

GM CROPS: THE NEED OF TOMORROW'S

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ABSTRACT

With a very high rate of increase in world population the food production has to be increased by 70% to meet the demands of the increasing population. Because of deleterious effect of pesticides and fertilizers used to enhance the crop yields the soil has get contaminated with the recalcitrant, so we need newer technologies to reduce the use of such deleterious substances and simultaneously increase the yield with compromising the quality. Biotech crops will ensure that there is plenty of food to feed the growing population and with enhanced nutritional qualities. With the increase in the knowledge about the GM crops the production has increased from 1.7 million hectares in 1996 to 179.7 million hectares in 2015. 17 to 18 million farmers have adopted GM technology making biotech crops the fastest adopted crop technology in recent times.

Keywords: Biotech crops, pesticides, transformation, transgenic.

Introduction

World population is expected to reach an estimated 9.2 billion by 2050, food production globally has to increase by 70% to feed the world, while total arable land, which has reached its maximal utilization, may even decrease. Because of the deleterious effects of pesticides on soil and human health, new technology is needed to increase the crop yield, improve nutritional quality of food and reduce crop losses (Qaim, 2010). Biotech crops will ensure that there is enough food with the aim of combining higher yields, improved food and feed quality with environmental friendly agronomic practices (Phipps and Beever, 2000). The term GMO (genetically modified organism) refers to plant or animal that has been modified by the genetic material that is not native to the target species i.e. foreign DNA. Plant genetic engineering methods were developed over 30 years ago, and since then, genetically modified (GM) crops have become commercially available and widely adopted. Chemical pesticides are used for preventing, destroying, repelling or mitigating any pest. Some examples of chemically-related pesticides are: Organophosphate Pesticides, Carbamate Pesticides, Organochlorine, Pyrethroid Pesticides etc. Pesticides are a special kind of products for crop protection. Crop protection products in general protect plants from damaging

influences such as weeds, diseases or insects. Companies selling genetically engineered seed that reduce the use of agricultural chemicals and promote biotechnology as necessary to feed the world.

There are many harmful effects of chemical pesticides:

1. Pesticides tend to remain active long after destroying the target and causes contamination of food materials, disruption of natural balance of ecosystem by killing non target species and gradual increase in the immunity of target organisms to these chemicals.
2. Bioaccumulation and bio magnification of pesticide: Continued use of huge amounts of different kinds of poisonous agricultural pesticides increase their concentration in the organism and multiplies through food chain and a phenomenon called biomagnifications is caused which moves up in the food chain and affects the apex species in the food pyramid. Man, also situated at the higher tropic level of food accumulates these poisons and many cases of food poisoning and contamination are reported.
3. Adverse effects are being noticed due to the excessive and imbalanced use of chemical fertilizers. This situation lead to identifying of various diseases such as hypertension, kidney failure, calcium deficiencies, stone formation in gall bladder and kidneys and bone disease.
4. Pesticides can contribute to air pollution. Pesticide drift occurs when pesticides suspended in the air as particles are carried by wind to other areas, potentially contaminating them.
5. Many of the chemicals used in pesticides are persistent soil contaminants, whose impact may endure for decades and adversely affect soil conservation.
6. Nitrogen fixation, which is required for the growth of higher plants, is hindered by pesticides in soil. The insecticides DDT, methyl parathion, and especially pentachlorophenol has been shown to interfere with legume-rhizobium chemical signalling.

Genetically modified crops

Genetically modified crops are used as an alternative to chemical pesticides. GM crops which have been altered using genetic engineering techniques, so basically using DNA molecules from different sources and combining them to create a new set of genes which are then transferred into an organism to give that organism a desired trait. The first genetically modified crop plant i.e. an antibiotic-resistant tobacco plant was produced in 1982 (Fraley *et al.*, 1983). In 1986, the first field trials occurred in France and the USA, when tobacco plants were engineered for herbicide resistance. In 1987, Plant Genetic Systems (Ghent, Belgium), founded by Marc Van Montagu and Jeff Schell, was the first company to genetically engineer insect-resistant tobacco plants by

incorporating insecticidal genes from *Bacillus thuringiensis* (Bt). Commercial sale of genetically modified foods began in 1994, when Flavr Savr (delayed ripening) tomato was approved by the FDA for marketing in the US. Polygalacturonase gene was inserted into the tomato genome in the antisense direction. The polygalacturonase enzyme degrades pectin causing the tomato fruit to soften. In 1994, the European Union approved herbicide (bromoxynil) resistant tobacco, making it the first commercially genetically engineered crop marketed in Europe (Debora, 1994). In 1995, the following transgenic crops received marketing approval in USA: canola with modified oil composition (Calgene), *Bacillus thuringiensis* (Bt) corn/maize (Ciba-Geigy), cotton resistant to the herbicide bromoxynil (Calgene), Bt cotton (Monsanto), Bt potatoes (Monsanto), soybeans resistant to the herbicide glyphosate (Monsanto), virus-resistant squash (Monsanto-Asgrow), and additional delayed ripening tomatoes (DNAP, Zeneca/Peto, and Monsanto). In 2000, scientists created golden rice, genetically modified food to increase its nutrient value for the first time (Potrycus, *et al.*, 2000). As in 2012, an apple that has been genetically modified to resist browning, known as the 'Nonbrowning Arctic apple' produced by Okanagan Specialty Fruits, a gene in the fruit has been modified such that the apple produces less polyphenol oxidase. In February, 2015 Arctic Apples were approved by the USDA, becoming the first genetically modified fruit approved for US sale (Pollack, 2015).

Methods of Transformation

The uptake of foreign DNA or transgenes by plant cells is called transformation. Genetically engineered plants are generated in the laboratory by altering their genetic makeup and are tested for desired qualities. This is usually done by adding one or more genes to the genome of plants using genetic engineering techniques. There are many methods for producing the transgenic crop but mostly genetically modified plants are generated by the biolistic method (particle gun) or by *Agrobacterium tumefaciens* mediated transformation.

Indirect method of transformation

***Agrobacterium* - mediated transformation**

These are natural plant parasites, which has their natural ability to transfer genes for the development of genetically engineered plants. This is achieved by engineering selected genes into the T-DNA of the bacterial plasmid in laboratory conditions so that they become integrated into the plant chromosomes when the T-DNA is transferred. It has unique advantages in plant transformation:

- i) The simplicity of *Agrobacterium* gene transfer makes it a 'poor man's vector.
- ii) A precise transfer and integration of DNA sequences with defined ends.

- iii) A linked transfer of genes of interest along with the transformation marker.
- iv) The higher frequency of stable transformation with many single copy insertions.
- v) Reasonably low incidence of transgene silencing.
- vi) The ability to transfer long stretches of T-DNA (> 150 kb).

In Planta Transformation

Transformation protocol requiring extensive periods of plant tissue culture have several disadvantages, including time, space and including somaclonal variations. Therefore, there is a considerable interest in developing transformation method that obviate the need for plant tissue culture. Methods for transformation *in planta* (vacuum infiltration, floral dip and spraying) utilize either *Agrobacterium* mediated transformation or direct gene transfer methods. One of the most promising method developed using *Arabidopsis* as the target plant is floral dip. In this method plants with young flowers are dipped (with or without vacuum being applied) into a culture of *Agrobacterium* which also contains a surfactant. The plant is sequentially allowed to set seeds, whereupon a small portion of seeds produced are transgenic.

Direct methods of transformation

PEG method of transformation

DNA uptake by protoplasts can be stimulated by chemicals like polyethylene glycol. PEG is used to stimulate the uptake of liposomes and improve the efficiency of electroporation. PEG at high concentration 15-20% will precipitate ionic macromolecules such as DNA and stimulate their uptake by endocytosis without any gross damage to protoplasts. This is followed by cell wall formation and initiation of cell division. *Petunia* and *Nicotiana* are some of the examples where this technology has been used.

Microinjection

A delivery system that involves the direct injection of foreign DNA into plant cells using minute needles. In this microinjection of DNA into the nuclei of isolated protoplasts could be an efficient means of gene transfer. Its limitation is that only one cell receives DNA per injection, and handling requires more skill and instrumentation.

Particle bombardment

In this method, DNA coated on pellets is forced down the barrel of a 'Particle Gun' by an explosive charge. The particles are forced through the cell wall where the DNA is released. This method has been applied successfully for many cultivated crops, especially monocots like wheat or maize.

Electroporation

This is a technique using electrical fields to make protoplasts temporarily permeable to DNA, and offers an effective alternative to vectors. It was first reported in rice and has been used to produce stably transformed cell lines & /or plants in several plant species, e.g., tobacco, petunia, maize, rice, wheat etc.

Liposome-mediated

Liposomes are small lipid bags in which large number of plasmids is enclosed. They can be induced to fuse with protoplasts using devices like PEG and therefore have been used for gene transfer. Petunia, carrot & tobacco have been transformed with this method.

Pollen mediated

Transformation can also be obtained by incubation of pollen with foreign DNA followed by pollination and fruit set (e.g. rice and wheat).

Silicon carbide fibers

This is a simple technique for which no specialized equipment is required. Plant material is introduced into a buffer containing DNA and silicon carbide fibers which is then vortexed. The fibers penetrate the cell wall and plasma membrane allowing the DNA to gain access inside the cell. This method has been utilized to transform maize.

Laser-Induced DNA Delivery

Laser has been successfully used for high frequency (10^{-3}) transfection. Lasers puncture transient holes in the cell membrane through which DNA may enter into the cell cytoplasm. Lasers have been used to deliver DNA into plant cells as well, but there is no information on transient expression or stable integration.

Comparison of some important DNA delivery techniques for plant cells.

DNA delivery methods

Feature	Agrobacterium mediated	Chemical and electrical	Microinjection	Particle gun
Size of DNA construct	≤ 50 kb	5-20 kb	16 kb	---
Range of plants	Limited	All plants	All plants	All plants
Efficiency	High	High	---	---
DNA insert integrity	Not affected by rearrangements	High degree of rearrangements	---	High degree of rearrangements
Number of copies integrated	Mostly single or few copies	High frequency of multicopy insertion	1-5	High frequency of multicopy insertion
Need of special equipment	No	No (chemical) Yes (electrical)	Yes	Yes
Regeneration protocol	Needed	Needed	Needed (often)	Not necessary (use of embryo, shoot tip, etc.)
Protoplast culture	Not Needed	Necessary	May be Needed	Not Needed
Applicable to organized meristem	Generally, No	No	Yes	Yes
Applicability to cereals	Applicable	Frequently used	Possible	High applicability
Chimaeric plants	No	No	Often (when meristems are used)	Often (when meristems are used)

Global Scenario of genetically modified crops

The global acreages under GM crops continued to expand through 2014. According to the International Service for Acquisition of Agri-Biotech Applications (ISAAA), acreage under GM

crops increased to 181.5 million hectares (448 million acres) in 2014, about five million hectares more than last years. But in 2015 the acreage under GM crops was 179.7 million hectares (444 million acres) a marginal decrease of 1% was observed. Under GM crops, US continued to be the largest country, accounting for 39% followed by Brazil accounting for 25% of the total planted area globally. Out of the 10 top biotech crop countries, listed by hectareage, 8 were developing. Out of 28 countries that planted GM crops last year, twenty were developing nations and eight were developed countries. For the fourth consecutive year, developing countries planted more biotech crops than industrial countries in 2015. Farmers from Latin American, Asian and African collectively grew 97.1 million hectares or 54% of the global 179.7 million biotech hectares (versus 53% in 2014) compared with industrial countries at 82.6 million hectares or 46% (versus 47% in 2014), equivalent to a gap of 14.5 million hectares in favor. India has overtaken Canada to emerge as the fourth largest country to grow genetically modified (GM) crops and farmers here planted *Bt* cotton in about 11.6 million hectares in 2015 and has become No. 1 in the production of cotton in the world. On the 20th anniversary of commercialization of biotech crops i.e. in 2015 up to ~18 million farmers, in 28 countries planted 179.7 million hectares (444 million acres), with a marginal decrease of 1% or 1.8 million hectares (4.4 million acres) from 2014. The 28 countries include Vietnam which commercialized stacked biotech maize in 2015 for the first time. Bangladesh, a small poor country doubled the commercial hectareage of the prized vegetable *Bt* brinjal/eggplant; it was grown on 25 hectares in 2015 compared with 12 hectares in 2014. Success with *Bt* brinjal has led Bangladesh to prioritize the field testing of a new late blight resistant potato (an important crop occupying ~0.5 million hectares in Bangladesh) which could be approved by 2017. The first biotech drought tolerant Maize was first planted in the US in 2013, increased more than 15-fold from 50,000 hectares in 2013 to 275,000 hectares in 2014 and 810,000 hectares in 2015, reflecting high farmer acceptance at 3-fold year-to-year between 2014 and 2015. In Argentina, two home-grown products were approved – a drought tolerant soybean and a virus resistant potato. In Brazil, approval was gained for cultivation of a 20% higher yielding home-grown eucalyptus, developed by FuturaGene/Suzano, plus commercialization of two home-grown crop products in 2016 – a virus resistant bean and a new herbicide tolerant soybean. In Myanmar, a new *Bt* cotton variety *Ngwe-chi-9* was commercialized in 2015. In Canada, there was approval of a higher quality non-browning apple.

Innate™ potato, another food crop, was approved in the US in November 2014. It has lower levels of acrylamide, a potential carcinogen in humans, and suffers less wastage from bruising; potato is the fourth most important food staple in the world. A safer product and decreased wastage in a vegetatively propagated and perishable crop, can contribute to higher productivity and food security. Also in November 2014, a new biotech alfalfa (event KK179) with up to 22% less lignin, which leads to higher digestibility and productivity, was approved for planting in the US.

Global Area of Biotech Crops in 2015: by Country (Million Hectares)**

Rank	Country	Area (million hectares)	Biotech Crops
1	USA*	70.9	Maize, soybean, cotton, canola, sugarbeet, alfalfa, papaya, squash, potato
2	Brazil*	44.2	Soybean, maize, cotton
3	Argentina*	24.5	Soybean, maize, cotton
4	India*	11.6	Cotton
5	Canada*	11.0	Canola, maize, soybean, sugar beet
6	China*	3.7	Cotton, papaya, poplar
7	Paraguay*	3.6	Soybean, maize, cotton
8	Pakistan*	2.9	Cotton
9	South Africa *	2.3	Maize, soybean, cotton
10	Uruguay*	1.4	Soybean, maize
11	Bolivia*	1.1	Soybean
12	Philippines*	0.7	Maize
13	Australia*	0.7	Cotton, canola
14	Burkina Faso*	0.4	Cotton
15	Myanmar*	0.3	Cotton
16	Mexico*	0.1	Cotton, soybean
17	Spain *	0.1	Maize
18	Colombia*	0.1	Cotton, maize
19	Sudan*	0.1	Cotton
20	Honduras	<0.1	Maize
21	Chile	<0.1	Maize, soybean, canola

22	Portugal	<0.1	Maize
23	Vietnam	<0.1	Maize
24	Czech Republic	<0.1	Maize
25	Slovakia	<0.1	Maize
26	Costa Rica	<0.1	Cotton, soybean
27	Bangladesh	<0.1	Brinjal/Eggplant
28	Romania	<0.1	Maize
	Total	179.7	

* 19 biotech mega-countries growing 50,000 hectares, or more, of biotech crops

** Rounded off to the nearest hundred thousand

Source: Clive James, 2015.

Indian Scenario

- ❖ In 2015, plantings of Bt cotton in India surpassed the historical milestone of 11.6 million hectares and has become the leading producer of cotton in the world.
- ❖ **Indian developed two Bt rice transgenic varieties** IR-64 & Pusa Basmati-1 (*Cry1a* & *Cry1b* under the control of maize ubiquitin promoter) using biolistics and *Agrobacterium* mediated transformation (Khanna *et al.*, 2001).
- ❖ **Researchers developed protein-packed Potato in India** *Ama1* (Albumin1) from *Amarantus*. 60% more protein & increased levels of amino acids. (Datta *et al.*, 2000)
- ❖ **Aphid repellent mustard in India** *Brassica juncea* was transformed with *Eβf* gene isolated from *Mentha arvensis*, mustard lines capable of emitting a sesquiterpene compound (E)-β-farnesene were developed. Farnesene is an alarm pheromone, and plants producing this volatile develop repellency to aphids (Verma *et al.*, 2015)
- ❖ **Cotton genotypes H 777 & HS 6** were transformed into cotton curl leaf virus resistant plants with antisense rep (replicate protein), sense coat protein & antisense coat protein gene constructs mediated through *Agrobacterium*.
- ❖ **Putative rice transgenic** was developed in cultivars Pusa Sungandh 2, IR 64 & BPT 5204 with DREB1a gene for drought tolerance, & Swarna with *cry1AB* for resistance to stem borer.
- ❖ **Transgenic mustard** expressing osmotin that confers tolerance to salinity.

- ❖ **Transgenic potato** expressing RB gene from *Solanum bulbocastaneum* that confers resistance to late blight disease.
- ❖ **Transgenic sorghum** expressing *cry1B* gene that confers resistance to stem borer are under field testing.
- ❖ Biosafety tests on cotton transgenics, Anjali & LRK 5266 with *cry1F* gene showing resistance for American bollworm were carried out in our country.
- ❖ *Bacillusthuringiensis* brinjal, popularly known as *Bt* brinjal, is right now in the middle of an environmental and health controversy in India. But it was grown in Bangladesh.

FIELD TRIALS OF GM CROPS IN INDIA IN YEAR 2013

S.No.	Crop	Company Name	Trail	Trait
1.	RRF Cotton	Maharashtra Hybrid Seeds Company Ltd.	BRL-I 2 nd Year	Herbicide Tolerance
2.	Corn	Syngenta Biosciences Pvt. Ltd.	BRL-I	Insect Resistance and Herbicide Tolerance
		Syngenta Biosciences Pvt. Ltd.	BRL-I 2 nd Year	Insect Resistance and Herbicide Tolerance
		Syngenta Biosciences Pvt. Ltd.	Seed Increase	Insect Resistance and Herbicide Tolerance
		Monsanto India Ltd.	BRL-I 2 nd Year	Insect Resistance
3.	Cotton	Bayer Biosciences Pvt. Ltd.	BRL-I (2 nd Season)	Herbicide Tolerance
	Cotton	Bayer Biosciences Pvt. Ltd.	BRL-I	Insect Resistance
4.	Maize	Monsanto India Ltd.	BRL-I 2 nd Year	Herbicide Tolerance

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Benefits of GM technology

- Productivity enhancement (prevents yield losses)
- Eco-friendly (reduced pesticide and fertilizer use)
- Positive effect on farming/food product
- Bio-energy (bio-diesel, ethanol etc.)

- Nitrogen use efficiency
- Increase in food availability
- Improvement in nutritional quality and health benefits
- Improved protein quality and quality of meat, milk and livestock
- Improved shelf-life and organoleptic quality of foods
- Biological defence against diseases, stresses, pests, weeds, herbicides, and viruses
- Bioremediation
- Increase in food carbohydrate content
- Manufacture of edible vaccines and drugs
- GM crops function as bio-factories and source of industrial raw materials
- Improved profitability to farmers
- Growth of seeds industry and job opportunities

Conclusion

The population of the world is increasing at a very fast rate the resources to feed such a huge population are limiting, and the area under cultivation is decreasing day by day as it is one of the most important limiting factor. So, to overcome this Biotech crops has to adopted and the misconceptions has to be overruled. If small countries like Bangladesh could adopt GM technology, why not others. Bangladesh being one of the smallest and poorest countries in the world, is an extra ordinary model of the importance of political will in the adoption of biotech crops. Developing countries are continuously plating more biotech crops than the developed countries for the fourth consecutive year. Farmers are benefited enormously by economic grains and at least a 50% reduction in the insecticide applications, and importantly contributed to a more sustainable environment and better quality of life. So, a complete knowledge should be spread about the GM technology and its full utilization should be made. Biotech crops is the only possible ways to meet the demands of the increasing population.

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