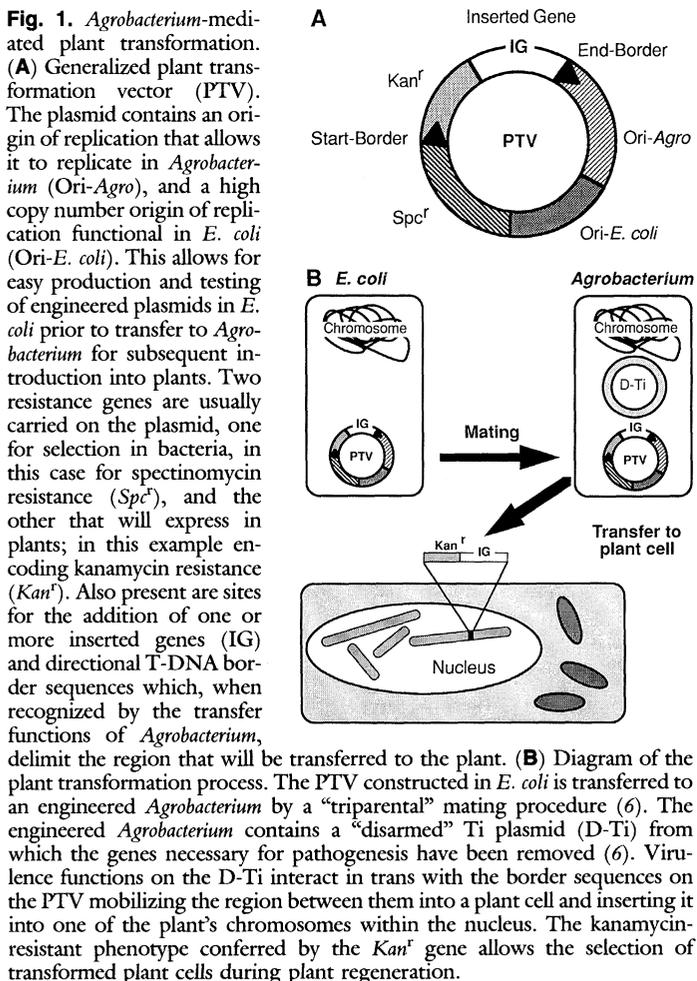
Agrobacterium constitutes an excellent system for introducing genes into plant cells, since (i) DNA can be introduced into whole plant tissues, which bypasses the need for protoplasts, and (ii) the integration of T-DNA is a relatively precise process. The region of DNA to be transferred is defined by the border sequences; occasional rearrangements do occur, but in most cases an intact T-DNA region is inserted into the plant genome (9). This contrasts with free DNA delivery systems in which the plasmids routinely undergo rearrangement and concatenation reactions before insertion and can lead to chromosomal rearrangements during insertion in both animal (10) and plant (11) systems. Sequencing of insertion sites shows that only small duplications or other changes occur in flanking sequences during T-DNA integration (12). The stability of expression of most genes that are introduced by *Agrobacterium* appears to be excellent. Published studies have shown that integrated T-DNAs give consistent genetic maps and appropriate segrega-

tion ratios (1, 13). Introduced traits have been found to be stable over at least five generations during cross-breeding and seed increase on genetically engineered tomato and oilseed rape plants (14). This stability is critical to the commercialization of transgenic plants. The list of plant species that can be transformed by *Agrobacterium* has been greatly expanded and now includes several of the most important broadleaf crops (Table 1).

Advances in other transformation technologies. In those systems where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer. Few monocotyledonous plants appear to be natural hosts for *Agrobacterium*, although transgenic plants have been produced in asparagus with *Agrobacterium* vectors (15) and transformed tumors have been observed in yam (16). Cereal grains such as rice, corn, and wheat have not been successfully transformed by *Agrobacterium*, despite encouraging evidence for T-DNA transfer in corn (17). Extensive efforts have consequently been directed toward the development of systems for the delivery of free DNA into these species. The first of these systems to give demonstrable transformation of plant cells relied on physical means similar to those used in the transformation of cultured animal cells. Transformation has been achieved in plant protoplasts through facilitation of DNA uptake by calcium phosphate precipitation, polyethylene glycol treatment, electroporation, or combinations of these treatments (18). These methods have allowed the production of transgenic cells for the study of gene expression in systems that cannot be transformed by other means (19).

The applicability of these systems to the production of transgenic plants is limited by the difficulties involved in regenerating plants from protoplasts. There have been significant advances in the regeneration of cereals (traditionally one of the most recalcitrant groups) from protoplasts. Several laboratories have succeeded in regenerating fertile rice plants from protoplasts (20). This advance was rapidly followed by the production of transgenic rice plants through the delivery of free DNA to protoplasts followed by regeneration (21). Progress in regeneration of corn has been more limited; one group demonstrated regeneration of mature plants from protoplasts and succeeded in producing transgenic plants (22, 23). However, all plants were sterile, apparently as a result of the necessary period in culture or the regeneration procedure. While this progress is encouraging, limitations remain in the application of this technology to cereal crop improvement. In corn and rice, the ability to form regenerable protoplasts appears to be primarily confined to a small number of varieties. Even if the fertility problems are overcome, introduction of the transferred genes into the broad range of commercial varieties in use today would require a lengthy period of backcrossing.

In parallel with the work on protoplast transformation, efforts to find novel ways to introduce DNA into intact cells or tissues have been emphasized. Regeneration of cereals from immature embryos or from explants is relatively routine (24). One of the most significant developments in this area has been the introduction of "particle gun" or high-velocity microprojectile technology. In this system, DNA is carried through the cell wall and into the cytoplasm on the surface of small (0.5 to 5 μm) metal particles that have been accelerated to speeds of one to several hundred meters per second (25-27). The particles are capable of penetrating through several layers of cells and allow the transformation of cells within tissue explants. Production of transformed corn cells (28) and fertile, stably transformed tobacco (26) and soybean (27) plants with particle guns has already been demonstrated. By eliminating the need for passage through a protoplast stage, the particle gun method has the potential to allow direct transformation of commercial genotypes of cereal plants. Intensive efforts to produce transgenic cereals by the



use of particle guns are currently under way in many laboratories around the world.

Other methods that have the potential to influence the production of transgenic cereals include gene transfer into pollen (29), direct injection into reproductive organs (30), microinjection into cells of immature embryos (31), and rehydration of desiccated embryos (32). There has been some demonstration of transient or stable gene expression through the use of each of these methods in some species, but the range of their applicability remains to be demonstrated.

Application of Genetic Engineering to Crop Improvement

The availability of efficient transformation systems for crop species is of intense interest to biotechnology, agrichemical, and seed companies for the application of this technology to crop improvement. Initial research has been focused on the engineering of traits that relate directly to the traditional roles of industry in farming, such as the control of insects, weeds, and plant diseases. Progress has been rapid, and genes conferring these traits have already been successfully introduced into several important crop species. Genetically engineered soybean, cotton, rice, corn, oilseed rape, sugarbeet, tomato, and alfalfa crops are expected to enter the marketplace between 1993 and 2000.

Weed control. Engineering herbicide tolerance into crops represents a new alternative for conferring selectivity and enhancing crop safety of herbicides. Research has largely concentrated on those herbicides with properties such as high unit activity, low toxicity, low soil mobility, and rapid biodegradation and with broad spectrum activity against various weeds. The development of crop plants that are tolerant to such herbicides would provide more effective, less costly, and more environmentally attractive weed control. The commercial strategy in engineering herbicide tolerance is to gain market share through a shift in herbicide use (33)—not to increase the overall use of herbicides, as is popularly held. Herbicide-resistant plants will have the positive impact of reducing overall herbicide use through substitution of more effective and environmentally acceptable products.

Two general approaches have been taken in engineering herbicide tolerance: (i) altering the level and sensitivity of the target enzyme for the herbicide and (ii) incorporating a gene that will detoxify the herbicide. As an example of the first approach, glyphosate, the active ingredient of Roundup herbicide, acts by specifically inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (34). Glyphosate is active against annual and perennial broadleaf and grassy weeds, has very low animal toxicity, and is rapidly inactivated and degraded in all soils (35). Tolerance to glyphosate has been engineered into various crops by introducing genetic constructions for the overproduction of EPSPS (36) or of glyphosate-tolerant variant EPSPS enzymes (37, 38). Similarly, resistance to sulfonylurea compounds, the active ingredients in Glean and Oust herbicides, has been produced by the introduction of mutant acetolactate synthase (ALS) genes (39). Glean and Oust are broad-spectrum herbicides and are effective at low application rates. Since both EPSPS and ALS activities are present in wild-type plants, the possibility of deleterious effects on crop performance or product quality due to their reintroduction is unlikely. The use of these herbicides in new crop applications may require reexamination of residues of the herbicides; however, since the residue safety levels for these two compounds in food crops have already been established, this is not an issue unique to genetically engineered plants.

Resistance to glufosinate (40) and bromoxynil (41) has been achieved by the alternative approach of introducing bacterial genes

encoding enzymes that inactivate the herbicides by acetylation or nitril hydrolysis, respectively. In field tests the glufosinate-tolerant plants have shown excellent tolerance to the herbicide (42). Evaluation of the biological activity of the specific herbicide conjugates and metabolites that may be present in the transgenic plants will be carried out according to existing chemical residue regulations.

Current crop targets for engineered herbicide tolerance include soybean, cotton, corn, oilseed rape, and sugarbeet. Factors such as herbicide performance, crop and chemical registration costs, potential for out-crossing to weed species, proprietary rights issues, and competing herbicide technologies must all be considered before final decisions on commercialization of specific herbicide-tolerant crops can be made.

Insect resistance. The production of insect-resistant plants is another application of genetic engineering with important implications for crop improvement and for both the seed and agrichemical industries. Progress in engineering insect resistance in transgenic plants has been achieved through the use of the insect control protein genes of *Bacillus thuringiensis* (B.t.). *Bacillus thuringiensis* is an entomocidal bacterium that produces an insect control protein which is lethal to selected insect pests (43). Most strains of B.t. are toxic to lepidopteran (moth and butterfly) larvae, although some strains with toxicity to coleopteran (beetle) (44) or dipteran (fly) (45) larvae have been described. The insect toxicity of B.t. resides in a large protein; this protein has no toxicity to beneficial insects, other animals, or humans (46). The mode of action of the B.t. insect control protein is thought to be exerted at the level of disruption of ion transport across brush border membranes of susceptible insects (47).

Table 1. Species for which the production of transgenic plants have been reported. Abbreviations: At, *Agrobacterium tumefaciens*; Ar, *Agrobacterium rhizogenes*; FP, free DNA introduction into protoplasts; PG, particle gun; MI, microinjection; IR, injection of reproductive organs.

Plant species	Method (reference)
<i>Herbacious dicots</i>	
Petunia	At (2)
Tomato	At (83)
Potato	At (84)
Tobacco	At (1), FP (85), PG (26)
<i>Arabidopsis</i>	At (86)
Lettuce	At (87)
Sunflower	At (88)
Oilseed rape	At (89), MI (31)
Flax	At (90)
Cotton	At (91)
Sugarbeet	At (92)
Celery	At (93)
Soybean	At (38), PG (27)
Alfalfa	At (94)
<i>Medicago varia</i>	At (95)
Lotus	At (96)
<i>Vigna aconitifolia</i>	FP (97)
Cucumber	Ar (98)
Carrot	Ar (99)
Cauliflower	Ar (100)
Horseradish	Ar (101)
Morning glory	Ar (102)
<i>Woody dicots</i>	
Poplar	At (103)
Walnut	At (104)
Apple	At (105)
<i>Monocots</i>	
Asparagus	At (15)
Rice	FP (21)
Corn	FP (23)
Orchard grass	FP (106)
Rye	IR (30)

Transgenic tomato, tobacco, and cotton plants containing the B.t. gene exhibited tolerance to caterpillar pests in laboratory tests (48). The level of insect control observed in the field tests with tobacco and tomato plants has been excellent; in one such test tomato plants containing the B.t. gene suffered no agronomic damage under conditions that led to total defoliation of control plants (49).

The excellent insect control observed under field conditions indicates that this technology may have commercial application in the near future. Early market opportunities for caterpillar resistance are leafy vegetable crops, cotton, and corn. Crop targets for beetle resistance are potato and cotton. Other types of insecticidal molecules are necessary to extend biotechnology approaches for controlling additional insect pests in these and other target crops. Plants genetically engineered to express a proteinase inhibitor gene are partially resistant to tobacco budworm in laboratory experiments (50); field tests will be necessary to determine the agronomic utility of this approach.

Disease resistance. Significant resistance to tobacco mosaic virus (TMV) infection, termed "coat protein-mediated protection," has been achieved by expressing only the coat protein gene of TMV in transgenic plants (51). This approach produced similar results in transgenic tomato, tobacco, and potato plants against a broad spectrum of plant viruses, including alfalfa mosaic virus, cucumber mosaic virus, potato virus X, and potato virus Y (52). One mechanism of coat protein-mediated cross protection appears to involve interference with the uncoating of virus particles in cells before translation and replication (53).

Transgenic tomatoes carrying the TMV coat protein gene have been evaluated in greenhouse and field tests and shown to be highly resistant to viral infection (Fig. 2) (54). The transgenic plants showed no yield loss after virus inoculation, whereas the yield was reduced 23% to 69% in control plants. The level of capsid protein in the engineered plants [typically 0.01% to 0.5% of the total protein (52)] is well below the levels found in plants infected with this endemic virus. This fact should facilitate registration and commercialization of virus-resistant plants. Virus resistance could provide significant yield protection in important crops such as vegetables, corn, wheat, rice, and soybean.

While limited success in engineering resistance to fungal diseases has been reported (55), genetically engineered resistance to fungal pathogens and to bacteria remains in the early research stages.

Key Advances in Expression and Gene Isolation Technology

Dramatic progress has been made in our understanding of and ability to alter the regulation of gene expression in plants and in techniques for the identification and isolation of genes of interest. In many cases, this progress has been facilitated by the availability of efficient gene transfer systems. The engineered plants discussed in the previous section generally depend on the use of continuously expressed promoters driving dominant single gene traits. Future plant genetic engineering will probably include alteration of traits that require subtle temporal and spatial regulation of gene expression and introduction or alteration of entire biosynthetic pathways.

Regulated gene expression. Genes that show precise temporal and spatial regulation in leaves (56), floral organs (57), seeds (58), and other plant organs have now been identified and isolated from a number of species of higher plants (59). Within the next few years, genetic engineers will have in hand a large battery of regulatory sequences that will allow for accurate targeting of gene expression to specific tissues within transgenic plants. In addition, a number of genes that respond to external influences, such as heat shock,

anaerobiosis, wounding, nutrients, and applied phytohormones, have been isolated and characterized (60). The control regions of these genes may also find utility in genetic engineering strategies.

The ability to decrease the expression of a gene in a transgenic plant also has potential utility in the study of plant gene expression and function as well as in crop improvement. Significant successes have already been achieved with genes that produce antisense RNAs to the messengers for polygalacturonase in tomato fruits (61) and chalcone synthase in petunia and tobacco plants (62). In all of these studies, substantial reductions (up to 90%) in the levels of the mRNA and protein products of the target genes were observed. Striking phenotypic alterations were observed in some of these transgenic plants (62). This method of constructing mutant phenotypes will significantly enhance biochemical and physiological studies on protein and enzyme function. In an alternative approach to reducing expression of a gene, the enzymatic regions derived from self-splicing RNA molecules are used to design RNA enzymes capable of specific RNA cleavage (63). In vitro studies have demonstrated the potential of this method, but it has yet to be applied in plants (63). Preliminary work on insertion of donor DNA into plant chromosomes by homologous recombination (64) indicates that it may also be possible to use this approach for the selective inactivation of a gene.

Gene tagging. Advances in methods for the identification and isolation of new gene coding sequences are of great importance to the engineering of improved plants. The cloning of transposon sequences has allowed the isolation of genes from several species by transposon-mediated gene tagging (65). The demonstration that mobile elements isolated from maize are able to transpose when introduced into dicot species (66) indicates that this powerful technique is applicable to any plant species for which transformation is possible. It has also been shown that under appropriate transformation conditions, the T-DNA of a plant transformation vector can itself serve as an insertional mutagen (67).

Gene mapping. Major efforts have been mounted to obtain high-resolution restriction fragment length polymorphism (RFLP) genetic maps in a number of plant species (68). The availability of such a map in tomato has already led to the resolution of several loci affecting quantitative quality traits (69). The RFLP mapping technique will be especially powerful in *Arabidopsis*, where the small genome size and lack of significant repetitive sequences (70) will simplify the process of genome "walking" from an RFLP marker to a closely linked gene. The availability of *Arabidopsis* genomic libraries in cosmids, which can also act as plant transformation plasmids (71), will allow direct testing of the isolated DNA for its ability to complement the mutation of interest at each step of the walking process. In addition, such libraries may be used in large-scale transformation experiments to directly rescue genes by complementing mutants with a selectable phenotype (71).

Key Issues Affecting Introduction of Genetically Engineered Plants

The advances in crop improvement by genetic engineering have occurred so rapidly that the initial introduction of these crops in the marketplace will be primarily influenced by nontechnical issues. These issues include regulatory approval, proprietary protection, and public perception.

Regulatory approval. In the United States, genetically engineered plants potentially come under the statutory jurisdiction of three federal agencies: the United States Department of Agriculture (USDA), Food and Drug Administration (FDA), and Environmental Protection Agency (EPA). The field testing of genetically

engineered crops has been less controversial than the introduction of other recombinant organisms into the environment. In the last 3 years there have been over a dozen tests of engineered crops in diverse locations across the United States (72)—by year end there will be over 30 such tests. All of these tests have been reviewed in detail by the USDA, with input from the other government agencies. The key consideration in approval of these tests has been a scientific evaluation of the risk and environmental impact of a particular field test experiment. Several studies and discussions of the issues and perceptions that surround the release of genetically engineered crops have produced a consensus that such engineered crops present virtually no direct risk to human or animal health (73). The specific knowledge of the introduced DNA sequences, the detailed understanding of the known functions of the gene products, and the high level of biological or physical containment were cited as key reasons for the inherent low risk to human and animal health.

The “success” of such small field tests, while important, has overshadowed other needs in the regulatory process. For example, many unanswered questions remain regarding the cost and regulatory requirements for large-scale multisite field tests. It is important that an approval process be developed to accommodate the rapid transition that will occur as testing of engineered crops goes from small, isolated field plots to large-scale, multisite testing; the development of genetically engineered crop varieties and hybrids will ultimately occur in the fields around the world—not in the research laboratory. The mechanism for FDA or EPA approval or endorsement of genetically engineered plants and food products remains undefined. Issues such as regulatory requirements, registration costs, and commercialization timelines are already becoming significant issues for companies attempting to develop improved genetically engineered crops for the mid-1990s. Several groups (74), such as the International Food Biotechnology Council (IFBC) and the Federation of American Scientists for Experimental Biology (FASEB) expert panel on criteria for determining the regulatory status of food and food ingredients produced by new technologies, consisting of academic scientists and representatives of major food, chemical, biotechnology, and seed companies, are working with government agencies to develop appropriate registration guidelines. The regulation of transgenic plants must be based on scientific principles that (i) meet the general public’s need for a safe and reasonably priced food supply and (ii) recognize the inherent low risk of gene transfer technology and the benefits afforded by genetically engineered crops to growers, food processors, and consumers.

Proprietary protection. Patent protection for genetically engineered plants is considered essential to offset the cost of developing crops with significant new traits. The Supreme Court decision in *Diamond v. Chakrabarty* (75) ruled that microorganisms were not unpatentable simply because they were living cells, and in 1985, the U.S. Board of Patent Appeals and Interferences ruled specifically that whole plants were patentable (76). Numerous companies have since filed patent applications that cover the genes, the processes of isolating genes, and making the genetically modified plants and seeds themselves. Patent protection provides a broader proprietary right than is provided under either the International Union for the Protection of New Varieties of Plants (UPOV) or the U.S. Plant Variety Protection Act (PVPA). The scope of the proprietary right of a patent on a plant is broadened by the absence of the “experimental use” exceptions found in protection afforded by plant varietal certification status. Although no one disputes that companies that have invested heavily in R&D to isolate, test, and commercialize genes are entitled to protection for their inventions, there is considerable debate within the seed industry concerning how much

protection is deserved and what impact patents will have on the cooperative nature of the seed industry itself (77). The concern has been voiced that patents on plants will favor large seed companies and reduce the overall number of companies. In contrast, while there were three private soybean seed companies before PVPA, now there are more than 40; patenting plants will likely create further incentive to invest in the seed industry in order to position it to meet the technological challenges and supply needs of the future. Much of this debate results from confusion surrounding the restrictions imposed by patent rights versus the incentive they provide for the competitive research and product development that stimulates innovation. Many of the conciliatory proposals, including patenting of genes (but not plants) and compulsory licensing in the event that plant patenting is permitted, if implemented, could significantly reduce the incentive for private industry funding in this field.

Lack of proprietary protection for genetically engineered plants outside the United States remains a serious limitation; plant and animal varieties are largely excluded from patent protection by European countries that signed the 1973 European Patent Convention. At this time only specific processes can be patented. The European Patent Office (EPO) is currently readdressing the patenting of plants and animals, but this seems certain to be appealed and it may be several years before the situation is clear, and only then will

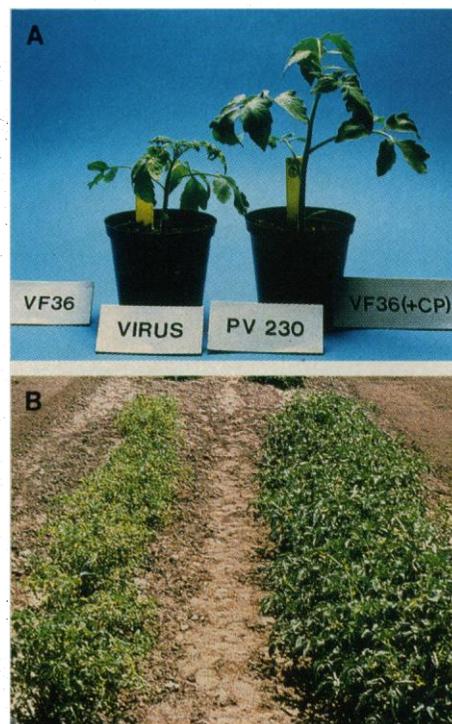


Fig. 2. Virus-resistant plants. (A) Greenhouse evaluation of tomato plants containing the TMV coat protein gene. Tomato cotyledons were transformed (83) with an *Agrobacterium* strain containing a TMV coat protein cDNA chimeric gene (51). Transgenic regenerates were screened for coat protein production by immunoblot analysis. The R₁ progeny of a representative plant that expressed high levels of coat protein were analyzed for virus resistance after inoculation with TMV. The control plant on the left is a segregant that lacks the TMV coat protein gene; the plant on the right has inherited the gene. (B) Field test (1988) of tomato plants containing TMV coat protein gene. Control and transgenic tomato seedlings were grown in a greenhouse and transplanted 6 weeks later to the field test site located in Jersey County, Illinois. The control (left) and engineered (right) plants were inoculated with the PV230 strain of TMV (10 µg/ml) 2 weeks after planting, and the photo was taken 4 weeks later. The fruit yield on the control plants was 19.6 kg per plot compared to 62.4 kg per plot for the engineered plants; the yield of the inoculated engineered plants was equivalent to that of noninoculated plants.

begin the wave of oppositions, appeals, and infringement actions that have marked the early pharmaceutical patents in the biotechnology area (78). Enforceability of plant patents in other countries, including Japan, China, and Eastern Bloc countries, is questionable. While there are numerous initiatives to harmonize both registration and proprietary protection throughout the key trading countries in the world, the outcome is not imminent and will be unlikely to have an impact on first-generation products.

Public perception. Genetically engineered crops are being developed at a time when a lack of understanding regarding the importance of agricultural research exists. Current issues, including concerns about (i) periodic, temporary production surpluses, (ii) changing farm infrastructure, (iii) inconsistency in farm policies, and (iv) a general distrust for new technologies, have at times overshadowed the long-term need for the provision of economical, high-quality food products for a growing world population. Currently, at the beginning of the 1989 cropping season, world reserves of grain are at their lowest level since the years immediately following World War II; another drought in 1989 could create a world food emergency (79).

Despite this background, recent polls conducted by the Office of Technology Assessment indicate that most people believe that the benefits of agricultural biotechnology research outweigh remote risks (72). In view of the initial public debate that has occurred over the last several years on field testing and environmental release of genetically engineered organisms, it would seem that agricultural biotechnology has indeed passed its first major public perception obstacle.

The next test of the public acceptance of this technology will come in several years when food products derived from genetically engineered crops enter the general food supply. The current focus on issues of risk and environmental release has heightened the need for increased science education and open discussion of issues. It is essential that the safety and benefits of agricultural biotechnology research and the critical role that it will play in providing for world food demand (80) be communicated and understood, so that informed decisions by the public are possible.

A Future Perspective on Genetically Engineered Plants

During the last 5 years, the availability of gene transfer systems has catalyzed a major refocusing on plants as a biological system; the use of genetically engineered plants as an analytical tool to explore unique aspects of gene regulation and development and the potential to produce novel commercial crop varieties has created a high level of scientific excitement and has driven research into many new areas. The breadth of information to be gained from the study of transgenic plants is serving as an important focus for unifying basic plant science research in plant breeding, pathology, biochemistry, and physiology with molecular biology. Regulation of gene expression is the fundamental basis for manipulating cellular metabolism, and this new research tool offers the possibility of extending physiological and genetic observations to a mechanistic level. In the next few years we can expect to see major advances in our understanding of basic plant processes.

These advances, in turn, will accelerate the application of genetically engineered plants in the seed production and agrichemical industries. The major crops that can currently be improved with genetic techniques are soybean, cotton, rice, and alfalfa (Table 1), and commercial introductions of genetically engineered varieties are likely in the mid-1990s. Rapid progress is being made in the genetic engineering of corn, and it is likely that genetically engineered corn

hybrids carrying traits for resistance to herbicides, insects, and viral diseases will reach the marketplace by the year 2000. The timing of commercialization of genetically engineered crops is ultimately determined by the need to address each of the following issues: (i) evaluation of field performance, (ii) breeding and seed increase for commercial-scale release, (iii) establishment of optimal agronomic practices, and (iv) regulatory approval and crop certification.

The worldwide agrichemical industry has been and will continue to be a leading sponsor of agricultural biotechnology research. All major agrichemical companies have R&D efforts in the area of biotechnology for crop improvement. These companies see opportunities to develop new products and extend the use of existing products, as well as to be positioned at the leading edge of new technologies that may have a significant impact on existing agrichemical businesses.

Genetic engineering of plants also offers exciting opportunities for the food processing industry to develop new products and more cost-effective processes. While many of the early successful examples of genetically engineered plants have focused on agronomic genes, it is possible that the food processing and specialty chemical industries may represent the greatest commercial opportunity for biotechnology. Examples of such applications include production of (i) larger quantities of starch or specialized starches with various degrees of branching and chain length to improve texture and storage properties, (ii) higher quantities of specific oils or the elimination of particular fatty acids in seed crops, and (iii) proteins with nutritionally balanced amino acid composition. The ability to reduce processing costs by the elimination of anti-nutritive or off-flavor components in foods is quite feasible with antisense nucleic acid technology. The enzymes and genes involved in biosynthesis of coloring materials and flavors are important to the food industry and to the consumer. Studies on the biosynthesis of some of these compounds have been hampered by the low quantities of enzymes present in the producing cells, but new techniques based on gene tagging may overcome these difficulties.

Enormous opportunity lies in the successful use of crops for both commodity and specialty chemical products. Plants have traditionally been a source of a wide range of polymeric materials. These range from starch and celluloses, which are carbohydrate-based, to polyhydrocarbons such as rubber and waxes. Many of these polymers have been replaced in the last two to three decades by synthetic materials derived from petroleum-based products. However, the cost, supply, and waste-stream problems often associated with petroleum-based products are issues that are focusing renewed attention on the use of biological polymers. Genetic engineering will significantly enlarge the spectrum and composition of available plant polymers.

Plants also offer the potential for production of foreign proteins with various applications to health care. Proteins such as neuropeptides, blood factors, and growth hormones could be produced in plant seeds, and this may ultimately prove to be an economical means of production. Several mammalian proteins have been produced in genetically engineered plants (81), and expression of pharmaceutical peptides in oilseed rape plants has been reported (82).

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