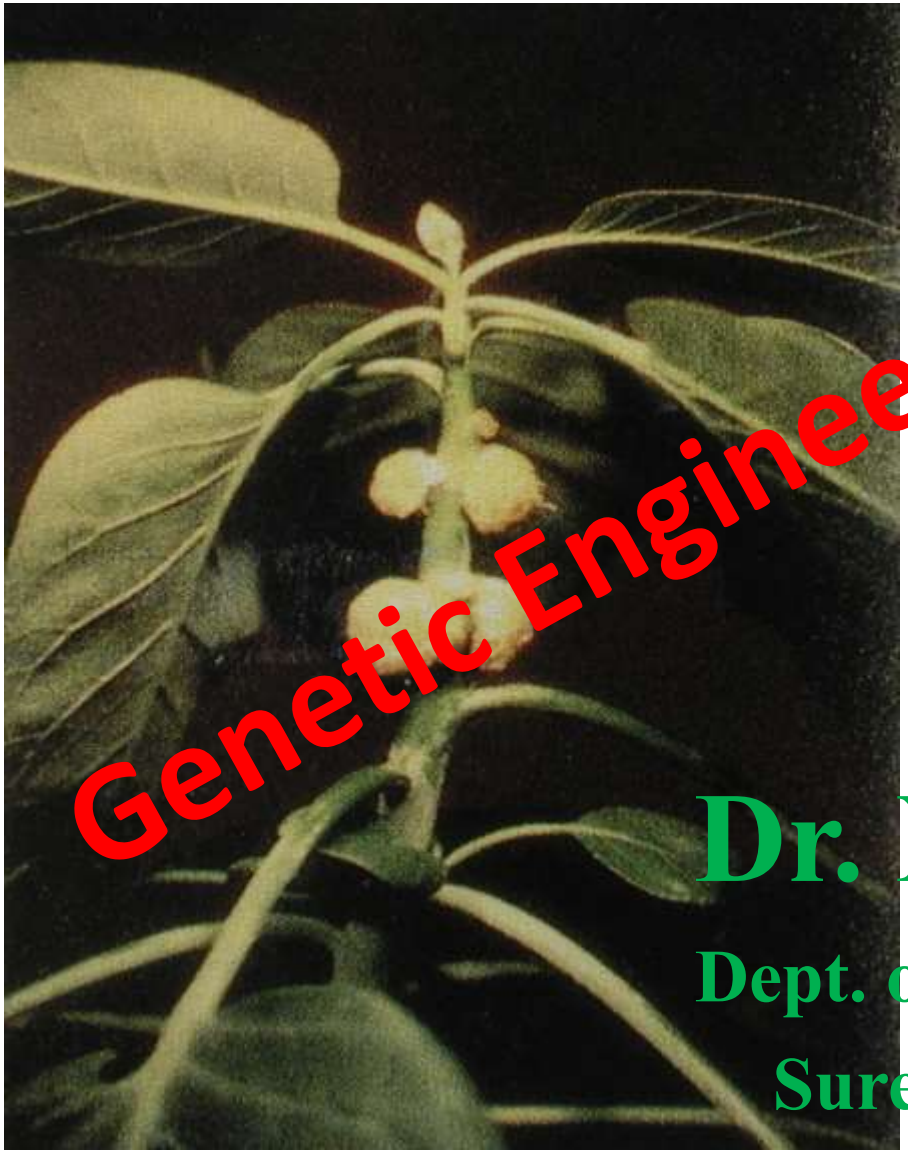
Ti Plasmid as Plant Transformation Vector



Genetic Engineering of Plants

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Surendranath College

MLB-G-DSE-A-5-1

Recombinant DNA Technology (4+2 = 6 credits)

MLB-G-DSE-A-5-1-TH (4 credits/50 marks)

Cloning: Cloning vectors (pBR322, pUC18/19, YACs), Bacteriophage lambda and M13 based vectors. Cosmids, Ti plasmid as transformation vector. Use of linkers and adaptors, Homopolymeric tailing, c-DNA synthesis and cloning. Genomic DNA and c-DNA libraries. (20 hours)

Restriction and Modification systems in bacteria: Types I, II and III. Mode of action, nomenclature, applications of Type II restriction enzymes in genetic engineering, Restriction Mapping, Restriction Fragment Length Polymorphism (RFLP). (10 hrs)

Enzymes used in Recombinant DNA techniques: DNA ligase, Polynucleotide Kinase, DNA Polymerase, Reverse Transcriptase, Terminal deoxynucleotidyl transferase, Phosphatases. (10 hours)

Polymerase Chain Reaction & qPCR, Electrophoresis & Blotting Techniques, Site- Directed Mutagenesis, DNA Sequencing, Reporter Gene Assays, DNA-Protein Interaction Assays, Protein-Protein Interaction Assays, DNA Fingerprinting. (20 hours)

Why genetically engineered plants?

- **To improve the agricultural or horticultural value of plants**
- **To serve as living bioreactors for the production of economically important proteins or metabolites**
- **To provide a renewable source of energy (biofuels)**
- **To provide a powerful means for studying the biological action of genes and gene products**

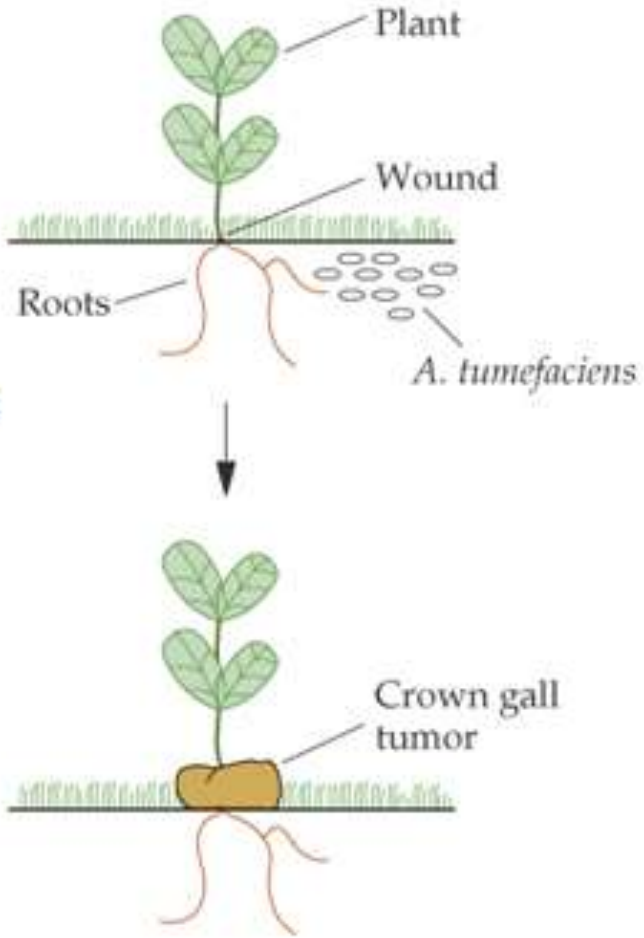
Plant transformation with the Ti plasmid of *Agrobacterium tumefaciens*

- ***A. tumefaciens*** **is a gram-negative soil bacterium which naturally transforms plant cells, resulting in crown gall (cancer) tumors**

- **Tumor formation is the result of the transfer, integration and expression of genes on a specific segment of *A. tumefaciens* plasmid DNA called the T-DNA (transferred DNA)**

- **The T-DNA resides on a large plasmid called the Ti (tumor inducing) plasmid found in *A. tumefaciens***

Formation of Crown Gall tumor in plants

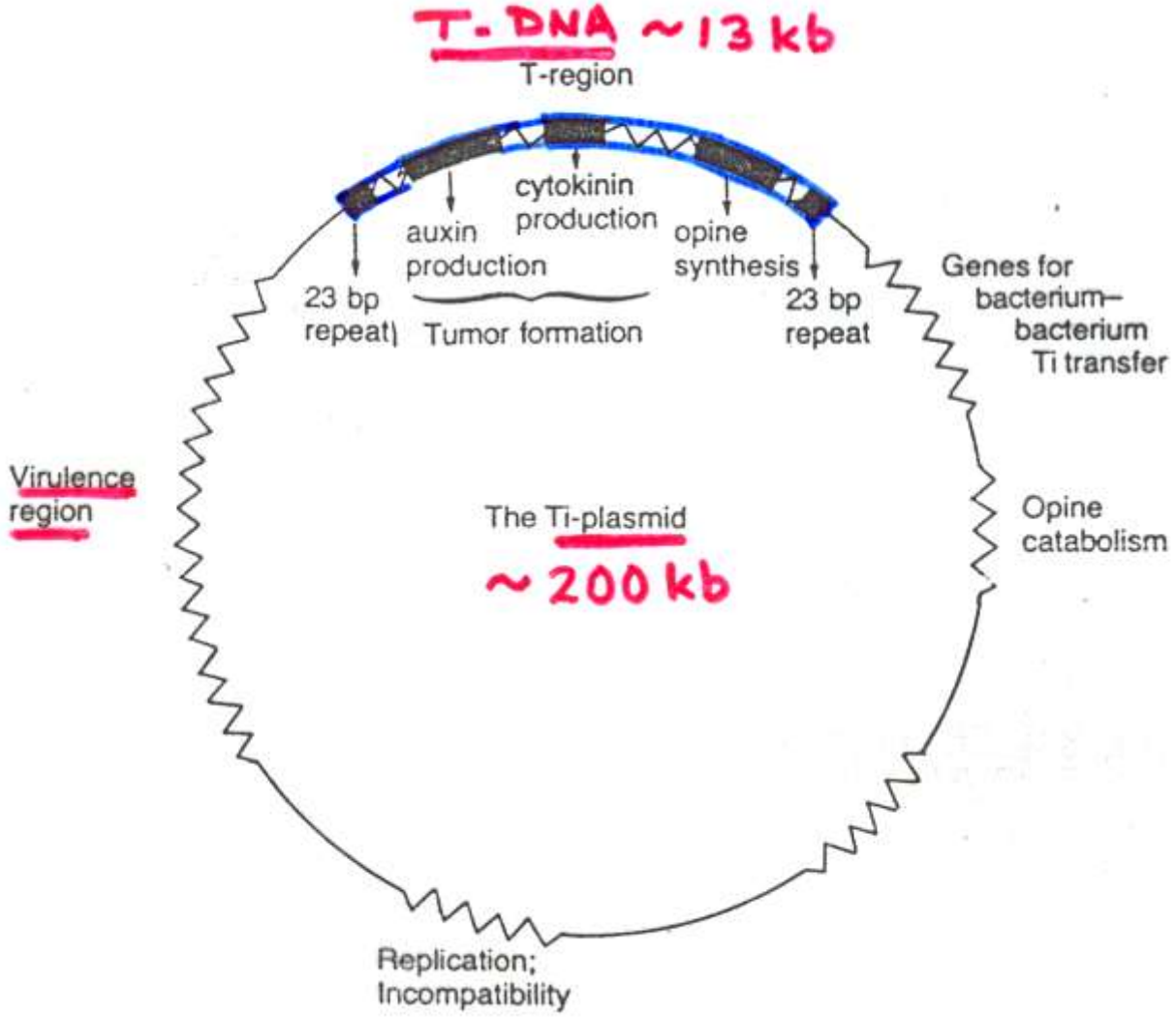


Infection of a plant with *A. tumefaciens* and formation of a crown gall tumor.

Infection of a plant with *A. tumefaciens* and formation of crown galls

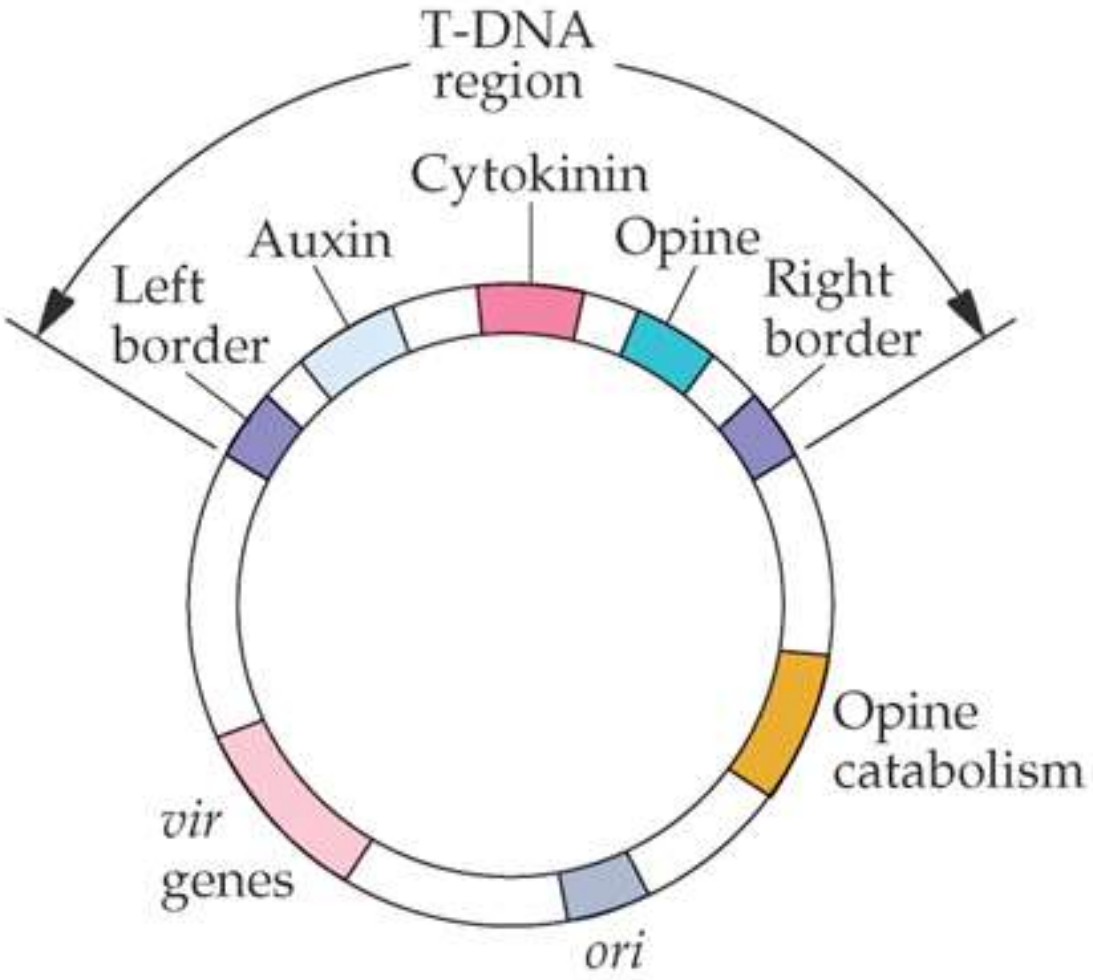
Crown Gall on Tobacco

The Ti plasmid of *Agrobacterium tumafaciens* and its T-DNA region containing eukaryotic genes for auxin, cytokinin, and opine production.

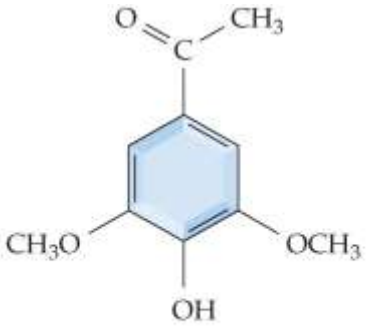


The Structure of Ti plasmid

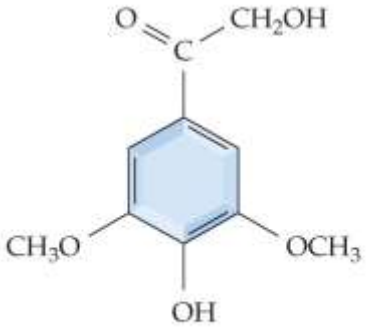
Ti plasmid structure



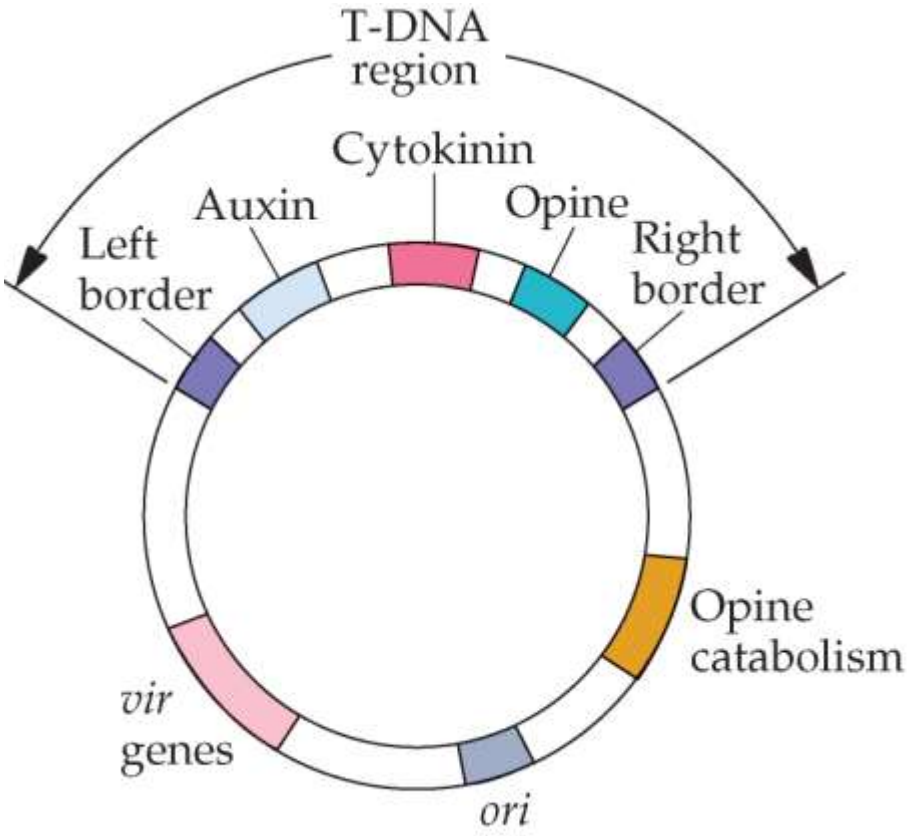
The wound-induced plant phenolics induce the *vir* genes on the Ti plasmid.



Acetosyringone



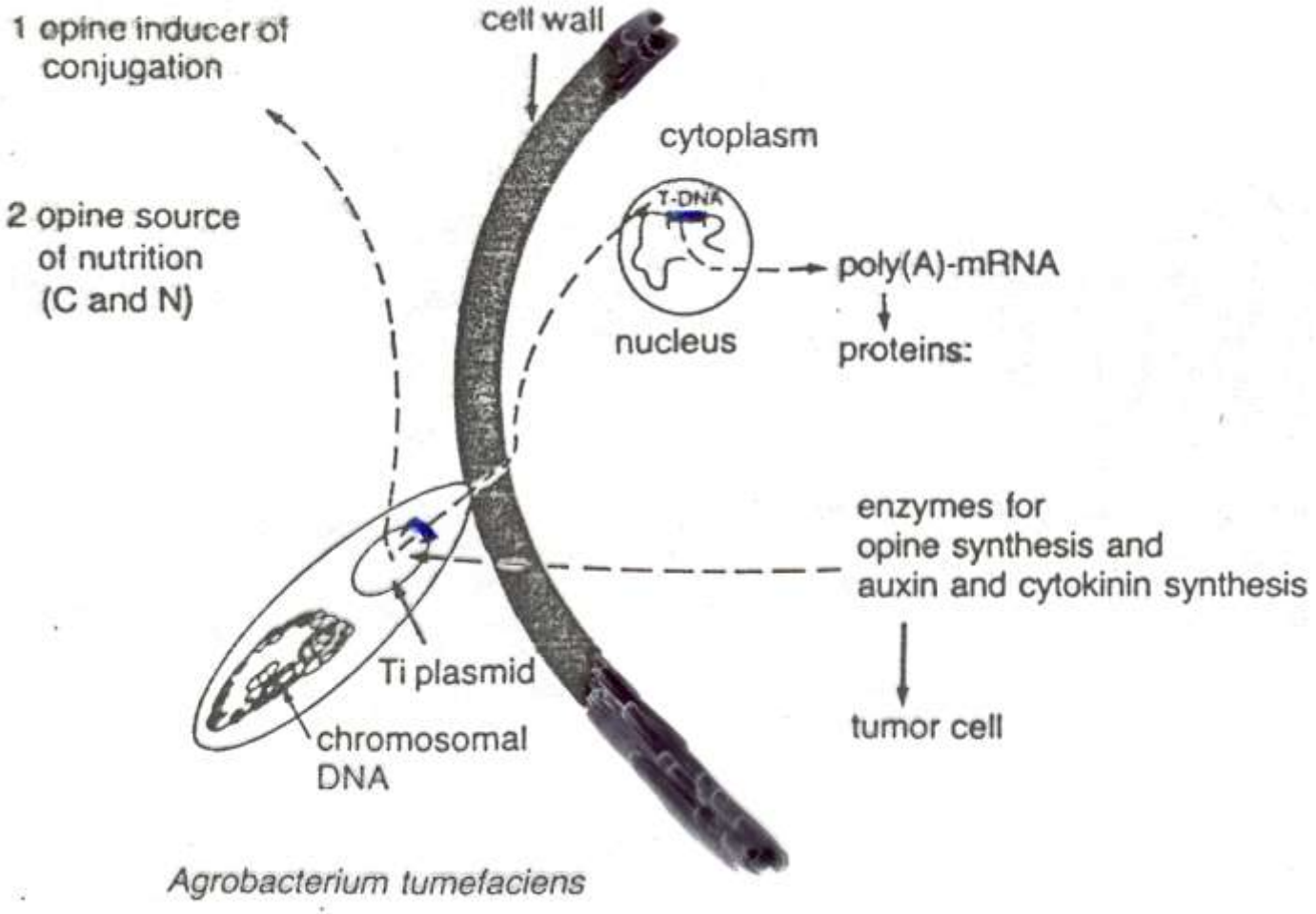
Hydroxyacetosyringone



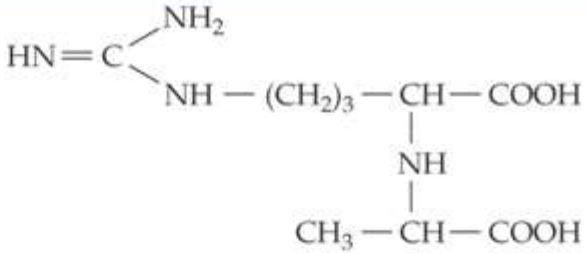
The infection process:

1. **Wounded plant cell releases phenolics and nutrients.**
2. **Phenolics and nutrients cause chemotaxic response of *A. tumefaciens***
3. **Attachment of the bacteria to the plant cell.**
4. **Certain phenolics (e.g., acetosyringone, hydroxyacetosyringone) induce vir gene transcription and allow for T-DNA transfer and integration into plant chromosomal DNA.**
5. **Transcription and translation of the T-DNA in the plant cell to produce opines (food) and tumors (housing) for the bacteria.**
6. **The opine permease/catabolism genes on the Ti plasmid allow *A. tumefaciens* to use opines as a C, H, O, and N source.**

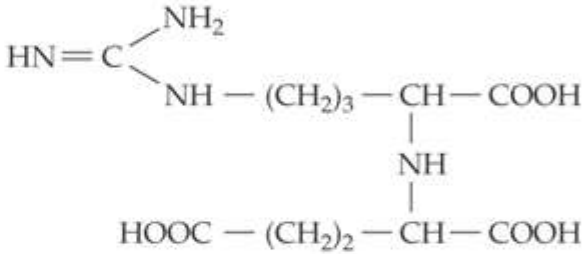
The Ti plasmid of *Agrobacterium tumafaciens* and the transfer of its T-DNA to the plant nuclear genome



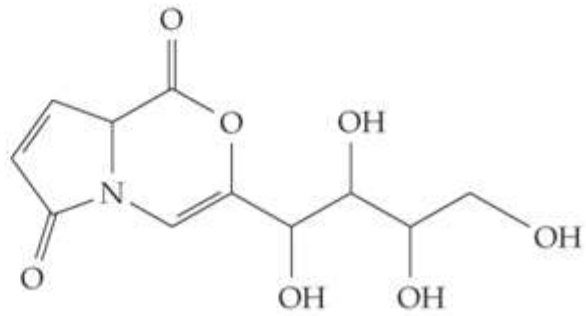
Chemical structures of three opines produced by plants.



Octopine

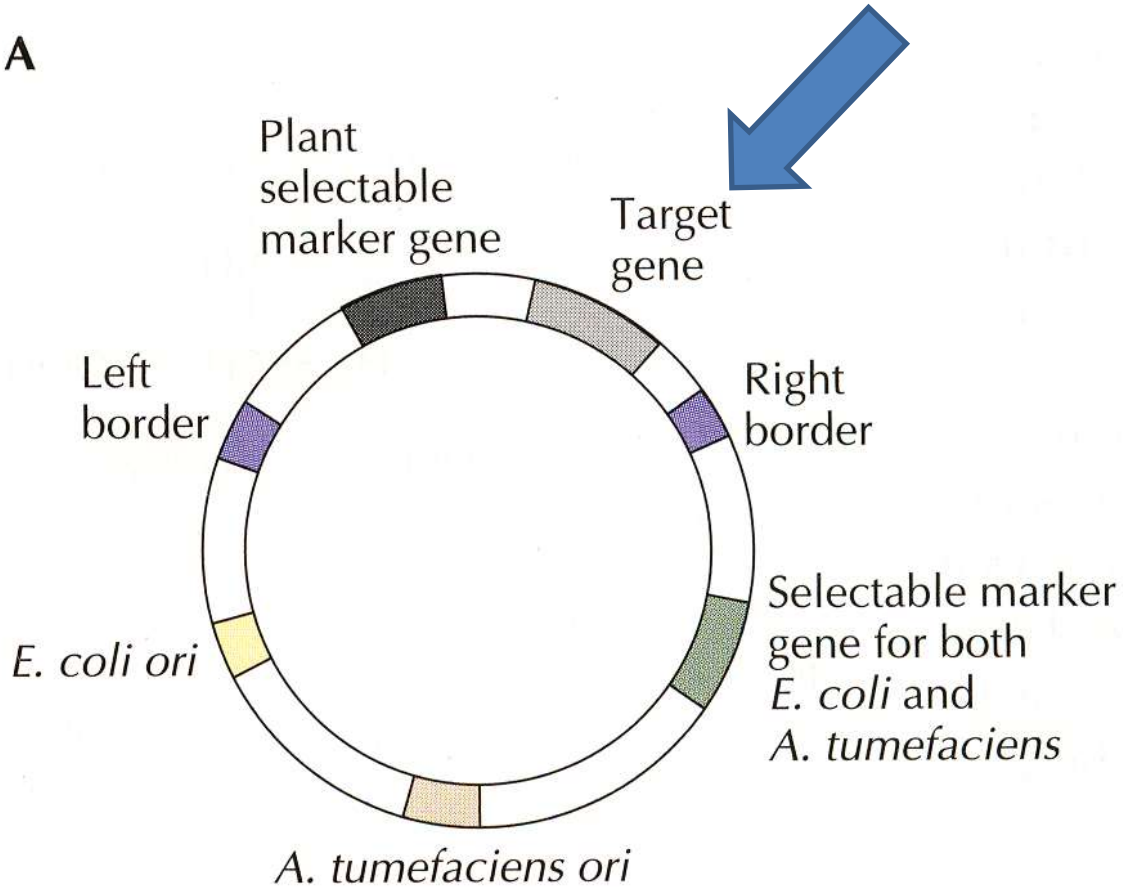


Nopaline

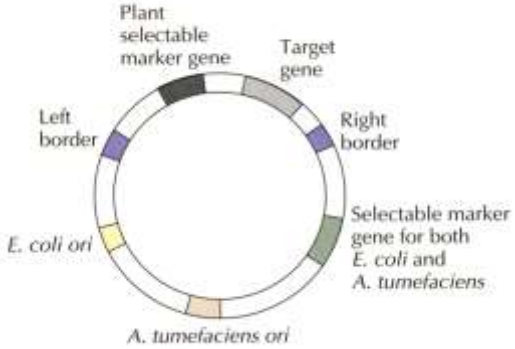


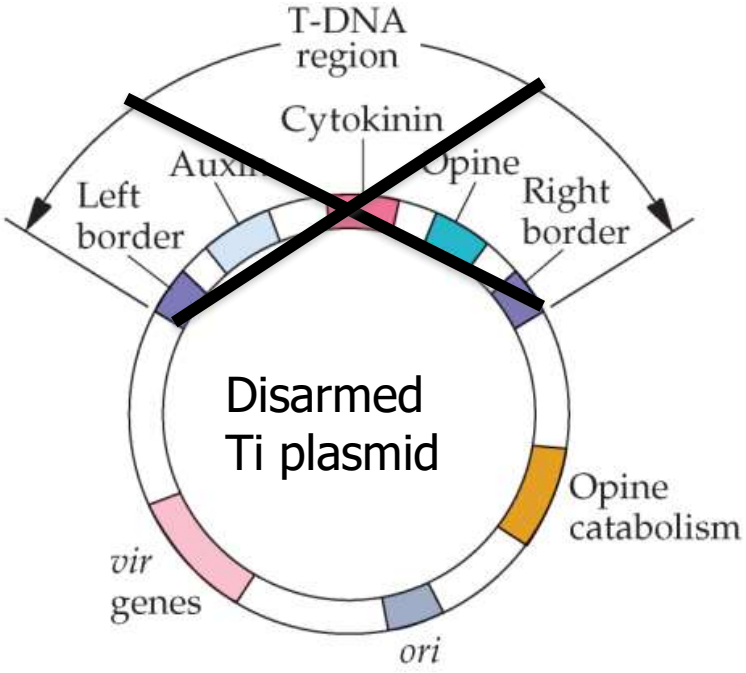
Agropine

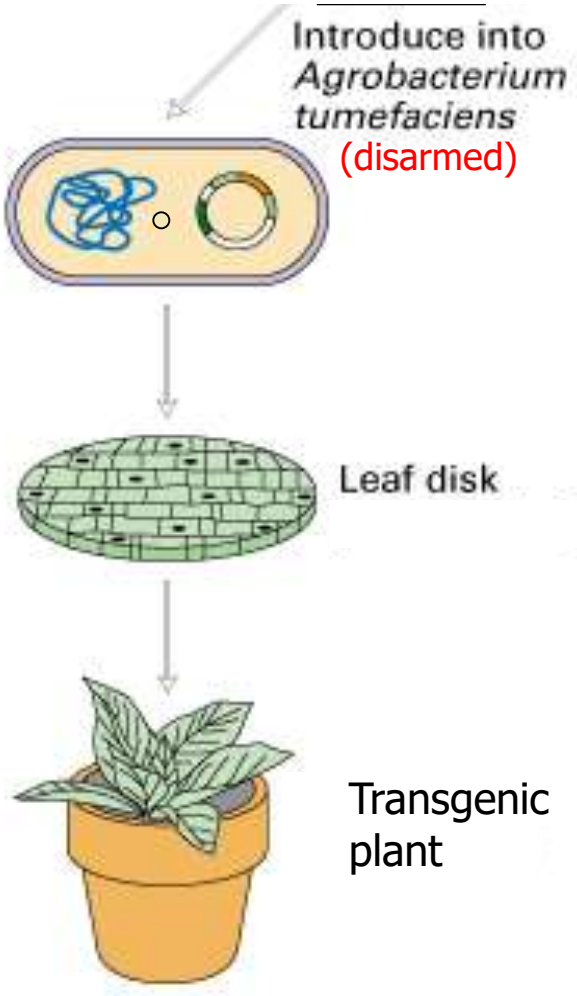
The binary Ti plasmid system involves using a small T-DNA plasmid (shown below) and a disarmed (i.e., no T-DNA) Ti plasmid in *A. tumefaciens*



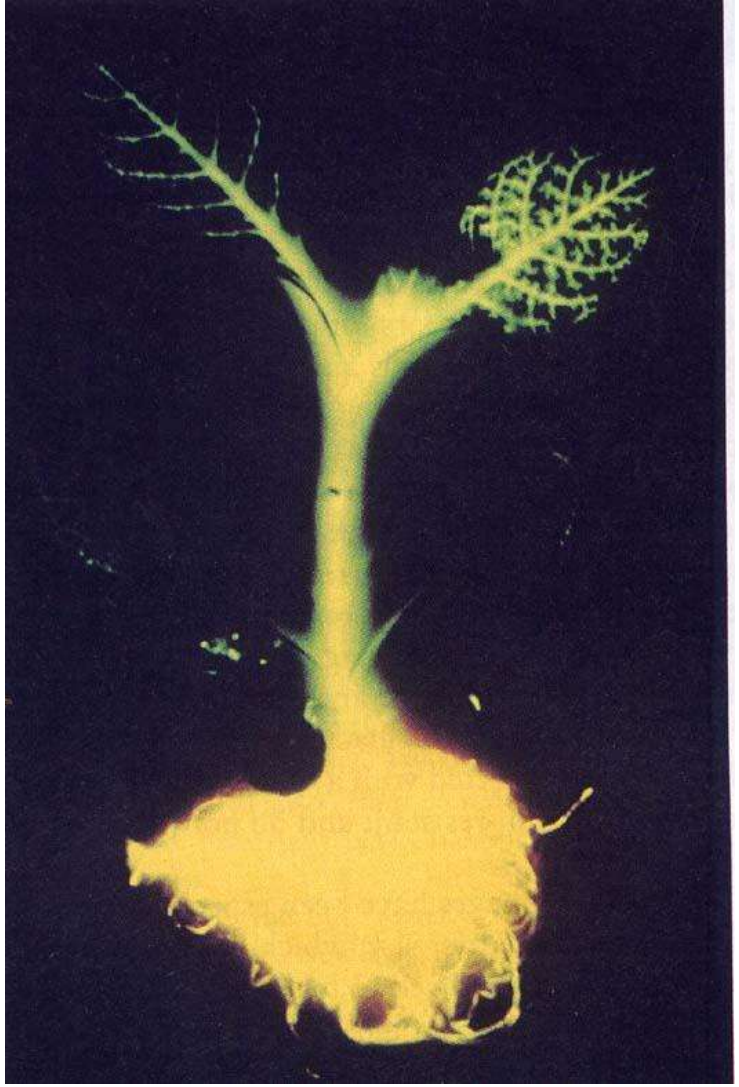
Clone YFG (your favorite gene) or the target gene in the small T-DNA plasmid in *E. coli*, isolate the plasmid and use it to transform the disarmed *A. tumefaciens* as shown.







Plant genetic engineering with the binary Ti plasmid system



A tobacco plant that has been transformed with the firefly luciferase gene. The green light emitted by the plant indicates the activity of luciferase. Note the concentration of the enzyme in the roots and stem.

List of Plants that have been genetically Transformed

TABLE 18.2 Plants that have been genetically transformed

Alfalfa	Carnation	Kiwi fruit	Papaya	Potato	Sunflower
Apple	Carrot	Lettuce	Pea	Red fescue	Sweet potato
Arabidopsis	Corn (maize)	Licorice	Peanut	Rice	Tall fescue
Asparagus	Cotton	Lily	Pear	Rye	Tobacco
Banana	Cranberry	Lotus	Pearl millet	Sorghum	Tomato
Barley	Cucumber	Norway spruce	Peony	Soybean	Wheat
Bean	Eggplant	Oat	Petunia	Strawberry	White spruce
Cabbage	Flax	Orchard grass	Plantain	Sugar beet	
Canola	Grape	Orchid	Poplar	Sugarcane	