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Transgenes is Approaches for Crop Improvement

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Abstract – Crop improvement is an activity in which useful characteristics dispersed in numerous individuals are picked and inserted together in host to produce a variety containing as many as desirable attributes as possible (10). Recognizing valuable traits and incorporating them into future generations is very important in plant breeding. Advances in biotechnology have made it possible to identify and modify genes controlling specific characteristics (52). Increasing world population and food demands require world agricultural production be increased by 50% by 2030. In the meantime, climate change and shrinking environmental resources are limiting agricultural production over the world (32). These challenges bring an urgent need to enhance crop productivity. To breed crops with increased yield and resistance to environment stresses, a pivotal consideration is how to effectively utilize genetic diversity. Traditional plant breeding uses crossing, mutagenesis and somatic hybridization for genome modification to improve crop traits. It introduces new beneficial alleles from crossable species. Traditional plant breeding is time-consuming due to crossing barriers and linkage drag and it requires several generations of breeding and selection (23).

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The complete set of genetic material of an organism, i.e., the entire DNA contained in an organism, is called a genome. The process of isolating gene(s) from the genome of one organism and inserting the same into the genome of another organism is known as Genetic Engineering. In nature, exchange of genes happens only between compatible or closely related species. However, the modern technique of genetic engineering facilitates the removal of group of genes from one species and insertion into another, there being no need for compatibility. The transfer process involves shifting the desired gene from the chromosome of a particular plant or animal or any other organism into a cell. This genetically modified cell is then regenerated to produce a 'genetically modified organism' (GMOs). The modified organism passes the new gene onto its progeny. Such methods are now being used to create GM plants, of desired quality, growth and strength. Basic idea is to have plant varieties with high yield, pest/ disease resistant, or other such qualities mainly for better marketability and durability. This is different from the processes of modifying crops/plants from their wild ancestors through selective breeding or mutation breeding, which have been practised by farmers as part of their regular farming activity.(62)

Genetic modification is a biological technique that effects alterations in the genetic machinery of all kinds of living organisms. GMO is defined as follows by WHO (World Health Organization): "Organisms (i.e. plants, animals or microorganisms) in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and/or natural recombination" (60). The genesis of DNA modification technology can be traced back to 1944, when scientists discovered that genetic material can be transferred between different species (62). The first genetically modified plants antibiotic resistant tobacco and petunias were produced by three independent research groups in 1983 (5,13,21). Scientists in China first commercialized genetically modified tobacco in early 1990s. In 1994 the US market saw the first genetically modified species of tomato with the property of delayed ripening approved by the Food and Drug Administration (FDA). Since then, several transgenic crops have received FDA approvals, including "Canola" with modified oil composition, cotton and soybeans resistant to herbicides, etc. GM foods that are available in the market include potatoes, eggplants, strawberries, carrots, and many more are in pipeline (4).

Transgenic breeding uses molecular cloning techniques to identify cloned or synthesized genes of interest and directly transforms the recipient genome. This process manipulates plant genomes through insertion of gene or genes from another species. An organism that is generated this way is considered to be a genetically modified organism (GMO). Transgenic plants have been created with better tolerance against natural stresses, and to produce biofuels, vaccines, and antibodies (1). Many plant species, including major crops such as rice, soybean, maize, cotton, canola, potato, cassava, squash, papaya, groundnut, oilseeds, and numerous vegetables and fruits have foreign genes inserted in their genomes. However, transgenic crops have brought considerable concerns about their safety and impact on health and the environment. This is because exogenous genes from other species such as bacteria, or synthesized DNA such as DNA borders are transferred to plant genomes, and the sites of insertion are random, which may have unpredictable side effects (23).Biotechnology as such could not be exploited to its full potential because of several reasons, the major one being its acceptance by the consumers due to biosafety issues. Scientists have been continuously looking for its alternatives where biosafety issues do not exist.

In order to generate transgenic plants, the aim is to introduce a new trait to the plant which does not occur naturally in the species. Examples in food crops include resistance to certain pests, diseases, or environmental conditions, reduction of spoilage, or resistance to chemical treatments (e.g. resistance to a herbicide), or improving the nutrient profile of the crop. Examples in non-food crops include production of pharmaceutical agents, biofuels, and other industrially useful goods, as well as for bioremediation. Transgenic plants are the ones, whose DNA is modified using genetic engineering techniques. The aim is to introduce a new trait to the plant which does not occur naturally in the species. A transgenic plant contains a gene or genes that have been artificially inserted. The inserted gene sequence is known as the transgene, it may come from an unrelated plant or from a completely different species. The purpose of inserting a combination of genes in a plant is to make it as useful and productive as possible. This process provides advantages like improving shelf life, higher yield, improved quality, pest resistance, tolerant to heat, cold and drought resistance, against a variety of biotic and abiotic stresses. Transgenic plants can also be produced in such a way that they express foreign proteins with industrial and pharmaceutical value. Plants made up of vaccines or antibodies (Plantibodies) are especially stricing as plants are free of human diseases, thus reducing screening costs for viruses and bacterial toxins (20). The first transgenic plants were reported in 1983. Since then, many recombinant proteins have been expressed in several important agronomic species of plants including tobacco, corn, tomato, potato, banana, alfalfa and canola (17). Tobacco plants were generally used, however potatoes and bananas are also considered, for the purpose of vaccines for human beings.

DEVELOPMENT OF TRANSGENICS:

Genetically engineered plants are generated in a laboratory by altering the genetic make-up. The nucleus of the plant-cell is the target for the new transgenic DNA. Genes for the development of the transgenics are transferred by indirect (vector mediated) and direct (gene gun, electroporation, microinjection etc.) methods Most genetically modified plants are generated by *Agrobacterium tumefaciens* mediated transformation or by the biolistic method (Particle gun method). (44)

Agrobacterium tumefaciens-mediated transformation:

Agrobacterium tumefaciens is a Gram-negative soil phytopathogenic bacterium which causes crown gall disease in plants and it can be grown in vitro in simple culture media (6). This disease is established by neoplastic growth caused by the integration of the transferred DNA (T-DNA) obtained from specific DNA fragments (Ti) plasmid into the plant nuclear genome (14). This feature is widely used in plant biotechnology, and *Agrobacterium* is the most important tool employed to produce transgenic plants (2). T-DNA integration for *Agrobacterium* includes two main steps: firstly, the T-strand is converted to a double-stranded form; and secondly, the host cell DNA repair machinery mediates the double-stranded T-DNA integration into double strand breaks in the host genome (14). Briefly, the following steps of host genetic transformation facilitated by *Agrobacterium* are:

- √ The induction of *Agrobacterium*'s virulence machinery results in expression and activation of the virulence genes (*vir* genes), some of these genes are important in the transfer of T-DNA from the bacterium to host cell, whereas others helps in targeting T-DNA to the nucleus and most likely to the precise integration site in the host cell for T-DNA (54).
- √ This mobilises a single stranded DNA segment from the (Ti) plasmid which usually contains one T-DNA region. However, in some cases, a Ti plasmid may comprise multiple T-DNA regions (38).
- √ The transferred DNA (T-DNA), bounded by two (25) bp direct repeat sequences defined as right and left borders (RB and LB), it is termed the T-strand, and the DNA present in between the border sequences is transferred to the receiver plant nucleus.
- √ In addition, VirD2, linked with VirD1, forms a nuclease able to remove the T-strand by a strand-replacement mechanism, at the completion of which VirD2 remains covalently linked to the 5-end (RB) of the T-strand (38).

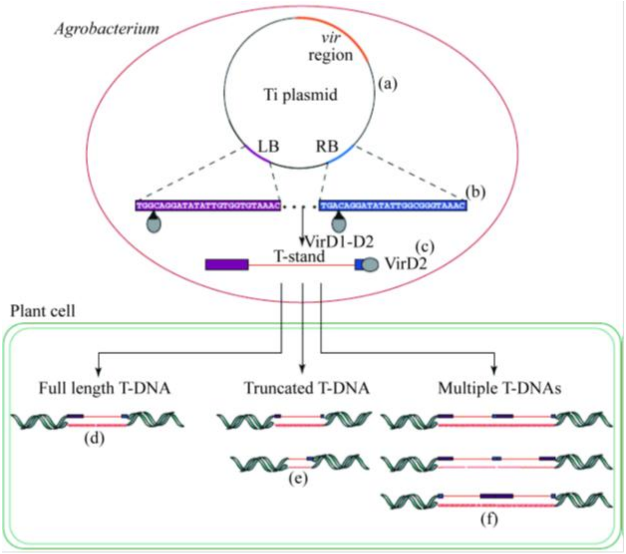

Fig: Structure representation of the *Agrobacterium* Ti-plasmid and integrated T-DNA molecules. (a) The T-DNA section includes left border (LB), right border (RB) and the virulence (*vir*) region. (b) The borders of T-DNA are 25-bp repeats and works as targets for the VirD1 - VirD2 endonuclease complex (c) As single-stranded DNA molecule, the (T-strand) is released and T-DNA typically take part into the host genome (d) In various orientationsas a single full-length or (e) truncated molecule in addition to (f) multiple molecules joined to each other. (45)

This method has been found to be challenging due to the low copy number and large size of Ti plasmids, leading to difficulties in plasmid manipulation and isolation, and it limited in the range of plant species that can be transformed because not all tissues or species are susceptible to *Agrobacterium* (53,58). This method may not be the ideal for transformation large DNA fragments. Beside some drawbacks for *Agrobacterium* transformation, this method still works in many labs and high transformation rate frequencies could be obtained.(45)

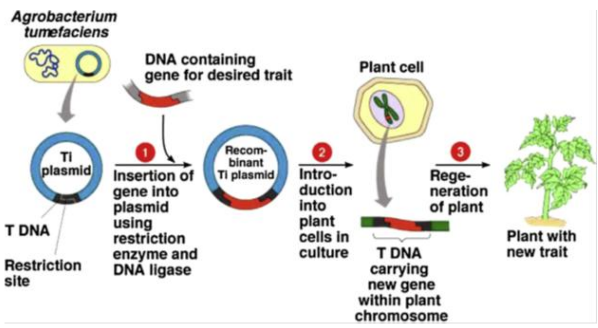

Fig: *Agrobacterium* mediated transformation (44)

DIRECT GENE TRANSFER

The development of novel direct gene transfer methodology, by-passing limitations imposed by *Agrobacterium*-host specificity and cell culture constraints, has allowed the engineering of almost all major crops, including formerly recalcitrant cereals, legumes and woody species. Direct gene transfer transformation methods are species and genotype-independent in terms of DNA delivery, but their efficiency is influenced by the type of target cell, and their utility for the production of transgenic plants in most cases depends on the ease of regeneration from the targeted cells, as most methods operate on cells cultured *in vitro*. As direct gene transfer referred methods such as particle bombardment, DNA uptake into protoplasts, treatment of protoplasts with DNA in the presence of polyvalent cations, fusion of protoplasts with bacterial spheroplasts, fusion of protoplasts with liposomes containing foreign DNA, electroporation-induced DNA uptake into intact cells and tissues, silicon carbide fiber-induced DNA uptake, ultrasound-induced DNA uptake, microinjection of tissues and cells, electrophoretic DNA transfer, exogenous DNA application and imbibition, macroinjection of DNA (Barcelo and Lazzeri, 1998· Walden and Schell, 1990).

Out of direct gene transfer techniques, particle bombardment and protoplast transformation are today the most widely used. The particle bombardment most closely satisfies the criteria of technical simplicity and reproducibility, although it requires a specialized particle gun, the commercial version of which uses relatively expensive consumables. Protoplast transformation can be highly efficient, but demands more complicated cell culture techniques and is limited by the difficulty of regenerating plants. Tissue electroporation is relatively simple, applicable to regenerable tissues and has produced stably transformed plants in several systems after only a relatively short period of development. These results suggest the method should receive further attention to evaluate its potential for wider application. Ultrasound and silicon carbide fiber-mediated techniques are newer methods, which are again technically quite simple. They have been tested in few laboratories and need more research to determine their limitations. Microinjection and laser-mediated transformation are specialized techniques, which are at present inefficient.

- **Particle bombardment**

Particle bombardment is a commonly used method for genetic transformation of plants and other organisms. Gene guns which are also known as biolistics or particle guns “shoot” target genes into plant cells. It is the most common method. DNA is bound to tiny particles of gold or tungsten which are subsequently shot into plant tissue or single plant cells under high pressure. The accelerated particles penetrate both the cell wall and membranes. The DNA separates from the metal and is integrated into plant DNA inside the nucleus. In a typical experiment, transient gene expression averaged nearly 8000 “hits” per bombarded plate. Five months after bombardment, there were nearly five putative transgenic embryos per bombarded plate. About half of the embryos were regenerated into confirmed transgenic plants. This method has been applied successfully for many cultivated crops, especially monocots like wheat or maize, for which transformation using *Agrobacterium tumefaciens* has been less successful. The major disadvantage of this procedure is that serious damage can be done to the cellular tissue.(42)

- **Electroporation**

Electroporation refers to a technique that utilizes short, high-intensity electric fields to permeabilize reversibly the lipid bilayers of cell membranes. It is widely believed that the electric pulse causes extensive compression and thinning of the plasmalemma. The resulting transient formation of pores permits free diffusion of DNA. As a method of DNA transfer, electroporation is convenient. In most cases it is more efficient than other methods designed for the same purpose, such as particle bombardment. In addition, it does not suffer from host-range limitations imposed by biology-based systems such as those employing *Agrobacterium tumefaciens* or toxicity problems sometimes encountered using a polyethylene glycol based procedure.(42)

- **Microinjection:**

When cells or protoplasts are used as targets in the technique of microinjection, glass micropipettes with 0.5-10μm diameter tip are used for transfer of macromolecules into the cytoplasm or the nucleus of a recipient cell or protoplast. The recipient cells are immobilized on a solid support (cover slip or slide, etc.) or artificially bound to a substrate or held by a pipette under suction (as done in animal systems). Often a specially designed micromanipulator is employed for microinjecting the DNA. Although, this technique gives high rate of success, the process is slow, expensive and requires highly skilled and experienced personnel. The microinjection method was introduced by two groups of scientist led by Crossway and Reich in l986. Recently a method known as "holding pipette method" was introduced. In this, the protoplasts are isolated from cell suspension culture are placed on a depression slide, by its side with a microdroplet of DNA solution. Using the holding pipette, the protoplast has to be held and the DNA to be injected into the nucleus using the injection pipette. After the micro injection the injected cells are cultured by hanging droplet culture method.

- **Other techniques for plant transformation:**

Plant Protoplast can be transformed with naked DNA by treatment with **Polyethylene Glycol** in the presence of divalent Cation (Ca2+). They both help in destabilizing the plasma membrane of plants and make it convenient for DNA penetration. Once the DNA enters the nucleus, it gets integrated into the genome. **In Silicon carbide fiber** plant material is introduces into a buffer containing DNA and silicon carbide fibers. The fibers penetrate the cell wall and plasma membranes allowing the DNA to gain access to inside of the cell (52).

ROLE IN CROP IMPROVEMENT:

It is very important to know about role of transgenics in crop improvement. Transgenics are made by inserting genes of other species into their DNA. Though this kind of genetic modification is used both in plants and animals, it is found more commonly in the former than in the latter. Genetically engineered crops can be grown at places with unfavourable climatic conditions too. A normal crop can grow only in specific season or under some favourable climatic conditions. Though the seeds for such foods are quite expensive, their cost of production is reported to be less than that of the traditional crops due to the natural resistance towards pests and insects. This reduces the necessity of exposing transgenic crops to harmful pesticides and insecticides, making these foods free from chemicals and environment friendly as well (4). There is clear evidence that the use of transgenic crops has resulted in significant benefits. These include:

√ Higher crop yields

√ Reduced farm costs

√ Increased farm profit

√ Improvement in health and the environment

These “first generation” crops have proven their ability to lower farm-level production costs. Now, research is focused on “second-generation” GM crops that will feature increased nutritional and/or industrial traits. These crops will have more direct benefits to consumers.

Examples include:

√ Rice enriched with iron and vitamin A

√ Potatoes with higher starch content

√ Edible vaccines in maize and potatoes

√ Maize varieties able to grow in poor conditions

√ Healthier oils from soybean and canola

Bt-based genetically modified crop plants developed for commercial use

Crop	Trade name	Bt-protein	Resistance to insect(s)
Cotton	Bollgard	Cry 1Ac	Cotton bullworm, tobacco budworm
Maize	YieldGard knockout	Cry 1Ab	European corn borer
Maize	Starlink	Cry 9C	European corn borer
Maize	Harculex I	Cry 1F	European corn borer
Maize	Bt-Xtra	Cry 1Ac	European corn borer
Potato	New-leaf	Cry 3A	Colarado beetie

A selected list of plant insectisidal (non-Bt) genes used for developing transgenic plants

Plant gene	Transgenic plant(s)	Encoded protein	Resistance to insect(s)
Protease inhibitor			
CpTi	Potato, apple, rice, sunflower, wheat, tomato	Trypsin	Coleoptera, Lepidoptera
CII	Tabacco, potato	Serine protease	Coleoptera, Lepidoptera
PI-IV	Tabacco, potato	Serine protease	Lepidoptera
OC-1	Tobacco, oilseed rape	Cysteine protease	Coleoptera, Homoptera
CMe	Tabacco	Trypsin	Lepidoptera
α-Amylase inhibitors			
α-A1-Pv	Pea, tobacco	α-Amylase	Coleoptera
WMAI-1	Tabacco	α-Amylase	Lepidoptera
Lectins			
GNA	Potato, rice, sugarcane, sweet potato, tobacco	Lectin	Homoptera, Lepidoptera
WGA	Maize	Agglutin	Lepidoptera, Coleoptera
Others			
BCH	Potato	Chitinase	Homoptera, Lepidoptera
TDC	Tabacco	Tryptophan decarboxylase	Lepidoptera

Example of gene transferred herbicide resistant plants

Herbicide	Gene transfer/mechanism of resistance	Transgenic crop(s)
Glyphosate	Inhibition of EPSPS	Soybean, tomato
Glyphosate	Detoxification by glyphosate oxidase	Maize, soybean
Phosphinothricin	*bar* gebe coding phosphinothricin acetyltransferase	Maize, rice, wheat, cotton, potato, tomato, sugarbeet
Sulfonylureas/imidazolinones	Mutant plant with acetolactate synthase	Rice, tomato, maize, sugarbeet
Bromoxynil	Nitrilase detoxification	Cotton, potato, tomato
Atrazine	Mutant plant with chloroplast *psb A* gene	Soybean
Phenocarboxylic	Monooxygenase detoxifaction	Maize, cotton
cyanamide	Cyanamide hydratase gene	Tobacco

A selected list of transgenic plants developed along with the genes transferred and the resistance provided against the pathogens

Crop	Gene (s) transferred	Resistance against pathogen
Pathogenesis-related (PR) proteins		
Tabacco	Chitinase from bacterium (*Serratia marcescens*)	*Alternaria longipes*
	Bean chitinase	*Rhizoctonia solani, Phytophthora parasitica*
	Chitinase and 1,3-β glucanase	*Cercospora nicotinae*
Rice	Chitinase	*Rhizoctonia solani*
Carrot	Chitinase and 1,3-β glucanase	*Alternaria dauci, A. radicina*
Tamato	Chitinase and 1,3-β glucanase	*Fusarium oxysporum*
Brassica napus	Chitinase	*Rhizoctonia solani*
Antimicrobial protins		
Tobacco	Barley ribosome inactivating protein	*Rhizoctonia solani*
Tobacco	Defensin from radish	*Alternaria longipes*
Tobacco	α-Thionin gene from barley	*Pseudomonas syringae*
Potato	Bacteriophage T-4 lysozyme	*Erwinia carotovora*
Phytoalexins		
Rice	Stilbene synthase	*Pyricularia oryzae*
Tobacco	Stilbene synthase	*Botrytis cinerea*

List of transgenic crops for commercial crops

Crop plant	Genetically altered trait	Product name
Cotton	Insect resistance	Bollguard
	Glyphosate resistance	Roundup ready
	Bromoxil resistance	BXN
Maize	Insect resistance	Yield Guard
	Insect resistance	Maximizer
	Glyphosate resistance	Roundup ready
	Glufosinate resistance	Liberty Link
Rice	Vitamin A enrichment	Golden Rice
Tomato	Delayed ripening	Flavr Savr
	Delayed ripening	Endless Summer
Soybean	Glyphosate resistance	Roundup ready
Potato	Insect resistance	New leaf
Oilseed rape (canola)	Glufosinate resistance	Innovator
	Glyphosate resistance	Roundup ready
	High lauric acid	Laurical
Squash	Virus resistance	Freedom II

Some examples of transgenic crop plants at the developmental stages

Plant	Gene transfer	Trait transferred/application(s)
For improving human health		
Tomato	Phytoene desaturase	Provitamin A (β-carotene) supplement
Canola	ϒ-Tocopherol methyl transferase	Vitamin E supplement
Sugar beet	Sucrose-sucrose fructosyl transferase	Fructans-low calorie alternatives to sucrose
Rice	Ferritin	Iron supplement
Potato	Antisense threonine synthase	Increased methionine levels
Potato	Seed albumin	Protein with all essential amino acids
Tomato	S-Adenosylmethionine decarboxylase	Increased lycopene levels
Tomato	Chalcone isomerase	Flavanols- act as antioxidants, reduce risk of cancer, heart diseases
Arabidopsis	Isoflavone synthase	Isoflavone- reduces serum cholesterol, and reduces osteoporosis.
Canola	Modified acyl-acyl carrier protein thioesterase	*cis*-Stearates- lower the risk of heart diseases
For increased crop yield		
Rice	Phosphoenol pyruvate carboxylase	Increased efficiency of photosynthesis
Tobacco	Phytochrome A	Avoids shades
Lettuce	Gibberellic acid (GA) oxidase	Inhibits GA accumulation and stem growth (dwarfing)
Potato	Phytochrome B	Increased photosynthesis and longer life span

TRANSGENIC PLANTS AND SAFETY:

The production of transgenic cultivars through genetic engineering is a new departure from conventional breeding to modern technology which raises safety concerns. Crops produced through genetic engineering are formally examined critically to ensure that they do not possess non-congenial characteristics before field testing or commercial release (1). The safety assessment of transgenic plants is a fascinating and challenging intersection of many disciplines including ecology, agronomy and molecular biology which mainly focus on food and environmental safety (9). Other potent risks considered in the assessment of transgenic plants particularly for insect or disease resistance traits include environmental consequences on worms, insects, birds, mammals and other organisms. Since 1986, a formal policy namely Coordinated Framework for Regulation of Biotechnology provides a system for evaluating products developed using modern protocols. The National Institutes of Health (NIH) has devised stringent rules and regulations on the judicious proper use and disposal of GM plants. In addition, other principal agencies to date are the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHS), the Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) which provide guidelines for the testing and commercial release of transgenic organisms (51). All government regulatory agencies should be fully responsible for ensuring that the GM crops do not harm the environment and human health.

CURRENT GM CROP STATUS IN INDIA:

India had a slight decrease (7%) in biotech cotton planting brought by a small reduction in the total cotton area (8%) in the 10 states of India. Adoption however increased from 95% to 96% indicative of acceptance by as much as 7.2 million farmers benefiting from the technology. Biosafety regulations in the country have been streamlined with revised guidelines on the monitoring of confined field trials of biotech crops. Biotech mustard expressing the barnase-barstar gene is under final review including public comments for environmental release in 2017. Mustard production and yields have remained stagnant for the past 20 years and the future introduction of the biotech mustard can potentially increase yield by as much as 25%, revive the mustard industry and be competitive with canola. Insect resistant chickpea and pigeon pea were approved for field trials by the government regulatory agency in 2016. India retained the title as the number one cotton-producing country in the world with cotton production surpassing 35 million bales despite the slowed down global cotton market.(25)

FUTURE PERSPECTIVE:

The advent of genetic engineering modifies the plant to produce reasonable amounts of the products rich in nutrition and safe to humankind and envionment. Transgenic plants have been generated for their enhanced tolerance to herbicides and pests. Some others have been developed for providing nutritionally rich food and biofuel production. Healthier oils, vegetables and fruits with low calorie sugars and enriched with vitamins are under development. Golden rice is a genetically modified crop. It is rich in provitamin A (β-carotene) and iron. Many parts of the world experience insufficient levels of essential vitamins and minerals such as vitamin A and iron. Golden rice is the promising crop to overcome this problem. Golden rice is being tested these days in India, Vietnam and the Philippines for its ability to effectively produce high levels of vitamin A and iron. High protein potatoes have also been developed in India by transferring a gene from an amaranth plant. Despite the controversies by many countries on transgenic crops agricultural biotechnology has yielded substantial economic benefits. According to a projection by Brookes and Barfoot (2011) the generation of GM crops has allowed to use 393 million kg less pesticides by the growers. This effect has a significant role in reducing greenhouse gas emission which in 2009 was equivalent to removing 7.8 million cars from the roads. Due to the present growing trend of transgenic crops, it is assumed that available transgenic crops in the future could boost crop yield, and the food produced from such crops will be nutritionally rich. Valuable proteins are expressed in transgenic plants that can be extracted and processed, which have many advantages over industrial proteins.

Though plant-based vaccines have shown promising results, the oral tolerance to plant vaccines is a very important problem that needs in depth research. The genetic engineered plants being used need strict safety evaluation. The plant biotechnologists should keep in mind that the transformants that they are going to develop should be safe enough. Apart from the success stories in many cases, many concerns are yet to be mitigated before plant based vaccines become a real boom. The world most dangerous diseases like HIV and malaria are very complex diseases. Plant-based vaccines have been found to be very promising in controlling these diseases effectively, but since all these studies have been carried out to a limited scale, so for their effective widespread use, up-scaling of these studies is essential. Furthermore, although a number of vaccines for many diseases are provided by the WHO, there are certain diseases for which the vaccines have to be purchased locally. For example, hepatitis-B/DTP combination vaccines are to be purchased from the local market and the cost of the vaccines is too high. Resultantly, thousands of children are deprived of vaccination and hence at the risk of this preventable disease.

To eradicate this problem transgenic plants may provide an excellent expression system and the vaccines can be fed directly to people in the form of edible vegetables, fruits etc. Plants like banana, tomato, potato, spinach, tobacco, rice, corn, etc. are being used to fight diseases like cholera, measles, hepatitis-B, Norwalk virus and rabies virus by inducting immunization edible vaccines. Edible plant vaccines are highly safe as well as cost-effective. Undoubtedly, there is a consistent increase in the use of genetically modified organisms for food or other essential commodities. The promoters of GM foods claim that they are environment-friendly, have no risk to human health, profitable for farmers as well as well regulated, many people are still of the firm view that GM foods can be injurious to human and animal health, because they have not been properly tested. Thus, every country needs to frame well defined rules and regulations for the utilization of GM organisms, although many developed and some developing countries have already formulated specific regulations.

SUMMARY

The classical methods of alien gene transfer by traditional breeding yielded fruitful results. However, modern varieties demand a growing number of combined traits, for which pre-breeding methods with wild species are often needed. Introgression and translocation breeding require time-consuming backcrosses and simultaneous selection steps to overcome linkage drag. Breeding of crops using the traditional sources of genetic variation by cisgenesis can speed up the whole process dramatically, along with usage of existing promising varieties. New biotechnology is making cisgenesis increasingly feasible in use of gene resources and precisely obtaining new agricultural traits without insertion of foreign genes or gene fragments. The generation of cisgenic crops is still very limited and has been reported in apple, in pear, in barley, and in potato. Application of cisgenic techniques enhances the possibility to introgress the preferred genes into the novel cultivars (mostly single gene in the first step), without disturbing their favorable characteristics. Thus, if we expand our area of research towards cisgenic approach and if it has been exempted from the regulatory framework of GM technology it is anticipated that cisgenesis may wipe out the likely uncertain outcomes and the social beliefs that public have in their mind regarding GM technology. Therefore, cisgenesis will be playing an important role in sustainable crop improvement.

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