Methods of Gene Transfer in Plants

Transgenic plants are those plants in which foreign genes have been introduced and stably integrated into the host DNA. It results in the synthesis of appropriate gene product by the transformed plants. The different methods of introducing foreign DNA into the plant genome have been grouped under two broad categories: A) Vector-mediated gene transfer and B) Direct gene transfer.

A) Vector-mediated gene transfer: (*Agrobacterium*-mediated gene transfer)

Agrobacterium tumefaciens and *Agrobacterium rhizogenes* are soil-borne, Gram-negative bacteria. These are phytopathogens (that cause infection in plants) and are treated as the nature's most effective plant genetic engineer. *A. tumefaciens* induces crown gall disease and *A. rhizogenes* that induces hairy root disease in plants.

Crown Gall Disease- Ti plasmid

Almost 100 years ago (1907), Smith and Townsend postulated that a bacterium was the causative agent of crown gall tumors, although its importance was recognized much later. As *A. tumefaciens* infects wounded or damaged plant tissues, it induces the formation of a plant tumor called crown gall. The entry of the bacterium into the plant tissues is facilitated by the release of certain phenolic compounds (acetosyringone, hydroxyacetosyringone) by the wounded sites.

Formation of a Crown Gall Tumor

Crown gall formation occurs when the bacterium releases its Ti plasmid (Tumor- inducing plasmid) into the plant cell cytoplasm. A fragment of Ti plasmid, referred to as T-DNA, is actually transferred from the bacterium into the host where it gets integrated into the plant cell chromosome (i.e. host genome). Thus, crown gall disease is a naturally evolved genetic engineering process. The T-DNA carries genes that code for proteins involved in the biosynthesis of growth hormones (auxin and cytokinin) and novel plant metabolites namely opines-amino acid derivatives and agropines-sugar derivatives.

The growth hormones cause plant cells to proliferate and form the gall while opines and agropines are utilized by *A. tumefaciens* as sources of carbon and energy. Thus, *A. tumefaciens* genetically transforms plant cells and creates a biosynthetic machinery to produce nutrients for its own use. As the bacteria multiply and continue infection, crown gall develops which is a visible mass of the accumulated bacteria and plant material. Crown gall formation is the consequence of the transfer, integration and expression of genes of T-DNA (or Ti plasmid) of *A. tumefaciens* in the infected plant.

Organization of Ti plasmid:

The Ti plasmids (approximate size 200 kb each) exist as independent replicating circular DNA molecules within the *Agrobacterium* cells. The T-DNA (transferred DNA) is variable in length in the range of 12 to 24 kb, which depends on the bacterial strain from which Ti plasmids come. Nopaline strains of Ti plasmid have one T-DNA with length of 20 kb while octopine strains have two T-DNA regions referred to as TL and TR that are respectively 14 kb and 7 kb in length.

The Ti plasmid has three important regions.

1. T-DNA region:

This region has the genes for the biosynthesis of auxin (aux), cytokinin (cyt) and opine (ocs) and is flanked by left and right borders. These three genes-aux, cyt and ocs are referred to as oncogenes, as they are the determinants of the tumor phenotype.

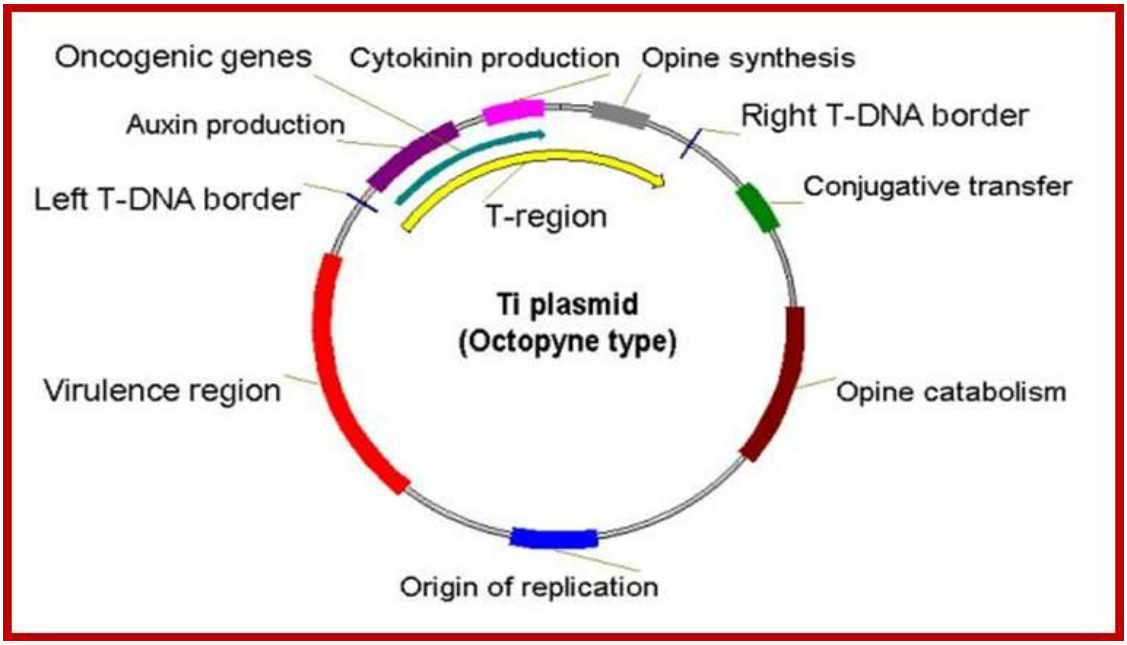
T-DNA borders — A set of 24 kb sequences present on either side (right and left) of T-DNA are also transferred to the plant cells. It is now clearly established that the right border is more critical for T-DNA transfer and tumori-genesis.

2. Virulence region or *vir* region

The genes responsible for the transfer of T-DNA into the host plant are located outside T-DNA and the region is referred to as *vir* or virulence region. *Vir* region codes for proteins involved in T-DNA transfer. At least nine *vir*-gene operons have been identified. These include *vir* A, *vir* G, *vir* B1, *vir* C1, *vir* D1, D2, D4, and *vir* E1 and E2.

3. Opine catabolism region:

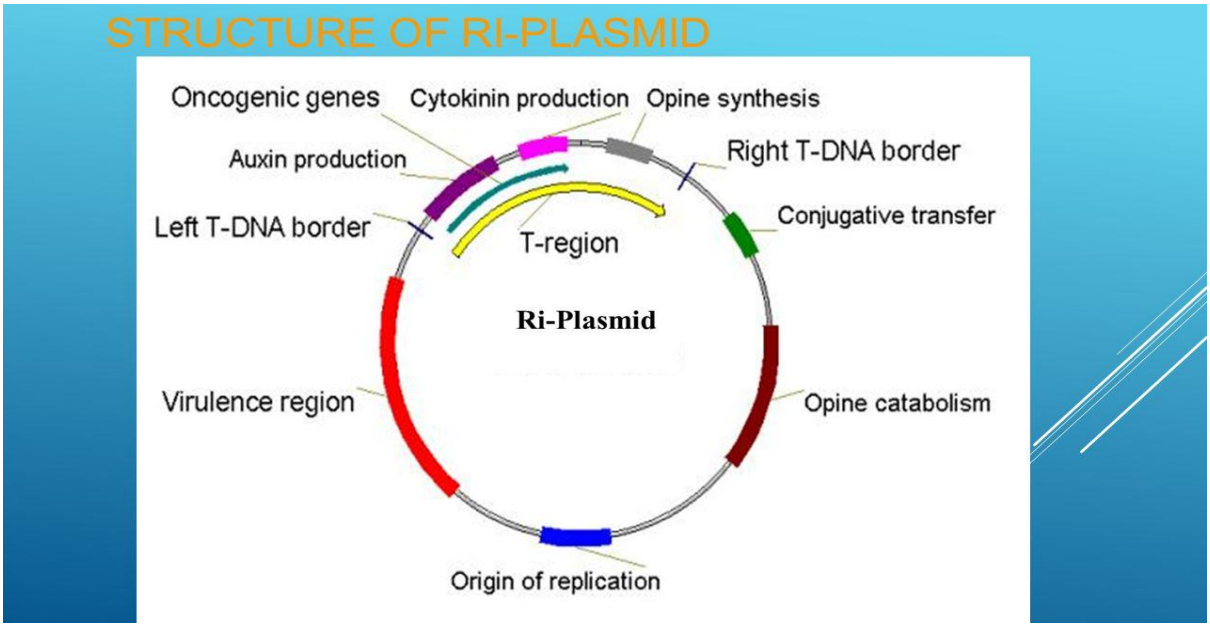
This region codes for proteins involved in the uptake and metabolisms of opines. Besides the above three, there is *ori* region that is responsible for the origin of DNA replication which permits the Ti plasmid to be stably maintained in *A. tumefaciens*.



Hairy Root Disease of *A. Rhizogenes* — Ri Plasmids:

Agrobacterium rhizogenes can infect plants that cause hairy root disease. The plasmids of *A. rhizogenes* are referred to as Ri plasmids, (Root-inducing plasmids). These are of different types. Some of the Ri plasmid strains possess genes that are homologous to Ti plasmid e.g. auxin biosynthetic genes.

Instead of virulence genes, Ri plasmids contain a series of open reading frames on the T-DNA. The products of these genes are involved in the metabolism of plant growth regulators which gets sensitized to auxin and leads to root formation.



T-DNA transfer and integration:

The process of T-DNA transfer and its integration into the host plant genome is depicted in Fig. 3 and is briefly described below:

1. Signal induction to *Agrobacterium*:

The wounded plant cells release certain chemicals- phenolic compounds and sugars which are recognized as signals by *Agrobacterium*. The signals induced result in a sequence of biochemical events in *Agrobacterium* that ultimately helps in the transfer of T-DNA of Ti-plasmid.

2. Attachment of *Agrobacterium* to plant cells:

The *Agrobacterium* attaches to plant cells through polysaccharides, particularly cellulose fibres produced by the bacterium.

3. Production of virulence proteins:

As the signal induction occurs in the *Agrobacterium* cells attached to plant cells, a series of events take place that result in the production of virulence proteins. To start with, signal induction by phenolics stimulates *vir* A which in turn activates (by phosphorylation) vir C. This induces expression of virulence genes of Ti plasmid to produce the corresponding virulence proteins (D1, D2, E2, B, etc.). Certain sugars (e.g. glucose, galactose, xylose) that induce virulence genes have been identified.

4. Production of T-DNA strand:

The right and left borders of T-DNA are recognized by vir D1/vir D2 proteins. These proteins are involved in the production of single-stranded T-DNA, its protection and export to plant cells. The ss T-DNA gets attached to vir D2.

5. Transfer of T-DNA out of *Agrobacterium*:

The ss T-DNA-vir D2 complex in association with vir G is exported from the bacterial cell. Vir B products form the transport apparatus.

6. Transfer of T-DNA into plant cells and integration:

The T-DNA-vir D2 complex crosses the plant plasma membrane. In the plant cells, T-DNA gets covered with vir E2. This covering protects the T-DNA from degradation by nucleases; vir D2 and vir E2 interact with a variety of plant proteins which influences T-DNA transport and integration.

The T-DNA-vir D2-vir E2-plant protein complex enters the nucleus through nuclear pore complex. Within the nucleus, the T-DNA gets integrated into the plant chromosome through a process referred to illegitimate recombination.

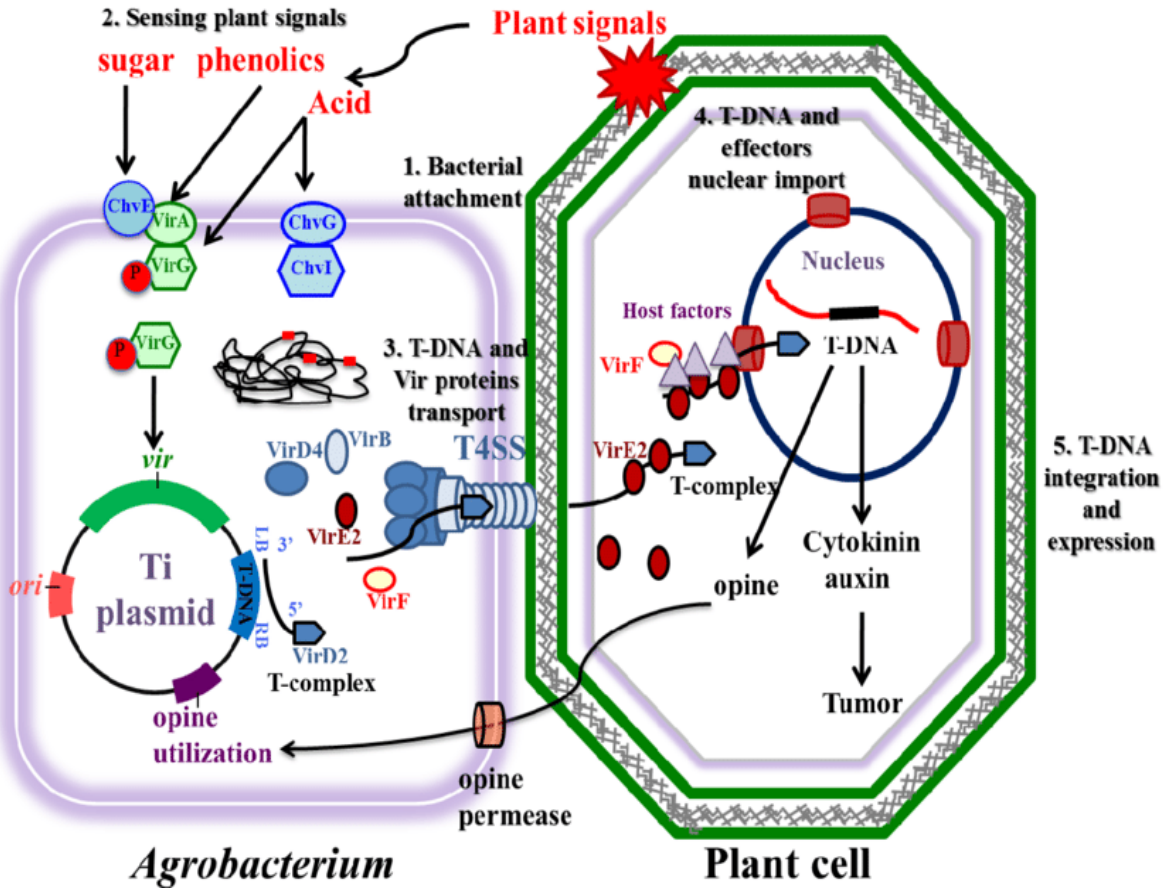


Figure 3

Modifications of Ti plasmid

Two types of Ti plasmid-derived vectors are used for genetic transformation of plants—

i) Co-integrate vectors and ii) Binary vectors.

i) Co-integrate vectors

In the co-integrate vector system, the disarmed and modified Ti plasmid combines with an intermediate cloning vector to produce a recombinant Ti plasmid.

Production of disarmed Ti plasmid:

In these Ti plasmids, the oncogenes located in the T-DNA region have been replaced by exogenous DNA.

Examples of these vectors include:

1. SEV series: the right border of the T-DNA together with the phytohormone genes coding for cytokinin and auxin are removed and replaced by a bacterial kanamycin resistance gene while the left border and a small part of the left segment (TL) of the original T-DNA (referred to as Left Inside Homology (LIH)) are left intact.
2. pGV series: the phytohormone genes are excised and substituted by part of pBR322 vector sequence. The left and right border sequences as well as the nopaline synthase gene of the Ti plasmid are conserved.

Construction of Intermediate vectors

These are small pBR322-based plasmids (*E. coli* vectors) containing a T-DNA region. They are used to overcome the problems derived from the large size of disarmed Ti plasmids and their lack of unique restriction sites. Intermediate vectors are replicated in *E.coli* and are transferred into *Agrobacterium* by conjugation. They cannot replicate in *A. tumefaciens* and therefore, carry DNA segments homologous to the disarmed T-DNA to permit recombination to form a co-integrated T-DNA structure.

Helper vectors

These are small plasmids maintained in *E. coli* that contain transfer (tra) and mobilization (mob) genes, which allow the transfer of the conjugation-deficient intermediate vectors into *Agrobacterium.*

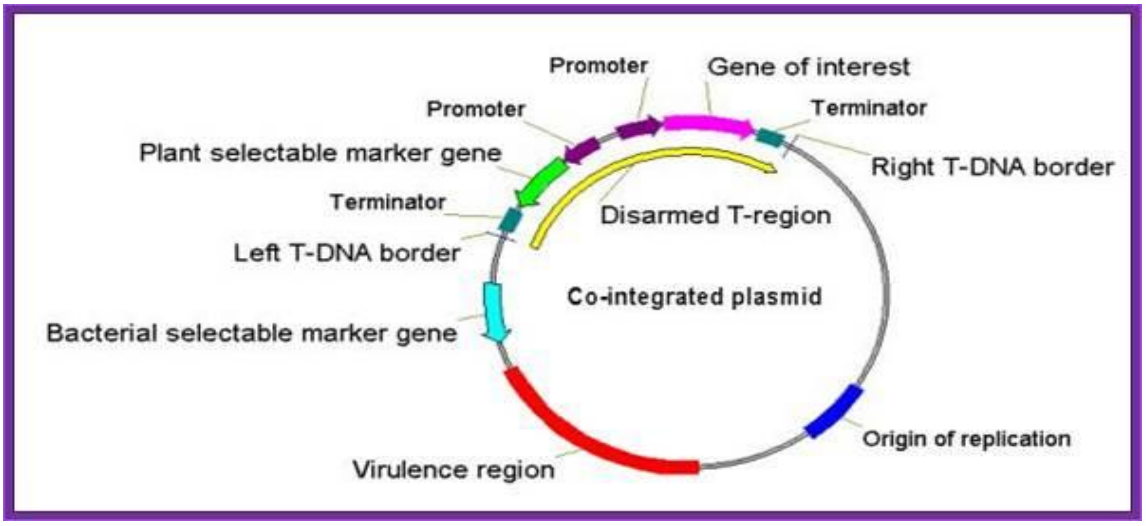
A resulting co-integrated plasmid assembled by *in vitro* manipulation normally contains:

1. the vir genes,

2. the left and right T-DNA borders,

3. an exogenous DNA sequence between the two T-DNA borders, and

4. plant and bacterial selectable markers.



Binary vector:

The binary vector system consists of an *Agrobacterium* strain along with a disarmed Ti plasmid called vir helper plasmid (the entire T-DNA region including borders deleted while vir gene is retained). It may be noted that both of them are not physically linked (or integrated). A binary vector with T-DNA can replicate in *E. coli* and *Agrobacterium.*

The binary vector has the following components.

1. Left and right borders that delimit the T-DNA region.

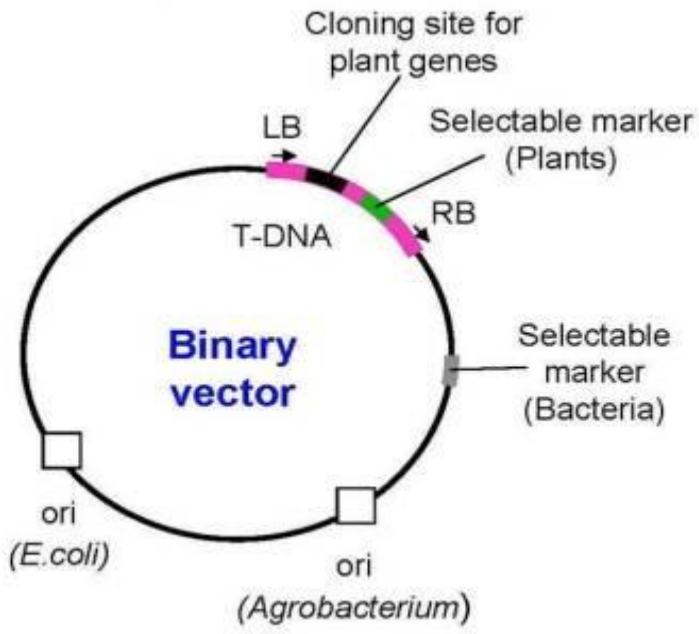
2. A plant transformation marker (PTM) e.g. npt II that confers kanamycin resistance in plant transformed cells.

3. A multiple cloning site (MCS) for introducing target/foreign genes.

4. A bacterial resistance marker e.g. tetracycline resistance gene for selecting binary vector colonies in E. coli and *Agrobacterium.*

5. oriT sequence for conjugal mobilization of the binary vector from *E. coli* to *Agrobacterium*.

6. A broad host-range origin of replication such as RK2 that allows the replication of binary vector in *Agrobacterium*.



Advantages of Agrobacterium mediated gene Transfer

1. Simple and comparatively less expensive

2. High transformation efficiency

3. Transgenic crops obtained have better fertility percentage

4. Protocols for both dicotyledons and monocotyledon are available

5. Relatively large length DNA segment can be transferred.