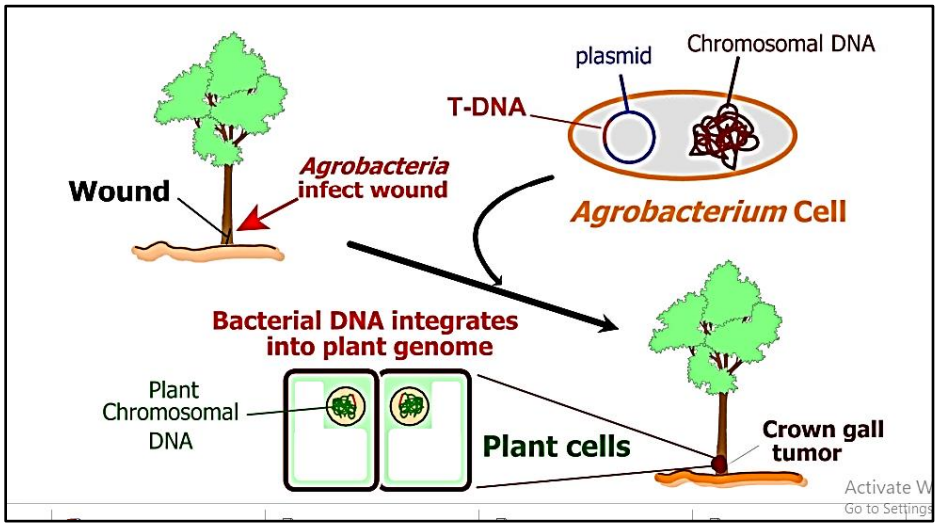
Indirect method of gene transfer : *Agrobacterium mediated* transformation

- Genetic transformation is a way to change genome by introducing genes from distant or unrelated species. *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* mediated transformation is the most commonly used method for obtaining transgenic plants.
- The host plant species for range A. tumefaciens include: Large number of dicots and some monocots and Gymnosperms.
- Agrobacteria are naturally occurring, ubiquitous soil borne gram (-) pathogenic bacterium *A. tumefaciens* causes crown gall disease (tumors) & *A. rhizogenes* causes root hair disease (hairy root) (Gelvin 2005).
- . *Agrobacterium tumefaciens* which normally induces disease called crown gall in plants by transferring a distinct portion of its DNA.
- *So, Agrobacterium* is also called a natural genetic engineer, has been successfully used for genetict ransformation of dicotyledonous plants and few monocots.



Ti plasmid has these regions

1. T-DNA .flanked by Right and left border (RB, LB) . These 2 are 25basepair regions TDNA transfer is dependent on presence of direct repeat and flanking sequence of. Called left border sequence and right border sequence (LB and RB)
1. vir region ; has the virulence genes which encode proteins for T-DNA transfer
2. Ori (origin of Replication) region
3. region which carries genes encoding opines.
4. region encodes genes for opine utilization/catabolism

I. TDNA

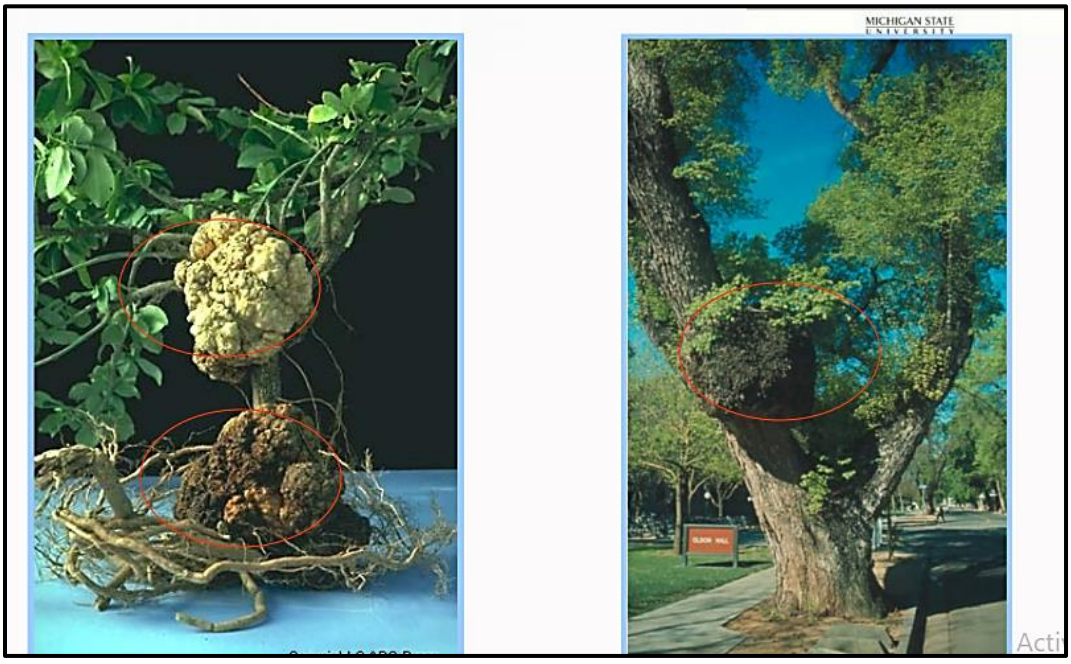
has short stretch which causes tumours in transformed plant, This stretch has 3 genes,

- which code for an auxin
- cytokinin
- opines : Opines are bacteria's carbon and nitrogen source

It is 15-40 kb region contains genes for synthesis of Auxins, Cytokinins and Opines. Auxins and cytokinin genes are expressed in plant tissue inducing tumour. Opines (unusual amino acids) produced by *Agrobacterium* cells are used as nutrients by it. T-DNA region is bordered on both sides by 25bp repeat which helps in its transfer to plant genome

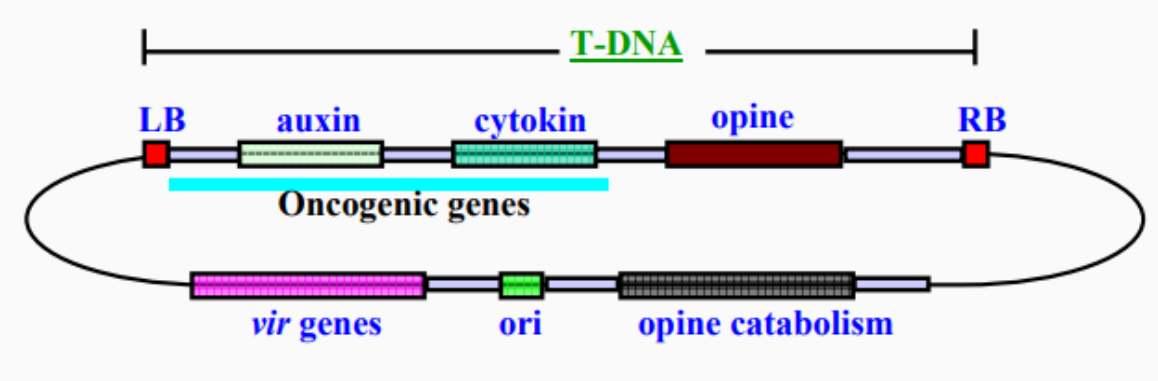
The Ti plasmids are classified into different types depending upon the specific opine being synthesized. (octapine/nopaline/Agropine).:

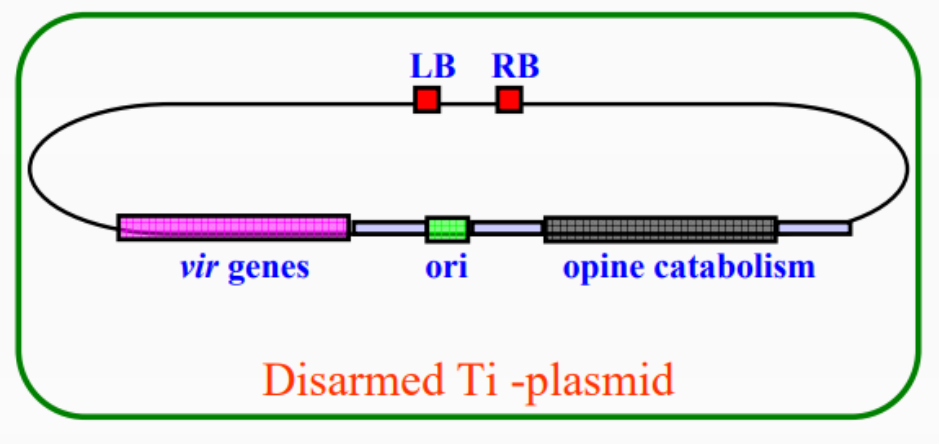
1. Agropine-type : Carry genes for agropine synthesis and catabolism
2. Octopine-type : Carry genes to synthesize octopine in the plant and catabolism in the bacteria.
3. Nopaline-type: Carry gene for synthesizing nopaline in the plant and for catabolism in the bacteria.



- *A. tumefaciens* plasmid transfers only T-DNA. So, it is possible to introduce any gene into plant genome by inserting in T-DNA

- In T DNA the region having genes for auxins nd cytokinins which causes tumour growth are removed , the process called disarming , and the plasmid is called disarmed plasmid





II. **Virulence Region**

- contains about 8 operons having about 24- 25 genes These genes help in transfer of T-DNA
- The vir genes of Ti plasmid are retained as such because they are crucial for T-DNA transfer
- Vir region contains 8 operons (VirA,B,C,D,E,F ,G &H) which together have 25 genes. Vir region mediates transfer of T-DNA into plant genome. It is itself not transferred. VirA and Vir G are constitutive operons encoding Vir A and VirG Proteins. Other Vir operons encode various proteins involved in T-DNA transfer

❖

Gene Transfer using *Agrobacterium*

Chromosomal and Vir Genes

Chromosomal and *vir* genes of bacterial cells are both involved in T-DNA transfer

Virulence genes	
vir A	Chemoreceptor, activator of *vir* G
vir B	Transmembrane complex
vir C	Host-range specificity
vir D	Site-specific endonuclease
vir E	T-DNA processing and protection
vir F	Host range specificity
vir G	Positive regulator of *vir* B, C, D, E, F
Chromosomal genes	Attachment to plant cell, *vir* gene regulation

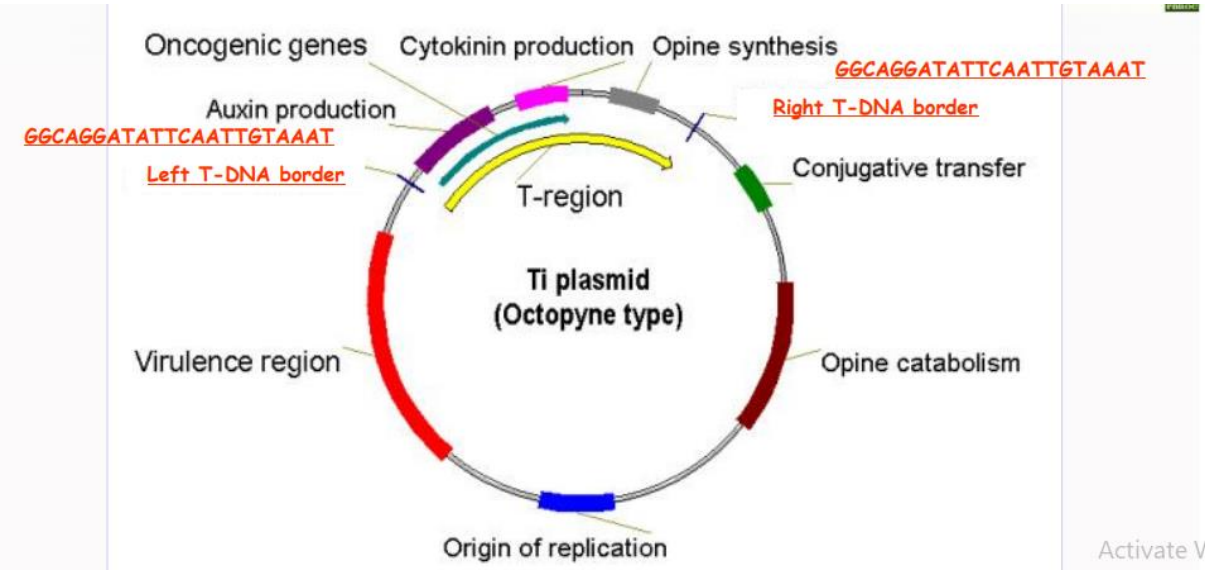
MECHANISM OF TRANSFER OF T-DNA : Transfer of T-DNA is a step wise process

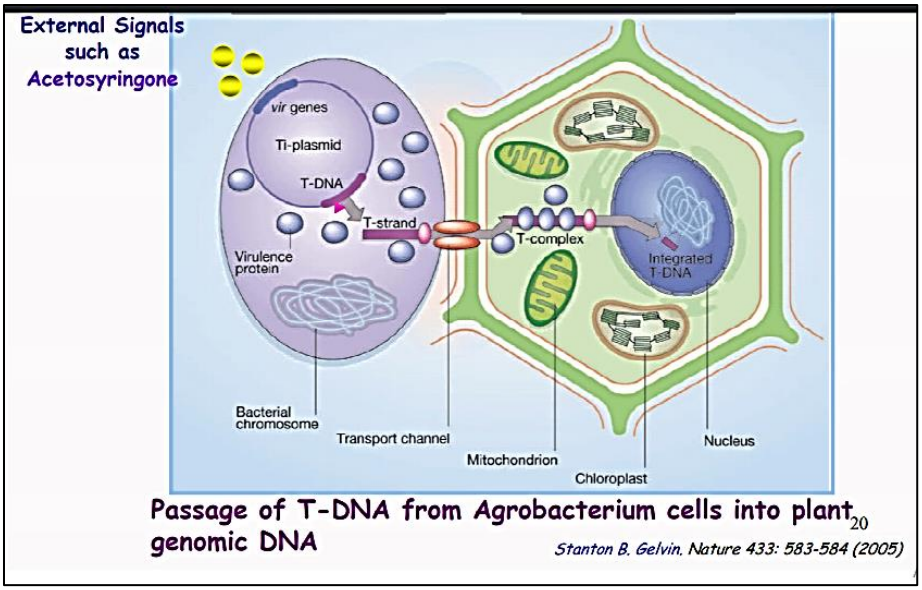
- . *Agrobacteria* attaches to plant cell surfaces at wound sites.
- . The plant releases wound signal compounds, such as acetosyringone. Vir region of Ti plasmid becomes activated by the phenolic signal molecules Acetosyringone released by wounded tissue of dicot plants
- Vir C and/or Vir F recognize the host plant cells.
- Acetosyringone bind with Vir A protein (located in the inner membrane) and activates it. It start functioning as autokinase to phosphorylate itself by ATP.
- Vir A activates vir G: Phosphorylated Vir A protein then phosphorylates Vir G protein which then dimerises
- Phosphorylated Vir G induces expression of rest of Vir operons including vir D and E.
- Vir D1 protein has topoisomerase and endonuclease activity. It binds to right border sequence of T-DNA and facilitate the action of Vir D2 protein which is also endonuclease and nicks at the right border and remains bound to 5'end so generated.

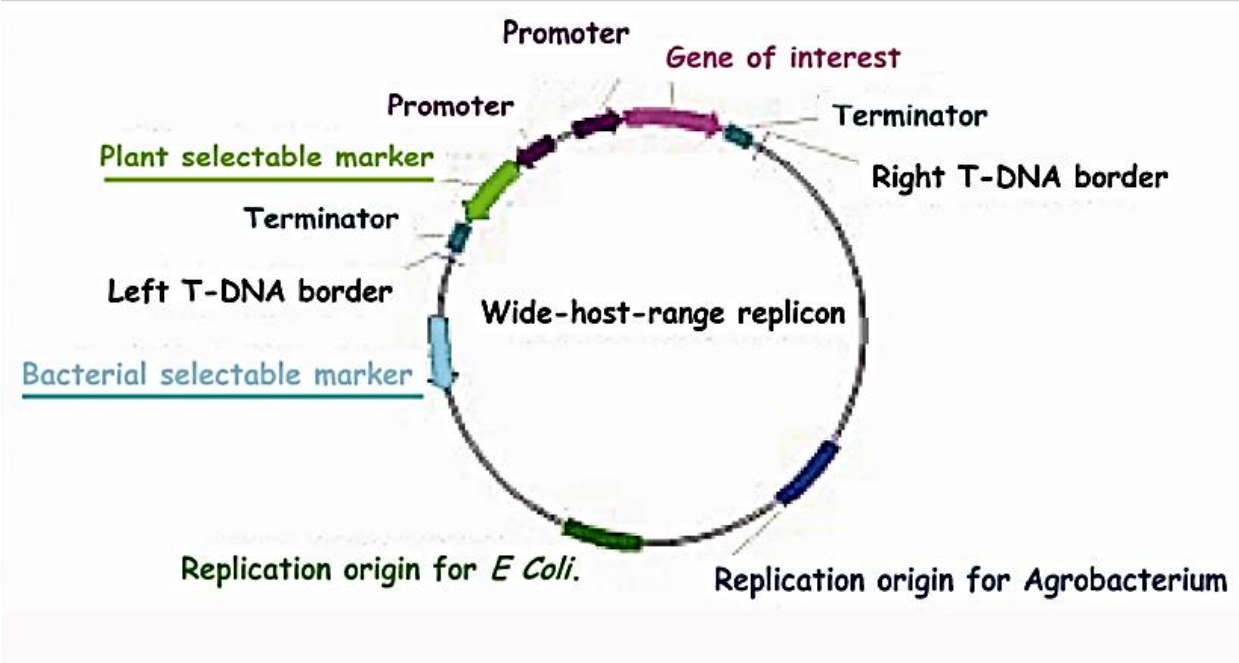
TRANSFER OF T- DNA by Vir GENES

- The next steps, are T-DNA transfer, and integration of the T DNA into the whole plant genome

- 3’end produced at the site of nick serves as a primer for DNA synthesis in 5’----3’ direction as a result of which one strand of T-DNA is displaced from the DNA duplex. The T-DNA strand is again nicked at the left border to generate a single strand copy of T-DNA. To this single strand copy Vir E 2 protein (single strand DNA binding proteins) bind for its protection against exonucleases
- Vir B operon consisting of 11 genes encode membrane bound Vir B proteins. These along with Vir D4 proteins participate in conjugal tube formation between bacterial and plant cells for transfer of T-DNA Vir D2 which remains bound to 5’end of T DNA has a signal sequence which drives it into the nucleus of plant cell.
  - Vir B, D, E + T-DNA as complex is transferred to the plant cell, where it integrates in nuclear DNA.
  - Vir E2 also has nuclear localization sequence and is responsible for transfer of T DNA into plant cell nucleus
  - T DNA is integrated into genome by homologous or non homologous recombination.
  - Since Ti plasmid gets transferred and integrated into the plant genome , plasmid has been used as a vector to transfer useful genes.







Binary Vector

Advantages of the Agrobacterium- mediated transformation

1. No destruction of plant cells in transformation.

2. T-DNA is delivered into the cell nucleus, and this significantly increases the chance of integration of target genes into the plant genome
3. Introduces a small number of copies of foreign DNA per cell as compared with other transformation methods.

Question Bank

Q How genetic engineers use Ti plasmid?

Genetic engineers employ different strategies for transfer of desired gene modified Ti plasmid (disarmed) is used : Ti plasmid with disarmed T-DNA region in which oncogenes and opine biosynthesis genes were replaced by desired (Foreign) gene and a selectable marker gene . Along with this some other sequences as unique restriction sites were added**.**

Q why natutal plasmid unsuitable for use in genetic enginnering?

The natural Ti plasmids are unsuitable to be used directly as vectors for invitro manipulation due to following reasons • Large size • Tumor induction (Oncogenic) property • Absence of unique restriction enzyme site Now it is also well known that disarmed TDNA ,left and right borders along with genes of Vir region are essential elements for designing of transformation vectors Now let us see the strategies involved for harnessing Agrobacterium for introduction of new/desired/foreign genes into plants