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Review Article

TRANSGENIC TECHNOLOGIES IN AGRICULTURE: FROM LAB TO FIELD TO MARKET

***Sanjay Mohan Gupta, Atul Grover and Mohammed Nasim**
Molecular Biology and Genetic Engineering Laboratory, Defence Institute of Bio-Energy Research (DIBER), DRDO, Haldwani-263 139, (Uttarakhand) India
**Author for Correspondence*

ABSTRACT
Transgenic technology has been the fastest growing technology in agriculture. However, various concerns, ranging from religious to environmental, and a number of taboos and fears in the minds of common man, have worked as obstacles towards rapid commercialization of this technology. Here, we intend to present an overall perspective on the acceptability and commercialization of 'Transgenic Technology in Agriculture', discussing the scientific merits of the technology, and as well as the 'fear of the unknown' attached with adoption of the technology.

Key Words: *Transgenic Development and Commercialization, Genetically Engineered Plants, Cis-Genesis, Containment and Confined Field Trials of Transgenics, Transgenic Controversies*

INTRODUCTION
Food and energy insecurities are currently two foremost problems being faced worldwide. The world population is likely to reach nine billion by 2050. To feed these nine billion mouths, food production will be required to be increased by more than two fold in this limited time period (Varshney *et al.,* 2011).

Box 1 Important terms and their definitions in the field of Transgenic Technology

Term	Definition
Cisgenesis	A genetic modification of a cell or organism in which only natural genes in the sense orientation under control of their own regulatory sequences derived from the same species have been transferred
Electroporation	A method for the direct transfer of macromolecules into the target cells by perforating the cell membrane with a short electric pulse and high potential gradient.
First generation transgenic crops	Transgenic crop plants with enhanced biotic stress tolerance.
Second generation transgenic crops	Transgenic crop plants with enhanced abiotic stress resistance, postharvest enhanced nutrition and edible vaccines etc.
Third generation transgenic crops	Transgenics for altered metabolic/physiologic functions and enhanced nutrition assimilation.
Transgenesis	Transfer of genes into a cell or organism from a foreign origin.
Transgenic founder	Organisms into which a gene (or DNA) has been inserted from a foreign source, and which are allowed to reproduce to successive generations.
Transgenic line	The direct progenies derived from a transgenic founder, and which contain stably inherited genetic element of foreign origin.
Transgenic plant	A plant into which foreign DNA has been transferred.
Transgenic technology	A set of techniques used for transferring desirable gene(s) across taxonomic boundaries.

In Indian scenario, it is estimated that by 2030, India would be most populated country in the world with a population of 1. 5 billion required to be fed (Jagadish, 2012). With rapid changes in land use from wild to

agricultural to urban and increasing degradation of cultivable land in terms of water availability and salinity, there is an urgent need to revolutionize the way we do farming and produce our food. Modern demands for agriculturally fit varieties include enhanced biotic and abiotic stress tolerance, higher nitrogen and water use efficiencies and yields (Pennisi, 2010).

Plant transgenic technology refers to a set of techniques used for transferring desirable gene(s) across taxonomic boundaries into a certain plant. The donor of the genes can be other plants, animals and microbes, or even artificial, synthetic or chimeric DNA. The term 'plant transgenic technology' further includes all those techniques which are required for subsequent stable integration of the introduced DNA into the plant genome, and its expression. The inserted gene sequence is known as transgene and the plant developed after a successful gene transfer is known as transgenic plant (or genetically modified plant or GM plant or genetically engineered plant) (**Box 1**).

Transgenic technology provides a possibility of not only bringing in desirable characteristics from other varieties of the plant, but also of adding characteristics from other unrelated species. These transgenes are introduced into plant cells, tissues, or organs by a variety of methods, allowing production of new plant variety, which is usually normal in appearance and differs from the parent only with respect to the function of the inserted transgene. Whether transgenics or cisgenics, the dependency on GM crops is now increasing and the reason is as simple as availability of a tool that permits need based accelerated evolution. With the increasing mouths to feed, longer and healthier life spans, reducing agricultural land, loss of biodiversity and germplasm and change in global climate patterns, there is an urgent need and increasing pressure to produce more and there are two ways to fulfill it- firstly; Increasing the yield and productivity of crops; and secondly by reducing losses due to abiotic and biotic reasons. Either way, existing breeder crops, hybrids, cultivars and varieties are unable to sustain the produce, and available germplasm does not permit conventional plant breeding principles and applications to fulfill the need. Joining hands with the modern biotechnological tools and development of GM crops is a preliminary and partial solution to the crisis. And hence researchers across the globe are into developing transgenic plants in food, feed, fibre, fuel, fruit and forest crops. Transgenic crops in plant biotechnology have come as a new solution to the age old problems.

Box 2 Primary aims for which plant transgenic technology is practiced.

S.No.	Description
1.	Improving production stability
2.	Giving nutritional benefits to the consumers
3.	Reducing environmental impacts of intensive agriculture
4.	Decreasing dependency on hazardous and expensive chemicals to overcome biotic stresses
5.	Providing sufficient food in order to meet the demand of growing population
6.	Overcoming problems of malnutrition
7.	Increasing yield from limited resources
8.	Providing access to food for small-scale farmers in developing nations
9.	Using non-food crops as renewable sources of oil
10.	Starch and other raw materials for industry
11.	Detoxifying hazardous compounds
12.	Increasing the amount of land available to cultivate crops
13.	Increasing the availability of polymers, pharmaceuticals and vaccines
14.	Increasing the understanding of the role and behaviour of plant genes

The aim of plant transgenic technology (Box 2) is to create diversity of plants serving human needs and hence to benefit producers, processors and consumers. Thus, the plant transgenic technology offers opportunities to introduce genes conferring resistance to insects, viruses, bacteria, fungi, herbicides and abiotic stress factors. Besides the strategy also aims to increase nutritional content of plants, manipulate biochemical pathway of flower pigmentation, delay fruit ripening, increase shelf-life, modify plant products, augment sweetness, alter lignin content of trees, increase efficiency of crop production and introduce male sterility.

Another major goal for production of transgenic plants is related to 'molecular farming', *i.e.*, the use of transgenic plants as bioreactors (or bio-factories) for the production of neutraceutical, therapeutic agents, antigens, monoclonal antibody (McAb) fragments, vaccines, biopolymers, *etc*. Transgenic plants are also produced for identification of regulatory sequences for many genes, using gene constructs with overlapping deletions.

In conclusion, transgenic technology has provided important tools for sustainable development of agriculture and forestry and can be of significant help in meeting the food needs of a growing and increasing urbanized population.

The potential benefit of transgenic plants is the applications for which they are being developed, the new traits being introduced. In addition, transgenics have potential to reduce usage of insecticides, herbicides and weedicides, improve tolerance to stress, and function as edible vaccine and biosensors. This review deals with the potential of transgenic technology for translational research, and makes an effort to justify that the transgenic technology has evolved from Lab to Field to Market in a short span of 20 years.

2. Transgenic Development: Methods and Potential

Currently a variety of techniques are available for easy and successful transformation of almost all the plant species, which provide the basis for the advances in plant biotechnology (Slater *et al.*, 2004). These techniques fall into two main categories namely, **(i) Vector-mediated gene transfer**, which involves plant gene vectors for the transfer of genetic information to plants from other organisms, and **(ii) Direct DNA transfer or vectorless gene transfer**, which are generally chemical or physical methods of gene introduction in plant cells. Physical gene transfer methods, also called DNA-mediated gene transfer (DGMT), are based on direct delivery of naked DNA to the plant cells.

This subcategory includes microprojectile bombardment, electroporation, microinjection, silicon carbide whiskers, ultrasonication, U.V. laser microbeam irradiation and liposome-mediated transformation. On the other hand, chemical gene transfer methods employ direct gene transfer with the aid of chemical agents, for example, calcium phosphate and polycations. In the following sections, a detailed description of successful transformation methods is provided.

2.1 Vector Mediated Genetic Transformation

2.1.1. Agrobacterium Mediated Genetic Transformation

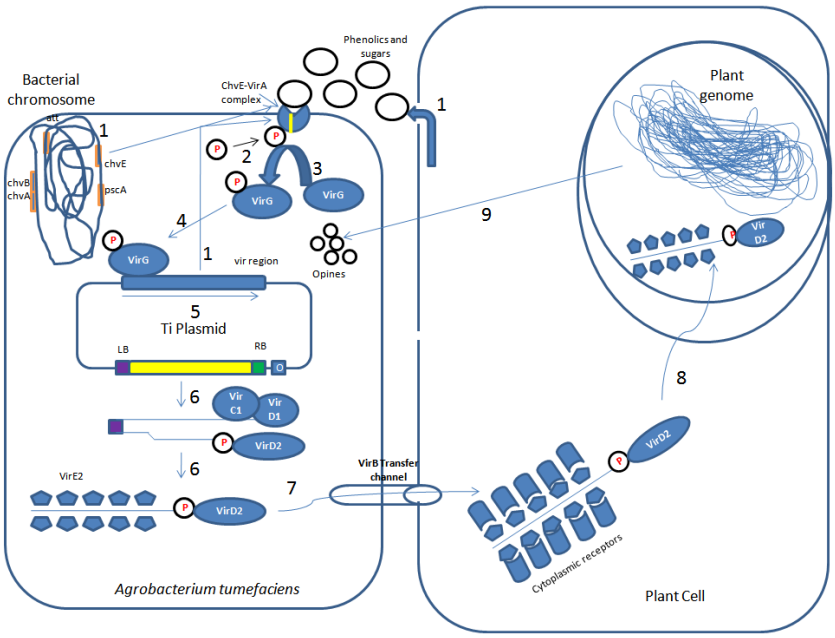
Classical genetic transformation system using co-cultivation of explants or calli with *Agrobacterium* cultures along with innovative in planta methods are currently the most widely used approaches for plant transformation.

These methods make use of natural genetic engineering ability of the gram negative soil bacteria *A. tumefacianes* and *A. rhizozenes* of family *Rhizobeaceae* in plant cell, through Ti and Ri plasmids respectively. Both the plasmids have 22 kb T-DNA, which is transferred and thus integrated into the plant genome after infection. In nature, Ti plasmid causes crown gall disease, while Ri plasmid infection results in hairy root disease. A detailed pictorial presentation of the entire process of 'genetic engineering' in nature is presented in figure 1.

Table 1: A quick review of most recent attempts of genetic transformation in plants, along with aim and methodologies used

S.No.	Gene and its source	Methodology used	Description of the transgenic	Reference
1.	*HVA1* from Barley	Biolistics	Drought and salt stress tolerant maize	Nguyen and Sticklen, 2013
2.	*SGTL1* from *Withania somnifera*	*Agrobacterium*-mediated transformation using floral dip method	Salt and healt tolerant and cold acclimating *Arabidopsis thaliana*	Mishra *et al*., 2013
3.	*NHX1* from *Arabidopsis thaliana*	*Agrobacterium*-mediated transformation using co-cultivation with explants	Salt tolerance in *Brasica napus*	Dorani-Uliaie *et al*., 2012
4.	Synthetic promoters containing pathogen and/ or defence signalling inducible cis-acting regulatory elements (RE) fused to fluorescent protein (FP) reporter	*Agrobacterium*-mediated transformation using floral dip method	Pathogen sensing transgenic tobacco and *Arabidopsis* plants	Liu *et al*., 2013
5.	Acetyl-transferases from *Aspergillus nidulans*	*Agrobacterium*-mediated transformation using floral dip method	Transgenic *Arabidopsis* and *Brachypodium* plants with decreased polysachharide acetylation and increased pathogen resistance	Pogorelko *et al*., 2013
6.	*Cry1Ab*	*Agrobacterium*-mediated transformation using co-cultivation with calli	Insect resistant transgenic rice	Qi *et al*., 2013
7.	*ß-Glucuronidase*	Biolistics	Transgenic triticale	Karadag *et al*., 2013
8.	Ribosome Inactivating Protein from Barley	*Agrobacterium*-mediated transformation using co-cultivation with leaf discs	Enhanced resistance to *Rhizoctonia solani* in transgenic potato	M'hamdi *et al*., 2013
9.	ß-fructofuranosidase (FFase) of *Aspergillus niger* ATCC 20611 c	*Agrobacterium*-mediated transformation using co-cultivation with leaf discs	Fructooligosaccharide production in transgenic tobacco plants	Fukutomi *et al*., 2013
10.	*bar* and the *gus-intron* genes	*Agrobacterium*-mediated transformation using co-cultivation with leaf discs	Transgenic peach	Soliman, 2013

a



b

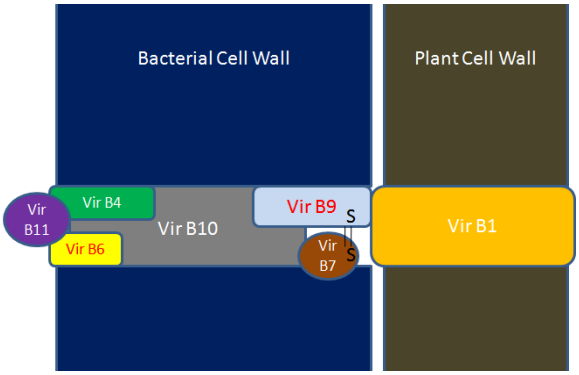


Figure 1 (a): A simplified scheme of *Agrobactierium*-mediated gene transfer into wounded plant cells that synthesize phenplic compounds as part of their denfense and wound-healing strategies. These phenols serve as attractants for the ubiquitously occurring soil bacterium *Agrobacterium tumefaciens* (Step 1), which is simultaneous to expression of chromosomal and plasmid genes involved in early virulence steps (*chvA, chvB, chvE, virA* and *virG*). Signaling moleculaes are recognized by the dimeric transmembrane receptor complex VirA-ChvE. Binding of phenolics to this dimeric complex leads to autophosphorylation of VirA (step 2), which further leads to phosphorylation of cytoplasmic VirG (Step 3). Activated VirG binds at the regulatory region of other *vir* operons, acting as transcriptional activator (Step 4), leading to the expression of downstream *vir* gene complex (*virC, virD, virE,virB, virF, virH*; step 5). VirD1/VIrD2 recognize 25 bp T-DNA borders (RB, LB), VIrC1 recognizes overdrive enhancer (O) at RB. VirD2 cleaves the bottom strand and remains covalently bound to the 5'-end of the T-DNA complex (Step 6). Single strand binding proteins (VIrE2) coat the T-DNA strand, so as to stabilize it (Step 6), while transferring it through VirB transfer complex, spread across the cell walls of bacteria and the plant (step 7). Plant cytoplasmic receptors recognize the nuclear location signals in VirE2 and VIrD2, thereby delivering the T-DNA complex (T-DNA bound to VirD2 and VirE2) to nucleus (Step 8). T-DNA integrates to plant genome by illegitimate recombination, facilitated by VirD2. Wild type T-DNA code for the production of unusual amino acids known as opines and also for plant hormone (auxin and cytokinin). The production of excess hormone induces gall to form at the site of infection and the bacteria can utilize the opine produced, as the carbon and nitrogen source (step 9).
Figure 1(b): Conceptual diagram on structure of VirB Transfer channel.

Due to large size, absence of unique restriction enzyme sites and tumor induction property does not allow Ti plasmids to be used as such, and several modifications have to be carried out for its suitable applications in a Plant Molecular Biology Laboratory. In fact, a series of vectors have been developed in which wild type opines and tumor inducing genes have been replaced by genes of interest, to be introduced in plants. This is known as disarming.

The removal of gene that code for phytohormone overproduction also means that phenotypically normal transformed cells can be regenerated. This process can be performed with any tissue explant that provides a good source for initiation of whole plant differentiation. Though the introduction of gene through *Agrobacterium* mediated approach is the most common and preferred method, it suffers with certain limitations including limited host range and poor efficiency. Nevertheless, detailed protocols for a number of dicot and monocot plants, alongwith fungi, algae, yeast and even mammalian cells are now available (Wang, 2006).

2.1.2 Virus Vectors

Similar to *Agrobacterium*, plant viruses exhibit natural tendency of gene transfer to plants and thus have the potential for use as stable plant transformation vectors. This probability is speculated due to several properties possessed by them, which include: Importantly, plant viruses can be easily transmitted by direct adsorption and introduction of their genome into intact plant cells. Besides this normal infection route, naked DNA or RNA is also infectious in many viruses. At present, three kinds of viruses-Caulimoviruses (double stranded DNA), Gemini viruses (single stranded DNA) and Tobacco mosaic virus (RNA) are being used for agroinfection (Gleba *et al.,* 2007).

2.2. Direct DNA Transfer or Vectorless Gene Transfer

2.2.1 Physical Methods

2.2.1.1 Microprojectile Bombardment (Gene Gun)

Microprojectile bombardment serves as one of the most important alternative to *A. tumefaciens* based DNA delivery systems for plants. The technique relies on the use of high-velocity microprojectiles (most commonly tungsten or gold coated micro-particles) to penetrate outer cell layers in order to introduce gnetic material into living cells. On their way through the cell, the DNA on the particle surface is stripped off and may then be inserted into the genome of the cell. The cells survive to express the introduced genes. A major benefit of the microprojectile method is that the transfer of gene does not require protoplasts, but is possible with intact tissues (Kahl, 2004). Microprojectile bombardment is currently one of the popular methods for transformation of genes in different plants (**Table 1**). It is also called 'biolistics', 'particle gun', 'particle bombardment', 'gene gun' and 'bioblaster'.

2.2.1.2 Electroporation

In principle, the **c**ell membrane is an electric capacitator that is unable to pass current (except through ion channels). Subjecting cell membranes to electrical pulses of high field strength reversibly permeates them, facilitating uptake of even macromolecules such as DNA. It has been hypothesized that the mechanism of DNA entry into the cell during electroporation may be either due to unspecific membrane process, or it may be specifically mediated by permease enzymes. The activation of permease may be due to high electric field and thus may lead to thinning of membrane. As a result pores may be created, which are stabilized by a favourable dipole interaction of lipid dipoles in an electric field. Macromoleculaes thus entering a cell may remain in cytoplasm or nucleoplasm, but may be degraded rapidly. However, a few DNA molecules may also get integrated covalently into the nuclear or organellar genome. This method is suitable for both monocot and dicot plant cells or protoplasts, and successful gene transfer using electroporation has been reported in tobacco, petunia, maize, rice, wheat, sorghum and carrot (Shillito *et al.,* 1985).

2.2.1.3 Microinjection

Microinjection method of transformation is used for mechanical introduction of DNA into a specific target under microscopic control (Crossway *et al.,* 1986). The principle involved in microinjection technique is that the appropriate volume of biological sample is injected directly into the cytoplasm or

nucleus of cell with the aid of gentle air–pressure applied to the capillary through a syringe (or micropipette). This technique was originally developed for animal cells, but is now applied successfully to plant cells as well. However, from this chimeric plant, transformed plants of single cell origin can be subsequently obtained. Although, this technique gives high rate of success, the process is slow, expensive and requires highly skilled and experienced personnel.

2.2.1.4 Ultrasonication

In this method, plasma membrane is disrupted by ultrasonication, leading to uptake of DNA molecules. Two hypotheses have been given for its explanation. By this technique, versatile cell types such as plant protoplasts, cells in suspension and intact pieces of plant tissues can be readily transformed (Zhang *et al.,* 1991). Furthermore, the equipment for ultrasound transformation is simple, cheap and multifunctional. This method has been successfully used to transform tobacco, wheat and sugar beet, and holds prospects for substantial future applications in plant transformation.

2.2.1.5 Lipofection

Liposomes serve as ideal carrier systems which can deliver exogenous DNA into plant protoplasts (Caboche, 1990). This use of liposomes as a transformation or transfection system is called lipofection. Liposomes represent special type of lamellar phase in which water is self-contained and the lipid molecules are disposed in bimolecular layers attached by their non-polar interfaces. Thus liposomes are closed; self-sealing, solvent-filled vesicles bound by a single bilayer, and possess many properties akin to those of biological membranes. These are easy to manipulate, their lipid contents can be varied at will, and many substances may be trapped inside the interlamellar spaces. Owing to their properties, a wide variety of molecules including DNA can be encapsulated within their aqueous interior, and can be delivered to cells in a biologically active form via endocytosis or membrane-membrane fusion. Liposome strategy is also useful for transferring DNA into vacuoles of walled plant cells.

2.2.2 Chemical Methods

2.2.2.1 Ca-DNA Coprecipitation (or Calcium Phosphate Coprecipitation)

In this method, DNA is mixed with $CaCl_2$ solution and isotonic phosphate buffer to form a DNA-calcium phosphate co-precipitate (also called Ca-DNA co-precipitate). On contact with protoplasts, protoplast / Ca-DNA complex is formed. In order to encourage the endocytic uptake of the co-precipitate, the protoplast / Ca-DNA complex is treated with polyvinyl alcohol and high pH calcium. A physiological shock with DMSO can also increase the efficiency of transformation to a certain extent. Upon several hours of incubation, the Ca-DNA co-precipitate is transferred across the plasma membrane in a calcium requiring process.

2.2.2.2 Polycation-mediated DNA Uptake

Direct DNA uptake by protoplasts can be stimulated by using extremely hydrophilic, high molecular weight, long chain polycations such as PEG with or without metal ions such as Zn^{2+}, Li^{+}, Cs^{+}, Rb^{+}, K^{+}, Na^{+}, Ca^{2+} or Mg^{2+} (Negruitiu *et al.,* 1987). When placed in a solution, PEG removes 'free water', *i.e.*, molecules that interact with charged (usually ionic) molecules soluble in water. Both protoplast membrane and DNA are normally negatively charged, and hence have a tendency to repel each other due to charge repulsion, thereby limiting the interaction between the two.

Potential of Transgenic Technology

Transgenic plant technology is currently being used in three major areas- expression of recombinant proteins to improve crop quality by improving tolerance to stress, optimizing crop productivity and yield by genetic manipulation of metabolic pathways and for large-scale cost effective production or recombinant proteins for industrial and therapeutic purposes.

2.3.1 Insecticide and Herbicide Free Agriculture

In recent decades, productivity of world agriculture has improved a great deal with applications of insecticides and herbicides. These agrochemicals protect crop plants from pests and weeds. Careful and judicious use of these chemicals generally deliver good benefits for farming community, as well as for

society, in large. However, insecticides and herbicides can be harmful to living organisms and therefore there are risks associated with their use.

Different strategies employed for generation of insect-resistant transgenic plants include introduction of genes encoding the following through recombinant DNA technology: (i) an insecticidal protoxin (Bt toxin or Cry protein encoded by *cry* gene) produced by one of several subspecies of *B. thuringiensis*, and (ii) proteins that protect plants against a variety of insects, which exert their action by interfering with insect development, for example protease inhibitors, α-amylase inhibitors, lectins, cholesterol oxidase, tryptophan decarboxylase and avidin. It is significant to note that a range of insecticidal crystal proteins has been isolated, and characterized from *Bacillus thuringiensis* strains, and presently Bt technology is the most effective, and the safest mode for the control of plant pests, and hence the best choice for pest control. The practical results obtained with *Bt*-crops have demonstrated that these are safe and beneficial, and few of the Bt-transgenic plants have been commercialized. A modified *cry* 3A gene was introduced in potato to provide resistance against Colorado beetle (Perlak et al., 1993). In 1995, this crop became the first transgenic insect-resistant crop to reach commercial production, as NewLeaf^{TM} potato marketed by Monsanto.

For development of herbicide free plants, common strategies include (i) endow plants with the capability to metabolically inactivate the herbicide, (ii) inhibit the uptake of the herbicide, (iii) overproduce the target protein, (iv) detoxify herbicide, and (v) reduce the ability of herbicide sensitive target protein to bind to a herbicide. One of the most common strategies used for the development of herbicide-tolerant transgenic plants is overexpression of herbicide resistant target protein in crop plants. Some examples include: Glyphosate is a glycine derivative and is a herbicide which is found to be effective against the 76 of the world's worst 78 weeds. EPSPS gene was isolated from Petunia and introduced in to the other plants. These plants could tolerate glyphosate at a dose of 2- 4 times higher than that required to kill wild type plants. It kills the plant by being the competitive inhibitor of the enzyme 5-enoyl-pyruvylshikimate 3- phosphate synthase (EPSPS) in the shikimic acid pathway (Heck *et al.,* 2005) i.e. "5-Enol-pyruvilshikimate synthase from *Agrobacterium* confers resistant to the nonselective sp.CP4 (CP4 EPSCS) herbicide glyphosate when expressed in transgenic plants of *Zea mays* (Heck *et al.,* 2005). Certain strategies were used to provide glyphosate resistance to plants (Chang *et al.,* 2003).

2.3.2 Disease-resistant Plants

Many phytopathogenic fungal or bacterial infections result simultaneously, or as a consequence of infestation of plants by insects, which often result in extensive damage, and loss of crop productivity accounting to enormous economic losses. Presently chemical agents such as fungicide or bactericide are used for the control of fungi or bacteria, respectively. However, the use of chemicals is disadvantageous because these may persist in the environment, and may pose threat to animals or humans. To overcome these problems associated with the application of chemicals, fungal- or bacterial pathogen-resistant transgenic plants have been developed as a simple, inexpensive, effective, and environment-friendly non-chemical means (Stuiver and Custers, 2001; Punja, 2001; Gilbert *et al.,* 2006). In this direction, several approaches have been tested, which are grouped into five categories: (i) expression of gene product that is directly toxic to pathogens or that reduces their growth.

These include pathogenesis-related proteins (PR proteins) such as hydrolytic enzymes (chitinases, glucanases), antifungal proteins (osmotin- and thaumatin-like), antimicrobial peptides (thionins, defensins, and lectin), ribosome inactivating proteins (RIP), and phytoalexins. (ii) Expression of gene product that destroys or neutralizes a component of the pathogen arsenal such as polygalacturonase, oxalic acid, and lipase. (iii) The expression of gene product that can potentially enhance the structural defenses in the plant, for example, elevated levels of peroxidase and lignin. (iv) The expression of gene product releasing signals that can regulate plant defenses, for example, production of specific elicitors, hydrogen peroxide (H_2O_2), salicylic acid (SA), and ethylene (C_2H_4). (v) The expression of resistance gene (R) product involved in the hypersensitive response (HR) and in interactions with avirulence (Avr) factors.

2.3.3 Virus-resistant Plants

There are several strategies for engineering plants for viral resistance and these utilize the genes from virus itself (e.g. the viral coat protein gene). The virus-derived resistance has given promising results in a number of crop plants such as tobacco, tomato, potato, alfalfa, and papaya. The induction of virus resistance is done by employing virus-encoded genes-virus coat proteins, movement proteins, transmission proteins, satellite RNA, antisense RNAs, and ribozymes. The virus coat protein-mediated approach is the most successful one to provide virus resistance to plants. The transgenic plant providing coat protein-mediated resistance to virus are rice, potato, peanut, sugar beet, alfalfa, citrus, maize, melon, orange, lettuce, *Prunus* etc. The viruses that have been used include alfalfa mosaic virus (AMV) (Xu *et al.,* 1999), cucumber mosaic virus (CMV), potato virus X (PVX) , potato virus Y (PVY), etc. (Savenkov and Valkonen, 2000), bean mottle virus (BMV), Citrus tristeza virus (CTV), lettuce mosaic virus (LMV), maize dwarf mosaic virus (MDMV), Nucleocapsid (NC), potato leaf roll virus (PLRV), plum pox virus (PPV), rice strip virus (RSV), tobacco etch virus (TEV), Zucchini yello maosic virus (ZYMV), etc. Rice yellow mosaic virus resistant transgenics were developed by Pinto *et al*. (1999).

2.3.4 Abiotic Stress-tolerant Plants

Abiotic stresses negatively influence the survival, biomass production and yields of vegetable crops up to 70% (Mizoi *et al.,* 2012). Since tolerance to abiotic stresses is multigenic and quantitative in nature, a massive challenge exists to understand the key molecular mechanisms by which plants perceive environmental signals and further their transmission to cellular machinery to activate adaptive responses is of critical importance for the development of transgenic strategies to impart abiotic stress tolerance in vegetable crops (Nakashima and Yamaguchi-Shinozaki, 2010). Timely modulation of specific sets of genes is critical for survival of plants during abiotic stresses, which further dictates accumulation of mRNAs and proteins and subsequently leads to overall physiological and biochemical changes in the plants. Although a number of transcription factors (TFs) have been discovered that undergo altered expression on exposure to abiotic stress and many of these like, DREB/CBF, AP2/EREBP, MYB, MYC, bZIP, AREB/ABF, SCOF-1, WRKY, CBL-CIPK, DELLA and NAC have been proposed to show their abiotic stress tolerance (Gupta *et al.,* 2009; Nakashima and Yamaguchi-Shinozaki, 2010; Aslam *et al.,* 2012; Gupta *et al.,* 2012a,b; Gupta *et al.,* 2013a,b,c; Patade *et al.,* 2013).

2.3.5 Improvement of Plant Nutritional Content

Improvement of plant nutritional content can be done in a variety of ways, for example, increase the amino acid content (specifically met and lys) of seed storage proteins, modify lipid (or fatty acid) composition of both edible and non-edible oil producing crops so that the oil becomes better suited for intended end use, synthesize vitamin E, and β-carotene, and increase the levels of available iron, *etc* (**Table 2**). Such modifications increase the nutritional status of foods, and may help to improve human health by addressing malnutrition, under-nutrition and micronutrient deficiencies. Traditionally plant nutritional improvement was achieved by plant breeding, but these approaches were difficult, slow, and intrinsically limited by the existing genetic content and cross-breeding strains.

Table 2: Quick overview of reports on transgenic plants with improved nutritional status

S.No.	Transgenic plant	Description	Reference
	Transgenic plants with improved amino acid/protein content		
1.	Transgenic canola and soybean seeds with increased Lysine	Feedback regulation system for lysine synthesis made insensitive	Falco *et al.,* 1995
2.	Transgenic lupins (*Lupinus angustifolius* L.) expressing a sunflower seed albumin gene	Enhanced methionine levels and increased nutritive value of seeds	Molvig *et al.,* 1997
3.	Transgenic potato plants with	Non-allergenic seed albumin gene	Chakraborty *et*

	increased protein content	*ama I* from *Amaranthus hypochondriacus*	*al.*, 2000
4.	Soybean seeds with enhanced methionine levels in seeds	Expresses feedback-insensitive cystathionine γ-synthase	Song *et al.*, 2013
	Transgenic plants with altered fatty acid composition		
5.	Transgenic canola having higher levels of 8:0 and 10:0 fatty acids	Overexpression of *FatB2 Cuphea hookeriana*	Dehesh *et al.*, 1996
6.	Transgenic rice plants with improved seed oil quality	Soybean microsomal omega-3 fatty acid desaturase gene expressing rice plants	Anai *et al.*, 2003
	Transgenic plants with altered starch content		
7.	Potatoes with freeze-thaw stable starch containing tubers	An amylose-free starch with short-chain amylopectin was produced by simultaneous antisense downregulation of three starch synthase genes	Jobling *et al.*, 2002
8.	High-amylose potatoes	Antisense gene targeting of two branching enzymes coding genes *sbeI* and *sbeII*	Schwall *et al.*, 2000; Hofvander *et al.*, 2004; Andersson *et al.*, 2006
9.	Sweetpotato plants with increased amylose content in starch	RNA interference of the starch branching enzyme II gene (*IbSBEII*)	Shimada *et al.*, 2006
	Micronutrients and functional metabolites		
10.	Canola plants with increased Vitamin E content (α-Tocopherol)	Increased expression of expression of γ-tocopherol methyltransferase	Shintani and DellaPenna, 1998
11.	Tomato fruits with increased β-carotene and lycopene	*β-Lcy* gene expression in tomato fruits modified	Rosati *et al.*, 2000
12.	Rice with increased iron content with increased bioavailability	Rice plants contained ferritin gene from *Phaseolus vulgaris* for increased iron content in rice grains, a thermotolerant phytase from *Aspergillus fumigatus* into the rice endosperm, for increased bioavailability and endogenous cysteine-rich metallothionein-like protein for increased absorption.	Lucca *et al.*, 2001
13.	Tomato fruits with enhanced aroma and flavor on engineering of terpenoid	*Overexpression of Clarkia breweri S*-linalool synthase (*LIS*) gene causes	Lewinsohn *et al.*, 2001

	metabolic pathway	increased accumulation of S-Linalool	
14.	Tomato fruits with increased flavonols	Overexpression of *Petunia chalcone* isomerase	Muir *et al.,* 2001
15.	Transgenic maize plants with increased Vitamin C	Wheat dehydroascorbate reductase (DHAR) gene over-expressed in maize	Chen *et al.,* 2003
16.	Enhanced zinc and iron accumulation in transgenic rice	Cloning and over-expression of soybean *ferritin* gene in rice	Vasconcelos *et al.,* 2003
17.	Corn plants with increased Vitamin E	Overexpression of barley homogentisic acid geranylgeranyl transferase (HGGT) resulted in increased tocotrienol and tocopherol	Cahoon *et al.,* 2003
18.	Higher vitamin E in Soybean seeds	*Arabidopsis* genes *At-VTE3* and *At-VTE4* (γ-tocopherol methyltransferase) expressed in soybean seeds	Van Eennemaan *et al.,* 2003
19.	Transgenic multivitamin corn	Increased accumulation of ascorbate, folate and β-carotene in endosperm	Naqvi *et al.,* 2009
20.	Transgenic tomato plants with increased carotenoid, tocopherol, phenylpropanoids, flavonoids, and anthocyanidins	Fruit-specific downregulation of the *DE-ETIOLATED1* (*DET1*) gene	Enfissi *et al.,* 2010
	Genetic manipulation of fruit ripening		
21.	Transgenic tobacco with altered ethylene production and perception	Silencing of ACS gene Over expression of RTE1	Knoester *et al.,* 1997; Zhou *et al.,* 2007
22.	Transgenic tomato fruits with altered cell wall softening	Silencing of *LeExp1* gene Silencing of PG gene	Brummell *et al.,* 2002; Smith *et al.,* 1988
23.	Transgenic fruits with altered sweetening	Over expression of β-fructosidase & Invertase gene	Klann *et al.,* 1993; Xie *et al.,* 2007
24.	Transgenic fruits with altered volatile production	Over expression of Geraniol synthase gene	Davidovich-Rikanati *et al.,* 2007
	Designer traits		
25.	Parthenocarpic eggplants	*DefH9-iaaM* overexpression in eggplant	Acciarri *et al.,* 2002

2.3.6 Altered Flower Pigmentation

Flower industry is continuously attempting to improve flower appearance. In this direction, traditional breeding techniques have been used to create thousands of new varieties differing from one another in color, shape and architecture. However, these techniques are slow, tedious and are limited by gene pool of a particular species. Now uniquely colored flowers have been developed by manipulation of genes encoding enzymes involved in anthocyanin and carotenoid biosynthetic pathway (Courtney-Gutterson *et al.*, 1994; Tanaka *et al.*, 1998). This is achieved by genetic manipulation using genes encoding chalcone synthase, dihydroflavonol-4-reductase, β-carotene ketolase and delphinidin.

2.3.7 Transgenic Plants for Detoxification of Toxic Metals

Toxic metals such as mercury, lead, cadmium and selenium pose a great threat to environment and mankind. To overcome the associated problems, systems for detoxification and/or chelation of such toxic compounds are required. Presently, phytoremediation, *i.e.*, the use of green plants to remove, contain, or render harmless environmental pollutants, offers an effective, environmentally nondestructive, and cheap remediation method. However, the use of genetic engineering to modify plants for metal uptake, transport, and sequestration has opened up new avenues for enhancing efficiency of phytoremediation (Eapen and D'Souza, 2005). Plants have also been engineered to detoxify toxic metals. For example, transgenic plants containing genes for bacterial detoxifying enzymes have been used for bioremediation of contaminated soils. Thus in a study, *Arabidopsis* plants engineered with *mer* A gene encoding mercuric reductase were found to be capable of growing on mercurium contaminated soils (Eapen and D'Souza, 2005).

2.3.8 Transgenic Plants for Better Photosynthesis

In terms of yield enhancement, photosynthesis is perhaps the most obvious target for genetic intervention because it determines the rate of carbon fixation, and hence the overall size of the organic carbon pool. Strategies used for increasing photosynthetic activity include the modification of light-harvesting phytochromes, and key photosynthetic enzymes. Attempts have been made to introduce components of the energy-efficient C4 photosynthetic pathway into C3 plants, which lose a proportion of their fixed carbon through photorespiration. In this direction, maize gene encoding phosphoenol pyruvate carboxylase (PEPC) of C4 pathway has been transferred into several C3 plants including potato and rice (Ku *et al*., 1999, 2000). This manipulation increased the overall level of carbon fixation. Transgenic rice plants expressing pyruvate orthophosphate dikinase (PPDK) and NADP-malic enzyme have also been produced.

2.3.9 Transgenic Plants as Biosensor

Various transgenic plant based models have been successfully used for bio-monitoring genotoxic pollutions, radioisotope levels around nuclear power plants and to detect jet fuel contaminants at military bases. They could also disclose the presence of certain other unwanted and especially dangerous substances in our environment of which presently there is no good way of monitoring. The idea to use organisms to detect TNT was first exemplified using GM bacteria by Dr. Robert Burlage and his coworkers at the Oak Ridge National Laboratory. The bacteria, *Pseudomonas putida*, had TNT inducible promoter fused to GFP and were tested on a faux minefield with surrogate landmines, detected landmines. The list of traits, however, does not end here. With time, newer traits and more applications are versioned and transgenics developed for same**.**

2.3.10 Molecular Farming

Another major goal for production of transgenic plants is their use as bioreactors (or biofactories) for production of specialty chemicals and pharmaceuticals including biopolymers, vaccines, recombinant antibodies, therapeutic agents, nutraceuticals, hormones and industrial enzymes, *etc*. Plant system is relatively more advantageous as compared to recombinant microbial system because of ease of growth and transformation, high-level expression and high yield, rapid scale-up, stability of exogenous DNA, formation of biologically active proteins, ease of storage and distribution and above all safety and economic viability. Only problem while using transgenic plants for large-scale production of high value

products is their purification from an enormous amount of harvested transgenic plant. However, this is easily achieved either by expressing foreign protein at a high-level or by ensuring its expression in a manner that facilitates its purification. These strategies include chloroplast targeting of foreign protein, directing the foreign protein to cell apoplast thereby facilitating its exudation or fusing foreign protein to plant oleosins. In view of various advantages of plant system, considerable attention has been drawn in using transgenic plants as bioreactors.

3. Biotechnology and Biosafety Laws Regulating Confined Field Trials of Transgenics

Adoption of genetically engineered (GE) or genetically manipulated (GM) transgenic crops in agriculture is rapidly on rise (Falck-Zepeda *et al.*, 2012), which is corresponding to the area dedicated to their commercial planting. Bulk of this land mass under cultivation of GM crops belongs to cotton, maize, soybean and canola that too for traits insect resistance (IR) and herbicide tolerance (HT). Of the 52 countries that have granted approvals for field trials of biotech crops, Japan tops the list followed by USA, Canada, South Korea, Australia, Mexico, the Philippines, New Zealand, the European Union and China. Benefits derived from cultivation of GM crops has motivated developing countries too to introduce these crops to their coutries, and many of these crops are presently under biosafety regulatory evaluation and approval stages in these countries (James 2010; Falck-Zepeda *et al.*, 2012). Biosafety is the safe use of GMOs in contained conditions (e.g., in the laboratory), in confinement (experimental field trials) and in general, unconfined introductions into the environment (Komen, 2012), as depicted in Figure 2.

Biotechnology and biosafety policies define intention of a country for adoption of GM crops. They dictate biosafety legislation, though in some countries, biosafety legislation precedes biosafety and biotechnology policies (Wafula *et al.*, 2012).

GM crops rightfully have to undergo highly regulated biosafety evaluations under containment and confined field trials, during which food, feed and environmental issues are studied rigorously. Several international agreements and treaties regulate advancements in agricultural biotechnology. These include access to genetic resources, as described under Convention on Biological Diversity (CBD), intellectual property rights, as defined under International Union for the Protection of New Varieties of Plants (UPOV) and biosafety, as defined mostly under the Cartagena Protocol.

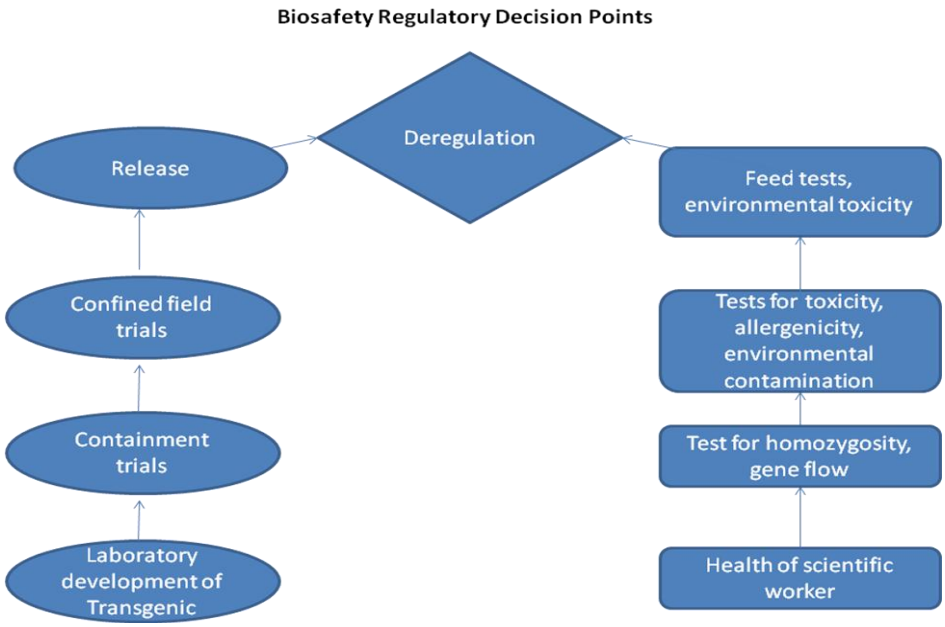


Figure 2: A conceptual diagram showing stages of development and release of a transgenic crop, alongwith various biosafety regulatory decision points

The Cartagena Protocol

There are no international laws governing genetically modified organisms (GMOs) but several respected World Organizations have regulations governing GMOs with formulation of Cartagena Protocol of Biosafety, the parties have been suggested to have natural law so as to harmonize with international regimes. Organizations such as FAO, WHO, WTO provide further regulation of the products of biotech through global agencies.

The Cartagena protocol on bio safety to the Convention on Biological Diversity (Biosafety Protocol) signed by 131 government/ parties on Jan 2000 in Montreal, Canada to protect biodiversity from potential environmental effects of the trans-boundary movement by GE living organism (LMO). The protocol assists developing countries in building their capacity for managing modern biotechnology, creates on advanced informed agreement (AIA) procedure asking exporters to seek consent for import of GMOs and establishes an internet based Biosafety clearing House to help countries exchange scientific, technical, environmental and legal info about GMOs. For most developing countries, Cartagena Protocol on Biosafety (CPB) is a starting point for formulation of their internal biosafety regulations and legislations (Komen, 2012; Wafula *et al.*, 2012). It is generally recognized that biosafety assessments is an important part of the sustainable development of human society. The policies designed in some countries are against unidentified risks, while other countries design the policies, so as to reap maximum benefits of the technology, while also manage actual and potential risks (Wafula *et al.*, 2012).

The Regulatory Framework in India

In India, field trials of transgenic crops started in 1995. To ensure the compliance of safeguards various levels, drafting of guideline and their implementation is indispensable. The activities and use of GE organism and products thereof are regulated under the "Rules for the manufactures, use/import/export and storage of hazardous organisms/ GE organism or cells notified under the Environment Protection Act, 1986, commonly referred as Rules, 1989. These rules and regulations are implements by the Ministry of Environment and forest (MOEF) & Department of Biotechnology (DBT) and state governments. Seven competent authorities and their composition have been provided for in the Rules to handle various aspects (http://www.dbtbiosafety.nic.in).

1. Recombinant DNA Advisory Committee (RDAC)
2. Review Committee on Genetic Manipulation (RCGM)
3. Genetic Engineering Approval Committee (GEAC)
4. Institutional Biosafety Committee (IBSC)
5. State Border Coordination Committee (SBCC)
6. Distict Level Committees (DLCs)
7. Monitoring and Evaluation Committee (MEC)

While RDAC is advisory in function, the IBSC, RCGM and GEAC are of regulatory functions; SBCC, DLC and MEC are for monitoring purpose. DBT has issued certain guideline for applicants seeking approval for environmental release of GE plants in India under Rules, 1989. Specifically these protocols address key elements of the safety assessment of foods and/or livestock feeds that may be derived from GM crops. The results are submitted to appropriate regulatory bodies i.e. RCGM and GEAC as required. DBT has reviewed these guidelines in 2008 and made them available as draft guidelines on its website. DBT has prepared five protocols each based on international best practices including guidance and peer reviewed publication from the codex Alimenatrius Commission, FAO, WHO and Organization for Economic Co operation and Development and the ILSI (http://www/dbtbiosafety.nic.in). These include;

1. Acute Oral safety Limit Study in rats or mice
2. Sub chronic Feeding study rodents
3. Protein thermal stability
4. Pepsin Digestibility Assay
5. Livestock Feeding Assay

Table 3: Description of some commercialized transgenic crops

SNo	Trade name and crop	Trait	Developer	Countries where commercialized
1	32138 SPT maintainer **Maize**	PCS	DuPont	USA
2	Amflora™ **Potato**	MPQ	BASF	EU
3	Atlantic NewLeaf™ **Potato**	IR	Monsanto	Australia, Canada, Mexico, New Zealand, USA
4	Bollgard™ **Cotton**	IR	Monsanto	Canada, Japan, New Zealand, USA
5	BT Shanyou 63 **Rice**	IR	Huazhong Agricultural University (China)	China
6	Bt Xtra™ **Maize**	IR	Monsanto	Australia, Canada, Japan, New Zealand, Philippines, South Korea, Taiwan, USA,
7	BXN™ **Cotton**	HT	Monsanto	Australia, Canada, Japan, Mexico, New Zealand, USA
8	CDC Triffid **Flax**	HT	University of Saskatchewan	Canada, Colombia, USA
9	Cultivance **Soybean**	HT	BASF	Argentina, Brazil, Mexico, Philippines, Russian Federation, USA
10	Elizaveta plus **Potato**	IR	Centre Bioengineering, Russian Academy of Sciences	Russian Fedretion
11	Enlist™ **Maize**	HT	Dow AgroSciences LLC	Australia, Canada, Mexico, New Zealand, South Africa, Taiwan, USA
12	Enogen™ **Maize**	MPQ	Syngenta	Australia, Canada, Japan, Mexico, New Zeland, Philippines, Russian Federation, South Korea, Taiwan, USA
13	Fibermax™ Liberty Link™ Bollgard II™ **Cotton**	HT, IR	Bayer CropScience	Australia, Japan, Mexico, New Zealand, South Korea
14	FLAVR SAVR™ **Tomato**	MPQ	Monsanto	Canada, Mexico, USA

15	Genuity® DroughtGard™ **Maize**	AST	Monsanto	Australia, Canada, Japan, Mexico, New Zealand, Taiwan, USA
16	Genuity® Roundup Ready™ 2 Xtend™ **Soybean**	HT	Monsanto	Australia, Canada, Japan, Mexico , New Zealand, USA
17	Glytol™ x Twinlink™ **Cotton**	HT, IR	Bayer CropScience	Brazil
18	Herculex XTRA™ **Maize**	HT, IR	Dow AgroSciences LLC and DuPont	Canada, EU, Japan, Mexico, Philippines, South Africa, South Korea, Taiwan, Turkey, USA
19	Hi-Lite NewLeaf™ Y **Potato**	DR, IR	Monsanto	USA
20	Huahui-1 **Rice**	IR	Huazhong Agricultural University (China)	China
21	Huanong No. 1 **Papaya**	DR	South China Agricultural University	China
22	Intacta™ Roundup Ready™ 2 Pro **Soybean**	HT, IR	Monsanto	Argentina, Brazil, EU, Mexico, Paraguay, South Korea, Uruguay
23	InVigor™ **Maize**	PCS	Bayer CropScience	Canada, USA
24	JK-1 **Cotton**	IR	JK AgriGenetics Ltd.	India
25	Laurical™ **Canola**	MPQ	Monsanto	Canada, USA
26	Liberty Link™ Independence™ Argentine **Canola**	HT	Bayer CropScience	Japan, USA
27	Liberty Link™ **Sugarbeet**	HT	Bayer CropScience	Canada, Japan, USA
28	Lugovskoi plus **Potato**	IR	Centre Bioengineering, Russian Academy of	Russian Fedretion

			Sciences	
29	Mavera™ YieldGard™ **Maize**	MPQ	Renessen LLC and Monsanto	Japan, Mexico, USA
30	Moondust™ **Carnation**	MPQ	Florigene Pty Ltd.	Australia, EU, Japan, Norway
31	Moonlite™ **Carnation**	MPQ	Florigene Pty Ltd.	Australia, Japan
32	NaturGard KnockOut™, Maximizer™ **Maize**	HT, IR	Syngenta	Argentina
33	Navigator™ **Canola**	HT	Bayer CropScience	Australia, Canada, China, Japan, New Zealand
34	New Leaf™ Plus Russet Burbank **Potato**	HT	Monsanto	Australia, Canada, Japan, Mexico, New Zealand, Philippines, South Korea, USA
35	Ngwe Chi 6 Bt **Cotton**	IR	Cotton and Sericulture Department, Myanmar	Myanmar
36	Optimum® Gly **Canola**	HT	DuPont	Canada, Mexico, USA
37	Optimum™ GAT™ **Maize**	HT	DuPont	Argentina
38	Phytaseed **Canola**	MPQ	BASF	USA
39	Power Core™ **Maize**	HT, IR	Monsanto and Dow AgroSciences LLC	Argentina
40	Rainbow, SunUp **Papaya**	DR	Cornell University and University of Hawaii	Canada, Japan, USA
41	Roundup Ready™ 2 **Maize**	HT	Monsanto	Argentina
42	Roundup Ready™ Bollgard™ II **Cotton**	HT, IR	Monsanto	Australia, Costa Rica, EU, Japan, Mexico, New Zealand, Philippines, South Korea

43	Roundup Ready™ **Canola**	HT	Monsanto	Australia, Canada, Chile, China, EU, Japan, Mexico, New Zealand, Philippines, South Korea, USA
44	Roundup Ready™ **Wheat**	HT	University of Florida	Australia, Colombia, New Zealand, USA
45	Seed Link™ **Chicory**	HT, PCS	Bejo Zaden BV	USA
46	Shepody NewLeaf™ Y **Potato**	DR, IR	Monsanto	Australia, Canada, Japan, Mexico, New Zealand, Philippines, South Korea, USA
47	Starlink™ **Maize**	IR	Bayer CropSciecne	USA
48	Superior NewLeaf™ **Potato**	IR	Monsanto	USA
49	TruFlex™ Roundup Ready™ **Canola**	HT	Monsanto	Australia, Canada, Japan, New Zealand, USA
50	TwinLink™ **Cotton**	HT, IR	Bayer CropScience	Brazil, Canada, South Korea, USA
51	VIPCOT™ Roundup Ready Flex™ **Cotton**	HT, IR	Syngenta and Monsanto Company	Costa Rica
52	Vistive Gold™ **Soybean**	HT. MPQ	Monsanto	Australia, Canada, Mexico, New Zealand, USA
53	Widestrike™ Roundup Ready **Cotton**	HT, IR	Monsanto and Dow AgroSciecnes LLC	Costa Rica, Japan, Mexico, South Africa
54	YieldGard™ Plus **Maize**	IR	Monsanto	EU, Japan, Mexico, Philippines, South Africa, South Korea, Taiwan, USA,

AST- Abiotic stress tolerance, DR- Disease resistance; HT- Herbicide tolerance, IR- Insect resistance, MPQ- Modified product quality, PCS- Pollination Control System

While submitting applications for field trials, all details of the method of production including details of vectors, expression cassettes, etc needs to be submitted. While conducting the field trials, minimum isolation distance between the GM and non GM versions has to be maintained, as per guidelines for a particular crop. Data needs to be collected for a minimum of two years, and all carcasses of the crops needs to be destroyed by burning. The land where GM crop is grown is required to be left fallow for one year after completion of trials.

4. Commercialization

As a result of consistent and substantial benefits during the initial years since 1996 of commercialization of transgenic or GM crops, farmers have continued to plant more biotech crops every single year. The rate of increase of acreage of GM crops is over 10% (James, 2010), and the most recent data suggests that GM crops being planted in more than 1.7 million ha land world over (Brookes and Barfoot, 2013). A database on field trials and commercialization of transgenic crops is maintained by Green Industry Biotechnology Platform (GIBP), an association of major European Plant Biotechnology Companies.

A partial list of prominent transgenics released so far are is provided in Table 3. Thirty four countries, at present have allowed commercial cultivation of GM crops (Table 3), with nineteen of them being developing nations. China was the first country to grow a commercial transgenic crop. Interestingly, more number of developing countries is rapidly joining the list (Table 3). The benefits have been drawn more by farmers in developing countries than in the developed countries (Brookes and Barfoot, 2011).

Commercialization of transgenic crops is often subject to independent approvals from several agencies, chiefly depending on the trait improved and ultimate use of the crop. In India, the jurisdiction of approval for commercialization rests on Genetic Engineering Action Committee (GEAC), jointly under Ministry of Science and Technology, and Ministry of Environment and Forests. In USA, such approvals are jurisdiction of different agencies like Environment Protection Agency (EPA) and Food and Drug Administration (FDA).

Indian Scenario

India, the largest cotton growing country in the world, where 60 million people are impacted by cotton, reported an average gain of 38% yield in cotton till 2011, translating to US$ 267 average income per hectare, by the use of GM cotton (Brookes and Barfoot, 2013). *Bt* cotton was first introduced for commercial cultivation in 2002, and has increased yield by up to 55%, reduced insecticide sprays by 63%, with environmental and health implications (Jagadish, 2012).

The story of Bt cotton in India is remarkable. With political will and farmer support in place, adoption is projected to continue increasing with Bt cotton plantings escalating to upto 92% (James, 2010). As a result, India ranked fourth on the list of largest GM crop growing countries in the world in 2010 (James, 2010).

Bt Brinjal became first GM vegetable crop in India to reach the approval stage for commercialization and consumption by humans (Kumar *et al.*, 2011).

Bt Brinjal carries the gene *cry1Ac* from *Bacillus thuringiensis* and has been developed by the Maharashtra Hybrid Seed Company Ltd., (Mahyco), the Tamil Nadu Agricultural University, Coimbatore, the University of Agriculture in Dharwad (Karnataka) and Indian Councils of Agricultural Research (ICAR).

The developers of *Bt* brinjal have carried out rigrous containment and confined field trials, as discussed by Kumar *et al.*, (2011). However, following aggressive protests by non government players (As discussed later), release of *Bt* brinjal in to the market has been put on hold for "indefinite time" by Government of India. If released, *Bt* brinjal, based on its potential to reduce the consumption of pesticide by 77%, and increase in yield by 116%, is likely to increase average income of the farmer by US$300 per hectare (James, 2010).

Other food crops like maize, sorghum, rice, cabbage, cauliflower, tomato, groundnut, etc. too are awaiting regulatory approvals for field trials and cultivation in India. However, their fate depends on the decision taken by the government based on the petitions filed both by supporters and protesters of GM crops in the country (Jagadish, 2012).

5. Acceptability by Society: Impact on Biodiversity, Environment and Ethical Issues

Ever since first commercial cultivation of transgenic crops, the landmass under GM crop cultivation has grown 87-fold (James, 2010), and still it constitutes only a fraction of the total cultivated land in the world. Nearly half of the transgenic crop area is located in the US. Maize covers 25% of the global transgenic crop area. Nowhere in the world, have GM crops received open armed reception. In fact, very few R&D products on GM crops have reached to the farmers, as listed in Table 3. Possibilities of gene flow from GM to non-GM crops have been the primary concern of the people (Foetzki *et al.,* 2012). Thus, labelling of GM food and other material has been made mandatory in several countries, as described above. Other major safety concerns related to GM crops include potential risks of toxicity and allergenicity in humans and other animals, effects of IR genes on non-target organisms, weediness, development of super bugs and super weeds, etc. Among indirect issues, public attitude, societal ethics, socioeconomic factors, religious beliefs, intellectual property rights, etc. are the primary concerns. Among socioeconomic factors, fear of losing a job by shutting down or disinvestment of an (agrochemical) industry is included. Thus, in addition to scientific, non-technical and non scientific factors too add up as hurdles to properly communicate risks and benefits of GM crops to the public (Jagadish, 2012). Opinion of common man on the issue of GM crops, as well as the public policy is thus governed by sentiments, at least in Indian scenario, than by scientific input. Despite the significant success of Bt cotton in India and food/feed biotech (GM) crops such as soy, corn, canola and papaya in other countries, common man in general is not ready to accept GM crops and food.

Nevertheless, financial gains from GM crops are so lucrative that many a times, farmers have chosen them as their crop, even if they had to violate the sovereign laws and the crop is unapproved in the country (Jagadish, 2012). In one particular case in France at least, the popularity of a GM crop, even before its approval by the state has led to a new dawn in the country for GM crops. Thus, public sentiment is the most determining factor on the fate of GM crops, and proper scientific communication with public is the determining factor to make the sentiment. Countries like Kenya have allowed importation of GM maize, to combat serious food shortage due to severe drought and massive refuge influx from the neighbouring Somalia (Prakash, 2011). There is a particular segment of people, which are even standing in support of artificial life through 'synthetic biology' (Moses, 2012b).

Over the years, developers of GM crops have attempted to negate all of the public concerns relating to safety and adoption of GM crops (Jagadish, 2012). These explanations need to be carefully assessed. Scientific data exists to show substantial equivalence between a biotech (GM) and a non-GM crop of the same kind (Jagadish, 2012), and potential risks to health of humans, animals, society and environment exists even by non GM crops and their cultivation practices. Same way data exists on beneficial aspects of GM crops on heath of humans and environment (Redick, 2012). As discussed above, GM crops play important roles in mitigating greenhouse gas. Herbicides and pesticides, commonly used in conventional agriculture can too be a source of hazard to humans and other animals. Therefore there are risks associated with their use as well.

With regards to gene flow, it is a natural process, and most cultivated plants mate with one or more wild relative in some portion of their geographic range and many crops are known to naturalize and persist as weed population. In case of transgenics, the introduced trait is necessarily dominant and monogenic. Thus, if required, it can easily be removed by out crossing. Regarding toxicity and allergenicity of the GM crops, commonly employed tests like rodent toxicity tests with cute oral administration of protein, *in vitro* digestibility assay of whole grain of GM crop and the recent bioinformatics assays are efforts to remove any suspicion of toxic substance production. Also relative compositional analysis in nutritional properties is done to check production of any ant nutritional entity in the altered genome.

The use of antibiotic resistant markers in the development of transgenic crops has raised concerns about whether transgenic food will play a part in our loss of ability to treat illness with antibiotic drugs, will it enhance resistance in soil bacteria by gene flow, and will it cause an undesirable increase in use of antibiotics? We eat DNA every time we eat a meal. Most of it is broken down into more basic molecules

when we digest a meal. A small amount is not broken and is either absorbed into blood stream or excreted in faeces. It is suspected that body's immune system destroys the DNA. The debate is still on and hot. Keeping in mind public perception, other markers like phosphomannose isomerase, wuschel gene or reporter genes or other strategies like cre/lox, FRP/FRT, co-transformation are now in use that either avoid use of antibiotic selectable marker or eliminate them in successive generation.

The result of evolution of germplasm with undesirable traits due to hybridization with transgenes is a potential risk to the evolutionary process too. Research indicates that crop traits may escape from cultivar and persist for many years in wild population. Under favorable conditions, they can then hybridize with their compatible species. The likelihood that transgenes will spread can be different for each crop in each area of world care can be taken not to grow a transgenic crop in its native origin place to avoid development of such undesirable gene flow pattern.

Human health and environmental sustainability are two of the most glorious societal objectives at present. Possible and hypothetical threat to these, however, in no way shall be presented as excuses to restrict or commercialization of GM crops. Instead, effort shall be drawn to ensure that present and emerging biosafety frameworks adequately address all scientific and social concerns in an effective manner. A balanced regulatory regime would be the one, which would evaluate the technology for safety and efficacy in an honest and scientific manner before deployment (Wafula *et al.,* 2012). The biosafety legislation should be science-based and decisions informed by the best available scientific evidence on a case-by-case basis (Wafula *et al.,* 2012).

6. Current Controversies: Case Studies

Bt *Brinjal in India*

As discussed above, *Bt* brinjal in India has been banned for indefinite time, following protests and resentment among various groups (Kumar *et al.,* 2011). Factually speaking, such protests were carried out against earlier introduction of *Bt* crops elsewhere in the world, but they all have been found inappropriate (Kumar *et al.,* 2011). As India is at the center of the origin of cultivated brinjal, transgenes can move to the wild germplasm through this and we will not be able to differentiate between *Bt* brinjal and non-*Bt* (Kumar *et al.,* 2011). Presently, there are three important issues raised by opponents of *Bt* brinjal in India- (1) Data on testing of chronic toxicity, (2) Independent tests that command credibility and don't only depend on data provided by the developers themselves and, (3) The need to have an independent regulatory system that will be in a position to study all aspects of GM technology in agriculture.

Among the non scientific issues, participation of big names in the field of Agricultural Biotechnology, i.e., Monsanto has been a major issue. However, it must be remembered that Tamilnadu Agricultural University, Coimbatore and the University of Agricultural Sciences, Dharwad have also participated and developed *Bt* brinjal varieties. Earlier experiences of growing *Bt* cotton in India has yielded good results, as discussed above. Resistance development, nevertheless, is a serious concern for monophagous pests, so there is need to develop baseline susceptility data of cry toxin on the fruits and shoot borer population from all the brinjal growing regions.

The government of India recently announced that Bt brinjal needs additional time for review. In view of controversies over Bt Brinjal, the Genetic Engineering Approval Committee (GEAC) decided to set up an 'Expert Committee' on Bt brinjal in 2006. In October 2009, GEAC declared Bt Brinjal safe and recommended its commercial approval to the environmental ministry who subsequently imposed a moratorium on commercial release of Bt Brinjal. An independent joint panel of India's GEAC and eminent scientists on gene technology favour lifting of the moratorium and allowing limited release of Bt Brinjal under strict monitoring during the first meeting of the expert panel held in April 2011 (Kumar *et al.,* 2011). Final approval and release of Bt brinjal will benefit not only the producers of the crop, i.e., the farmers, it will also help grow the Indian economy. It will also result in reduced pesticide residues in soil and water, less air pollution and local environmental pollution due to decreased use of insecticides, protection of naturally occurring predators and parasitoids and other beneficial organisms due to reduced use of insecticides, reduction in soil and ground-water contamination, and safeguard soil microflora and

invertebrates from damage. After extensive field trials, safety and adverse reaction studies, Bt brinjal was found to be safe for humans as well as the environment.

Losing Political Will in Favour of GM Crops

There has been a strong resentment against GM crops throughout the world. Several political and non political agencies throughout the world have been lobbying against the stakeholders of GM, to the extent that damages caused to GM designing companies to losses amounting in millions of dollars. Recently in Germany, BASF, world's largest chemical company abandoned GM research work in the country. The company has also withdrawn its Amflora potato from the European market within two years of its approval by EU, and transferred all its GM development work to North America (Moses, 2012a). Amflora had taken thirteen years to gain approval, during which time the company was continually beset with protests, vandalism, violent attacks on personnel and political fights. Even before Amflora potatoes were approved by EU, the general trend in Europe was against the GM crops. There were more numbers of opponents to GM than supporters (Moses, 2011).

Because of the restrictive political conditions and the destruction of field trial plots, the Plant Science Garden in Üplingen, Saxony-Anhalt, which has been visited by thousands interested in genetically modified plants in the past few years, has remained shut in 2012 (Moses, 2012c). This situation, which has already led to the loss of a large part of the national scientific and economic resources in a future-oriented industry, has made it impractical to continue showing the latest worldwide developments in modern plant breeding to the public. GM crop trials are diminishing generally in Europe as the continent continues to try to come to grips with GM technology. There is also a report that efforts by the current Danish presidency of the EU to authorise Member States to refuse the cultivation of GM-crops on their national territories is making no progress (Moses, 2012c). Poland has decided to ban all plantings of Monsanto's MON810. (Moses 2012c). Similar trends have been seen in Bulgaria too. Political unrest has been seen in Ireland too over the issue of GM crops (Moses, 2011).

Vandalism During Trials

Vandalism during trials of GM crops is another common problem throughout the world. For example, in July 2011, GM high amylase wheat plants, undergoing field tests by CSIRO, were destroyed by anti-GM activists of Greenpeace in Canberra, Australia (Prakash, 2011). In a similar activity, almost at the same time in Germany, GM fungal resistant wheat trials, GM maize and GM potatoes with modified product quality were destroyed Gross Lüsewitz, near Rostock and Üplingen, Saxony-Anhalt (Prakash, 2011). In the same month, GM papaya farms were also vandalized in Hawaii, USA (Prakash, 2011).

Loss to R&D on GM Crops

Plant research at universities and other public institutions too has been discouraged due to increasing number of protests everywhere. Scientists have either turned to less contentious areas or they have emigrated, predominantly to the Anglo-Saxon countries (Moses, 2012a)

To Label or Not to Label of GM Food Material in USA

As discussed earlier, labelling of GM food material is mandatory in Europe, but there is no such regulation in USA. However, there has been a long debate both at Federal as well as state level to label or not to label the GM food. Connecticut announced mandatory labelling; other states still undecided, some even sorting to polling. While some parties think the labelling is necessary considering vast diversity of immune system in human population worldwide, other feel labelling will unnecessarily invite additional money and costs on GM foods (Moses, 2012a,b,c).

Changing Trends in Public Perception

Despite the cases discussed above, an increasing numbers of newspaper editorials around the world are not gradually switching over their view in favour of GM technology (Moses, 2012c). Even the public perception on GM crops is changing. For example, in a survey in Chicago showed that food safety not GM crops was of most concern to consumers (Rich, 2011). In India, after the exit of environment minister Jairam Ramesh, the general perception is that things will fall in line with faster GM crop commercialization (Prakash, 2011).

Illegal and Unapproved Cultivation of GM Crops: The French Case

Following several years of successful cultivation of *Bt* maize in Spain, French farmers near the Spanish border reportedly purchased GM seeds deom Spain, grew the *Bt* maize in France and then went back to Spain to sell their produce (Moses, 2012a). As a result of this popularity of GM maize among French farmers, French government formally approved it for cultivation. However, with the change of regime in France, in May 2007, the official view on GM crops changed, and in early 2008 the cultivation of GM maize in France was banned. Soon thereafter, highest French Court and European Court of Justice ruled the ban illegal, citing the reason that the government had not produced enough evidence to back its claims that the GM crop posed a significant risk to health or the environment. Despite that the French Government maintained ban on GM maize in their country.

CONCLUSION

With depleting natural resources and changing global climate, conventional agricultural practices alone are unable to sustain the quality and quantity of the produce. With advent of modern biotechnology, newer tools permitting gene transfer across the species: transgenics, opened an avenue for solving the age old problem. Genetically modified crops can be produced by several ways be it a physical or chemical delivery method of inserting gene of choice. The aim of gene transfer are – breeding crops with improved traits – as well as molecular farming, the production of pharmaceuticals, therapeutic molecules or specialty chemicals for industry. Transgenics have potential to reduce usage of insecticides, herbicides and weedicides, improve tolerance to stress, and function as edible vaccine and biosensors.

Like any known technology, Genetic manipulation of crop genome has its advantages and disadvantages. Potential risks associated with GM crops include gee flow, allergenic, toxic, anti nutritional effects use of ARMGs, undesirable evolutionary traits and change in biodiversity. Though genetic engineering is known as simple extension of conventional breeding, the developed crops are analyzed and evaluated for the potential risks by following the protocols mentioned in the Codex Alimentarius Commission report. The Cartagena protocol of Biosafety is internationally acknowledged as it harmonizes the issues of international regime in relation to GM crops.

Each country has a regulatory framework to monitor the stepwise development and release of GM crops. The Department of Biotechnology, India has a six tier regulatory framework that permits R&D in transgenics and monitors their release. Sustained global increase of 12 % in area under cultivation by GM crops was recorded I 2007, including 23 countries with India at rank five. The global value of GM crop market is projected US $7.5 billion for 2008. India grew GM cotton in 6.2 million hectares of land and the GM gave a 50% increase in yield. The result is, India is now an exporter rather than importer of cotton. The Indian Genetic Engineering Approval Committee (GEAC) had approved field trials of 14 crops for the Kharif season in 2007 with four GM crops from the universities and institutes. The number of GM crops from academia and R&D organizations is on the rise.

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