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Research Article

OVERVIEW OF PROSPECTS AND OPPORTUNITIES OF GM CROPS & MOLECULAR FARMING WORLDWIDE

Anjula Chaudhary* and Dr Abha Vashistha

IMS Ghaziabad University Courses Campus Adhyatmik Nagar, Ghaziabad- 201015

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ABSTRACT

Genetically modified or genetically engineered crops are one of most important and attracting field now these days. Such crops are produced by adding a transgene or an unrelated gene into the crop for a particular feature like insect, pest resistance, herbicide tolerance and also to increase yield for molecular farming for making edible vaccines etc. In relation to socio-economic impacts, GM crops have increased income for large- and small-scale commercial and subsistent farmers with associated downstream impacts through investments. Increased gross margins are due to higher revenues and reduced costs in relation to pest management. Issues such as debt problems caused by seed purchase occur but are no more extensive than for conventional crops; the question of crop monopolies by multinational companies remains though it must be acknowledged that such companies have invested cash billions in development and that they have little to gain by pricing farmers out of the market. Health benefits have also been achieved, especially through a reduction in pesticide use. Additional potential benefits of GM crops are discussed including possibilities for the improvement of human health by augmenting specific nutrients i. ebio fortification. But as every new technology has some positive and some negative affects it also have some. Like gene escape, environmental impact etc. but GM crops can help a lot to fulfil the demand of today's increasing population. Likewise crop plants produce large amounts of biomass at low cost and require limited facilities. Since plants have long been used as a source of medicinal compounds, molecular farming represents a novel source of molecular medicines, such as plasma proteins, enzymes, growth factors, vaccines and recombinant antibodies, whose medical applications are understood at a molecular level. Bio-pharming promises more plentiful and cheaper supplies of pharmaceutical drugs, including vaccines for infectious diseases and therapeutic proteins for treatment of such things as cancer and heart disease.

INTRODUCTION

With the advent of modernization agricultural lands are reducing and population is increasing, natural and genetic resources are also reducing, and climate is rapidly changing, the crops production faces unprecedented challenges, which need to be tackled with utmost precision. Biotechnological approaches including genetically modified crops are used in agriculture to tolerate abiotic or biotic stress and they can also speed up the breeding program. Examples in GM crops include pest, diseases, or environmental conditions reduction, resistance to chemical treatments (e.g. resistance to herbicide), or improving the nutrient profile of the crop.GM crops with desirable traits are developed through gene integration by the use of recombinant DNA technology, which is not possible through conventional breeding methods.GM crops, thus consists of "foreign genes" or "transgenes genes" which after proper integration expresses stable and confers either a novel trait to the plant, which was not initially present in the native form or to enhance the already present trait. A transgene consists of a promoter, which governs the expression of a gene, the insert, conferring a specific trait to the host plant; a marker gene that allows the selection of transformed plants and a terminator which functions as a stop signal. The main advantage of GM food crops is their potential promise of future food security, especially for small-scale agriculture in developing countries (Hossein, 2009). The main arguments of GM supporters are safe food security, improved food quality, and extended shelf-life as the reasons why they believe in GM crops which will benefit not only both consumers and farmers, but also the environment (Wisniewski *et al.*2002). Asdiscuss (Belcher *et al.* 2006), a critical question is what impact(s) biotechnology companies should take into their account. For example, in corn, the productivity impact is mainly yield

Corresponding author:* **Anjula Chaudhary
IMS Ghaziabad University Courses Campus Adhyatmik Nagar, Ghaziabad- 201015

increase, and in soybeans the GM technology allows saving on inputs of chemicals and labor.

Methods of making transgenic plants

There are mainly two modes of gene transfer in plants, named as,

Indirect Method or Agrobacterium mediated method

Transfer between Agrobacterium tumifaciens and certain plants have provided scientists with the most powerful tool to genetically engineered plants. Agrobacterium mediated transformation is very convenient.

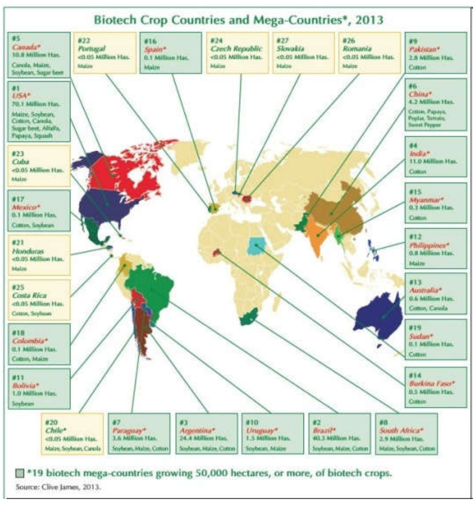
Agrobacterium Mediated Plant Transformation has three Main Phases

a. Creation of Agrobacterium stain having Ti plasmid with the gene that is transferred to plant.
b. Plant tissue is co-cultured with bacterium to transfer DNA.
c. Regenerating a plant from the transformed cells at the same time as the non- transformed cells are being killed by anti-biotics.

Direct Methods

There are various methods under this like microprojectiles or gene gun or particle gun, electroporation method, liposome mediated gene transfer, calcium phosphate precipitation method, transformation by ultrasonication, transformation using pollen or pollen tube and incubation of dry seeds etc.

Careful application of genetic engineering will make life better, improve human health and welfare, and save time and money (Uzogara, 2000). However, before any intervention in farming systems, antibiotic resistance genes used in GM crops must be scrutinized to see whether or not they can be substituted for with other similar effective selection methods to care both human and animal health and avoid any potential risks (Hileman, 2008). Remarkable progress has been made for the development and adoption of GM crops in more than 27 countries, with area of 175 million hectares under GM cultivation in 2013. Soybean, maize, cotton and canola are the major GM cultivation in 2013. Soybean, maize, cotton and canola are the major GM crops commercially cultivated globally, covering a range of traits such as insect resistance and herbicide tolerance.



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Beside this are also some potential benefits of GM crops like

Herbicide tolerance

Many herbicide tolerant plants have been produced such as tomato, tobacco, potato, soybean, cotton, corn, petunia etc. Two approaches have been used for the development of herbicide resistant plants:

a. Over production of target enzyme
b. Mutation resulting in enzymes that are less sensitive to the inhibitor

Under the trade name Round-up ready Glyphosate is one of the most potent broad spectrum herbicide. By blocking the action of enzyme EPSPs (5-enolpyruvyl shikimate -3-phosphate synthase) glyphosate kill the plants. EPSPs is an essential enzyme in the biosynthesis of amino acids containing aromatic ring like Tyrosine, tryptophan, and phenylalanine. By transferring EPSPs gene transgenic plants were developed which are resistant to glyphosate.

From *Salomonellatyphimurium* or E.coli a gene aro A was isolated and transferred to tomato and potato. Transgenic tobacco plants expressing a mutant ALS (acetolactate synthase) gene from Arabidopsis were produced that were tolerant to sulphonylurea herbicide.

Herbicides should only kill weeds not the crop plants so there must be the process of detoxification and degradation. Various detoxifying enzymes have been found in plants as well as microbes. Some of these detoxifying enzymes include:

1. GST glutathione-s-transferase it detoxifies herbicide Atrazine in maize and other plants
2. Nitrilase which detoxifies the herbicide bromoxynil
3. PAT or phosphinothricin acetyl transferase coded by bar gene detoxifies the herbicide PPT (phosphinothricin).
4. Thus transgenic plants reduce the use of weeding labour, farmers cost and improves the yield.

Insect pest Resistance in Transgenic Plants

Bt toxin has been isolated from *Bacillus thrugiensis* (a gram-negative soil bacterium) and by Agrobacterium mediated transfer method used for the transformation of tobacco, tomato and cotton plants. Bt toxin is used as biological insecticide.
CPTI (cow pea trypsin inhibitor) gene which is responsible for resistance against major storage pest of cowpea seeds, isolated and introduced with CamV35S in tobacco and other major crops, making them resistant against many insects. The CPTI gene is stably inherited and there is no serious "yield penalty".

Resistance Against viral Infection

In tropical areas there is serious crop loss caused by viruses. The ultimate result of virus infection is fully inhibited photosynthesis and diminished crop yield. Various strategies have been used to control viral infection. They are

a. By the use of coat protein
b. Using gene for nucleocapsid
c. Satellite RNA

Plants resistant to Cucumber mosaic virus (CMV) and Tobacco ring spot virus (ToMV) are prepared by the use of satellite RNA.

Viruses are submicroscopic pockets of nucleic (DNA or RNA) enclosed in a protein coat, and can multiply with a living cell.

From tobacco mosaic virus (TMV), coat protein gene has been transferred to tobacco making it nearly resistant against TMV. With the use of nucleocapsid protein resistance has been created in plants like tomato, tobacco, lettuce etc.

Use of satellite RNA makes many transgenic plants resistant to Cucumber mosaic virus (CMV), tobacco ring spot virus (ToMV).

From *Serratia marcescens* chitinase geneis obtained and introduced in tobacco, to make it resistant to *Alternaria longipes* which causes Brown spot disease. Acetyl transferase gene is introduced in tobacco making resistant to *Psuedmonas syringae*, a causal agent of wild fire disease.

Resistance against stress

Various types of genes which are responsible for making plants resistant against stresses such as heat, cold, salt, heavy metals, phytohormones and nitrogen have been identified. In current studies it was found that metabolites like proline and betaines are helpful in stress tolerance.

Many plants respond to drought stress by synthesizing a group of sugar derivatives called polyols. Plants having more levels of polyols are more resistant to stress. Using a bacterial gene that is an enzyme capable of synthesising mannitols is introduced in plants which raise level of mannitol very high, thus making plants resistant to drought.

Male Sterility and Fertility Restoration in Transgenic Plants (Suitable for Hybrid seed Production)

Transgnic plants with male sterility and fertility restoration genes have become available in *Brassica napus*. This facilitates production of hybrid seed without manual emasculation and controlled pollination as often practiced in maize.

In 1990 Mariani and others from Belgium have successfully used a gene construct having another specific promoter and bacterial coding sequence for a ribonuclease for production of transgenic plants in *Brassica napus*. The results were spectacular in the sense that the transferred gene prevented normal pollen development leading to male sterility.

Transgenic plants in Monocotyledons:

Transgenic Plants in Maize

For the production of transgenic plants in maize a reporter gene for neomycin phosphotransferase (NPT II) associated with 35 S promoter region of cauliflower mosaic virus (CMV) was used.

Lysine rich corn has also been produced through genetic engineering.

In USA biotechnology company Monsanto recently produced insect resistant transgenic maize plants using Kurstaki gene derived from *B. thruingiensis*. They are resistant against European corn borer.

To date Bt maize has benefitted people and environment worldwide. Use of Bt maize varieties has resulted in an estimated 7,000 metric tons/year decrease in pesticide while enabling a nearly 1.6 million ton increase in production.

Transgenic plants in wheat

Transgenic were produced in wheat (1992) by a non-resident scientist Indra K. Vasil and his co-workers. An herbicide resistant gene (bar) against herbicide PPT along with gus marker gene and Ca MV 35S promoter + ADh 1 intron was introduced into wheat plants. Expression of gus activity and resistance to herbicide PPT was observed in transgenic wheat plants.

Molecular Farming

Molecular farming is a novel technique for exploitation proteins or other metabolites useful for medicine in plants traditionally used in agricultural setting. Molecular farming is the production of pharmaceutically important and commercially valuable proteins in plants (Franken *et al*. 2006). The aim is to provide safe and economic modes for the bulk production of recombinant pharmaceutical proteins. In a typical way in molecular farming a protein with a desirable therapeutic activity is identified its protein and DNA sequenced and finally expressed in heterologus host. Generally the recombinant proteins which are selected are of therapeutic importance. The main aim of Molecular farming is the production of pharmaceutical protein and not altering the expression system.

Plants: as Production Systems

The demand for safe recombinant pharmaceutical proteins is increasing day by day. Both the amounts of proteins needed and protein complexity are increasing as novel pharmaceutical activities are identified or designed into macromolecules. The natural ability of plants to make human and animal proteins make them suitable for the production system.The potential of using plants as a production system for recombinant pharmaceuticals was established between 1986 and 1990 with the successful expression of a human growth hormone fusion protein, an interferon and human serum albumin (Barta *et al*. 2009;Zoeten *et al*.1989;Sijmons *et al*. 1990). A crucial advance came with the successful expression of functional antibodies in plants in1990 (Hippe *et al*.1990).This was a significant breakthrough for it showed that plants had the potential to produce complex mammalian proteins of medical importance. By analogy to the production of insulin in bacteria, the production of antibodies in plants had the potential to make large amounts of safe, inexpensive antibodies available. Plant expression systems are attractive becausethey offer significant advantages over the classical expression systems based on bacterial, microbial and animal cells. Firstly, they have a higher eukaryote protein synthesis pathway, very similar to animal cells with only minor differences in protein glycosylation (Macheteau*et al*.1999). Contrastingly, bacteria cannot produce full size antibodies nor perform most of the important mammalian post-translational modifications. Secondly, proteins produced in plants accumulate to high levels (Verwoerd *et al*.1995; Ziegler *et al*. 2000) and plantderived antibodies are functionally equivalent to those produced by hybridomas (Hiatt *et al*. 1989; Voss 1995). Thirdly, concerns about contamination of expressed proteins with human or animal pathogens (HIV, hepatitis viruses) or the co-purification of blood-borne pathogens and oncogenic sequences, are entirely avoided by using plants.

Transgenic plants as a Machinery for Production of Biopharmaceuticals

Another major target of biotechnology industry is to use transgenic plants for manufacturing of special chemicals and pharmaceuticals. Molecular farming can produce enzymes for food processing and other uses:-

a. Transgenic tobacco plants with higher mannitol level are made through transfer of mannitol dehydrogenase gene from E.coli.
b. The production of pharmaceutically active compounds like enkephalines was achieved in transgenic oil seed rape.
c. Cellulase can also be produced in transgenic plants and used as animal feed additive.
d. Castor contains ricinolic acid which makes it extremely versatile natural oil. The industrial uses of castor oil include the synthesis of nylon and manufacture of lubricants.

Plant Edible Vaccines

Edible vaccines are those in which vaccines are eaten in a fruit or raw vegetable. The procedure of generation of edible vaccines is very simple. From the pathogen the orally active antigenic protein is isolated and a suitable gene-construct for constitutive or tissue specific expression of gene is prepared. And when the gene is introduced into the plant species it suitably integrated into the plant genome and its expression produces the antigen. The suitable plants parts having the antigen are fed in raw form to animals or humans in order to immunize them. Such vaccines don't require not require cold storage like recombinant proteins or sophisticated expertise for their distribution and use throughout the developing countries. The principle of edible vaccine activity was proven for transgenic potatoes producing the enterotoxigenic *E. coli* heat labile enterotoxin B subunit (Haq *et al*.1995). An effective edible hepatitis B vaccine has been generated using transgenic lupin and lettuce plants expressing the hepatitis B surface antigen. Mice and humans fed transgenic plant material produced hepatitis B specific antibodies (Kapusta *et al*. 1999). It's a fact that animals develop certain level of tolerance to the components provided to them in their feed so they become non-immunogenic to them. So edible vaccines should not be used in animal or human food as a regular component they should be provided in such an amount that they can generate immunogenicity.

Production of Plastics by Plants

Polyhydroxybutyrate (PHB) that accumulates as natural storage material in many species of bacteria. PHB is used by industry as a renewable source of manufacturing biodegradable plastics. Synthesising PHB only requires three enzymes 3- ketothiolase, Acetoacetyl L CoA reductase and PHB synthase. The first enzyme is already present in plants. The research team introduced the genes encoding the two other enzymes into Arabidopsis thaliana and found that transgenic plants synthesised PHB and that plastic accumulated as small granules (0.2- 0.5 µm) in the cytoplasm nucleus and vacuoles of cell.

Bt Cotton

Cotton is one of the major crops of the global significance. It is cultivated in tropical and subtropical regions of more than eighty countries of world occupying nearly 33 m ha with annual production of 19 to 20 million tonnes of bales. In India, cotton is being cultivated in 9.0 m ha and stands first in acreage. The crop is grown in varied agroclimatic situation across nine major states viz. Maharashtra, Gujarat, Madhya Pradesh, Punjab, Haryana, Rajasthan, Andhra Pradesh, Karnataka, and Tamil Nadu. Introduction of Bt has provided growers with a new tool for managing bollworms in cotton. The most significant use of Bt cotton for growers is the reduction in insecticide use to control boll worms (Nathaniel *et al*. 2006).

It also results improvement of yield levels and also improves margin profit to farmers.

Rank	Country	Area (million hectares)	Biotech crops
1.	USA*	70.1	Maize, Soybean, cotton, Canola, Sugar beet, Alfa alfa, Papaya, Squash
2.	Brazil*	40.3	Soybean, Maize, Cotton
3.	Argentina*	24.4	Soybean, Maize, Cotton
4.	India*	11.4	Cotton
5.	Canada*	10.8	Canola, Maize, Sugarbeet, Soybean
6.	China*	4.2	Cotton, Papaya, Poplar, Tomato, Sweet pepper
7.	Paraguay*	3.6	Soybean, Maize, Cotton
8.	South Africa*	2.9	Maize, Soya Bean, Cotton
9.	Pakistan*	2.8	Cotton
10.	Uruguay*	1.5	Soybean, Maize
11.	Bolivia*	1.0	Soybean
12.	Philippines*	0.8	Maize
13.	Australia*	0.6	Cotton, Canola
14.	Myanmar*	0.3	Cotton
15.	Mexico*	0.1	Cotton, Soybean
16.	Columbia*	0.1	Cotton, Maize
17.	Chile	<0.1	Maize, Soybean, Canola
18.	Portugal	<0.1	Maize
19.	Cuba	<0.1	Maize
20.	Costa Rica	<0.1	Maize, soy bean

*Biotech mega countries **Area under GM crops in various countries**

But these GM crops sometimes may pose serious threats to environment. And they also have impacts on the ecology of soil. One of the least understood areas in the environmental risk is their impact on soil and plant associated microbial communities. This briefing identifies three main areas of concern:

- ✓ Genetic contamination of the soil and associated micro-organisms as a result of horizontal gene transfer.
- ✓ Changes to the soil ecosystem through the changed characteristics of GM plants.
- ✓ Soil contamination through GM seeds remaining in the soil after harvest.

It also highlights the current evidence that GM technology poses unacceptable risks to the health and fertility of the soil - one of our most precious natural resources.

Soil Fertility

The fertility of soil is greatly is greatly influenced by soil microorganisms .In a single gram of productive soil there is a complex amount that can exceed over 100 million micro-organisms that may represent over 1000 species. The main components are bacteria, fungi, algae, protozoa's, nematodes, earthworms, and insects. Out of these, bacteria and fungi alone can constitute more than 80%, the proportions of these two depending on soil type. There is a complex ecological interdependence between all soil organisms.

After the association with transgenic plants microbial diversity can also be altered, but these effects can also be both variable as well as transient. The changes in microbial population associated with growing transgenic crops are relatively variable and transient in comparison with some other well accepted agricultural practices such as crop rotation, tillage, tillage, herbicide usage, and irrigation. Since, minute changes in the diversity of microbial population, as appearance or removal of specific functional groups of bacteria such as rhizobacteria, phytopathogenic organisms or main organisms responsible for the process of nutrient cycling which may also affect soil health and functioning of ecosystem (Carpenter 2011).

Gene Escape

Further concern about GM crops is gene could “escape” and through cross pollination mix with non GM crops or their weedy relatives. For example, an herbicide tolerant gene could be transferred to weeds in wild habitats, turning them into “super weeds”.

Transgenic crops modified to be resistant to a particular pest or disease may have a negative effect on non-target species that are harmless or beneficial. For example, Bt maize pollen may be toxic to the Monarch butterfly. GM Bt maize varieties can release the activated GM Bt toxin through their roots where it binds with soil particles and persists in the soil. The GM Bt toxin remains toxic to some soil insects for very long periods.

Pest Resistance

Pest resistance is another major concern that, pest resistance can occurs with frequent use of any pest control product. Insects can develop resistance to toxins such as the Bt bacterium, reducing the effectiveness of this control method. Bt crops have proven to be unstable and ineffective; some insects, which survive Bt, transmit genetic resistance to their immediate offspring.

Herbicide Resistance

GM crops engineered to be resistant to specific herbicides enable farmers to spray weeds without damaging crops. Weeds are developing resistance to these herbicides, and rogue GM plants that grow after a harvest have appeared and spread widely. For example, the crops being used in the current farm-scale trials are resistant to the herbicide glufosinate but according to one report our knowledge of the effects of this product on soil micro flora is “extremely limited”. Glyphosate is major formulation of “roundup ready” crops and is now world’s best -selling “total” herbicide. With the introduction of GM round -up ready crops human and environmental exposure to the herbicide is expected to increase. However, there is strong evidence that glyphosate-containing products are acutely toxic to animals and humans. GM crops open up opportunities in order to stabilize and ensure food supply for poor subsistence farmers (Ellstrand, 1992).

Impact on Genetic Diversity

GMOs could impact on genetic diversity. The increased competitiveness of GMOs could cause it to damage biologically-rich ecosystems. Transgenic cropscould encourage biodiversity loss through the establishment of monoculture agriculture which replaces traditional crops and other established varieties. The main potential cause of loss of biodiversity is agricultural expansion, which destroys habitats.

Potential Hazards

Ecological and health hazards are also posed by genetic use restriction technologies (GURT) which are commonly known as terminator technology. These organisms do not flower and fruit and therefore provide no food for the multitude of insects, birds and mammals that feed on pollen, nectar, seed and Fruit, and will inevitably have huge impacts on biodiversity.

Economic Concerns

To introduce GM crop to market is quiet expensive process and of course agri-biotech companies wish to have profits on their investment. Many new plant genetic engineering processes and GM crops have been patented, and patent infringement is a big concern of agribusiness. The long term implications for farmers wishing to stay GM-free, and indeed on food production as a whole, are unknowable (www.Green peace.org.uk). Yet consumer advocates are worried that patenting these new plant varieties will raise the price of seeds so high that small farmers and third world countries will not be able to afford seeds for GM crops, thus widening the gap between the wealthy and the poor.

One way to combat possible patent infringement is to introduce a "suicide gene" or “terminator technology” into GM plants. These plants would be viable for only one growing season and would produce sterile seeds that do not germinate. Farmers would need to buy a fresh supply of seeds each year. However, this would be financially disastrous for farmers in third world countries who cannot afford to buy seed each year and traditionally set aside a portion of their harvest to plant in the next growing season. It is unlikely that the consumer reaction against GM technology will extend to molecular farming because the public is reluctant to harshly criticize medically related research or attempts to provide safer supplies of medicines (Garcia, 2005). Such advantages of GM crops would mitigate public hesitation about GM technology (Sharma, 2003).

CONCLUSION

Genetically-modified crops have the ability to sort out many of the world's hunger and malnutrition problems, and to help protect and preserve the environment by increasing yield and reducing reliance upon chemical pesticides and herbicides. However, we must proceed with care to avoid causing unintentional harm to human health and the environment as a result of our enthusiasm for this great technology.It is indeed hard to give a straight answer or simple solution on how food insecurity is being solved. Due to the possibilities of fered by GM technology in this new century, societies will need to make some important choices about the type of world that they wish to build up. The politicians in the developing countries like India are recently faced by acrucial question on how GM technology should be viewed in relation to their release for normal use. This review identified and scrutinized the potential benefits of GMO still unexplored/unidentified. Although GM food is important and beneficial, it should be adopted under conditions that avoid potential risks. Time and effort must be devoted to on-farm trials before any interventions in this regard.

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