

## General Application of Biotechnology in Agriculture

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### Abstract

New agricultural technologies, in general, need to cover two societal requirements-ensuring a safe, nutritious, and affordable food supply for the rapid increasing population, and at the same time, minimizing the negative environmental impacts of food production itself. Current agricultural technologies such as plant breeding and agrochemical research and development (R and D), environmentally sensitive and economic farm management practices will continue to play a major role in assuring a plentiful and safe food supply. Advances in all these areas will be required to meet world food production needs. Plant biotechnology complements plant breeding efforts by increasing the diversity of genes and germplasm available for incorporation into crops and by significantly shortening the time required for the production of new cultivars, varieties and hybrids. From an economic perspective, plant biotechnology offers significant potential for the seed, agrochemical, food processing and pharmaceutical industries to develop new products and manufacturing processes. Perhaps the most compelling attribute of the application of biotechnology to agriculture is its relevance to helping ensure the availability of environmentally sustainable supplies of safe, nutritious and affordable food and to providing a readily accessible, economically viable technology for addressing primary food production needs. Keeping in view, the article focusses on the biotechnological approaches for building a new tool which can significantly impact crop productivity in a sustainable, environmentally sound manner.

**Keywords:** Biotechnology; Agriculture; Crop Productivity

### Introduction

Agricultural biotechnology has been practiced for a long time, as people have sought to improve agriculturally important organisms by selection and breeding. Today, this technology has reached a stage where transgenic plants have been developed as a result from genetic engineering experiments in which genetic material is moved from one organism to another, so that the latter will exhibit a desired characteristic [1].

The common plant transformation techniques are as follows:

#### Agrobacterium Mediated Gene Transfer

This method of transformation is the most widely used to introduce foreign genes into plant cells. *Agrobacterium tumefaciens* is capable to transfer a particular DNA segment (T-DNA) of the tumor-inducing (Ti) plasmid into the nucleus of infected cells where it is subsequently stable integrated into the host genome and transcribed, causing the crown gall disease. The T-DNA contains two types of genes: the oncogenic genes, encoding for enzymes involved in the synthesis of auxins and cytokines and responsible for tumor formation; and the genes encoding for the synthesis of opines. Outside the T-DNA, are located the genes for the opine catabolism, By removing the tumor inducing genes and replacing them with the genes of interest, transformed plasmid can be obtained. Thus, making the bacteria an excellent vector for the transfer of foreign DNA.

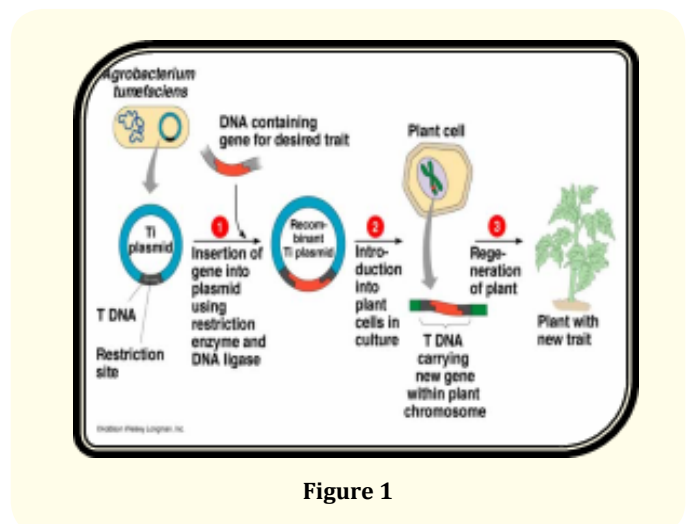


Figure 1

#### Biolistics

Some cells, tissues and intracellular organelles are impermeable to foreign DNA, especially plant cells. To resolve this problem in gene transfer, the gene gun machine is used for the transfer of desired gene. In this method, DNA or RNA adheres to biological inert particles i.e. gold or tungsten. DNA-particle complex is put on the top location of target tissue in a vacuum condition and accelerated by powerful shot to the tissue and then DNA will be effectively introduced into the target cells.

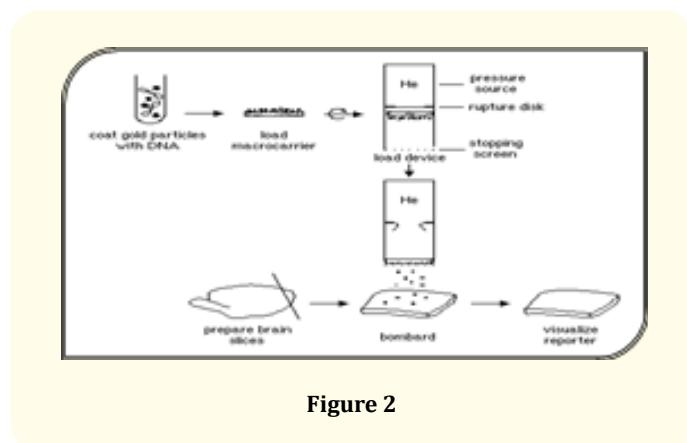


Figure 2

### Electroporation

Electroporation is the process where cells are mixed with a DNA construct and then briefly exposed to pulses of high electrical voltage. The cell membrane of the host cell is penetrable thereby allowing foreign DNA to enter the host cell. Some of these cells will incorporate the new DNA and express the desired gene.

### Microinjection

Microinjection is the direct mechanical introduction of DNA under microscopical control. A target can be a defined cell within a multicellular structure such as embryo, ovule and meristematic cells or a defined compartment of a cell. By examination with a microscope, a cell is held in place with gentle suction while being manipulated with the use of a blunt capillary. A fine pipet is then used to insert the DNA into the cytoplasm or nucleus. This technique is effective with plant protoplasts and tissues.

### PEG Mediated Gene Transfer

Liposomes are artificial lipid vesicles surrounded by a synthetic membrane of phospholipids, which have been used in animal cell culture for the drug delivery system. This process generally involves three steps:

1. Adhesion of liposomes to the protoplast surface,
2. Fusion of liposomes at the site of adhesion and,
3. Release of plasmid inside the cell.

### Strategies

Strategies are adopted to obtain the best quality and quantity of the desired product. Following three strategies are

1. **Plant gene expression:** Depending upon the purpose for which the product is to be made, required gene expression can be used among the following three methods of gene expression.

**Transient expression:** With transient expression (TE), a gene sequence is inserted into plant cells, without incorporation of the new genetic material into the plant chromosome. TE systems can be rapidly deployed and can produce large amounts of protein, but because non-chromosomal DNA is not copied with the process of mitosis or meiosis, gene expression is neither permanent nor heritable. While TE systems are very useful for research and development,

and may be useful for drug production, they require the fresh production of transformed plants with each planting and may be less attractive for long-term or high-volume protein production.

**Stable gene expression:** Alternatively, the primary plant chromosome can be altered to allow for the permanent and heritable expression of a particular protein. While permanent modification of the plant genome is more costly and time-consuming, it offers the clear advantage of stable, ongoing protein production with repeated planting alone.

**Chloroplast transformation:** Finally, systems exist that modify chloroplast DNA in plants and that can lead to heritable changes in protein expression. These provide advantages over nuclear transformation, particularly given that each cell may carry hundreds or thousands of such organelles, resulting in the ability to sustain very high numbers of functional gene copies.

2. **Location of Trans gene expression:** Consideration must also be given to where within the plant a pharmaceutical protein is to be produced. While production in green mass can produce large amounts of protein, green matter is highly physiologically active and protein levels may be poorly preserved if materials are not rapidly dried or otherwise inactivated. Thus, unless a protein or peptide is highly stable, green matter production may result in poor protein recovery and usually requires immediate processing. Tuber or root production, while feasible, shares many of the characteristics of green matter production systems. Unlike green matter, seeds generally contain fewer phenolic compounds and a less complex mixture of proteins and have specifically evolved to provide for stable, long-term storage of proteins and other materials in order to assure successful, delayed germination.
3. **Selection of plant species and characteristic:** It is also necessary to decide which plant species to transform for production of a specific pharmaceutical product. While nearly any plant could theoretically be transformed, practical considerations suggest the use of plants with which we are most familiar, and which already have well-established techniques for genetic transformation, high volume production, harvest, and processing. For green matter production, tobacco has usually been the material of choice, largely because of its highly efficient production of biomass, although other systems such as alfalfa and even duckweed show promise. For seed production, a plant optimized for large seed and high protein production is clearly preferred. Food crop plants have been bred specifically to produce highly productive stands of high-protein seed for which harvesting, processing, and storage technologies are already available.

### Transgenic Crops: For enhanced quality and quantity

Various crops have been genetically modified such as to have following beneficial aspects (Table 1).

Crop	Quality character/Controlled pathogen	Gene
Soybean	High oleic acid content	Fad2 (fatty acid desaturase) from <i>Borage officinalis</i>
Golden rice	Vitamin A	Phytoene synthase (Phy) from daffodil and phytoene desaturase and Zeta-carotene desaturase (Crt1) from <i>Erwinia uredovora</i> and lycopene β-cyclase from daffodil
Tomato	Increased sucrose level	Maize sucrose phosphate synthase (SPS)
Tobacco	High methionine content	2S albumin of Brazil nut under the promoter of phaseolin gene.
Tomato (Flavr Savr)	Shelf life	Antisense PG (Polygalacturonase)
Tomato (Endless Summer)	Shelf life	S-adenosyl methionine (SAM) hydrolase from T3 bacteriophage
Tobacco	Tolerance to salinity	Mannitol-1-phosphate dehydrogenase (mt/D) gene from <i>E. coli</i>
Tobacco	Resistance to drought	SacB from <i>Bacillus subtilis</i>
Tobacco	Oxidative and chilling stress tolerance	Cu/Zn superoxide dismutase from rice.
Cotton	Against lepidopteran	Bt gene from <i>B. thuringiensis</i>
Rice	Coleopteran ( <i>Sitophilus zeamais</i> )	Corn cysteine
Sugarcane	Sugarcane borer ( <i>Diatraea saccharalis</i> )	Bt cry I
Tobacco	<i>Spodoptera litura</i>	Trypsin inhibitor gene (sweet potato)
Potato	<i>Phytophthora infestans</i>	1,3-β glucanase
Tobacco	<i>Alternaria longipes</i>	Bacterial chitinase from <i>Serratia marcescens</i> .
Potato	<i>Erwinia carotovora</i>	Bacteriophage T4 lysozyme gene.
Tobacco	<i>Botrytis cinerea</i>	Stilbene (Phytoalexin-resveratol) synthase gene (STS) from peanut
Tobacco	Tobacco Mosaic Virus	Coat protein gene of TMV (interfere with uncoating; virus spread)
Canola (Roundup Ready)	Herbicide Glyphosate	aroA ( <i>Agrobacterium</i> sp.) encode 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme
Cotton (BXN)	Bromoxynil	bxn gene ( <i>Klebsiella ozaene</i> ) converts herbicide into 3,5-dibromo-4-hydroxy benzoic acid

**Table 1:** Transgenic crops and their transgene for various beneficial aspects.

**Potential improvements in nutritive value of plants**

In an attempt to get better human health, mainly in immature countries, balancing of human nutrition has been carried out by supplying balanced sources of nutrition by creating hereditarily distorted foods that hold nutrients known to help fight disease or starvation. The major component of human diet is oil and fat, in which saturated fatty acid compounds are present, which are hazardous to health. GM foods have been developed with reduced levels of saturated fatty acid components in soybean and castor.

**Improved crop productivity**

A lesser quantity of than 20% of the earth is arable land but some crops have been hereditarily altered to make them more liberal of conditions like salinity, cold and drought. The detection of genes in plants in charge for sodium uptake has lead to growth of knock-out plants able to grow in high salt environments. Up- or down-regula-

tion of record is usually the method used to alter drought-tolerance in plants. A global perspective suggests that some progress toward increased productivity has been made as insect-resistant, draught resistant and herbicide-tolerant transgenic varieties are reducing the risk of crop losses. One of the developments is the identification of a plant gene, At-DBF2, from *Arabidopsis thaliana*. *Arabidopsis thaliana* (tiny weed) shows tolerance to salt, drought and the heat and cold in plants. When this gene was inserted into tomato and tobacco cells, these cells withstood these conditions far better than ordinary cells similarly, Pizzuti and Daroda [2], also investigated the recombinant proteins from the root exudates of transformed tobacco for the major environmental concerns.

**Improved resistance to pesticides/herbicides**

Intensive agriculture adversely affects the environment because farmers are using chemical inputs for optimising soil nutrient conditions and pesticides for controlling insects, pathogens

and weeds. As pesticide resistant plants are broadminded of pesticides, allow farmers to selectively kill nearby weeds with no harming their crop. The most well-known example of this is the Roundup-Ready technology, urbanized by Monsanto. First introduced in 1998 as GM soybeans, Roundup-Ready plants are unaffected by the herbicide glyphosate, which can be applied in copious quantity to get rid of any other plants in the field. The decreased requirement of herbicides (10 - 40%) on herbicide tolerant varieties results in better control of weeds, improved yields, no-carryover of herbicide residues, better soil, water and air quality with savings in time and costs associated with conservative tillage to reduce weeds, or multiple applications of different types of herbicides to selectively eliminate exact species of weeds.

**Improved texture or appearance of food**

Modified fruit can ripen longer on the plant and then be transported to the consumer with less risk of spoilage, and a still-reasonable shelf life. The first genetically modified food product was a tomato which was transformed to delay its ripening.

**Better flavor**

Flavor can be altered by enhancing the activity of plant enzymes that transform aroma precursors into flavoring compounds. Transgenic peppers and melons with improved flavor are currently in field trials.

**Insect/Pest resistance**

Most of the current commercial applications of modern biotechnology in agriculture are on reducing the dependence of farmers on agrochemicals [3,4]. Insect/pest resistance crop provide more effective targeting of insects protected within plants for longer season protection. At the same time reduces the pesticide use and their exposure to farmers, labourers and non-target organisms e.g. Bt cotton: Engineered to contain and express the genes for Bt toxin, which they produce in its active form. When a susceptible insect ingests the transgenic crop cultivar expressing the Bt protein, it stops feeding and soon thereafter dies as a result of the Bt toxin binding to its gut wall. Bt corn: Engineered to control corn borer (a lepidopteran insect), which is otherwise controlled by spraying (a more difficult process).

**Disease resistance**

Biotechnology has helped to increase crop productivity by introducing qualities as disease resistance. For example, researchers from the University of Hawaii and Cornell University developed two varieties of papaya resistant to papaya ringspot virus by transferring one of the virus' genes to papaya to create resistance in the plants. Seeds of the two varieties, named 'SunUp' and 'Rainbow', have been distributed under licensing agreements to papaya growers since 1998.

**Transgenic crops: For molecular farming**

Plant Molecular Farming (PMF) consists of using transgenic plants as production platforms for the synthesis of compounds for pharmaceutical or industrial purposes [5,6]. Insulin, produced by bacteria engineered with recombinant human DNA, was the first biopharmaceutical to be introduced to the market in 1982. Andrew, et al. [7] in 1989 reported production of plantibody IgG. The first recombinant plant-derived pharmaceutical protein (PDP) was human serum albumin, initially produced in 1990 in transgenic tobacco and potato plants.

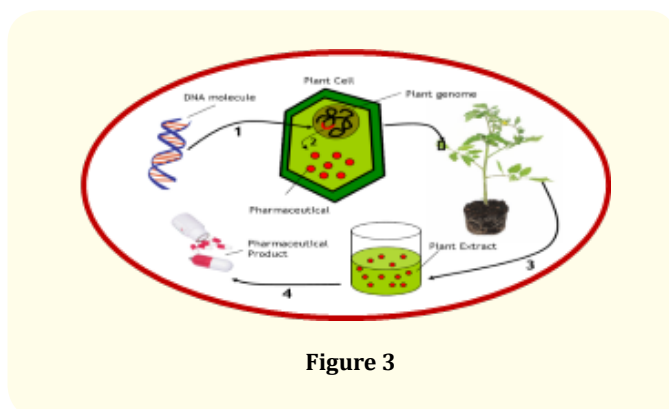


Figure 3

**Vaccines**

Oral vaccines have been in the works for much existence as a likely solution to the increase of disease in immature countries, where costs are excessive to extensive vaccination. Hereditarily engineered crops, frequently fruits or vegetables, planned to carry antigenic proteins from transferable pathogens that will activate an immune reply when injected. An example of this is a patient-specific vaccine for treating cancer [8]. An anti-lymphoma vaccine has been made using tobacco plants carrying RNA from cloned malignant B-cells. The resultant protein is then used to vaccinate the patient and boost their immune system beside the cancer [9]. Tailor-made vaccines for cancer treatment have shown substantial promise in preliminary studies.

Plant made vaccine: Transgenic plants are developed to express the antigens [10,11]. Human or animal vaccines and expressed by transgenic plants:

Origin	Plant	Recombinant protein
Enterotoxigenic <i>E. coli</i>	Tobacco/potato	Heat-labile enterotoxin B
<i>Vibrio cholera</i>	Potato	Cholera CtoxA and Ctox B subunits
Hepatitis B virus	Potato/lettuce	Envelope surface protein
Rabies virus	Tomato	Rabies virus glycoprotein
Foot-and-mouth disease	Arabidopsis/alfalfa	Virus epitope VP1

### Antibiotics

Plants are used to create antibiotics for both human and animal use [12]. An expressing antibiotic protein in stock feed, fed straight to animals, is less expensive than traditional antibiotic production, but this practice raises many bioethics issues, because the result is widespread, possibly needless use of antibiotics which may encourage expansion of antibiotic-resistant bacterial strain [13]. Quite a few rewards to using plants to create antibiotics for humans are condensed costs due to the larger quantity of product that can be produced from plants versus a fermentation unit, ease of purification, and condensed risk of contamination compared to that of using mammalian cells and culture media.

Production of plantibodies (antibody produced by genetically modified crops)

Plant	Antibody	Application
Tobacco	slgA (hybrid)	<i>S. mutans</i> (dental caries)
Tobacco	IgG (guy's 13)	<i>S. mutans</i> (dental caries)
Tobacco	IgG Co 17-1A	Surface antigen(colon cancer)
Soybean	IgG (anti HSV-2)	Herpes simplex virus
Tobacco	scFv (38C13)	Lymphoma

Production of pharmaceuticals: Following table represents range of proteins of pharmaceutical interest that have been expressed in plants

Recom binant protein	Origin (gene)	Plant (protein production)	Application
Protein C	Human	Tobacco	Anticoagulant
Hirudin	<i>Hiruda medicinalis</i>	Canola	Anticoagulant
Somatotrophin	Human	Tobacco	Growth hormone
Beta-Interferon	Human	Tobacco	Treatment for hepatitis B + C
Serum albumin	Human	Tobacco	Burns/fluid replacement
Glucocerebrosidase	Human	Tobacco	Gaucher's disease
$\alpha_1$ Antitrypsin	Human	Rice	Cystic fibrosis, haemorrhages
Aprotinin	Human	Maize	Transplant surgery
Trichosanthin- $\alpha$	<i>Trichosanthes kirilowii</i>	Tobacco	HIV therapy, cancer

Source: Drake and Christou, 2003

### Production of enzymes

Following table summarises recombinant enzyme production in plants.

Enzyme	Use
Avidin	Diagnostic kits
$\beta$ - Glucuronidase	Diagnostic kits
Trypsin	Pharmaceuticals, wound care
Cellulase	Ethanol production from cellulose waste
Xylanase	Biomass processing
Phytase	Phytase breakdown, improved phosphate utilisation
$\alpha$ - Amylase	Food processing
(1-3) (1-4) $\beta$ - Glucanase	Brewing
Lignin peroxidise	Paper manufacture

Source: Drake and Christou, 2003

Production of carbohydrates, lipids and their derivatives

Compound	Origin of genes	Origin of genes	Transgenic plant
Amylose -free starch	Potato	Food, industrial	Potato
Cyclodextrins	<i>Klebsiella pneumonia</i>	Food, pharmaceutical	Potato
Polyhydroxybutyrate	<i>Alcaligenes eutrophus</i>	Biodegradable plastics	Soybean

Source: Christou P and Harry K, 2004

### Regulatory system for production and release of transgenic products

**USA regulatory system:** Applicant issues the notification, if APHIS (Animal and Plant Health Inspection Service) of USDA (U.S. Department of Agriculture), did not have any objection, the later was allowed to conduct the trial. If the crop is used for feed/food purposes, it requires an additional clearance from Food and Drug Administration (FDA).

**EU regulatory system:** Application for small scale release is submitted to Joint Regulatory Author (JRA). If approved dossier is reviewed by relevant bodies (government and non-governmental), if accepted small- scale release allowed. After monitoring and assessment of small scale release, if found safe dossier is submitted to Member State for marketing release. If accepted dossier is reviewed and assessed by Member State. Finally dossier is forwarded to European Commission for consideration by all Member States. If considered safe approve marketing release.



**India regulatory system:** In India, for handling the GMOs and their product the Ministry of Environment and Forests (MOEF), the Central Government had enacted environment protection laws under the Environment Protection Act (EPA).

For trial permits, the Department of Biotechnology (DBT) of the Ministry of Science and Technology prepared biosafety guidelines. Under which, every organisation involved in R and D using GMOs is required to set up its Institutional Biosafety Committee (IBC) with a nominee from DBT. This committee is the nodal point for interaction with the government through national committee called the Review Committee for Genetic Manipulation (RCGM), which function under the charge of DBT. Experiments are monitored by the RCGM besides the IBSC. On the recommendation of RCGM, trial permits are issued by DBT.

### Potential human health concerns

#### Potential risk of introducing allergens into food crops

A major safety concern raised with regard to genetic engineering technology is the risk of introducing allergens and toxins into otherwise safe foods. Example: the consumption of transgenic soybean (containing methionine coding gene from Brazil nut) trigger allergic response in those sensitive subjects who were allergic to Brazil nuts.

Another example is of celery that was resistant to wilting after harvest was found to be the cause of skin rashes in people handling in its packing sheds. The new variety consist of increased amounts of a natural product, a psoralen, which confers resistant to wilting and also causes skin irritation.

When the introduced gene has been derived from the source of allergen there is an obvious need for testing of the novel food in people who are known to be allergic to the donor organism The Food and Drug Administration (FDA) checks to ensure that the levels of naturally occurring allergens in foods made from transgenic organisms have not significantly increased above the natural range found in conventional foods. Transgenic technology is also being used to remove the allergens from peanuts, one of most serious causes of food allergy.

#### Transfer of antibiotic resistance markers

Transfer of antibiotic resistance marker gene from genetically modified crop to intestinal bacteria is another concern. The WHO has judged antibiotic marker genes to be safe but the outcome of their use might be hazardous if they represent a major source of resistance to a wide class of antibiotics. This issue was raised during the commercialisation of Flavour Savor tomato but it was conferred that enzyme is produced at such a low level that it has absolutely

no effect on the consumed antibiotics. Nevertheless, to be on the safe side, FDA has advised food developers to avoid using marker genes that encode resistance to clinically important antibiotics.

#### Risk of toxicity

Farm workers may be exposed to unhealthy levels of a bio-pharmaceutical by absorbing products from leaves through their skin, inhaling pollen, or breathing in dust at harvest.

### Potential environmental concerns

#### Gene escape

One of the most contentious issues is the fear that a transgenic crop will become a potential conduit for transmission of genes to non-GMO plants of the same or a closely related species. Therefore, may produce unpredictable effects when inserted into other species without rigorous scientific control. Example: development of superweed (Kudzu) by acquiring herbicide tolerance gene.

Further, if gene transfer occurs from the transgenic to a non-GMO crop line, it could adversely affect farmers who sell to customers and entities who wish to consume non-GMO crops.

#### Ecosystem disruption

Even without gene transfer into wild species, transgenic plants might disrupt an ecosystem. For instance, an introduced gene could confer pest or herbicide resistance in the field in order to improve overall crop yield. Ecosystems involve complex, integrated connections among organisms in the environment. The introduction of a new variable could be significant enough to affect non-target organisms living in the same environment as the transgenic crop. Often these consequences are not studied or elucidated until after the crop has already been introduced into an ecosystem. Bt corn, for instance, produces a very specific pesticide intended to kill only pests that feed on the corn. In 1999, however, researchers at Cornell University found that pollen from Bt corn could kill caterpillars of the harmless Monarch butterfly. When they fed Monarch caterpillars milkweed dusted with Bt corn pollen in the laboratory, half of the larvae died. But follow-up field studies showed that under real-life conditions Monarch butterfly caterpillars are highly unlikely to come into contact with pollen from Bt corn that has drifted onto milkweed leaves-or to eat enough of it to harm them.

#### Effect on productivity and genetic diversity

GM crops could encourage monoculture cropping contributing to further decline in land productivity and genetic diversity. The introduced gene or its product may have negative effects on the natural environment. For example, wildlife feeding on the crop may ingest harmful levels of the PMP, or soil micro-organisms

may be inhibited by decomposing crop residue or substances exuded from roots of PMP plants. If gene for insecticide production spread into wild plants there could be serious effects on a wide range of insects including loss of some beneficial species. Example: Corn originates from Mexico which holds the greatest biodiversity of corn species in world but now it has been reported that wild corn variety located in some areas of Mexico have been contaminated by some genetically modified organism genes.

### Resistance or tolerance of target organisms

Adaptation by insect population to an environmentally related pest controlled technique could result in use of chemical pesticides with higher toxicity.

### Generation of new live viruses

The existence of virus resistant plants could encourage viruses to grow stronger or give rise to new and stronger variant that can infect plant.

### Potential economic concerns

#### Increasing dependence on industrialized nations by developing countries

The IPR system intrinsic to the WTO is heavily biased against developing countries. Not only does it provide MNCs the right to seize and patent genetic resources without any/adequate compensation, but it also prevents farmers from saving and reusing the modified seeds. Therefore, being forced to come back to seed giants year after year, for seed purchases would be financially unviable for them, leading to increased vulnerability. Since 1990, Monsanto has sued 145 farmers for "patent infringement". Monsanto claims that farmers are using their GMO plants without paying for them.

#### Biopiracy, or foreign exploitation of natural resources

Plant breeding relies on genetically diverse germplasm for progress to be made and maintained, which in traditional agriculture is regarded as a common resource of great value and is freely available. It is unethical to treat such traditional forms of agriculture as markets to be conquered by private interests.

#### Poor farmers might become dependent on international corporations for seed

Some transgenic crops are designed with terminator technology that produces infertile seeds. This ensures a steady stream of income to the company that designed and sells the seeds. Because farmers they become dependent on buying seed every year rather than saving seed from one year's crop to plant the following year. Only large scale farmers are benefitted.

GM seeds enable farmers to practise precision agriculture and narrow row farming, for small farmers, having fragmented land holdings with few technological inputs it is very difficult to achieve. Only the rich farmers would be able to afford these more expensive seeds. Moreover, these crops have led to increased flexibility in agricultural practices, such as simplified weed control, conservation tillage, broad spectrum control etc., all of which have led to reduced labor requirements. Thereby, having adverse economic impact on labor intensive developing country (India agricultural sector employs 64% of workforce).

### Commingling of PMP crops and food or feed crops

Commingling of PMP crops and food or feed crops may occur. This could happen through improper labelling, mixing of seed in planting, harvesting, transportation, or processing. In a 2001 case, ProdiGene failed to eliminate volunteer bio-pharm corn plants from a soybean crop planted later in the same field as the PMP corn. The company was fined \$250,000 by USDA and was required to reimburse the government \$3 million for expenses related to destruction of 500,000 bushels of potentially contaminated soybeans.

### Social and ethical concerns

- Violation of natural organisms' intrinsic values.
- Tampering with nature by mixing genes among species.
- Suffering of animal.
- Using animals for production of pharmaceutical proteins- animals are just reduced to mere factories.
- GE takes mankind into realms that belong to God and God alone.
- Strict vegetarians might object to gene sequences from animals being introduced into plants.
- There are different social and ethical issues confined with the involvement of sacred and religious trees and animals in transgenic research.

### Suggested Safeguards

- **Physical containment:** Growing GM plants in physical structures (plastic tunnels; greenhouse) would help to avoid contamination of the environment or of the food/feed chains production facilities.
- **Spatial containment:** Includes several strategies aiming mainly at minimizing cross-fertilization between pharma/industrial crops and other crops.
- **Dedicated land:** Involves cultivating molecular farming crops in regions where similar non-farming crops are not grown or in locations that are far removed from areas where non-farming crops are grown.

- **Restricted use:** Refers to the prohibition against the planting of a food crop the year after the land was used for biopharming.
- **Plastid transformation:** Since the pollen of many crop species does not contain chloroplasts, the transgene may not be transferable via cross pollination.
- **Male sterility:** Chase [14] reported that inducing male sterility in GM plants provide means by which transgenes are prevented from transferring to other plants.
- **Prevent release from roots:** It consists of blocking release or diffusion of the product from the roots of transgenic plants thus reducing “protein pollution” represent hazard for soil and rhizosphere microbial communities.
- **Postharvest inducible expression:** In this approach, the transgene is not expressed at all in the plants in the field but the molecular farming product will only be formed when the harvested plant material is removed and exposed to a chemical or environmental stimulus that activates expression of the transgene.

## Conclusion

International agreements proliferated, responding to calls for biodiversity preservation, biosafety, intellectual property protection, and international trade. Financiers promoted biotechnology as a radically new technology and hyped it to investors as a road to easy wealth. Large corporations came to believe that agricultural biotechnology offered the potential for dramatic business growth and began acquiring biotechnology start-ups and seed companies. Some observers raised ethical issues about the transfer of genetic material across species, and social justice advocates became concerned about the concentration of seed production in the hands of a few companies; substantial opposition arose to genetically engineered crops as food sources.

General attitudes appeared to be an important determinant of the expectation of consuming food produced by genetic engineering. The general public should see biotechnology as a safe tool for scientific crop improvement because it helps in the fight against hunger and poverty. Therefore, research funding should be allocated accordingly to long-term plant breeding programs, which include biotechnology as one of its tools. In this way, we may effectively face the serious challenge of feeding the rapidly growing world population in the next millennium.

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