Gene Transfer Mechanism

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A plant in which a gene has been transferred through genetic engineering is called a transgenic plant. Transformation is the genetic alteration of a cell resulting from the introduction, uptake and expression of foreign genetic material (DNA or RNA).

This is a common technique in molecular biology. The effect was first demonstrated in 1944 by Oswald Avery, Collin Macleod, and Maclyn McCarty, who showed gene transfer in *Streptococcus pneumoniae*. Avery, Macleod and McCarty call the uptake and incorporation of DNA by bacteria transformation.More generally the term is used to describe mechanisms of DNA and RNA transfer in molecular biology. For example the production of transgenic plants like transgenic maize requires the insertion of new genetic information into the maize genome using an appropriate mechanism for DNA transfer.

The general approach for genetic engineeting in plants may be outlined as follows

1. Introduction of the gene of interest into the cells of concerned plant species.
2. Integration of this gene into the nuclear/organellar genome of the plant cells
3. Expression of the trassferred gene in the new genetic background
4. Regeneration of whole plants freom the genetically modified cells and
5. Transmission of the transferred gene to the sexual progney of these plants.

It may be noted that gene transfers in plants are primarily based on tissue cultureand that the itetgration and expression of the preoducrd genes must be stable to be transmitted through the sexual process.

Methods of gene transfer

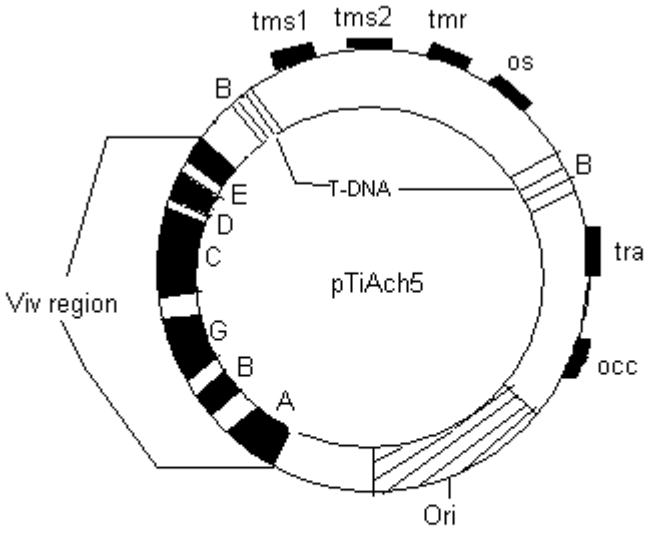
The DNA segment of the gene to be transferred may be introduced into the plant cells through one of the following methods:

1. Ti or Ri plasmids of agrobacterium used as vectors
2. Caulimoviruses or gemini viruses used as vectors,
3. Direct DNA uptake by cells
4. Polyethylene glycol (PEG) induced DNA uptake
5. Electroporation
6. Microinjection
7. Particle gun
8. Calcium phosphate precipitation and
9. Liposome mediated transfer

Of these Ti and Ri vectors have been the most successful: free DNA uptake, particle gun and microinjection have also been successful.

1. Ti plasmid: this plasmid is ofund In *Agrobacterium tumfaciens* which produces crown gall in a large number of dicot species similarly, the Ri plasmid occurs in *Agrobacterium tumfaciens* which produces the hairy root disease of several dicots and monocots. The Ti (tumour indicating) and Ri (root inducing) plasmids are essentially similat, they carry the genes for the production of tumors and roots, respectively.

Both Ti and Ri plasmids have a T- reion (the region transferred into the host genome: generally called T-DNA) and a Vir=Virulence region. The T-DNA is flanked on both the sides by the border of direct repear sequences each of 25 bp. The T-DNA has four important genes; two genes tms 1 and tms 2, govern the synthesis of IAA; one gene,tmr, determines the production of a cytokinin; while the remaining gene, *os* is concerned withn opine synthesis. The genes tms 1, tms 2, and tmr are involved in turour production ; these genes have to be removed from the T-DNA of a Ti plasmid before it can be used as a vector. The process of removal of the three tumour producing genes from T-DNA is called disarming of the Ti plasmid , and the resulting plasmid is referred to as *disarmed*.



a schematic representation of the Ti plasmid of A. tumefaciens.the T region is borded on both the sides by a direct repeat sequence of 25 bp; it contains genes tms1, tms2, tmr and Os. the vir region has at least sis operons; Vir D operon codes for an endonclease responsible for the excision of T-DNA during its integration in the host genome.

The disarmed Ti plasmid may itself be used as a vector, or it may be fused with another plasmid,eg. pBR 322, to yield a binary vector pGV 3850. Binary vectors provide certain advantages over the Ti plasmid vectors, and a number of such vectors eg. pMon 505 etc., have been created.

2. Plant viruses: techniques are being developed to use cerain DNA and RNA plant viruses eg.caulimo virus, gmeini viruses, etc. the cauliflower mosaic virus is a DNA virus. A DNA segment may be integrated into the viral DNA which suitably modified to serve as a vector and the host plants may then be infected with this especially constructed virus. Following infection the virus spreads systematically into the host plant; plant cells ussually have a high copy number of the virus (up to 10^5 virions/cell.). Therefore,each cell will also have a very high copy number or the reansferred gene.

3. Direct DNA uptake: DNA is taken up directly by plant cells, plant protoplasts, pollen grains, mature embryos and even whole seeds; in many cases a transient expression of foreign genes has also been reported. Genetic transformation through direct DNA uptake by protoplasts was first reported in tobacco. Polyethylent glycol promotes DNA uptake by protoplasts as well as the frequency of genetic transformation up to 10^{-3} .

4. Electroporation is another way to make holes in cells, by briefly shocking them with an electric field of 100-200V. Now plasmid DNA can enter the cell through these holes. Natural membrane-repair mechanisms will close these holes afterwards.A plasmid DNA molecule will usually contain an antibiotic resistance gene which is placed in a bacterial strain that has no antibiotic resistance. Therefore, only transformed bacteria can grow on a media with the antibiotic (this is known as a selection medium).

One example of this is putting in a plasmid that contains the encoding for the protein ß-lactamase, which makes bacteria resistant to ampicillin. This is called the *bla* gene. The bacterial colony is then treated with ampicillin, thus weeding out those bacteria who did not take up the plasmid with the *bla* gene. Another selection medium is bioluminescence, using a gene taken from jellyfish.In bacteria the term transformation is not normally applied to genetic changes arising by Transduction or Conjugation, in which transfer of DNA is mediated by genetic parasites (phages and conjugative plasmids respectively).

5. Particle bombardment: Coat small gold or tungsten particles with DNA and shoot them into young plant cells or plant embryos. Some genetic material will stay in the cells and transform them. This method also allows transformation of plant plastids. The transformation efficiency is lower than in agrobacterial mediated transformation, but most plants can be transformed with this method.

6. Viral transformation: Package your genetic material into a suitable plant virus and then use the modified virus for infection of the plant. Genomes of most plant viruses consist of single stranded RNA which replicates in the cytoplasm of infected cell. So this method is not a real transformation, since the inserted genes never reach the nucleus of the cell and do not integrate into the host genome. The progeny of the infected plants is virus free and also free of the inserted gene.

7. Microinjection: in this technique, the DNA introduced directly into the nuclei of plant cells/ protoplasts/pollen grains with the help of a microsyringe. It may give a very high rate of transformation eg. 14-16% in case of alfa alfa and tobacco protoplasts, but it is very tedius requires great skill and is very slow.

8. Calcium phasphate precipitation: DNA is carried with Ca++ ions through the plasma lemma into the cytoplasm, where Ca++ is precipited as calcium phosphate. The DNA may now brigh further refined and is supposed to have a great potential in the transformation of plant cells.

9. Liposome Mediated DNA uptake: the DNA is enclosede within artificial lipid vesicles, which are taken up by plant protoplasts either through fusion or phagocytosis. The technique is still in the developing phases: DNA has successfully been introduces into protoplasts of carrot, tobacco, using this technique.

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Thank you

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