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Commercialization of Genetically Engineered Plants in the United States: Overview, Examples, and Future Prospects

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The impact of plant genetic engineering, a technology born in the early 1980's, is beginning to be felt across the world in the 1990's. The first wave of engineered plant products are reaching consumers in the supermarket and many more are destined to follow. Transformation technology now exists for most plants, including the four staple crops-maize, wheat, rice, and soybean. Early targets of genetic engineering include plants possessing insect resistance and herbicide tolerance, with future goals set on increasing harvestable yield, improving nutritional quality, and making specialty products. This review describes some of the milestones in plant biotechnology, the U.S. regulatory agencies, field trial numbers and deregulated plants, commercialization criteria, examples of commercialized plants, and future prospects of plant biotechnology.

Advances made in modern agricultural practices have been impressive in terms of variety development and increased harvestable yield of high-quality fruits, vegetables, and grains. Some of the key developments in the last several decades include directed, aggressive breeding programs, hybridization of inbred varieties, implementation of highly effective chemical pesticides, and dramatic increases in fertilizer usage. These developments have contributed to a significantly better standard of living in many countries and are paramount to feeding an ever-growing human population worldwide. Continued improvements are anticipated through modernization and innovation in farming techniques and as the scientific community gains further knowledge in the areas of plant physiology, biochemistry, and genetics. Molecular marker technology is becoming an increasingly important component in the breeding of many crop plants. Large collections of ESTs (expressed sequence tags) are being generated for many plants to identify the vast array of genes being expressed in various tissues and at different developmental stages. The entire sequence of a plant's genome (the model crucifer, *Arabidopsis thaliana*) will be determined within a few years and may provide important insights about genomic organization, gene structure, and protein functions. Genetic engineering has recently entered into the spectrum of strategies used to manipulate and improve crop plants for commercial benefit. This powerful technology significantly impacts both the agricultural and chemical pesticide industries by facilitating the introduction of specific, highly desirable traits that could not be easily obtained through conventional breeding.

Milestones in Plant Biotechnology

The inception and development of plant genetic engineering has been marked by numerous milestones in technical achievement and commercial application. Applied plant biotechnology began in 1983 when researchers reported that the Ti plasmid of *Agrobacterium tumefaciens* could be disarmed and foreign genes inserted into its T-DNA for transfer into the plant genome (Fraley et al., 1983: Zambryski et al., 1983). In addition, it was demonstrated that an antibiotic resistance marker, the neomycin phosphotransferase gene, could be used for selection of transformed cells which were ultimately regenerated into normal, fertile plants (Fraley et al., 1983: Herrera-Estrella et al., 1983: Bevan et al., 1983). The production of transgenic plants rapidly became an important tool for the study of gene function and regulation, and transformation methods for a wide variety of crops were subsequently established (Klein et al., 1987: Umbeck et al., 1987: Hinchee et al., 1988: Toriyama et al., 1988: Gordon-Kamm et al., 1990: Vasil et al., 1992). Many of the technological breakthroughs in the laboratory, including coat protein-mediated resistance (Abel et al., 1986), B.t.-based insect resistance (Vaeck et al., 1987: Fischhoff et al., 1987), herbicide tolerance (De Block et al., 1987: Stalker et al., 1988: Comai et al., 1985: Shah et al., 1986), ripening control in tomatoes (Sheehy et al., 1988, Smith et al., 1988, Oeller et al., 1991: Good et al., 1994), engineered male sterility and restoration (Mariani et al., 1990 & 1992), modified carbohydrate composition (Visser et al., 1991: Stark et al., 1992), and modified fatty acid profile (Voelker et al., 1992: Knutzon et al., 1992) are being translated into commercial products at the supermarket. Some of the key milestones in plant biotechnology are listed in Table 1 (adapted from Leemans, 1993).

Given the relatively recent advent of plant genetic engineering, the breadth of accomplishments listed in Table 1 is quite impressive. The first genetically engineered food from plants, the Flavr Savr™ tomato, was developed, approved, and sold in supermarkets approximately ten years after the first description of plant transformation using *Agrobacterium*. This product established important groundwork for the testing and approval of transgenic plants by various U.S. regulatory agencies (Redenbaugh et al., 1995). Continued advancements in technology and regulatory procedures have shortened the time from laboratory bench to supermarket shelf. Successful transformation of maize was reported in 1990 (Gordon-Kamm et al., 1990) and the first generation of transgenic maize products were cleared for sale in 1996. Based on the number of field trials being carried out and the variety of phenotypic traits under analysis, the list of milestones presented in Table 1 should continue to grow rapidly in the foreseeable future.

Table 1. Milestones in Plant Biotechnology.

	Technical	Commercial
1983	Ti plasmid disarmed, foreign genes inserted	
	First selectable marker for plants	
1985		U.S. allows plant patents
1986	Coat protein-mediated virus resistance	First U.S. and European field trial approved
1987	B.t.-based insect resistance	USDA/APHIS proposed guidelines
	Herbicide-tolerant plants	for field testing
	Particle bombardment technology	
	Cotton tranformation	
1988	Soybean and rice transformation	
	Antisense in plant	
	Delayed softening tomatoes	
1989	Antibody production in transgenic plants	
	RFLP mapping	
1990	RAPD analysis	EC directive on deliberate release
	Corn transformation	and commercialization
	Engineered male sterility	
	Co-suppression in plants	
1991	Ripening control in tomatoes	Revised UPOV convention
	Modified carbohydrate composition	accomodates biotech. products
1992	Wheat transformation	>400 field trials worldwide
	Modified fatty acid profile	USDA deregulates softening-
	Engineered fertility restoration	controlled tomato
	Biodegradable plastic in plants	USDA/APHIS proposes simpler
		Notification & Petition Processes
1993	Viral vectors for high-level, transient expression in plants	US patent on insect-resistant plant
	Plastid transformation in tobacco	
1994		First sale of genetically engineered food from plants (Flavr Savr™ tomato, Calgene)
		Virus-resistant squash deregulated by FDA, USDA, EPA
1995	Oral immunization by antigen expression in transgenic plants	First sales of genetically engineered canola oil (Laurical™, Calgene)
	Cloning of plant disease resistance genes	APHIS proposes expanded Notification process
1996		First sale of B.t.-protected cotton (Bollgard™, Monsanto)
		First sale of genetically engineered soybeans (Round-Up Ready™, Monsanto)
		First sale of genetically engineered corn (Maximizer™, Ciba-Geigy)
1997		>2740 U.S. field trials at >11,290 sites since 1987

U.S. Regulatory Process

The development and testing of genetically engineered plants and plant products is closely monitored by the United States Department of Agriculture (USDA), the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), and most state governments. The ultimate goals of these agencies are to validate and inform the public about the safety of products that will be used or consumed and to monitor and protect the environment from introduced chemicals and organisms.

APHIS, the Animal and Plant Health Inspection Service, regulates interstate movement, importation into the U.S., and field testing of organisms and products (including those modified through genetic engineering) which are known or have the potential to become plant pests. Since extensive field testing has shown that a given genetically engineered plant has no more "pest-like" qualities than its nonengineered parents, a large number of engineered plant varieties have been granted non-regulated status. Specific permits must be filed with APHIS to move any potential plant pest into the U.S. or between states, to release or field test any engineered crop in the environment, or to move or field test an engineered plant that is not regulated by the agency. A petition for USDA exemption must also be filed before a genetically engineered crop can be sold commercially. Two alternative procedures were introduced by APHIS in 1993 to accelerate and simplify regulatory procedures. The Notification Process allows movement and field testing of six crop plants (corn, soybeans, cotton, potatoes, tomatoes, and tobacco), based on their safe history of U.S. field trialing, through simple notification rather than petition to APHIS. The Petition Process allows anyone to request a written determination by APHIS that a regulated plant should no longer be regulated. Based on a review of the scientific information provided, solicited expert opinion and public comment, APHIS can approve or deny such a request.

The FDA has broad authority to regulate the introduction of new foods and new food additives and holds producers legally responsible for the safety and wholesomeness of the foods they bring to market. A decision by the FDA in 1992 states that foods derived from new plant varieties produced by genetic engineering will be regulated essentially the same as foods created by conventional means, unless special circumstances apply. Triggers for food safety evaluation by the FDA include products that are encoded by uncharacterized or unstable genetic elements, contain significantly higher levels of known toxicants than the range found naturally in edible varieties of that species, have significantly altered nutrient levels, differ significantly in composition from substances currently found in foods, contain allergenic proteins, contain antibiotic resistance markers, produce specialty nonfood substances, or have significant changes in nutrients or toxicants compared to unaltered animal feed. For food additives, only genetically engineered crops that contain substances significantly different from those already in consumer's diets need to gain FDA approval. Currently, the FDA does not require genetically engineered food products to be labeled as such simply because they involved genetic engineering. After thorough review of compositional analyses and safety data, the FDA has found no information that would distinguish genetically engineered foods as a class from foods developed through other methods of plant breeding.

The EPA regulates pesticides, sets tolerances, and establishes exemptions for pesticide residues in or on food crops. Environmental exposures to pesticidal substances produced in transgenic plants will be regulated to insure no adverse affects on the environment or nontarget, beneficial organisms. Triggers for EPA review include pesticides not derived from a known food source, pesticides now consumed in a way different than previously ingested in the diet, and pesticides having different structure, function, or composition than those already present in foods. Engineered plants containing pesticides that do not require further EPA review may be granted exemptions or have tolerance levels set for safe consumption.

States also have the right to monitor the development of any genetically engineered product and to limit or regulate its sale. The general public, under the Freedom of Information Act, can access APHIS permit applications and field test assessments. Overall, U.S. consumer response to genetically engineered foods and plant products has been very positive.

Field Trials and Phenotypes

The USDA and APHIS provide up-to-date information to the public on both field testing and commercialization of new agricultural crop varieties. Their statistics indicate that the pace of field testing in the U.S. continues to increase dramatically, approximately doubling each year. As of April 1997, APHIS approved or acknowledged 2,745 field trials at 11,291 field sites. Derivatives of 48 different plant species have been field tested, with a wide range of modifications that can be grouped into the six broad categories shown in Table 2.

Apparent from the data in Table 2 is that the strongest driving force behind applied plant biotechnology has been the agrochemical industries and their interest in improving agronomic performance of crops. For example, the development of herbicide-tolerant crops through genetic engineering is an important vehicle for companies to gain market share for a particular chemical herbicide. Half of the products approved or under review by regulatory agencies for commercial sale are related to herbicide tolerance. Monsanto, the manufacturer of Round-Up™ herbicide, has made a major commitment to developing and commercializing Round-Up Ready™ canola, soybean, corn, and cotton varieties.

Table 2. U.S. Field Releases from 1987 to 1997 (as of 2/28/97).

Category	Permits Issued and Notifications Acknowledged
Herbicide Tolerance	795 (27.7%)
Product Quality	759 (26.5%)
Insect Resistance	693 (24.2%)
Viral Resistance	305 (10.6%)
Fungal Resistance	109 (3.8%)
Other (marker genes, selectable markers, bacterial resistance, nematode resistance)	204 (7.1%)

Other companies have similar efforts for engineered tolerance to phospinothricin, bromoxynil, and sulfonylurea herbicides.

The other high profile target of plant biotechnology today, in addition to herbicide tolerance, is B.t.-based insect resistance. Monsanto, Ciba-Geigy/Mycogen, DeKalb, Calgene, and Northrup King all have B.t.-containing crops for control of various insect pests. Agronomic performance of these varieties in field trials has been excellent, so a key determinant of their technology payback will be a resolution of the intellectual property rights surrounding B.t. and B.t.-variant proteins.

The majority of the commercially-viable, genetically engineered plants developed to date are the result of the expression of a single protein that does not and would not be predicted to have negative pleiotropic effects on plant growth and physiology. Manipulation of more fundamental processes, such as carbohydrate or nitrogen metabolism, without having negative effects on yield or morphology represents a greater challenge, as does the simultaneous expression of multiple genes to produce a desired trait.

Deregulated Plants

Researchers from academia and industry can petition the USDA that a given transgenic plant variety should no longer be regulated. The petition must include, among other things, scientific details about the genetics of the plant, the nature and origin of the genetic material used, and information about effects on other plants. If the USDA determines that a particular field-tested organism has no potential for plant pest risk, the genetically modified plant can be grown and plant products introduced more quickly into the marketplace. The current list of U.S. deregulated crop lines in shown in Table 3.

Table 3. USDA Deregulated Plant Varieties.

Crop	Trait	Institution	Approval
Cotton[ab]	Bromoxynil tolerant	Calgene	2/94
Soybean[ab]	Glyphosate tolerant	Monsanto	5/94
Tomato[a]	Altered fruit ripening	Calgene	10/94
Rapeseed[a]	Altered Oil Profile	Calgene	10/94
Squash[ab]	WMV2, ZYMV virus resistant	Asgrow/Upjohn	12/94
Tomato[a]	Altered fruit ripening	DNA Plant Techn.	1/95
Potato[ab]	Coleopteran insect resistant	Monsanto	3/95
Corn[ab]	Lepidopteran insect resistant	Ciba-Geigy	5/95
Tomato[a]	Altered fruit ripening	Zeneca & Petoseed	6/95
Corn[a]	Phosphinothricin tolerant	AgrEvo	6/95
Cotton[ab]	Lepidopteran insect resistant	Monsanto	6/95
Cotton[a]	Glyphosate tolerant	Monsanto	7/95
Corn[ab]	Lepidopteran insect resistant	Monsanto	8/95
Tomato[a]	Altered fruit ripening	Monsanto	9/95
Corn[a]	Phosphinothricin tolerant	DeKalb	12/95
Corn[ab]	Lepidopteran insect resistant	Northrup King	1/96
Cotton[a]	Male sterile/sulfonylurea tolerant	DuPont	1/96
Corn[a]	Male sterile/phosphinothricin tol.	Plant Genetic Systems	2/96
Tomato[a]	Altered fruit ripening	Agritope	3/96
Potato[ab]	Colorado potato beetle resistant	Monsanto	6/96
Squash	CMV, WMV2, ZYMV resistant	Asgrow	6/96
Soybean	Phosphinothricin tolerant	AgrEvo	7/96
Papaya	PRV virus resistan	Cornell University	9/96
Corn[a]	Phosphinothricin tolerant	DeKalb	12/96
Corn[a]	European corn borer resistant	DeKalb	3/97

[a] U.S. FDA deregulated.
[b] U.S. EPA deregulated.

Factors Influencing Commercial Success

Due to the large investment of time, money, and other resources that is necessary to development and market a genetically engineered plant product, certain important criteria should be considered before taking an engineered plant down this difficult path. Some of the key questions influencing commercial success include: 1) Does the plant contain a stable, heritable, significantly improved or unique trait? If the introduced gene(s) is unstable or is not transmitted to future generations in a predictable manner (excluding transient expression systems like viral vectors), then the trait will be of little value. If the phenotype of the engineered plant is only marginally different from the parent plant or the same result can be achieved through conventional breeding, then the cost of development and regulatory approval may not be warranted. 2) Is the engineered trait economically competitive and durable? If the costs required to cultivate and process an engineered plant for its beneficial trait are excessive or if alternative sources of a product are available and competitively priced (for example, through microbial fermentation), then the engineered plant may not be economically viable. Also, the trait must be consistent under the extremes of environmental variability and be durable against the possibility of resistant pests rapidly developing. 3) Does the developer of the new variety have a favorable patent or licensing position? Failure to gain patent rights for an invention or to obtain license agreements for key technologies used in the development of the genetically engineered plant may lead to costly lawsuits, lengthy royalty arrangements, or even the inability to market a product. 4) Do the modifications created through genetic engineering present any significant regulatory issues? Production of allergens, alterations in nutrient or toxicant levels, changes in overall food composition, possibility of weediness or spread through outcrossing, and increased selective pressure for resistant pests are concerns that can limit or delay the release of an engineered crop. 5) Does the engineered variety suffer any yield or performance drag or require any significant changes in cultivation practices? Farmers will be reluctant to grow new varieties that do not perform up to the standards they are used to observing or require complicated procedures for handling, pest management, or chemical use. Conversely, hardier, better-yielding, more resistant varieties that simplify the grower's tasks and decrease the use or health-concern of various pesticides will be readily welcomed. 6) Does the developer of the genetically-modified variety have the means and infrastructure to capture maximum value from the product? The engineered trait should be introduced into elite, high-quality germplasm to be competitive with existing varieties in different growing regions. Relationships with seed companies, producers, shippers, packers, processors, and retailers must be established for effective and maximal delivery of a product to the marketplace. As an example, Monsanto has invested in AgriPro Seeds Inc., Asgrow Agronomics, Calgene Inc./Stoneville Pedigreed Seed Co., DeKalb Genetics Corp., Delta Pine and Land, Holden's Foundation Seeds Inc., Corn States Hybrid and International, and Monsoy as a means to access key seed businesses for delivery of genetically engineered traits. Rigid control of the production chain from seed to supermarket is also essential if a given product is to be identity-preserved.

Examples of Commercialized Products

Flavr Savr™ Tomato

Development of the Flavr Savr™ tomato by Calgene, Inc. was pioneering work for the introduction and regulatory approval of a food obtained from genetically engineered plants. Antisense inhibition of polygalacturonase expression delays the softening of tomato fruits, allowing longer time on the vine and greater opportunity to develop full flavor. The engineered plants were shown by molecular analyses, genetic analyses, biochemical analyses, nutritional analyses, and extensive field testing for horticultural traits and plant pest risk to be substantially equivalent to non-engineered tomatoes. Data was also presented to show that the *kan* resistance gene is highly unlikely to move from the plant genome into microorganisms by horizontal gene transfer, that if such transfer could occur the impact would be minimal, that the neomycin phosphotransferase enzyme in transgenic plants would not compromise antibiotic use in humans or animals, and that this protein is not a toxin or allergen. Details of the extensive FDA and USDA consultation starting in 1989 until the first sale of Flavr Savr™ tomatoes in 1994 have been published (Redenbaugh et al., 1995). Commercial success of this engineered tomato can be debated. Reviewing the criteria mentioned above, the technology provides a stable, heritable trait having no regulatory concerns with the fruit as a food or the *kan* resistance marker as a food additive. A patent dispute concerning the use of antisense technology in plants was eventually resolved in Calgene's favor. The molecular traits provided by the technology (fruit firmness, enhanced resistance to certain pathogens) are not overly dramatic and impact certain varieties more than others (Kramer et al., 1992). The key driver for commercial appeal, enhanced flavor through vine ripening, is determined to a large extent by the germplasm or varieties into which the antisense gene is introduced. This factor, along with tremendous differences in varietal performance in different growing regions, forced Calgene to expend tremendous effort and resources on developing numerous transgenic varieties. In addition, to carefully control the growing, picking, packing, and sale of its premium tomatoes, Calgene decided to build a vertically-integrated business of its own. The costs associated with this undertaking, and the expense of hand-picking and careful packaging only high-quality premium tomatoes, were very high. An additional factor, the introduction of firmer *rin*-based (or LSL) tomatoes through conventional breeding, dramatically affected the tomato business as a whole. Though criticized for lack of flavor, these LSL varieties deliver many of the benefits of the Flavr Savr™ tomato, such as firmness and disease resistance, at a lower cost. Consumer response to the Flavr Savr™ tomato has been very positive, and sales are limited only by the availability and associated costs of growing the fruit. Fresh market tomatoes containing the Flavr Savr™ technology are still being sold in the U.S., and the #1 processing lines in the U.K. (owned by Zeneca/Peto Seeds and having increased solids content due to the transgene) utilize the antisense-PG technology.

Laurical™ Canola Oil

The first genetically engineered plant oil product, LauricalTM, was sold commercially in 1995. Laurical™, or laurate canola, is the first of a series of products being developed by Calgene, Inc. through the modification of fatty acid chain length, unsaturation, or triglyceride structure via genetic engineering technology. A key driver for this product was the perceived need by the >$400 million/year U.S.

market for a stable, reliable source of lauric oils which are currently imported primarily from southeast Asia. Laurate is a key raw material for the manufacture of soaps, detergents, and industrial oleochemical products, and has a variety of applications in the food industry. Canola, whose seeds normally contain greater than 40% oil but have no laurate, were modified by genetic engineering to express a new thioesterase enzyme in their seeds (Voelker et al., 1992). The thioesterase gene that was used came from the California Bay tree, a choice based on the fact that the oil from Bay seeds contains over 60% laurate. High-level expression of the Bay thioesterase in developing canola seeds can result in an oil with 40% or greater laurate content. Additional modifications will be needed to achieve the 50% level present in coconut and palm kernel oil, such as penetration into the *sn*-2 position of triglycerides.

The modified oil composition of Laurical™ is a stable, heritable trait at the 40-50% level and creates a novel, high-value canola variety. Its economic strength remains to be determined and will be influenced by future advances made in oil manipulation, production costs, breadth of applications, and pricing and availability of lauric oils from existing sources. Calgene has intellectual property rights for the Bay thioesterase gene and for the napin promoter used to drive seed-specific expression. USDA and FDA approval for Laurical™ has been granted. To date, Calgene has sold one million pounds of Laurical™ oil and is currently planning for at least 70,000 acres of commercial production in 1997. The processed oil has interesting properties in terms of food applications and many opportunities in this area are being explored (Kridl and Shewmaker, 1996: Del Vecchio, 1996). Breeding of the trait into high-performing varieties has been ongoing. Identity preservation of the specialty oil is possible through agreements with growers, shippers, and processors. Ownership of an oils processing facility, Calgene Chemical Co., provides a means for quality assurance and control of all Laurical™ endproducts.

Freedom II Squash

An early entry into the arena of genetically engineered food products was the virus resistant squash variety, Freedom II, developed by Asgrow Seed Co. This yellow crookneck squash hybrid has engineered resistance to two of its most damaging viral diseases caused by zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus II (WMVII) (Meeusen, 1996). Despite heavy insecticide use to control viral vectors, U.S. squash growers typically lose between 20% and 80% of their crop to disease caused by four major viruses - ZYMV, WMVII, cucumber mosaic virus, and papaya ringspot virus. Natural resistance alleles exist but are difficult to breed into a single hybrid for good, comprehensive protection. In addition, transgenic varieties with coat protein-mediated resistance appear to have better protection from viruses than that provided by the natural resistance alleles. Future varieties under development by Asgrow will protect plants from all four of the major viruses. Thus, through genetic engineering, Asgrow is able to provide significantly improved and much-needed viral resistance traits. Growers of these new varieties will most likely reduce the use of insecticides significantly, thereby producing a safer and more cost-effective product. USDA, FDA, and EPA clearances were obtained in 1994 and commercial sales began in 1995. USDA approval was significant, in that this was the first crop under review for which there are populations of interbreeding wild relatives in the major areas of squash cultivation in the U.S. While in theory the engineered squash plants and any outcrossed progeny could have altered fitness in the wild, the USDA concluded from extensive analyses that the engineered variety had no increased potential for weediness compared to standard varieties in cultivation. Asgrow conducted 46 field tests across 10 states in support of its application, and the USDA solicited expert opinions from researchers in the areas of plant virology and cucurbit ecology to help make its final determination. The commercialization strategy chosen for the introduction of Freedom II squash has been described as a "defensive" one (Meeusen, 1996). Since Asgrow currently holds a dominant position in the small but volatile yellow crookneck squash market, its new premium-priced varieties are expected to maintain Asgrow's market share and business revenues on a reduced growing acreage.

Bollgard™ Cotton

Monsanto's Bollgard™ cotton was the second genetically engineered cotton product to achieve commercial sales and represents a growing number of plant varieties being developed with B.t.-based insect resistance technology. Expression in plants of a gene obtained from the bacterium *Bacillus thuringiensis* results in the production of protein crystals that are toxic to insect pests. A large variety of B.t. genes exist, each with a different spectrum of inhibitory activity against various insects such as caterpillars and bollworms, but having no toxic affects on humans, animals, birds, or beneficial insects such as honeybees. Farmers who grow B.t.-protected plants can significantly reduce the amount of chemicals needed to control their major insect pests and may observe higher yields due to reduced insect or pesticide damage. B.t. technology provides a stable, heritable trait for resistance that is currently addressed only through heavy insecticide use. It can be very competitive economically because it decreases the costs associated with the use and application of pesticides. In 1996, U.S. farmers saw an average yield improvement of 7% with the new Bollgard™ varieties and saved approximately $33 per acre with enhanced yield and reduction in costs for insect control (Monsanto Co., personal communication). Appropriate pricing of the high-tech seeds and durability of the resistance provided are issues that will be determined in the coming years (Altman et al., 1996). A resolution of the conflict surrounding intellectual property rights of B.t.-based technology will also be important. Extensive scientific testing and evaluation of Bollgard™cotton has led to its approval for deregulated status by the USDA, FDA, and EPA. Strategies are in place to address the possibility of insects developing resistance to the specific B.t. protein being used. Four out of five growers of Bollgard™ cotton in 1996 said they were either "satisfied" or "very satisfied" with the cotton's overall performance, and farmland with Bollgard™ cotton is projected to reach 2 to 3 million acres in 1997 (Monsanto Co., personal communication). Monsanto has made investments in Delta Pine & Land Co. and Stoneville Pedigreed Seed Co. (via Calgene, Inc.) as vehicles to get its technology into the most competitive, highest-yielding cotton varieties available. Relationships and regulatory approval for the sale of Bollgard™ cotton in Mexico, Australia, China, and Africa are being aggressively pursued by Monsanto to capture value on a global scale from its engineered cotton plants.

Round-Up Ready™ Soybeans

The first genetically engineered soybean product to reach the marketplace for commercial sale is Monsanto's Round-Up Ready™ soybeans. Monsanto, producer of the very successful Round-Up™ and Round-Up Ultra™ herbicides, began to explore the use of genes that would confer tolerance to their herbicide back in the early 1980's. Success was achieved through the expression of an enzyme, 5-enolpyruvylshikimic acid-3-phosphate synthase, that is insensitive to the active *herbicide ingredient*, glyphosate. The Round-Up™ tolerance trait allows farmers to apply a broad-spectrum herbicide over a wide application window to the growing soybean crop. This post-emergence control of many difficult weeds, coupled with the rapid biodegradability of Round-Up™, makes this combination of plant and herbicide an attractive one for soybean growers. Current soybean weed control costs could be reduced by one-third or more by using Round-Up™ herbicide and Round-Up Ready™ soybeans (Monsanto Co., personal communication). In addition, growers gain the safety, flexibility, and greater weed spectrum associated with Round-Up™ as opposed to other herbicides. A key to the rapid development of these new soybean varieties was the active collaboration between Monsanto and Asgrow Seed Co. Access to elite germplasm, extensive field trialing, and cooperative registration with the EPA for seed sales and Round-Up™ use on soybeans positioned the two companies to enter the market quickly with varieties that could be grown across a wide geographical range (Meeusen, 1996). All available seed was planted on roughly one million acres in the U.S. in 1996, and projections for 1997 are that eight to ten million U.S. acres will be planted with Round-Up Ready™ soybeans. Soybean growers who used the new varieties reported extremely high satisfaction with the performance of the technology (Monsanto Co., personal communication). Monsanto's acquisitions of Asgrow and Monsoy, as well as its investment in DeKalb Genetics Corp., will facilitate the development and sale of engineered soybean products on a large scale. USDA and FDA approval for six new soybean varieties was obtained in 1994, and clearance by the EPA was received in 1995. "Substantial equivalence" of the genetically engineered beans to non-engineered varieties was demonstrated, and the levels of introduced protein in processed oil and meal were found to be negligible. The engineered plants do not contain an antibiotic resistance selectable marker. Sale of products derived from these new varieties has been hampered in some countries by requests from consumer groups for labeling of all foods containing the genetically engineered beans (Robinson, 1997). Goverment agencies will need to decide the proper course of action regarding the sale of genetically engineered food products, since the logistics and economics of identity-preserving a food ingredient, from growing seeds to processing plants to tracking all downstream food uses, would present some major challenges.

Maximizerv™ Corn

The commercial sale of genetically engineered corn was marked in 1996 by the introduction of insect resistant varieties from Ciba-Geigy/Mycogen. Capitalizing on the highly effective and specific activities of B.t. proteins against various insects, Ciba/Mycogen have developed transgenic maize plants that are resistant to the very destructive European corn borer (ECB) (Koziel et al., 1993). Left unchecked, each borer can cause between 3% and 7% yield loss per plant by its feeding activities. Chemical control is difficult because of the limited time the borer spends on exposed plant tissues and the fact that the insect has at least two generations, or broods, each year. Expression of an insecticidal protein within the plant tissues can protect the crop from both generations of the borer, including the chemically-inaccessible and preferred feeding sites deep within the stalk. Ciba researchers have tested a variety of constitutive and tissue-specific promoters to drive expression of a maize codon-optimized synthetic B.t. gene and have identified transgenic lines providing excellent ECB control. Use of these new varieties should allow farmers to reduce the amount of insecticides applied to maize plants and may result in higher yields per acre due to less insect and pesticide damage. This techology provides a significant and important trait, ECB resistance, that has not been obtainable through conventional breeding and can only be addressed through multiple insecticide applications. As with other insect-resistant and herbicide-tolerant varieties developed through genetic engineering, the farmer's savings in chemical costs will be partially offset by the higher price commanded by these premium seeds. Durability of B.t.-based insect resistance, like insect resistance to chemical insecticides, is an issue that will be monitored carefully and may be addressed by additional biotech. strategies currently in development (Koziel et al., 1996). USDA, FDA, and EPA deregulation of the new corn varieties was granted in 1995. A concern expressed by some countries about these genetically engineered maize varieties is the presence of a bacterial gene conferring resistance to ampicillin antibiotics (Robinson, 1997). Transfer of the *amp* resistance gene into the transgenic maize plants was a byproduct of the transformation process used, and the gene with its prokaryotic promoter is not expressed in plants. The possibility of horizontal gene transfer from maize plants or plant products into bacteria, thereby rendering a strain ampicillin resistant, was determined to be extremely low by the FDA. This is also a concern only for unprocessed maize products, since none of the introduced genes are intact after processing. Marketing of the new maize varieties was initially blocked in four European countries until the EU Scientific Committees on Pesticides, Animal Feedstuffs and Food determined that the risks of transfer were so low as to be unlikely to be a significant cause of increased antibiotic resistance in either farm animals or humans. The EU Council of Ministers subsequently gave marketing consent in December of 1996.

Future Prospects

The age of genetically engineered commercial crops is now. After a decade or more of development, consumers are beginning to see the first wave of products created by researchers from academic and industrial laboratories. To some individuals, including many investors in plant biotechnology, the pace of development has seemed slow. Yet, to look at the scientific and regulatory advances made in this area and the number of field trials conducted with genetically engineered plants, one could characterize the application of this technology as an "explosion." Some experts predict that plant biotechnology will become a 6 billion dollar business by the year 2005. Major agrochemical companies in the U.S. are battling to be the first to develop, patent, and sell the products of their research efforts. Key targets today are herbicide tolerance and insect resistance in a variety of crops, including corn, soybean, cotton, and canola. In the near future, a tremendous diversity of potential products will be investigated and developed. Transgenic plants are being viewed as possible low-cost bioreactors for the production of various oils, carbohydrates, pharmaceutical proteins and drugs, industrial enzymes, biodegradable plastics, and nutritional supplements (Goddijn and Pen, 1995). Industrialized as well as developing countries are expected to benefit from the higher yields and reduced pesticide use associated with plants engineered to resist insect, fungal, bacterial, and viral pests. The abundance, safety, and nutritional value of foods and animal feed are expected to be improved through plant biotechnology. Engineered plants may also serve as oral vaccines for various disease agents and as feedstocks for industrial synthesis (Mason and Arntzen, 1995: Flavell, 1995). A common hope is that solutions offered by plant biotechnology will lead to more sustainable agricultural systems and less reliance on petrochemicals. The opportunities created by this very young technology are vast and hold great promise for agriculture and human health. Reaching its full potential will require careful and innovative scientific research, unification and synergy among regulatory agencies across the world, and realized benefits at the level of the farmer and the consumer. The products we are seeing now represent the beginning of a productive pipeline from bench to commercialization in plant biotechnology.

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Flavr Savr, Laurical, Freedom II, Bollgard, Round-Up Ready, and Maximizer are trade names protected under U.S. law.

REFERENCES

Abel PP, et al. (1986) Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science **232**: 738-743

Altman DW, Benedict JH, Sachs ES (1996) Transgenic plants for the development of durable insect resistance: case study for cotton and *Bacillus thuringiensis* genes. *in* Collins GB, Shepherd RJ, eds. Engineering Plants for Commercial Products and Applications, Vol. 792, Annals of the New York Academy of Sciences, New York, New York pp. 106-114

Bevan MW, Flavell RB, Chilton MD (1983) A chimaeric antibiotic resistance gene as a selectable marker for plant cell transformation. Nature **304**: 184-187

Comai L, et al. (1985) Expression in plants of a mutant *aro*A gene from *Salmonella typhimurium* confers tolerance to glyphosate. Nature **317**: 741-744

De Block M, et al. (1987) Engineering herbicide resistance in plants by expression of a detoxifying enzyme. EMBO J **6**: 2513-2518

Del Vecchio AJ (1996) High-laurate canola: how Calgene's program began, where it's headed. INFORM **7**: 230-242

Fischhoff DA, et al. (1987) Insect tolerant transgenic tomato plants. Bio/Technology **5**: 807-813

Flavell RB (1995) Plant biotechnology R & D-the next ten years. TIBTECH **13**: 313-319

Fraley RT, et al. (1983) Expression of bacterial genes in plant cells. Proc Natl Acad Sci USA **80**: 4803-4807

Goddijn OJM, Pen J (1995) Plants as bioreactors. TIBTECH 13:379-387

Good X et al. (1994) Reduced ethylene synthesis by transgenic tomatoes expressing S-adenosylmethionine hydrolase. Plant Mol Biol **26**: 781-790

Gordon-Kamm WJ, et al. (1990) Transformation of maize cells and restoration of fertile transgenic plants. Plant Cell **2**: 603-618

Hamilton AJ, Lycett GW, Grierson D (1990) Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. Nature **346**: 284-287

Herrera-Estrella L, Depicker A, Van Montagu M, Schell J (1983) Expression of chimaeric genes transferred into plant cells using a Ti-plasmid-derived vector. Nature **303**: 209-213

Hinchee MAW, et al. (1988) Production of transgenic soybean plants using *Agrobacterium*-mediated DNA transfer. Bio/Technology **6**: 915-921

Klein TM, Wolf ED, Wu R, Sanford JC (1987) High-velocity microprojectiles for delivering nucleic acids into living cells. Nature **327**: 70-73

Knutzon DS, et al. (1992) Modification of Brassica seed oil by antisense expression of a stearoyl-acyl carrier protein desaturase gene. Proc Natl Acad Sci USA **89**: 2624-2628

Koziel MG, et al. (1993) Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. Bio/Technology **11**: 194-200

Koziel MG, et al. (1996) Transgenic maize for the control of European corn borer and other maize insect pests. *in* Collins GB, Shepherd RJ, eds. Engineering Plants for Commercial Products and Applications, Vol. 792, Annals of the New York Academy of Sciences, New York, New York pp. 164-171

Kramer M, et al. (1992) Postharvest evaluation of transgenic tomatoes with reduced levels of polygalacturonase:processing, firmness, and disease resistance. Postharvest Biol Technol **1**: 241-255

Kridl JC, Shewmaker CK (1996) Food for thought: improvement of food quality and composition through genetic engineering. *in* Collins GB, Shepherd RJ, eds. Engineering Plants for Commercial Products and Applications, Vol. 792, Annals of the New York Academy of Sciences, New York, New York pp. 1-12

Leemans J (1993) Ti to tomato, tomato to market. Bio/Technology **11**: S22-26

Mariani C, De Beuckeleer M, Truettner J, Leemans J, Goldberg RB (1990) Induction of male sterility in plants by a chimaeric ribonuclease gene. Nature **347**: 737-741

Mariani C, et al. (1992) A chimaeric ribonuclease-inhibitor gene restores fertility to male sterile plants. Nature **357**: 384-387

Mason HS, Arntzen CJ (1995) Transgenic plants as vaccine production systems. TIBTECH **13**: 388-392

Meeusen RL (1996) Commercialization of transgenic seed products: two case studies. *in* Collins GB, Shepherd RJ, eds. Engineering Plants for Commercial Products and Applications, Vol. 792, Annals of the New York Academy of Sciences, New York, New York pp. 172-176

Oeller PW, et al. (1991) Reversible inhibition of tomato fruit senescence by antisense RNA. Science **254**: 437-439

Redenbaugh K, Hiatt W, Martineau B, Emlay D (1995) Determination of the safety of genetically engineered crops. *in* Engel KH, Takeoka GR, Teranishi R, eds. Genetically Modified Foods: Safety Aspects, ACS Symposium Series 605, Washington, DC pp. 72-87

Robinson C (1997) Genetically modified foods and consumer choice. Trends in Food Sci and Technol 8:84-88

Shah DM, et al. (1986) Engineering herbicide tolerance in transgenic plants. Science **233**: 478-481

Smith CJS, et al. (1988) Antisense RNA inhibition of polygalacturonase gene expression in transgenic tomatoes. Nature **334**: 724-726

Stalker DM, McBride KE, Malyj LD (1988) Herbicide resistance in transgenic plants expressing a bacterial detoxifying gene. Science **242**: 419-423

Stark DM, Timmerman KP, Barry GF, Preiss J, Kishore GM (1992) Regulation of the amount of starch in plant tissues by ADP glucose pyrophosphorylase. Science **258**: 287-291

Toriyama K, Arimoto Y, Uchimiya H, Hinata K (1988) Transgenic rice plants after direct gene transfer into protoplasts. Bio/Technology **6**: 1072-1074

Umbeck P, Johnson G, Barton K, Swain W (1987) Genetically transformed cotton (*Gossypium Hirsutum* L.) plants. Bio/Technology **5**: 263-266

Vaeck M. et al. (1987) Transgenic plants protected from insect atack. Nature **328**: 33-37

Vasil V, Castillo AM, Fromm ME, Vasil IK (1992) Herbicide resistant fertile transgenic wheat plants obtained by microprojectile bombardment of regenerable embryogenic callus. Bio/Technology **10**: 667-674

Visser RGF et al. (1991) Inhibition of the expression of the gene for granule-bound starch synthase in potato by antisense constructs. Mol Gen Genet **225**: 289-296

Voelker TA, et al. (1992) Fatty acid biosynthesis redirected to medium chains in transgenic oilseed plants. Science **257**: 72-74