

Biotechnology has opened unprecedented avenues for exploring biological systems. One of the key techniques in genetic engineering is gene transfer, which involves transfer of recombinant DNA into plant cells to generate transgenic plants. Genes and genomes from a wide range of organisms are being manipulated for the benefit of mankind. Application of genetic engineering in agriculture has produced significant achievements such as yield improvement of major food crops.

Introduction

Gene is the fundamental unit of heredity in any organism. Mendel first proposed this in 1865 but he called it 'factor'. Later, in 1909 Johansson renamed 'factor' as gene. Gene is an active segment of DNA. The isolation and manipulation of an organism's genome for the betterment of mankind is termed genetic engineering, a fast growing science in the field of biotechnology (*Box 1*). One among the products of genetic engineering is transgenics.

Simply, *transgenics* is transferring foreign *genes* from one organism to another organism of interest. The transgenic plants and animals derived thus are frequently referred to as genetically modified organisms (GMOs). The first transgenic plant was tobacco — developed in 1983. There are more than 50 other plant species where foreign genes have been transferred viz., tomato, potato, sunflower, cotton, carrot, grapes, etc. (*Box 2*). Initially, the production of transgenics was restricted to dicotyledonous plants for the reasons detailed below. But, now it has been extended to several monocots like wheat, maize, rice, oats, etc.

Why Transgenics?

Classical genetics, as applied to plant breeding, cannot be separated from other improvements in agricultural production.

Transgenics is transferring foreign genes from one organism to another organism of interest.



Box 1. Milestones in Transgenic Science

- 1951 First amino acid was sequenced by F Sanger and H Tuppy.
- 1953 JD Watson and F H C Crick proposed that DNA is a double helix.
- 1958 Discovery of DNA polymerase by Kornberg's group and DNA replication studies began.
- 1959 Isolation of RNA polymerase.
- 1970 Discovery of reverse transcriptase showed that RNA can act as the template for the transcription of DNA and opened the way for cloning using cDNA libraries.
- 1970 General method was developed by M Mandel and A Higa for introducing DNA into cells by calcium dependent method.
- 1970 G H Khorana synthesised an artificial gene from DNA nucleotides.
- 1971 K Danna and D Nathans first used restriction endonucleases for mapping DNA.
- 1972 The recombinant DNA Era begins. J Mertz and R W Davis used T4 ligase to join DNA molecules.
- 1973 A DNA fragment containing the Kannamyin-resistance gene was cut out of plasmid using EcoR1 and ligated into the unique EcoR1 site of the plasmid pSC101 that was tetracycline resistant. Doubly resistant clones were isolated.
- 1973 Cohen and his co-workers invented cloning.
- 1975 E M Southern detected specific DNA sequences in gels.
- 1976 cDNA cloning of eukaryotic genes – the rabbit β -globin gene.
- 1977 A M Maxam, W Gilbert and Sanger and others gave the first DNA sequencing method.
- 1983 Ti plasmid are used as vectors for transformation of plant cells by Herrera–Estrella and co-workers.
- 1983 First plant species (*Nicotiana*) was transformed using *Agrobacterium tumefaciens* system by Baston and others.
- 1984 Biolistic process was invented by E D Wolf, N K Allen and J C Sanford.
- 1984 First transformation was performed in tobacco using direct gene transfer technique by Paszkowski and others.
- 1985 The polymerase chain reaction (PCR) technique was first used by R K Saiki, G Mullies, F Faloona and their co-workers to artificially multiply DNA sequences.

It has been estimated that genetic improvement alone accounts for 50% of the increased harvest.

Conventional breeding techniques aim to introduce genetic diversity into desired plants so as to evolve superior plants. This involves many time-tested procedures. However, evolving elite varieties takes comparatively a very long time, even up to 8 years. Further, in conventional breeding, the transfer of desirable genes from unrelated or wild relatives is limited for any of the reasons below:

- failure of pollen germination



Box 2. A list of few transgenic plants

Crop	Genes transferred	Purpose
Tobacco	<i>B. thuringiensis</i>	Insect resistance
	CpTI	Insect resistance
	Chitinase gene	Disease resistance
	<i>aro A</i>	Herbicide resistance
	Farnesyl diphosphate synthase gene of <i>Saccharomyces cerevisiae</i>	Increase in sterol and carotenoid synthesis
Tomato	<i>Bt</i>	Insect resistance
	<i>aro A</i>	Herbicide resistance
	Sucrose phosphate synthase	Increased sucrose and reduced starch
Potato	<i>Bt</i>	Insect resistance
	Bacteriophage T-4 lysozyme	Disease resistance
	Bacterial ADP-GPPase	Increased starch and amino acid
Cotton	<i>Bt</i>	Insect resistance
Rice	<i>Bt</i>	Insect resistance
Wheat	CP4 – EPSPS and <i>gox</i>	Herbicide resistance
	High molecular weight glutenin gene	Starch content modified
Maize	<i>Bt</i>	Insect pest resistance
Sugarcane	<i>Bt</i>	Insect pest resistance
Soybean	CP4 – EPSPS	Herbicide resistance
Sugarbeet	CP4 – EPSPS	Herbicide resistance

EPSPS : 5- enolpyruvyl shikimate-3-phosphate synthase;
 CpTI : Cowpea trypsin inhibitor;
gox : glyphosate oxide-reductase;
 ADP-GPPase : Adenine diphosphate glucose pyrophosphorylase



The addition of molecular biology and genetic engineering techniques to conventional methods allows the plant breeders to accomplish objectives that are impossible through conventional plant breeding.

- reduced seed set due to slow pollen tube growth
- sexual incompatibility
- abnormality of seed development
- hybrid inviability (seedling lethality)
- hybrid sterility and
- transfer of undesirable characters from the wild species.

Genetic engineering serves as a promising alternative tool for overcoming the above limitations. The addition of molecular biology and genetic engineering techniques to conventional methods allows the plant breeders to accomplish objectives that are impossible through conventional plant breeding. The tools of biotechnology are unlikely to replace any of the time-tested methods of crop improvement. Rather, biotechnology aims to increase the efficiency of various methods of conventional breeding.

Development of transgenic plants involves the following three steps:

1. Construction of Recombinant DNA

The first step involves the purification of the desired genes (DNA sequence) and construction of a recombinant DNA. First the DNA from the donor organism is located and isolated. Isolation of genes is generally done by probing a library for genes of interest (GOI). Other methods used are T-DNA tagging, transposon tagging, positional cloning, etc. Passenger DNA is sometimes chemically synthesized from nucleotides deduced by the amino acid sequence of proteins. Normally, GOI thus identified cannot be directly transferred into the plant tissues but needs a vehicle.

The GOI have to be cloned with any one of the vectors like plasmids, bacteriophages, yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), etc. with introducing certain specific promoter and terminator sequences. Thus the recombinant DNA is constructed. All these steps involve splicing and joining of DNA molecules, which are made possible by



restriction endonucleases and ligases, respectively.

2. Transformation of Gene of Interest into Plant

Transformation of the gene of interest into a plant may be done using any one of the following methods (Figure 1).

Agrobacterium-mediated transformation: *Agrobacterium tumefaciens* is a soil bacterium and was the first to be used to transform plant tissues. The bacterium causes tumour in wounded gymnosperms and dicot angiosperms as they possess a single copy of large tumour inducing (Ti) plasmid in the cell. The Ti plasmid also contains transfer DNA or T-DNA, which transfers itself to the host and causes tumour. These tumour-causing genes are removed and our gene of interest is inserted into the T-DNA. When *A. tumefaciens* with the recombinant DNA is made to infect plant cells, the DNA gets transformed into the plant genome and expresses itself later.

Direct gene transfer: Direct gene transfer is the direct delivery of the recombinant DNA into plant protoplasts or calli either physically or chemically.

In the chemical method, the recombinant DNAs are transferred into plant protoplasts under the presence of calcium phosphate or polyethylene glycol (PEG). In the physical method called electroporation, electrical impulses of high field strengths are used to reversibly permeabilize cell membranes to facilitate the uptake of large molecules, including foreign DNA.

Another technique that has attained major importance is the biolistic approach (particle gun bombardment) developed by Sanford and others (1983). In this approach, microscopic tungsten or gold particles (4 mm) carrying the recombinant DNA are accelerated to 400 ms^{-1} and allowed to penetrate intact cell walls of calli or protoplasts.

3. Reproduction and Regeneration of Transgenic Plant Cells

The transformed cells are selected and cultured on an artificial

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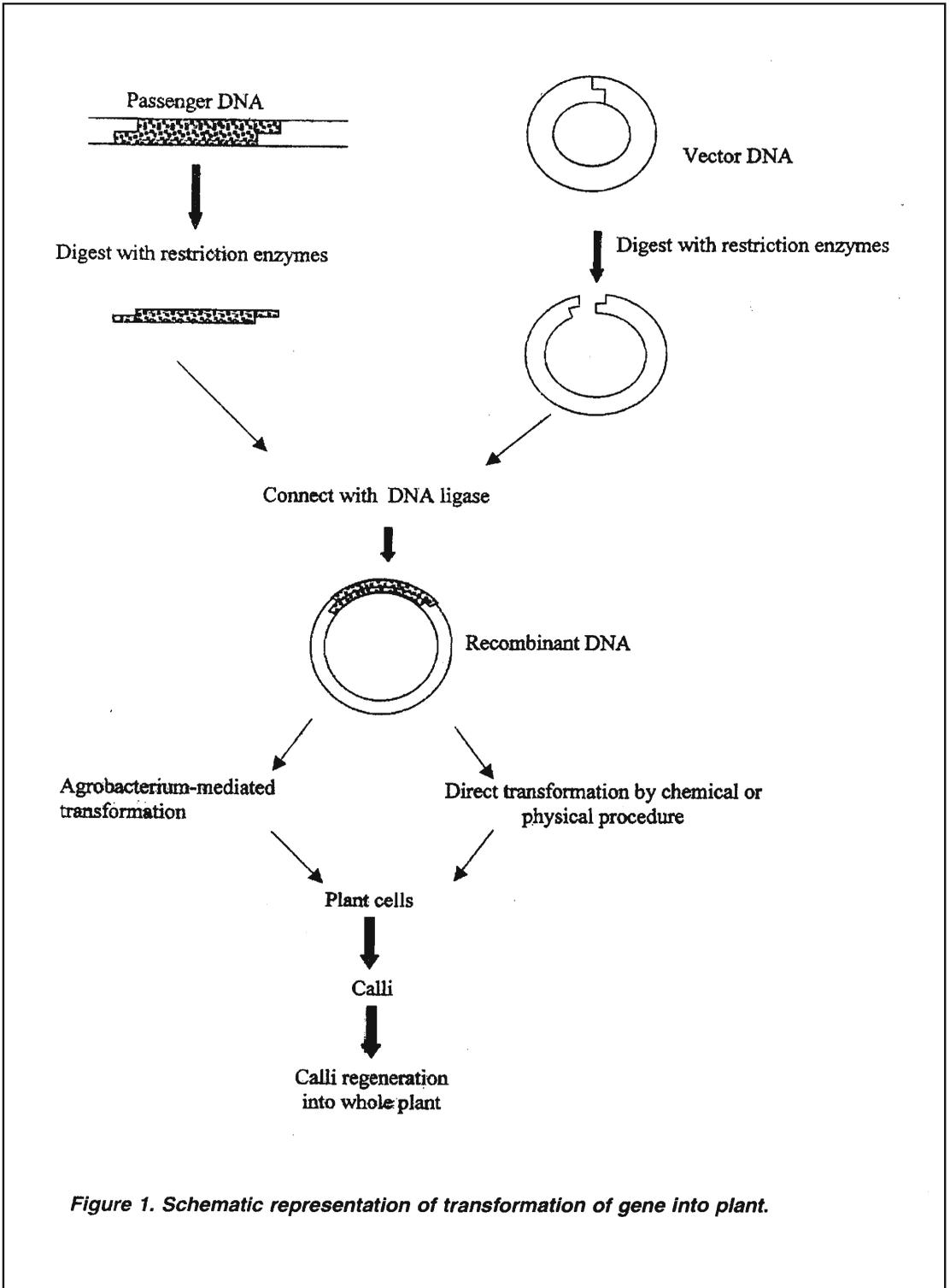


Figure 1. Schematic representation of transformation of gene into plant.

medium to grow into callus. Under the appropriate ratio of auxin and cytokinin, several plant species can be regenerated into whole plants from the calli, the phenomenon called *totipotency*.¹

Utilization of Transgenic Plants in Agriculture

Many economically important genes were engineered into crop plants worldwide. But a major contribution has been made in the field of crop protection. Here, plants transformed for insect and herbicide resistance alone are dealt in some detail.

a. *Insect-resistant Transgenic Plants Expressing Bt Toxin*

Bacillus thuringiensis (universally abbreviated as *Bt*) is a gram positive soil bacterium. The bacterium produces crystalline toxins on sporulation which are coded by *cry* genes. When consumed by the insect larvae the toxins affect the midgut epithelium resulting in death of the larvae. Different strains of *Bt* produce toxins effective against different insect species. Lepidopteran larvae are the most susceptible followed by Coleoptera and Diptera.

To produce an insect-resistant transgenic plant, the DNA sequence coding for the toxin is to be removed from the bacterium. With suitable promoter and terminator sequences recombinant DNA is constructed. This gene construct is introduced into plants using *Agrobacterium tumefaciens* as vector. Transgenic tobacco, potato and tomato plants resistant to various pests have thus been transformed.

b. *Herbicide-resistant Transgenic Plants*

Broad spectrum and non-selective herbicides are used to kill unwanted plants or weeds in agriculture. But these are equally capable of killing crop plants. Hence, crop plants resistant to broad-spectrum herbicides are to be developed so that we can spray herbicides on all weeds growing between crops. Transgenic plants resistant to many herbicides have been developed of which glyphosate is important.

All plant cells retain the ability to use all of their genes and thereby can produce any type of tissue and eventually whole plants. This ability to generate any cells from such starting tissue is the property of totipotency.

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The quality of agricultural produce has been improved utilizing the transgenic technique in many instances.

Glyphosate is a non-selective herbicide. It is a specific inhibitor of 5-enol pyruvyl shikimic acid-3-phosphate synthase (EPSPS) which catalyses one of the steps in the shikimic acid pathway. This is the key pathway, present only in plants and microorganisms, leading to the synthesis of many amino acids playing a vital role in protecting the plants against pest and diseases. As a result of inhibition by the herbicide massive accumulation of shikimic acid and the lethal depletion of end product aromatic amino acids occur.

To evolve crops resistant to glyphosate, insertion of EPSPS-insensitive genes was considered. Mutagenesis of yeast, *Saccharomyces typhimurium*, yielded a glyphosate-resistant mutant possessing an insensitive EPSPS encoded by *aroA* gene. A gene construct with *aroA* gene and octopine or monopinine synthase promoters was transferred into tobacco and tomato mediated by *Agrobacterium*. Transformed plants showed significant tolerance to glyphosate.

c. Transgenics for Improved Quality

The quality of agricultural produce has been improved utilizing the transgenic technique in many instances. Some examples are:

The shelf life and solid content of tomato fruits has been altered by inhibiting ethylene production or by decreasing polygalacturonase activity by antisense RNA technique.

Starch content of potato tubers has been increased by heterologous expression of mutated ADP-glucose pyrophosphorylase gene from *E. coli*.

In chrysanthemum, flower colour was improved using cloned pigment-biosynthesis genes.

The expression of synthetic genes encoding proteins rich in essential amino acids like lysine and methionine, along with normal protein production within the potato tuber, may increase the overall nutritional quality of the potato.

In chrysanthemum, flower colour was improved using cloned pigment-biosynthesis genes.



Limitations of Transgenics

Transgenic technology can be very attractive and has many advantages compared with other conventional procedures. But it has certain drawbacks too. Some of these are listed below.

Technical Limitations

- Transgenic expression is generally unpredictable and varies depending on the transgene copy number and site of integration.
- Transgene inactivation and silencing by the host system has emerged as a potential problem in the use of heterologous system.
- The introduced transgene sometimes suppresses endogenous gene expression by a phenomenon called co-suppression.
- Expression of the transgene may turn cytotoxic and inhibitory to host growth and metabolism.
- Somaclonal variation occurs to a certain degree in all trans-genes due to the stress induced by tissue culture and transformation procedures.
- There is a lack of technique for transferring multiple genes by transformation.
- The phenomena of recalcitrance in certain plant species limits transformation, transgene integration and regeneration of the plants.

Social Limitations

- Toxicity of the marker gene/gene product.
- Vector-mediated horizontal gene transfer and recombination to create new pathogenic bacteria.
- The spread of transgenic crops by pollen transfer to sexually compatible species may contaminate the native genetic information.
- Genetically modified plants with marker genes do limit

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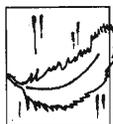


non-target insect life that comes in contact with plant exudates, such as sap or pollen.

- There are chances for transfer of genes from herbicide-resistant crops to wild or semi-domesticated relatives which would create *super weeds*.
- Insect pests could quickly develop resistance to transgenic crops.
- There are possibilities for vector recombination to generate new virulent strains of virus, especially in transgenic plants engineered for viral resistance with viral genes.
- Transgenic plants with foreign genes would surely affect various trophic level interactions through the food chain.

Suggested Reading

- [1] H S Chawla, *Biotechnology in Crop Improvement*, International Book Distributing Co., Lucknow, 1998.
- [2] R W Oldand and S B Primrose, *Principles of gene manipulation – An introduction to Genetic Engineering*, Blackwell Scientific Publications, London, 1989.



“Curiosity and the urge to solve problems are the emotional hallmarks of our species; and the most characteristically human activities are mathematics, science, technology, music and the arts – a somewhat broader range of subjects than is usually included under the ‘humanities’. Indeed, in its common usage this very word seems to reflect a peculiar narrowness of vision about what is human. Mathematics is as much a ‘humanity’ as poetry. Whales and elephants may be as ‘humane’ as humans”.

*Carl Sagan
The Dragons of Eden*