

GENE TRANSFER IN PLANTS, STRATEGIES & AIMS

INTRODUCTION

- It is defined simply as a technique to efficiently and stably introduce foreign genes into the genome of target cells.
- These methods are also classified in two groups : Direct method and Agrobacterium mediated gene transfer.
- Direct Gene transfer relies on the delivery of large amounts of DNA while the plant cell is transiently permeabilized by Particle bombardment, Electroporation, ultrasound, etc. It is mostly used for gene transfer in cereal crops (monocotyledonous and dicotyledonous crops).
- Agrobacterium mediated gene transfer are based on utilizing Agrobacterium, a pathogen of dicotyledonous (broad leafed) plants that transfers genes into the plant genome.



Physical

Microinjection
Pressure
Biolistics - gene gun/
particle bombardment
Electroporation
Silica/carbon fibers
Lazer mediated

Chemical

PEG
Calcium phosphate
Artificial lipids
Proteins
Dendrimers

Biological

A. tumefaciens
A. rhizogenes
Virus-mediated



STEPS

- ❖ **Advantage:** Some plants are PEG sensitive so electroporation may be of choice, method is fast, less costly and high percentage of stable transformants can be produced.
- ❖ **Application:** it has been used to produce stably transformed cell lines and plants of several species like tobacco, maize, rice, wheat, etc. In tobacco frequency is as high as 2-8% in presence of 7% PEG.

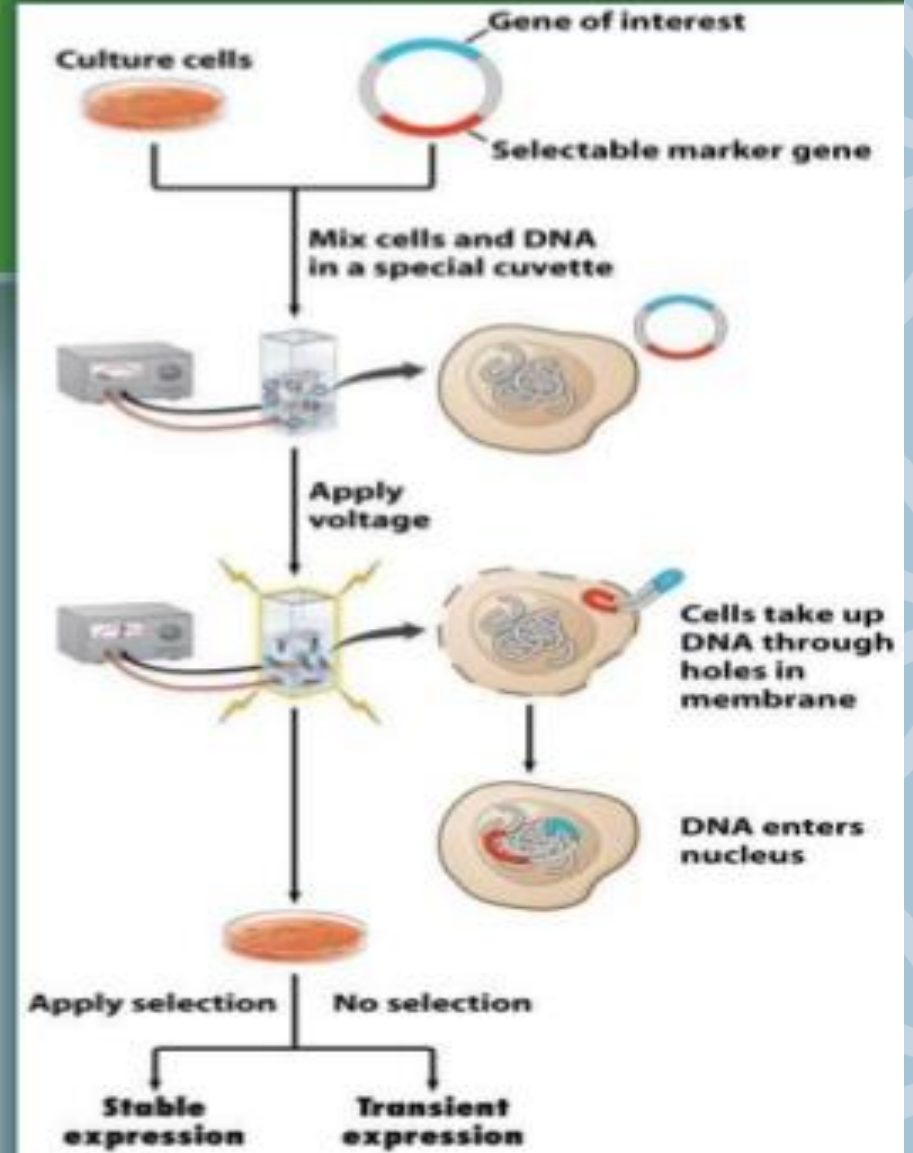


Figure 6-8
Genes and Genomics: A Short Course (3e)
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TI & RI PLASMID

WHAT IS T-DNA?

- T DNA stands for transfer DNA
- It derives its name from the fact that the bacterium transfers this DNA fragment into the host plant's nuclear DNA genome.
- It is a part of Ti plasmid (Tumor inducer plasmid) of the bacillus *Agrobacterium tumefaciens*
- *Agrobacterium tumefaciens* is a phytopathogenic, gram-negative, non sporing, motile bacillus.
- It is found in the rhizosphere
- It causes crown gall disease in plants, a disease of dicotyledonous plants characterized by a tumorous phenotype.



T1 PLASMID AND VIRULENCE GENES

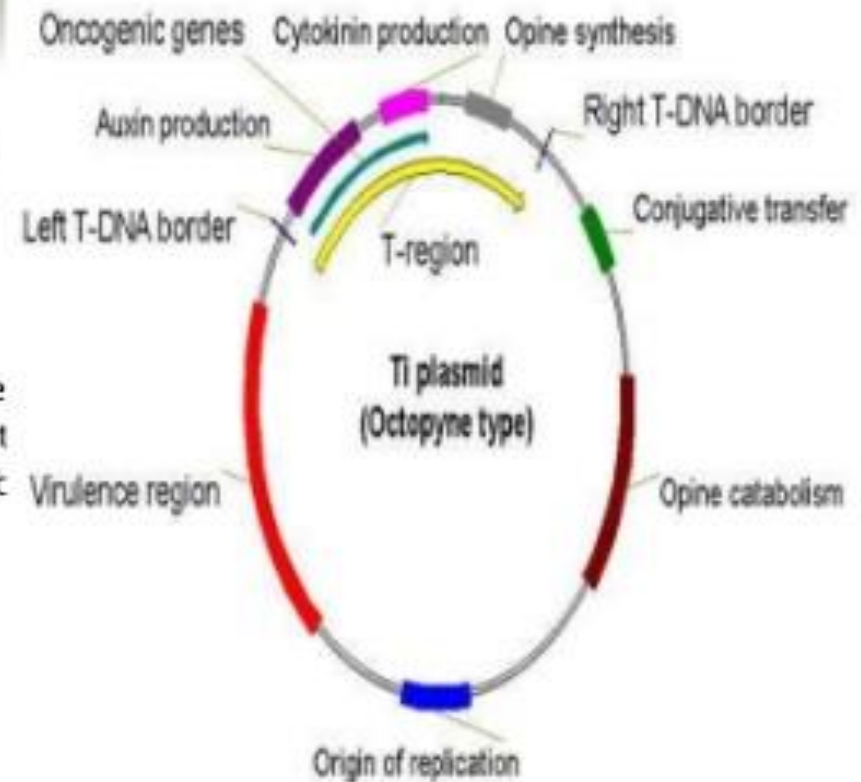
● A Ti plasmid is a circular piece of DNA found in almost all *Agrobacteria*.

● The size of plasmid is around 200 kb.

● Only T-DNA incorporates with the host genome and it is 24 bp small fragment.

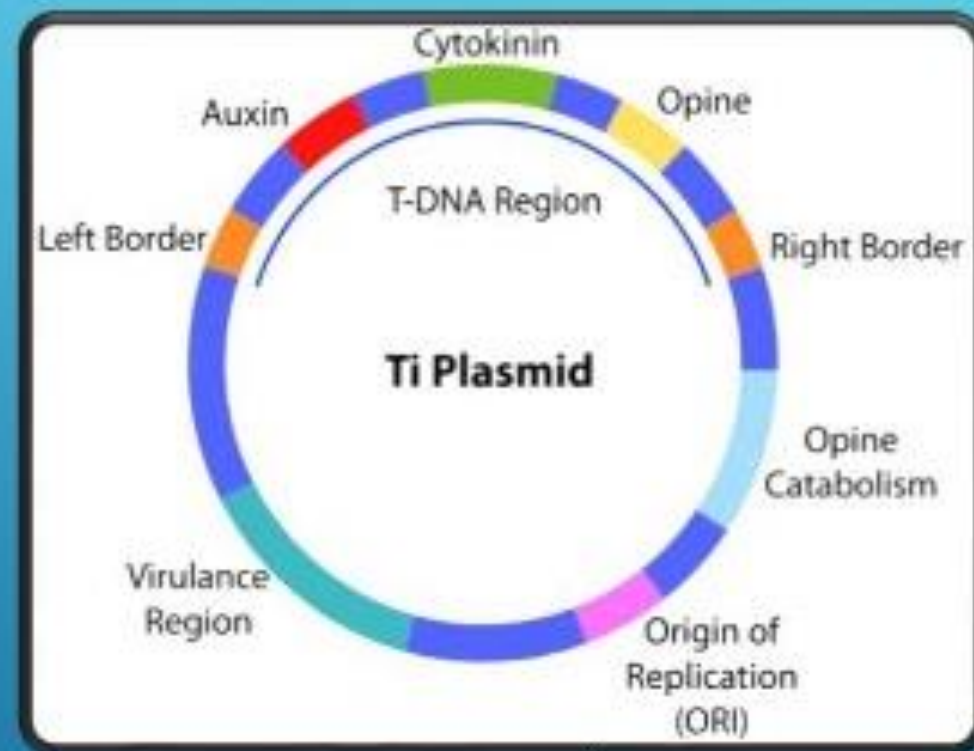
● Three main regions

1. T-DNA region (Between right and left T-DNA border one or more- 24bp imperfect repeats and has right and left border. Any DNA within the borders will be transferred into host genome.)
2. Virulence region (*vir*)
3. Opine catabolism region
4. Origin of replication
5. A region enabling conjugative transfer.



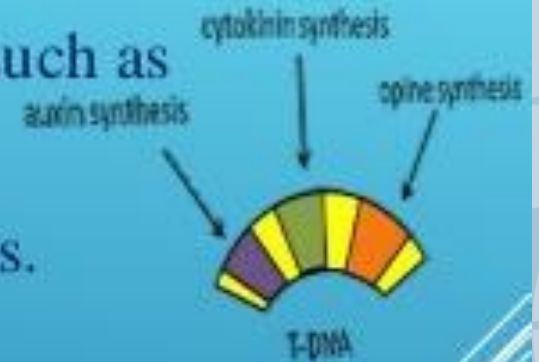
FEATURES OF TI PLASMID

- ❖ It is responsible for crown gall disease
- ❖ It has 200 kb
- ❖ It has 8 genes (*vir*, *shi*, *roi*, *nos*, *noc*, *ocs*, *occ* and *ori V*)
- ❖ A part of Ti-plasmid, T DNA, integrates into the plant chromosomal DNA
- ❖ The T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the right border and terminated at the left border and requires the *vir* genes of the Ti plasmid.



MECHANISM OF T DNA

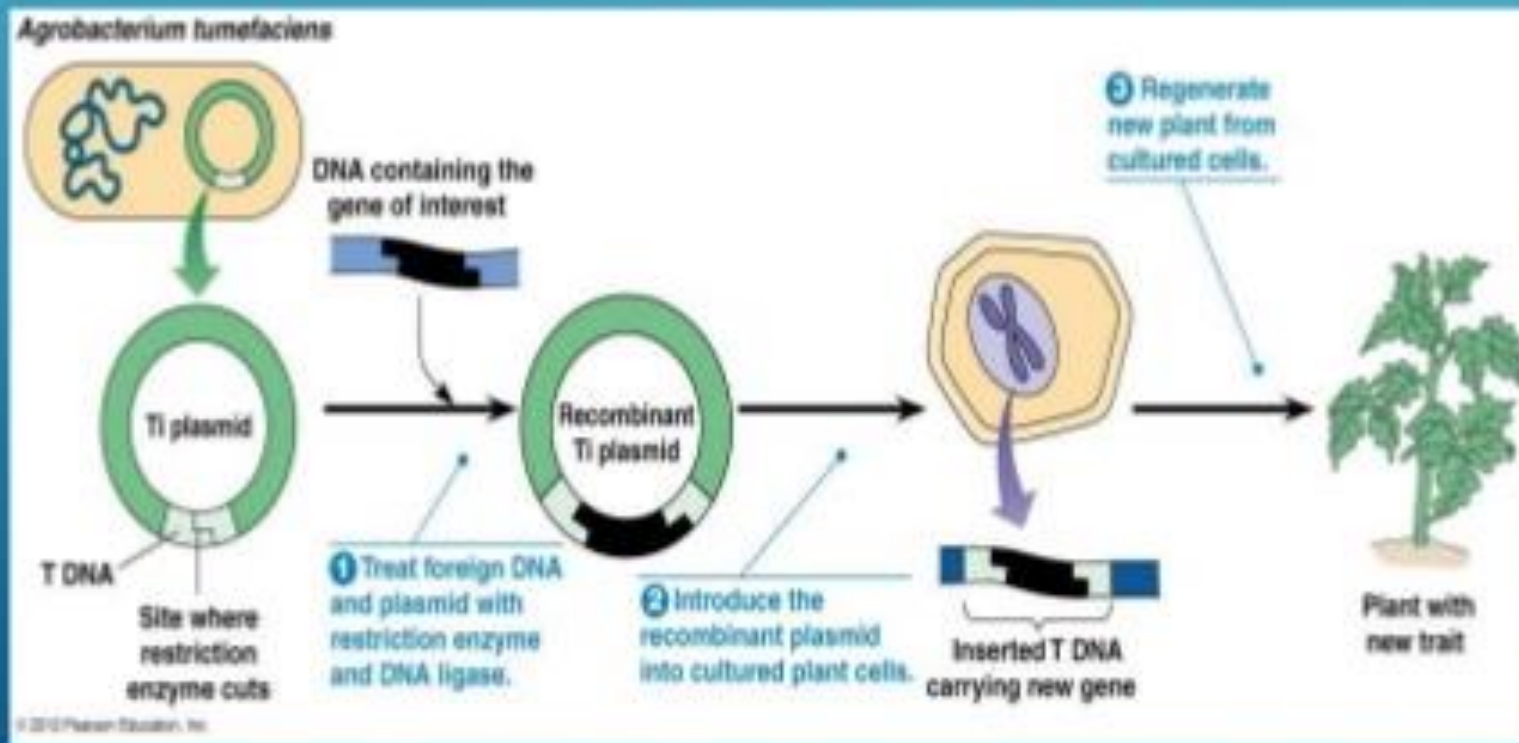
- ❑ A. Tumefaciens is attracted towards wound site by chemotaxis
- ❑ The wound induces the production of phenolic compounds such as acetosyringone
- ❑ The lipopolysaccharides aid in attachment of A. Tumefaciens.
- ❑ Ti plasmid code for chemotactic receptors
- ❑ The processing of T-DNA transfer is mediated by the vir (virulence) region. The vir gene encode a set of proteins responsible for the excision, transfer and integration of the T-DNA into the plant nuclear genome: VirB, VirC, VirD, VirE. Vir A and vir G proteins constitute a two component regulatory system that controls vir gene expression.



MECHANISM OF T DNA

- ❖ It binds tightly on T-strand which results in the unfolding of the ssDNA, i.e T-strand, thus reducing its diameter to 2nm facilitating its transfer through membrane channels.
- ❖ The T-strand along with VirD2 & VirE2 is called as T complex
- ❖ The T complex exit the bacterial cell membranes and cross the plant cell membranes and enter the plant cell.
- ❖ Once inside the cell, T complex targets the plant nucleus and cross the nuclear membrane where it integrates into the plant DNA.

- ▶ T-DNA transfer capabilities are used in genetic engineering to introduce foreign genes into desired plants: the tumor-promoting and opine-synthesis genes of T-DNA are removed and replaced by genes of interest. Such plants are then called transgenic plants.



BASIS OF TUMOR FORMATION

- *Agrobacterium tumefaciens* causes tumor in plants i.e. crown gall disease.
- *Agrobacterium rhizogenes* causes hairy root disease.
- The tumor formation results from the transfer of particular set of Ti & Ri – plasmid into plant chromosome.
- Tumor formation occur by transfer of small segment of Ti – plasmid. (T- DNA).

Ri- PLASMID

- The virulence plasmid of *A.rhizogenes* is commonly known as **Ri- Plasmid (pRi)**.
- *Agrobacterium rhizogene* is a soil borne , gram negative bacterium.
- It causes hairy root disease in plants .
- All strains of *A.rhizogenes* are known to produce agrocinopine.

