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Genetically Modified Crops: An Overview

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Abstract: Genetically modified transgenic crops also referred to as a Genetically Modified (GM) crops are produced by when the genetic material of an organism (either DNA or RNA) is altered by use of recombinant DNA technology and the modification can be replicated and/or transferred to other cells or organisms. The recently understood recombinant DNA technology has potential, via genetic engineering, to incorporate a specific gene which controls a particular trait, without co-transfer of undesirable genes from donor species as occurs in conventional breeding. In recent year, the globally cultivated area of transgenics crops has increased more than 81 million hectares. Most GM crops grown today have been developed to resist certain insect pests. There are GM plants being developed today to produce specific vitamins, resist plant viruses and even produce products for medicinal uses.

Key words: Transgenic crops, genetic material, particular trait, transgenics crops

INTRODUCTION

Genetic Modification (GM) occurs where the genetic material of an organism (either DNA or RNA) is altered by recombinant DNA technology (i.e., Agrobacterium-mediated transformation or direct gene transfer methods) (Griffiths *et al*., 2005) that does not occur in nature and the modification can be replicated and/or transferred to other cells or organisms. Typically, GM involves the removal of gene from any living organism, its manipulation outside the cell and reinsertion into the same or another organism and given appropriate regulatory signals and codon usage, can be expressed efficiently in a cell. The purpose of doing this is that when this cell is cultured or allowed to develop into a complete organism the genetic modification will have resulted in it having new characteristics. Often genetic modification involves isolating the DNA encoding a single gene from one organism and inserting it into the genetic material of another organism. The organism which has been modified is referred to as a Genetically Modified Organism (GMO). GMOs may be plants, animals or micro-organisms.

In 1996, GM crops were first introduced into the commercial market in the United States and were rapidly adopted by farmers. Great success was achieved in increasing agricultural productivity to fulfill human needs during the 20th century due to the introduction of GM crops (Yan and Kerr, 2002; Rommens *et al*., 2004). Crops and foods produced using recombinant DNA techniques have been available for fewer than 12 years and no long-term effects have been detected to date. These foods are substantially equivalent to their conventional counterparts (ISB News report 2001; Pandey *et al*., 2010).

The first commercially grown genetically modified whole food crop was the tomato which was made more resistant to rotting by Californian Company Calgene (Martineau, 2001). Since then, more than 50 other genetically engineered crops have been developed and released in the market by the different laboratories. This includes insect-resistant cotton and herbicide-tolerant soybeans both of which were commercially released in 1996. GM crops have been widely adopted in the United States. They have also been extensively planted in several other countries (Argentina, Brazil, South Africa, India and China) where the agriculture is a major part of the total economy. Other GM crops include insect-resistant maize and herbicide-tolerant maize, cotton and rapeseed varieties.

METHODS OF PLANT GENETIC ENGINEERING TECHNIQUES

In the last decades Plant breeders are using genetic engineering techniques to transfer useful segments of genetic material between systematically unrelated species into crops to improve plant characteristics. For this

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various methods have been developed and some are easier to transform than others but if there are sufficient economic reasons to fund research and development then for almost any crop transgenic plants can be produced. The procedures are briefly described in the literature.

***Agrobacterium* mediated transformation:** Many plants have been transformed successfully by utilizing *Agrobacterium*, a pathogen of dicotyledonous plants that transfer genes into the plant genome. The soil bacterium *Agrobacterium tumefaciens* can infect wounded plant tissue, transferring a large plasmid, the Ti plasmid, to the plant cell. Part of the Ti plasmid apparently randomly integrates into the chromosome of the plant. The integrated part of the plasmid contains genes for the synthesis of food for the bacterium and plant hormones. Genes from the Ti plasmid that are integrated in the plant chromosome are expressed at high levels in the plant. Overproduction of the plant hormones leads to continuous growth of the transformed cells, causing plant tumors. Rapid, cancerous growth of the transformed plant tissue obviously is advantageous to the bacterium, more food gets produced.

The Ti plasmid has been genetically modified ("disarmed") by deleting the genes involved in the production of bacterial food and of plant hormones and inserting a gene that can be used as a selectable marker. Selectable marker genes generally are coding for proteins involved in breakdown of antibiotics, such as kanamycin. Any gene of interest can be inserted into the Ti plasmid as well. In principle, one can thus transform any plant tissue and select transformants by screening for antibiotic resistance. However, unfortunately there are some complications like it has proven difficult to transform some monocots (grasses, etc.) by *Agrobacterium* and regeneration of plants from tissue culture or leaf discs is not always possible (Pati *et al*., 2008; Kutty *et al*., 2011).

Particle bombardment method: Particle bombardment (biolistic) method is used widely for the stable transformation of plants. This is the most effective and important gene transfer method in regular use. In this technique, tungsten or gold particles are coated with the DNA that is to be used to transform the plant tissue. The particles are propelled through DNA delivery device is named "Gene Gun" at high speed into the target plant materials, where the DNA that is released within the cell and can integrate into the plant genome. Over the last several years, use of the gene gun has become a very common method to transform a wide variety of plants including the economically important cereals and legumes and many woody species.

Electroporation: In this method, the foreign DNA (gene) migrates through high voltage induced pores in the plasma membrane and integrates into the plant genome. Electroporation has been successfully used to transform all the major cereals i.e., rice, wheat and maize. However, it is often very difficult to regenerate fertile plants from protoplasts of cereals. Nonetheless, significant advances in overcoming these practical difficulties have been made over the years. Electroporation also has the advantage that all the cells are in the same physiological state after transformation, unlike the situation with particle bombardment where transformed cells may be at a disadvantage due to damage from the transformation procedure.

PEG mediated transformation: Plant protoplast can be transformed with naked DNA by treatment with PEG (polyethylene glycol) in the presence of divalent cations (Ca^{++}). The PEG and divalent cations destabilize the plasma membrane of the plant protoplast and render it permeable to naked DNA. Once inside the protoplast the DNA enters the nucleus and integrates into the genome. However, DNA used for transformation is also susceptible to degradation and management. Despite these limitations, the techniques does have the advantages that protoplast can be isolated and transforms in large numbers from wide range of plant species.

Silicon carbide fibers: This is the simple technique using silicon carbide fibers without any specialized equipment. Plant materials (e.g., cells in suspension culture, embryo and embryo derived calluses etc.) are introduced into a buffer containing DNA and silicon carbide fibres which is vortexes vigorously. The fibres which are 0.3 to 0.6 μm in diameter and 10 to 100 μm long penetrate the cell wall and plasma membrane which facilitate the DNA to access to the inside of cells. The drawback of this technique is availability of suitable plant materials and the inherent dangers of fibres which requires careful handling.

A variety of techniques are available for plant transformation/genetic modification to the plant biotechnologist. These technique can be divided into two groups, *Agrobacterium* mediated transformation and second is direct gene transfer method which includes particle bombardment, Electroporation, PEG mediated and Silicon carbide fibres of which particle bombardment method is widely used in transformation (Table 1). These transformation techniques provide the basis for the advances in plant engineering and plant transform with the help of these techniques are routinely used in many laboratories around the world (Slater *et al*., 2008).

Table 1: Gene transfer methods

Methods	Significance
Agrobacterium mediated gene transfer	It is widely used method but it induce less rearrangement of the transgene and result in a lower transgene copy number than direct DNA delivery methods.
Direct gene transfer method	Very successful method. Risk of gene rearrangement and high copy number. Useful
• Practice bombradment	for transient expression.
• Electroporation	Low efficiency. Require careful optimization.
• PEG mediated transformation	Require optimization with a regenerable cell suspension that may not be available.
• Silicon carbide fibers	Simple technique. Requires regenerable cell suspension.

GENETICALLY MODIFIED CROPS

Since long, breeders have modified the genetic make up of plants and animals through conventional breeding methods. Breeders have developed new crop varieties using the existing genetic variability or by creating new variability which is the prerequisite for any breeding programme. Conventional breeding methods have the disadvantage of thousands of genes getting transferred in each cross which may or may not be of use along with the desired ones in the target species. Another major limitation in conventional breeding includes the barriers for gene transfer through incompatibility and species differences.

Genetic engineering has some advantages over other techniques used in plant breeding. It allows genes to be introduced into a crop plant from any source, it is relatively precise in that single or small numbers of genes can be transferred or genes can be manipulated in the laboratory before insertion into a plant, the safety of genes and their products can be tested extensively in the laboratory before use in a breeding program. These advantages have led to genetic modification becoming established as a new tool for plant breeders (Fig. 1).

Increased shelf life: In 1994 the USA released the first commercial GM plant varieties were tomato that had been modified to slow down the ripening process, giving them a longer shelf life. A major problem in fruit production is that consumers want to buy ripe fruit but ripening is often followed quite rapidly by deterioration and decay. Fruit ripening is a complex process that brings about the softening of cell walls, sweetening and the production of compounds that impart color, flavor and aroma. The process is induced by the production of a plant hormone, ethylene. Genetic modification has been used to slow ripening or to increase the shelf life of ripe fruit by interfering either with ethylene production or with the processes that respond to ethylene.

The development of these varieties went hand in hand with the invention of techniques that enabled scientists to use genetic modification to reduce the activity of (or silence) a specific plant gene. Initially it was done with antisense gene silencing (Grierson, 1996; Soliman *et al*., 2008) after that co-suppression method. Gene silencing turned out to be a natural defense mechanism employed by plants against virus infection. It involves the production of small, antisense RNAs, 25 nucleotides in length that interfere with the processing, transport and translation of RNA molecules produced by a target gene. The third method of gene silencing by genetic modification, called RNA interference (RNAi), involves inducing the plant to synthesize a double-stranded RNA molecule derived from the target gene (Dhakar *et al*., 2010). This has been done by splicing part of the gene sequentially in a head-to-tail formation downstream of a promoter. Introduction of such a gene into a plant causes the production of an RNA molecule that forms a hairpin loop which is cleaved by enzymes naturally present in plant cells into short molecules, each 23 nucleotides long.

Antisense and co-suppression were used in the first GM tomato varieties to reduce the activity of a gene encoding polygalacturonase (PG), an enzyme that contributes to cell wall softening during ripening. Calgene in the USA used an antisense technique while Zeneca in collaboration with Grierson's group used co-suppression. The Calgene product was a fresh fruit variety called 'Flavr Savr'. It was first grown on a large scale in 1996 but was not a commercial success and was withdrawn within a year.

Zeneca chose to introduce the trait into tomatoes used for processing and this proved to be much more successful. These tomatoes have a higher solid content than conventional varieties, reducing waste and processing costs in paste production and giving a paste of thicker consistency. This product went on the market in many countries and proved very popular in the UK from its introduction in 1996 until 1999 when most retailers withdrew it in response to anti-GM hostility.

In Australia transgenic long vase-life carnation have been commercialized, with ethylene production inhibited by down regulation of the ACC synthase gene. ACC has also been targeted using a gene from a bacterium, Pseudomonas chlororaphis, that encodes an enzyme called ACC deaminase which breaks down ACC. A similar strategy has been adopted to break down another of the precursors of ethylene, S-adenosyl methionine (SAM), using a gene encoding an enzyme called SAM hydrolase.

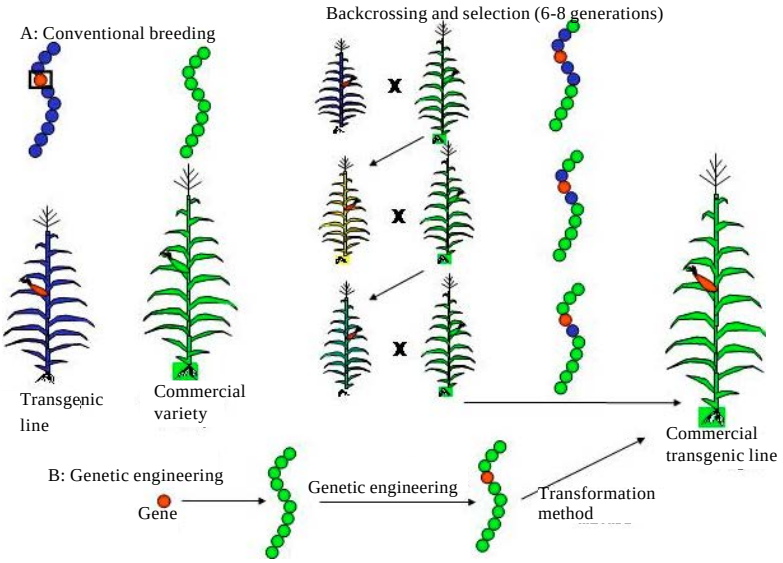

Fig. 1: Schematic diagram of making of GMO crop A: via conventional breeding, B: via genetic engineering method

Genetic modification to delay ripening and improve post-harvest shelf life is also being used in papaya, mango, pineapple and other fruits but there are no commercial varieties available yet.

Herbicide tolerance: Weeds have a significant effect on yield and quality of crops, as a result of competition for light and nutrients contamination of the harvested crops and because weed populations harbor pest and disease (Slater *et al*., 2008). Thus weeds are one of the three classes of biotic stress that have a major impact on the production of the world crop yield available for human consumption. Modern agriculture has developed a range of effective herbicides to control the effect of weed on crop yield. However, these can only be used at a time when the crop is not itself vulnerable to herbicide action. Thus herbicide tolerant GM crops were produced to simplify and cheapen weed control using herbicides. However, some crops are naturally resistant to certain herbicides and that tolerant strain may appear through the normal process of mutation and natural selection. Thus, the concept of herbicide tolerant crops is not unique to GM technology. Herbicides have been used since long before the advent of genetic modification. In 1941, the first modern herbicide, 2, 4-dichlorophenoxyacetic acid (2, 4-D), was synthesized and released in 1946. They are now an essential part of weed control for farmers in developed countries. However, herbicides pose a number of problems for farmers including health risk to farmers and some that are persistent in the soil, making crop rotation difficult.

Glyphosate is a broad spectrum herbicide that is reputedly effective against 76 of the world's worst 78 weeds. The soybean variety known as 'Round Up Ready' marketed by Monsanto was the first to carry this trait (Padgette *et al*., 1995). Glyphosate is a simple glycine derivative, relatively safe to use, does not persist long in the soil because it is broken down by microorganisms and is taken up through the foliage of a plant, so it is effective after the weeds have established. Its target is an enzyme called 5-enolpyruvoylshikimate 3-phosphate synthase (EPSPS). EPSPS catalyzes the formation of 5-enolpyruvoylshikimate 3-phosphate (EPSP) from phosphoenolpyruvate (PEP) and shikimate 3-phosphate (S3P). This reaction is the penultimate step in the shikimate pathway (Fig. 2) which results in the formation of chorismate which in turn is required for the synthesis of many aromatic plant metabolites including the amino acids phenylalanine, tyrosine and tryptophan. The gene that confers tolerance of the herbicide is from the soil bacterium *A. tumefaciens* and makes an EPSPS that is not affected by glyphosate. It has been introduced into commercial varieties of soybean, maize, cotton and oilseed rape, while glyphosate-tolerant varieties of many other crops, from wheat and sugar beet to onion, have been produced but not released yet (Halford, 2006).

There are two other broad-range herbicide-tolerant GM systems in use, involving the herbicides gluphosinate (or glufosinate) and bromoxynil. Gluphosinate, the scientific name for which is phosphinothricin, is a competitive inhibitor of glutamine synthetase (GS), an enzyme required for the assimilation of nitrogen into

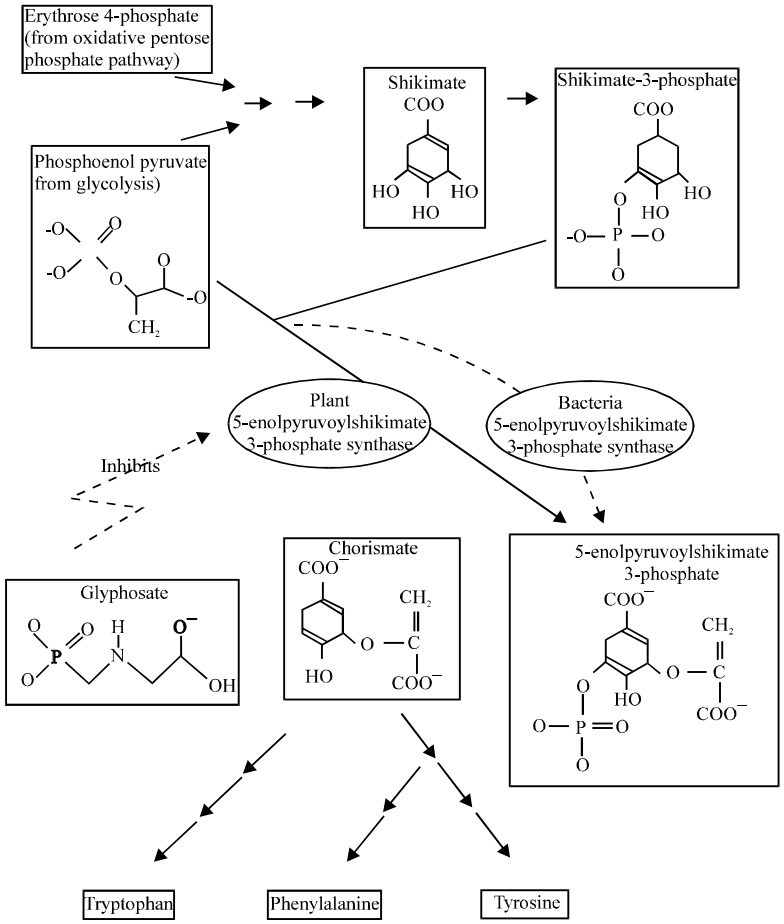

Fig. 2: The Shikimate pathway (Halford, 2006)

the amino acid glutamine. The gene used to make plants resistant to gluphosinate comes from the bacterium *Streptomyces hygroscopicus* and encodes phosphinothricine acetyl transferase (PAT), an enzyme that detoxifies the herbicide by converting phosphinothrycin to acetylphosphinothrycin (Fig. 3) (Thompson *et al*., 1987). Crop varieties carrying this trait include varieties of oilseed rape, maize, soybeans and cotton and the trait has also been introduced into fodder beet and rice. The primary mode of action for bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) is to inhibit photosynthesis by binding to the photosystem II complex of chloroplast membranes and blocking electron transport; a gene isolated from the bacterium *Klebsiella pneumoniae ozanae* confers tolerance. This gene encodes for an enzyme called nitrilase which converts bromoxynil into 3, 5-dibromo-4-hydroxybenzoic acid, a non-toxic compound (Fig. 4). So far this has only been used commercially in Canadian oilseed rape.

Beside these range of herbicides available including imidazalonones (IMI), protoporphyrinogen oxidase inhibitors ('Acuron'), triazines, 2-4-D, cholrsulfuron/ sulfonylurease and isoxazoles. Herbicide tolerance has now been engineered into many other crop species (Table 2) and is undoubtedly the most successful GM trait to be used so far. In the USA in 2003, 81% of the soybean crop, 59% of the upland cotton and 15% of the maize were herbicide tolerant (Benbrook, 2003). Herbicide- tolerant soybeans have been adopted even more enthusiastically in Argentina and now account for 95% of the market, while herbicide tolerant oilseed rape has taken 66% of the market in Canada. This success is due to the factors such as simplified and safer weed control, reduced costs and more flexibility in crop rotation.

Insect resistance: Chemical control of insect pest is both expensive and environment unfriendly. The bacterium produces a protein called the *Cry* (Crystal) protein (known

Table 2: Herbicide tolerant GM crops

Gene	Crop	Resistance to	Originating company	Reference
CP4.EPSPS	Soybean	Glyphosate	Monsanto	Schwember (2008)
CP4.EPSPS, GOX V247	Canola	Glyphosate	Monsanto	Schwember (2008)
Pat	Canola	Phosphinothricin	Bayer Crop Science	Schwember (2008)
CP4.EPSPS	Alfalfa	Glyphosate	Monsanto	Schwember (2008)
Bar gene	Citrus	Basta (Glufosinate resistance)	-	Piestun *et al.* (2000) and Li *et al.* (2002)
CP4.EPSP	Strawberry	Glyphosate	-	Morgan *et al.* (2002)
Als gene	Apple	Triasulfuron and metsulfuro-methyl	-	Yao *et al.* (1995)

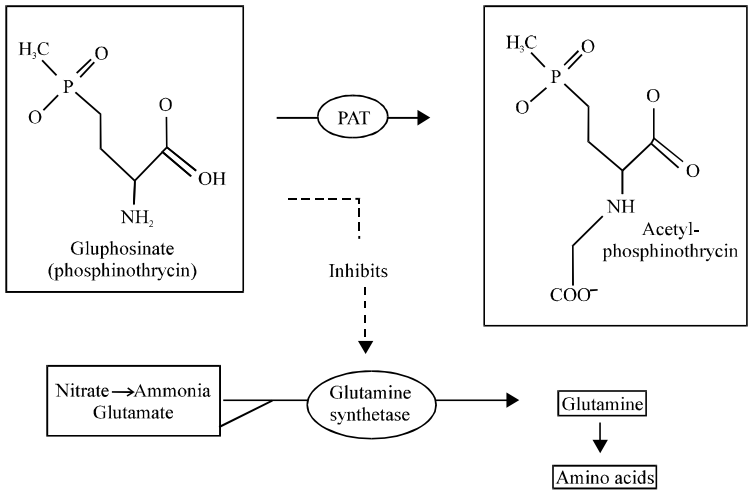

Fig. 3: The action of gluphosinate (competitive inhibitor of glutamine synthetase) on amino acid synthesis and the detoxifying action of phosphinothricine acetyl transferase (PAT) (Halford, 2006)

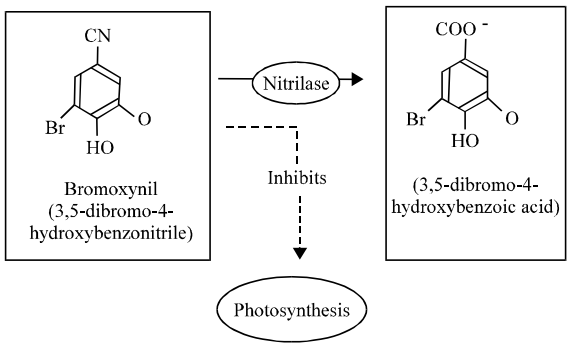

Fig. 4: Nitrilase converts bromoxynil into non-toxic compound 3, 5-dibromo-4-hydroxybenzoic acid (Halford, 2006)

as Bt protein); different strains of the bacterium produce different versions of the protein and these can be assigned to family groups, *Cry*1-40 (and counting), based on their similarity with each other. The *Cry* proteins are δ_endotoxins and they work by interacting with protein receptors in the membranes of cells in the insect gut. This interaction results in the cell membrane becoming leaky to cations, causing the cell to swell and burst. The interaction is very specific and different forms of the *Cry* protein affect different types of insects. *Cry*1 proteins, for example, are effective against the larvae of butterflies and moths, while *Cry*3 proteins are effective against beetles. The toxicity of all the *Cry* proteins to mammals, birds and fish is very low.

The fact that pesticides based on *B. thuringiensis* (Bt pesticides) had been used for a considerable length of time and had a good safety record, coupled with the fact that the insecticidal properties of the bacterium were imparted by a single protein, encoded by a single gene, made the Bt system an obvious target for adaptation for use in crop biotechnology. The first crop variety to carry the trait was a maize variety containing the *Cry*1A gene that was produced by Ciba-Geigy (now part of Syngenta) and first grown widely in 1996. Varieties of maize and cotton carrying the *Cry*1 gene are also now marketed by Monsanto, Bayer, Mycogen and DeKalb. Aventis, subsequently acquired by Bayer, produced a maize variety called StarLink which carried the *Cry*9C variant, while Monsanto introduced the *Cry*3A variant into potato, marketing varieties carrying the trait as NewLeaf and NewLeaf Plus, the latter also carrying a gene for resistance to a virus. Monsanto has also introduced the

*Cry*3B variant into maize but this variety is not yet on the market. The *Cry*1A and *Cry*9C proteins are effective against the European corn borer, a major pest of maize in some areas, while *Cry*1A is also effective against tobacco budworm, cotton bollworm and pink bollworm, three major pests of cotton. The *Cry*3A protein that was introduced into potato is effective against the Colorado beetle and the *Cry*3B protein against corn rootworm (Table 3) (Halford, 2006).

Bt varieties have been successful in many parts of the USA and Bt cotton in particular is gaining ground in Australia, China, India and the Philippines. Farmers who use Bt varieties cite reduced insecticide use and/or increased yields as the major benefits (Gianessi *et al*., 2002). A further, unexpected benefit of Bt maize varieties is that the Bt grain contains lower amounts of fungal toxins (mycotoxins) such as aflatoxin and fumicosin (Dowd, 2000). Not all Bt varieties have been successful. NewLeaf and NewLeaf Plus potato were withdrawn in the USA due to reluctance to use them in the highly lucrative fast food industry. Farmers have adopted broad-range insecticides instead to combat the Colorado beetle. StarLink maize was an even more costly failure; it was not approved for human consumption because of doubts over the allergenicity of the *Cry*9C protein but inexplicably given that maize is an outbreeding crop, the Environmental Protection Agency approved it for commercial cultivation for animal feed in 1998. Inevitably, crosspollination occurred between StarLink and maize varieties destined for human consumption and StarLink had to be withdrawn.

Some other genes that are being used in these studies include those that encode inhibitors of digestive enzymes, including trypsin, other proteases and α-amylase and originate from a variety of plant sources. Although they occur naturally in many crop species, some are potentially toxic or allergenic to humans and their use in crop biotechnology may not be practical. Another group of proteins that have insecticidal properties like the plant lectins. These proteins occur naturally in many kinds of beans, but most are toxic to animals, causing the clumping of erythrocytes, reduced growth, diarrhea, interference with nutrient absorption, pathological lesions and hemorrhages in the digestive tract, amongst other symptoms. However, not all lectins are toxic to animals and one such that retained its insecticidal properties would have potential in biotechnology. Another group of proteins that are being investigated for their use in imparting insect resistance are the chitinases, enzymes that degrade chitin. Chitin is a polysaccharide present in fungal cell walls and chitinases are believed to have evolved as a defense against fungal attack. However, chitin is also present in the exoskeleton of insects and although naturally occurring chitinases are not present in sufficient quantities to kill a grazing insect, it might be possible to increase their level by genetic modification to the point where they would cause lesions in the midgut membrane. However, the above approaches are being developed and tested in plant but none have yet been used in a commercial crop variety.

Now more than 40 different genes containing insect resistance have been incorporated into transgenic crop with several commercialize in different countries such as USA and Australia. However, two major concerns over the use of insect resistance crops first is it is mandatory to set aside non transgenic refuges when growing Bt cotton varieties, maize and potato and second concern is their potential effect on non target organism.

Plant virus resistance: Viruses cause significant losses in most major food and fibre crops worldwide. To control virus infection a range of strategies have been used including chemical treatment to kill virus vectors, identification and introduction of natural resistance genes from related species and use of diagnostics and indexing to ensure propagation of virus free starting material. Commonly two methods have been used for developing virus resistance plants; the first of these arose from studies on the phenomenon of cross protection, in which infection by a mild strain of a virus induces resistance to subsequent infection by a more virulent strain. Modifying a plant with a gene that encodes the viral proteins (coat protein, replicase and defective replicase) has been found to mimic the phenomenon.

Table 3: Insect-pest resistance GM crops

Gene	Crop	Resistance to	Originating company	References
Cry3A	Potato	Colorado leaf	Monsanto	Schwember (2008)
Cry1Ac	Cotton	Tobacco budworm	Monsanto	Schwember (2008)
Cry1Ac + *Cry2Ab*	Cotton	Cotton bollworm, Pink bollworm	Monsanto	Schwember (2008)
Cry1Ab	Corn, Rice	European corn borer, striped stem borer (*Chilo suppressalis*)	Monsanto	Schwember (2008) and Kiani *et al.* (2009)
Cry1Fa	Corn	European corn borer	Pioneer Hi-Bred International	Schwember (2008)
Cry3Bb1	Corn	Corn rootworm	Monsanto	Schwember (2008)
Cry1Ac	Walnut	Insect resistance	-	McGranahan *et al.* (1988), Dandekar *et al.* (1989,1994) and
Cry1Ac and *ICP* gene	Apple	Insect resistance	-	Dandekar (1991)

A good practical application of this technology comes from the papaya industry in the Puna district of Hawaii (Ferreira *et al*., 2002; Gonsalves, 1998). After an epidemic of papaya ringspot virus (PRSV) in the 1990s almost destroyed the industry, growers switched to a virus-resistant GM variety containing a gene that encodes a PRSV coat protein. The second method used to impart virus resistance is to use antisense or co-suppression techniques to block the activity of viral genes when the virus infects a plant. The New Leaf Plus potato variety discussed above, for example, carried a replicase gene from potato leaf role virus (PLRV) in combination with the Bt insect-resistance trait. This technology is being applied to many other plant virus diseases and just one example of resistance being achieved, at least under trial conditions, is with potato tuber necrotic ringspot disease (Racman *et al*., 2001) (Table 4). It has tremendous potential for developing countries where losses to viral diseases are the greatest and have the most severe consequences.

Resistance to fungal pathogen: Plants react to attack by fungal and other pathogen by activating a series of defence mechanisms, both locally and systematically throughout the plant. Local resistance may appear as a hypersensitive response in which a local necrotic lesion restricts the growth and spread of a pathogen. Systemic resistance which may take several hours or days to develop provides resistance to pathogens in parts of the plant remote from the initial site of infection and longer term resistance to secondary challenge by the initial pathogen and also unrelated pathogens. The strategy used to genetic engineer resistance to fungal pathogens often depends on the nature of host-pathogen interaction. For example in biotrophic fungal pathogen a specific R gene approach can be used since there is often a gene for gene interaction between pathogen and host and natural or modified R gene may be transferred to other genotypes of the same species or to other species which may confer resistance to the race of pathogen which they recognized in the host plant. However, necrotrophic fungal pathogens which kill tissues in advance of hyphal invasions, other approach are required. These include induction of systemic acquired resistance, production of a range of antifungal proteins (Broekaert *et al*., 1997) or introduction of gene which can degrade fungal toxins. Examples

- Genes for toxins inactivation (e.g., HM 1)
- Gene encoding antifungal proteins
- Gene encoding PR proteins (e.g., Chitinase, β 1, 3-glucanase)

Table 4: Plant virus resistance GM crops

Gene	Crop	Resistance to	Reference
Cp gene	Grape	Arabis mosaic virus (ArMV)	Golles *et al*. (2000), Spielmann *et al*. (2000)
Cp gene	Citrus	Citrus Tisteza Virus (CTV)	Piestun *et al*. (2000), Moore *et al*. (2000)
PRSV replicase gene	Papaya	Papaya ringspot virus (PRSV)	Chen *et al*. (2001)
Cp gene	Papaya	Papaya ringspot virus (PRSV)	Cheng *et al*. (1996), Yeh *et al*. (1998)
Cp gene	Apricot	Plum Pox Virus (PPV)	Da Camara Machado *et al*. (1992, 1994)

- Genes that will activate the systemic acquired resistance response
- Artificially induced hypersensitive reaction

In general, the approaches which involve transformation of plants with genes for anti fungal proteins do not give complete resistance to fungal pathogens. As a result, it is envisaged that stacking of such resistance gene will be required to provide effective fungal resistance. This may be achieved by multiple transformations or by joining the coding sequences of different antifungal protein genes with linkers for peptide recognized by proteases, such that the anti fungal proteins are translated as one polyproteins and subsequently cleaved to their separate active constituents by protease digestion (Table 5).

Modified oil content: Plant oils are normally stored as triacylglycerols, with fatty acid and glycerol separated in downstream processing. Oil crops are second in importance to cereal food source for human and provide many industrial products. Lauric acid, for example, is used in cosmetics and detergents. Palmitic acid, stearic acid and oleic acid are used in foods, while linolenic acid is used in health products. Erucic acid is poisonous but is used in the manufacture of plastics and lubricating oils. GM crop varieties with modified oil content are already on the market in the USA. Calgene, subsequently taken over by Monsanto, genetically modified an oilseed rape variety to produce high levels of lauric acid in its oil. It contains a gene from the Californian Bay plant that encodes an enzyme that causes premature termination of growing fatty acid chains. The result is an accumulation of the 12-carbon chain lauric acid to approximately 40% of the total oil content, compared with 0.1% in unmodified oilseed rape. Lauric acid is a detergent traditionally derived from coconut or palm oil.

The other major crop that has been modified to increase the value of its oil is soybean. The GM variety was produced by PBI, a subsidiary of DuPont; it accumulates oleic acid, an 18-carbon chain fatty acid with a single unsaturated bond (a monounsaturate) to

Table 5: Fungal resistance GM crops

Gene	Crop	Resistance to	Reference
Chitinase gene	Apple,Guava	Apple scab	Mehlenbacher (1995), Hanke *et al.* (2000) and Gupta *et al.* (2010, 2011)
AMP (antimicrobial peptide) gene	Apple	Apple scab	De Cubber *et al.* (2000)
PD (Plant defensin) gene	Apple, Pear, Guava	Fungal/microbial resistance	Dolgov *et al.* (1999), Gupta (2011) In press and Gupta *et al.* (2011),
RCC 2 (rice chitinase gene)	Grape	Powdery mildew and Anthracnose	Yamamoto *et al.* (2000)

approximately 80% of its total oil content, compared with approximately 20% in non- GM varieties. In conventional soybean, relatively little oleic acid accumulates because it is converted to linoleic acid, an 18-carbon chain fatty acid with two double bonds (a polyunsaturate), by an enzyme called a Δ^{12}-desaturase. Some of the linoleic acid is further desaturated to linolenic acid, a polyunsaturated with three double bonds. In the GM variety, the activity of the gene producing this enzyme is reduced so that oleic acid levels are increased while linoleic and linolenic acid levels are decreased.

Oleic acid is very stable during frying and cooking and is less prone to oxidation than polyunsaturated fats, making it less likely to form compounds that affect flavor. The traditional method of preventing polyunsaturated fat oxidation involves hydrogenation and this runs the risk of creating trans-fatty acids. Trans-fatty acids contain double bonds in a different orientation to the cis-fatty acids present in plant oils. They behave like saturated fat in raising blood cholesterol, contributing to blockage of arteries. The oil produced by high-oleic acid GM soybean requires less hydrogenation and there is less risk of trans-fatty acid formation. Relatively small amounts of these GM oilseed rape and soybean varieties are grown on contract, but those farmers who can get into this business benefit from a premium price for their crop (Halford, 2006).

Edible vaccines: Edible vaccines producing by plants hold great promise as a cost-effective, easy-to-administer, easy-to-store and socioculturally readily acceptable vaccine delivery system, especially for the poor developing countries. This is possible by cloned gene encoding immunogenic subunits of pathogen proteins to express in transgenic plants. These transgenic plants have a permanent capacity to express the vaccines. Transgenic material, in the form of seed or fruit, can be easily stored and transported from one place to another without fear of its degradation or damage. Furthermore, a large amount of bio-mass can be easily produced by cultivation in fields with relatively few inputs. Edible vaccines are currently being developed for a number of human and animal diseases.

Hiatt *et al.* (1989) attempted to produce antibodies in plants which could serve the purpose of passive immunization. Though the first report on production of edible vaccine appeared in 1990 in the form of a patent application (Mason and Arntzen, 1995), the concept of edible vaccine got impetus after Mason *et al.* (1992). Expressed hepatitis B surface antigen in tobacco in 1992 to produce immunologically active ingredient via genetic engineering of plants. Various foreign proteins including serum albumin, human a -interferon, human erythropoetin and murine IgG and IgA immunoglobulins have been successfully expressed in plants[6]. In recent years, several attempts have been made to produce various antigens and antibodies in plants (Mason and Arntzen, 1995; Ma and Hein, 1995). Antigens or antibodies expressed in plants can be administered orally as any edible part of the plant, or by parenteral route (such as intramuscular or intravenous injection) after isolation and purification from the plant tissue. The antigens in transgenic plants are delivered through bio-encapsulation, i.e., the tough outer wall of plant cells which protects them from gastric secretions and finally break up in the intestines. The antigens are released, taken up by M cells in the intestinal lining that overlie peyer's patches and Gut-associated Lymphoid Tissue (GALT), passed on to macrophages, other antigen-presenting cells; and local lymphocyte populations, generating serum IgG, IgE responses, local IgA response and memory cells which would promptly neutralize the attack by the real infectious agent.

Oral tolerance is an accepted mechanism leading to immune tolerance (Faria and Weiner, 2005). Other attempts to create an edible vaccine were examined using potato and banana fruits with expression of the hepatitis B surface antigen (Richter *et al.*, 2000; Kumar *et al.*, 2005). Takagi *et al.* (2005) reported GM rice expressing two T-cell epitopes derived from *Cry* j I and *Cry* j II as a fusion protein with the seed protein glycine to counter Japanese cedar (*Cryptomeria japonica*) pollen allergy. Oral feeding of GM rice to mice prevents the production of allergen-specific IgE and IgG antibodies andinhibits the productionof allergen-induced Th2 cytokine, IL-4, IL-5 and IL-13. Histamine release level was also low when compared those in mice with non transformed rice. Ma *et al.* (2005) reviewed pharmaceutical proteins for potential medical use derived from plant. Vaccines for diarrhea, hepatitis B and rabies and antibodies for nn-Hodgkin's lymphoma, colorectal cancer and dental caries have been submitted for phase I or phase II clinical trials in human.

BIOSAFETY REGULATIONS OF GMOs

The countries participating on the Earth Summit in 1992 have agreed upon the fact that biotechnology can offer indubitable benefits to sustainable development, world food supplies and economic prosperity. In order this potential to be largely applied worldwide and with particular emphasis in developing countries and thus facilitating the alleviation of poverty, the countries joined their efforts in preparation of international rules which would both ensure the further development of biotechnology for the benefit of the society at large and the conservation of genetic resources, especially in the centers of origin (mostly situated in the third world). The international rules reflected both in the Cartagena protocol on biosafety and the WTO agreements are built on scientific basis and promote the case-by-case approach, i.e., every transgenic event should undergo separate risk assessment and potential hazards, specific to this event should be identified and specific risk management measures assigned. The specific international agreements that threat different aspects of the products of modern biotechnology (GMOs) however, as any other international instruments, are results of negotiations and compromises. In the last years, there are some tendencies in the negotiations of a few international instruments e.g., the Cartagena protocol on biosafety and the Aarhus convention on public participation that diverge from the initial idea of the Earth Summit, by taking into consideration only the eventual negative effects that might be associated with the deliberate release into environment of the products of modern biotechnology. Some countries and nongovernmental organizations have expressed their willingness for stricter liability regimes that are in the position to hinder the development of public research in the countries, particularly developing countries and countries with economies in transition. The policy makers in these countries should take into account the fact that public research is always oriented respond to a specific problem in the country' s agriculture or medicine and has a clear social benefit driven feature. Moreover, the scientific problematic of the public research is not addressed by the multinational biotech companies which products are mostly in commodity crops that are able to bring fast and considerable profits. In the past years several international instruments that consider different aspects of the trade, transboundary movement and potential adverse effects for the environment of GMOs have been agreed. In most of the cases, closer interaction and cooperation, as well as further harmonization among these agreements would be recommendable. Unified stricter regimes may lose on flexibility and would not be able to satisfy the needs and interests of every country, particularly developing countries. Other than relying on international instruments e.g., the Cartagena Protocol on Biosafety, the countries all over the world are highly encouraged to develop their own national biosafety frameworks (GEF project) that would better reflect the countries' needs in terms of import- export of the products of modern biotechnology. There are several models of such national regulations, overviewed in this paper that may be effective in building up a workable biosafety system. Which model to be chosen depends on the policy of the given country and should be in accordance with its international and regional obligations. It is commonly understood that international and regional harmonization, in addition to synchronizing the national regulatory frameworks, should focus on the issues of strengthening capacities and information sharing for biotechnological safety. Many countries, especially in the developing world, need to acquire the technology and the capacities necessary to sustainably handle the results of modern biotechnology. Therefore, public awareness, education and technology transfer play an important role. A number of international organizations such as FAO, WHO, UNEP, UNIDO, OECD, ICGEB and CGIAR, as well as the shown examples of regional cooperation are in the position to offer necessary assistance in capacity-building and dissemination of information on biosafety.

ENVIRONMENTAL BENEFITS AND RISKS

Genetically modified plants have been rapidly adopted globally in the past seven years. Planting transgenic plants bring huge benefits to the society and the environment, by increasing yield and protecting environment, by reducing usage of toxic chemicals, efficient use of renewal resources, efficient use of arid land and improving environment and monitoring, detecting detoxifying environmental pollution. But before release into commerce, genetically modified crops are first assessed for possible risk, including risks to the environment. Potential environmental risks associated with gene flow, risks associated with the allergy or toxic to human being, beneficial insects or non target organisms, risk associated with directly switching transgenic plants to superweeds, risk associated with increasing use of herbicides and the pest resistant to Bt plant are the major concern of the day. Both the benefits and the risk of transgenic plants may very spatially and temporarily on a case-by-case basis and to compare transgenic plants with traditional plants and other agricultural practices for elucidating the relative benefits and risk of the transgenic plants.

CONCLUSION

Recombinant DNA technology opens the door to changing agricultural crops in ways not previously possible. These changes can results in plant that are better able to survive insect-pest attack and abiotic stress, can be enhanced nutritional value, or can be used to immunize human and animal. Over the past 12 years, biotech crop area has increased more than 67-fold, making GM crops one of the most rapidly adopted farming technologies in modern history (James, 2007).

Herbicide tolerant crops and Bt crops cover almost all the global area cultivated with GM crops. Glyphosate is the world's most used herbicide due to its safety and effectiveness at controlling hundreds of different kinds of weeds. Other herbicide resistant GM crop such as glufosinate are not get success as glyphosate resistant crops, probably because the herbicide is more expensive and less effective at killing a broad range of weeds. Bt crops reduced insecticide usage, providing benefits for human health and the environment. Yields of Bt cotton and Bt corn have been increased, especially in developing countries. Apart from herbicide-tolerant and insect resistant GM crops, other genetically engineered agronomic traits are currently being developed, such as fungal resistance, drought tolerance, salt tolerance and nematode resistance. But acceptance of transgenic crops for routine production has to do assurance of safety. The speculations about risk of transgenic are not always true. The transgenic risk is low and can be further reduced if proper bio-safety regulations are followed prior to release of transgenics on commercial scale. Thus, the genetic engineering is powerful tool and has a great potential in upgrading the genetic potential of crops. However, the new techniques of biotechnology must be considered as an important supplement to the existing technologies for plant breeders and in no way a substitute to conventional breeding.

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