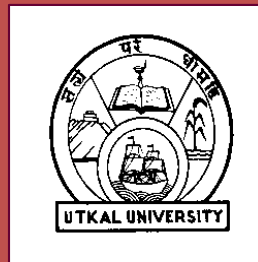


Plant Biotechnology and Transgenics



Chinmay Pradhan

P.G Department of Botany

Vani Vihar, Bhubaneswar-751004

WHY BIOTECHNOLOGY???

- **Biotechnology = Interaction between Science of Biology + Technology**
- **It consists of a variety of techniques, designated to genetically improve and/or exploit living systems or their components for the benefit of human beings.**
- **PLANT BIOTECHNOLOGY**
Manipulating or modifying plants to improve agriculture or to generate a “new” or improved commercial product.

GENETIC ENGINEERING

Using molecular biology methods to modify the genetic information of an organism.

- **To learn about the biology of an organism**
- **To generate a new or improved commercial product**

GENETICALLY MODIFIED ORGANISMS (GMOS):

Organisms are modified by genetic engineering to express desirable traits.

Acceptance of GM Crops in India

- ✚ 2002: Cultivation of GM crops started
- ✚ 2003: Cultivated in 50,000 hectares
- ✚ 2009: Cultivated in 83 Lakh hectares
(Increased by 168 times)
- ✚ In agriculturally advanced states like Maharashtra, Karnataka, Punjab and Haryana, GM crops have gained acceptance.
- ✚ In Odisha- Farmers of Western districts like Sambalpur, Balangir, Bargarh are growing BT Cotton for last couple of years.

Demonstrations against GM food



What are GM crops?

✚ GM plants are produced in laboratory whose DNA is manipulated (by adding one or two genes) using genetic engineering techniques.

✚ Genes from any source (bacteria, virus, fungi, algae, etc.) can be transferred to a plant.

✚ Terms such as Transgenic Plants or Genetically Engineered (GE) plants are also used.

✚ Any organism which is genetically modified is called a GMO.

Methods of production of GM crops

 Two commonly used methods are:

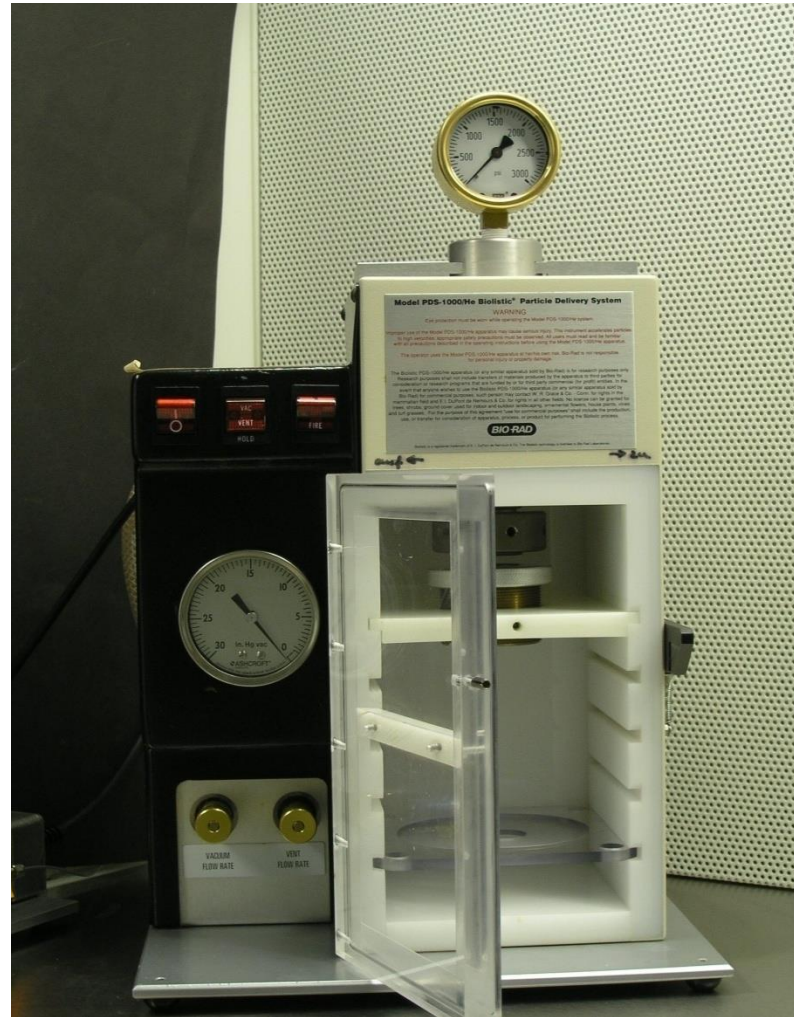
- **Biolistic (Particle gun/ Microprojectile) method**
- ***Agrobacterium* method**

(There are also several other methods such as micro-injection, Electroporation, PEG method etc.).

Biolistic Method

- + Very small gold or tungsten particles are coated with DNA.**
- + These are fired at a very high speed (> 1000 km/hr) to the target tissue.**
- + The apparatus required is called a particle gun.**
- + The propelling force is provided by compressed air, helium or gun powder.**

Particle Gun

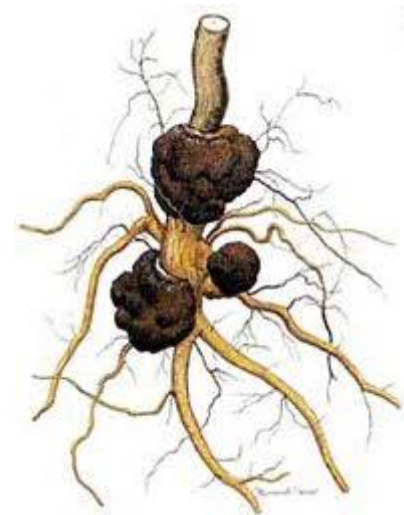


***Agrobacterium*-mediated**

- ***Agrobacterium tumefaciens*** is a soil bacterium.
- In nature, it causes “**crown gall**” disease in many dicots, Gymnosperms, lower plants. But not in monocots.
- While it infects cells, it transfers some of its own genes to the plant cell. Non-self limiting agents.
- So they are called natural plant genetic engineers.

Crown Gall Disease

- *A. tumefaciens* causes “Crown Gall” disease.
- The disease is characterized by a lump of undifferentiated cells (Tumor) at the crown region (Junction of the stem and the root).

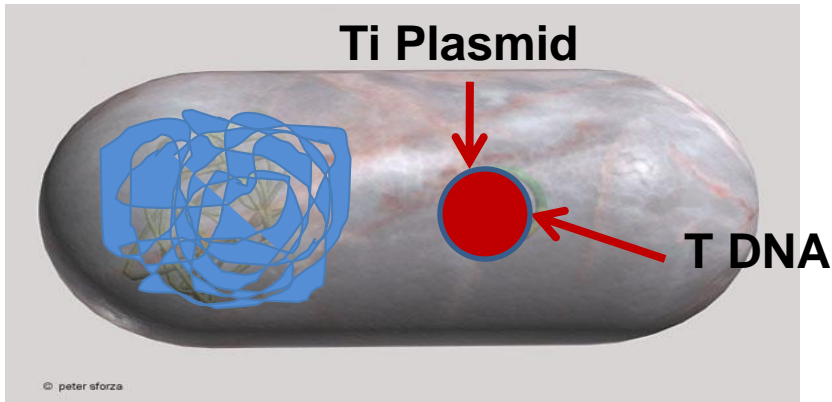


Agrobacterium rhizogenes

Hairy Root or Woolly Knot Disease

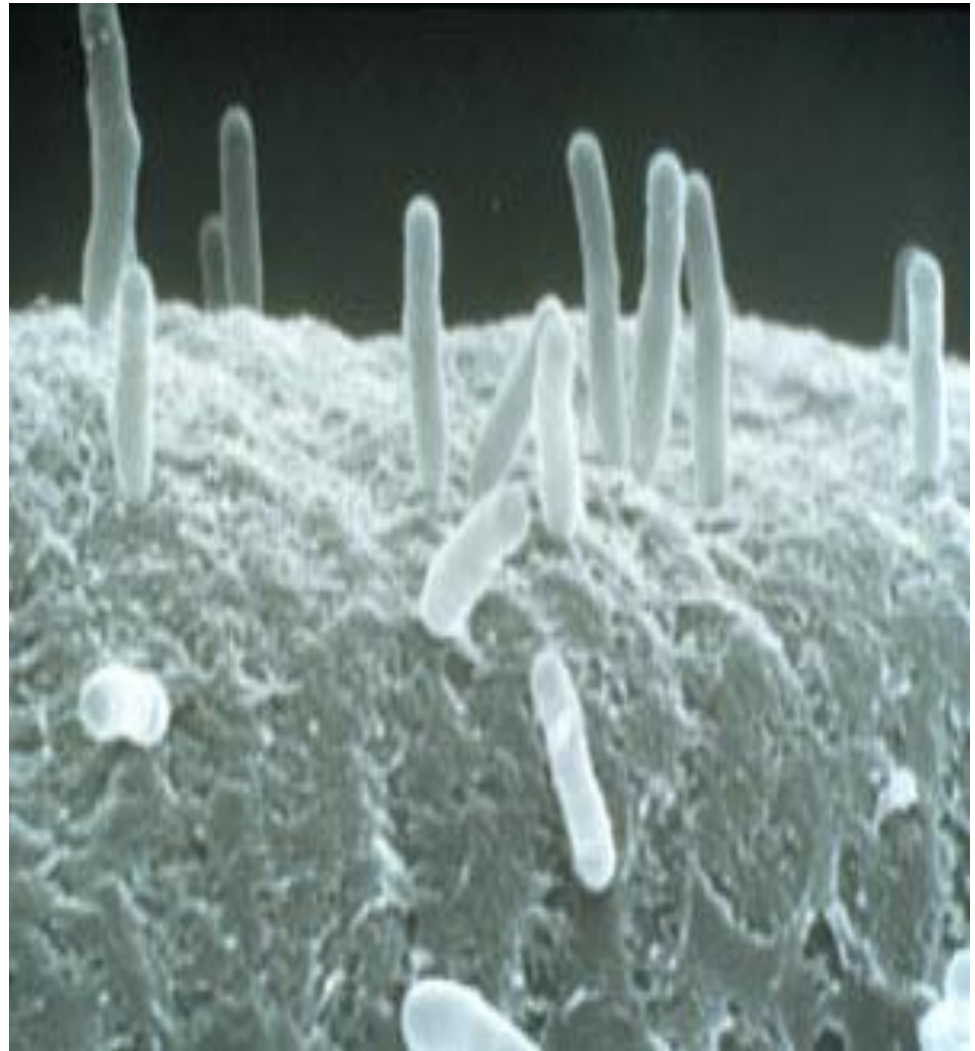


Agrobacterium

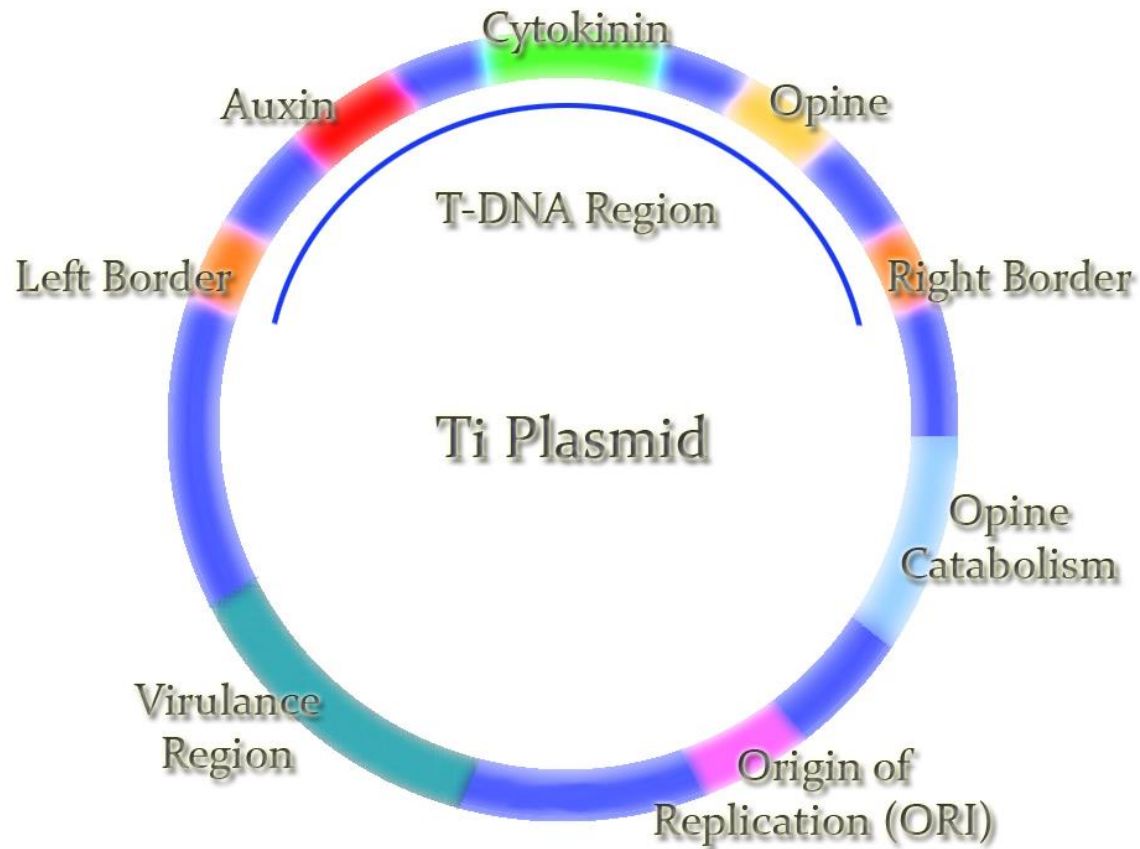


**Wounded Plant Cells release
Acetosyringone to the
hydrosphere.**

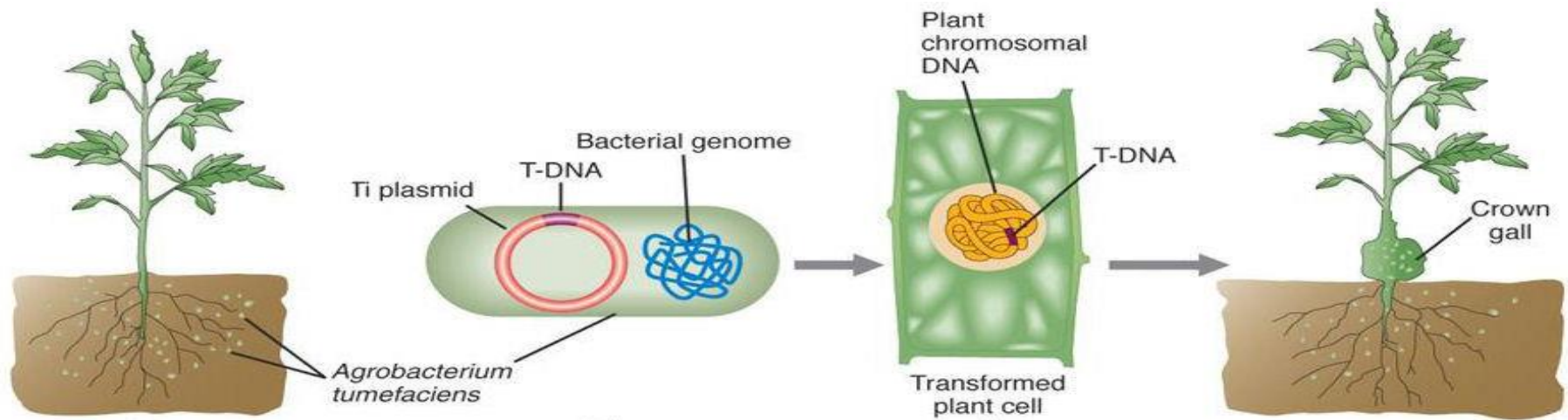
**These phenolic compounds
attract agrobacteria
(Chemotaxis)**



Ti Plasmid



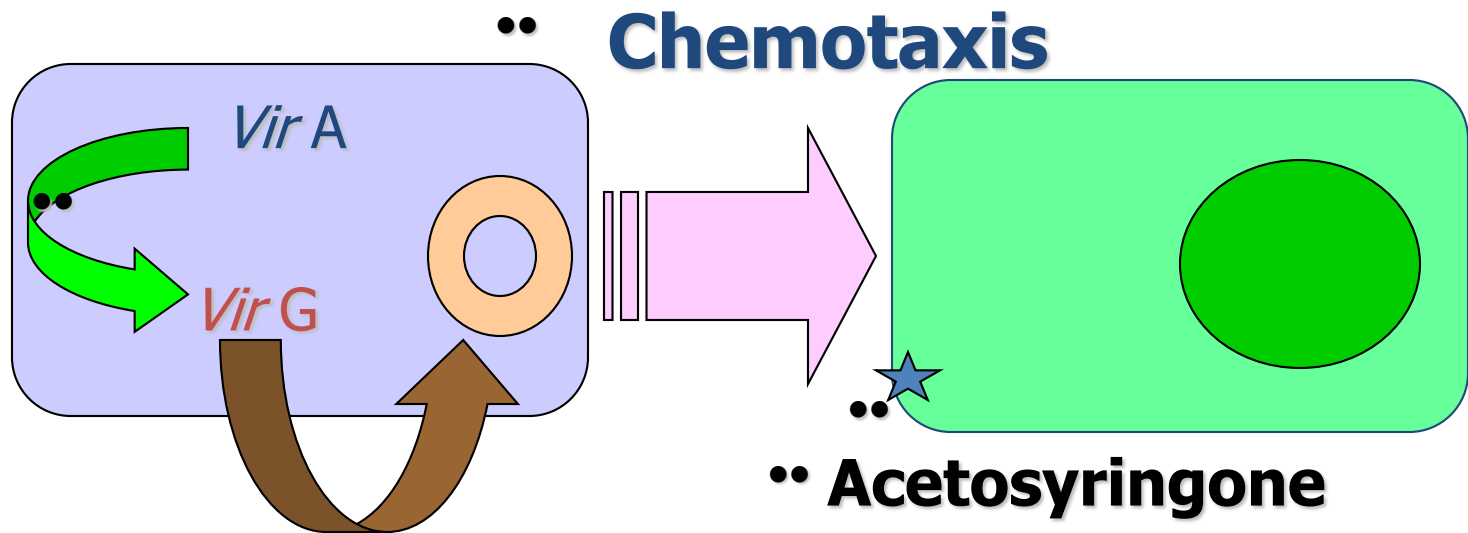
Agrobacterium-mediated Transformation



T-DNA Transfer

Agrobacterium

Wounded Plant Cell

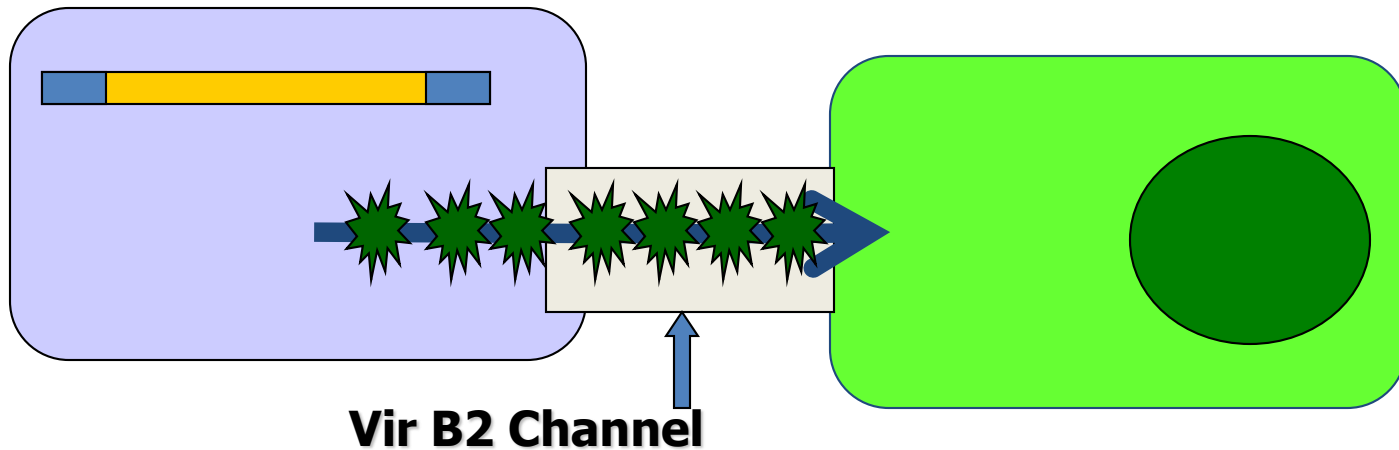


Activated Vir G induces transcription of other genes

T-DNA Transfer

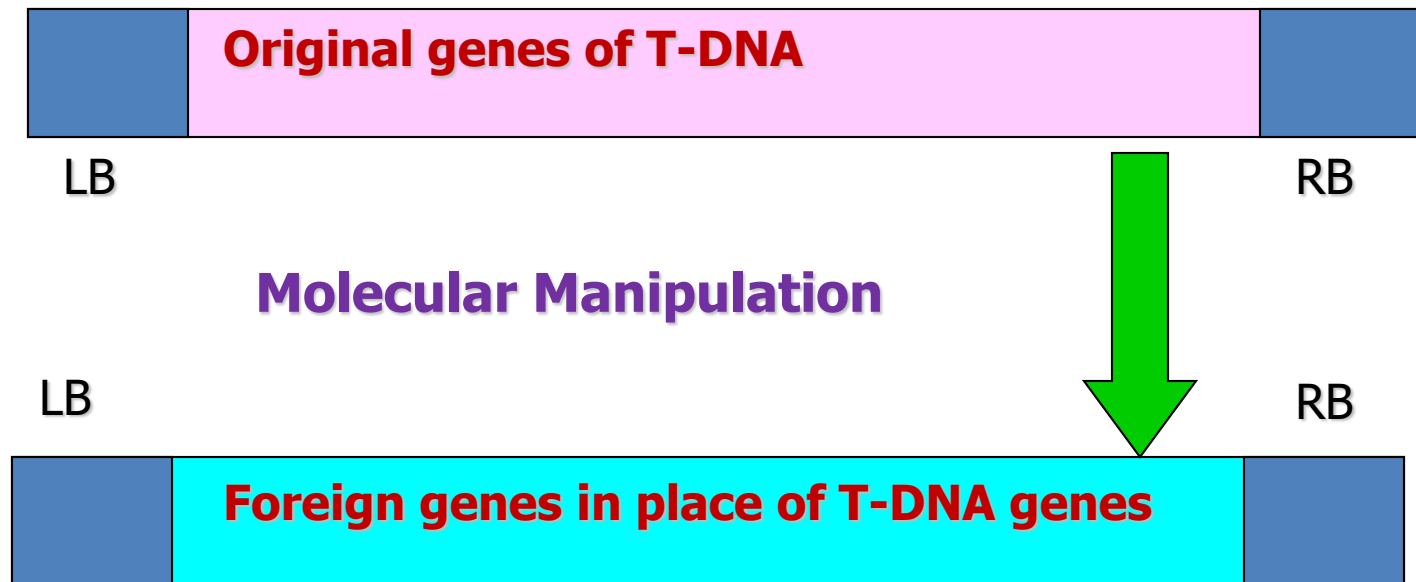
Agrobacterium

Wounded Plant Cell



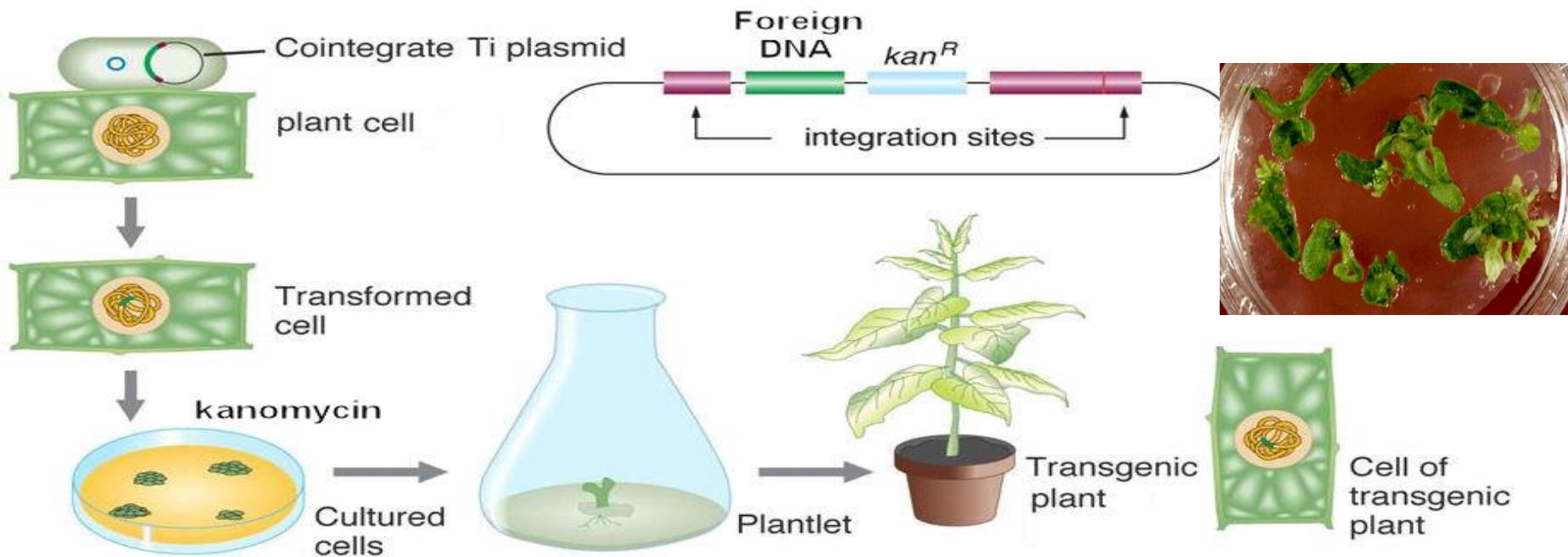
Principle

Any foreign gene can be placed between LB & RB and can be transferred to host plant.



Transgenic Plant Production

Explant is treated with *Agrobacterium*.
Co-cultivation on Callusing medium 24h.
Transferred to SHT medium containing
Cefotaxime, Carbanicillin, Kanamycin



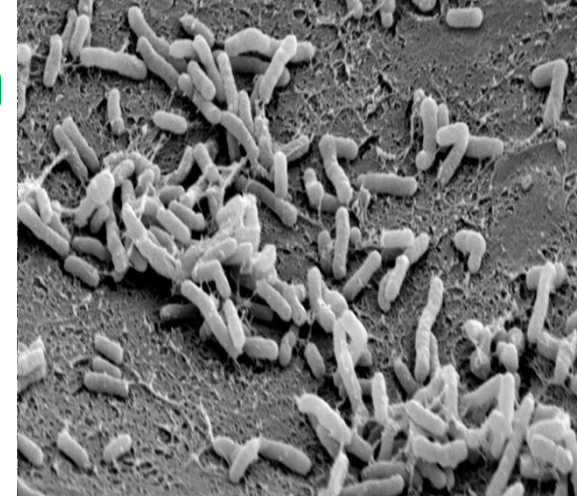
ESSENTIAL COMPONENTS OF GENETIC ENGINEERING

- **A source of DNA fragment (Specific genetic information) containing desirable traits**
- **Methods of introducing the DNA into the host plants**
- *A number of new techniques involved for the introduction of a specific genetic information (DNA) from one organism to another (Gene transfer).*
- *Gene Transfer in plants*
 - *Vector mediated gene transfer*
 - *Vector less or Direct gene transfer*

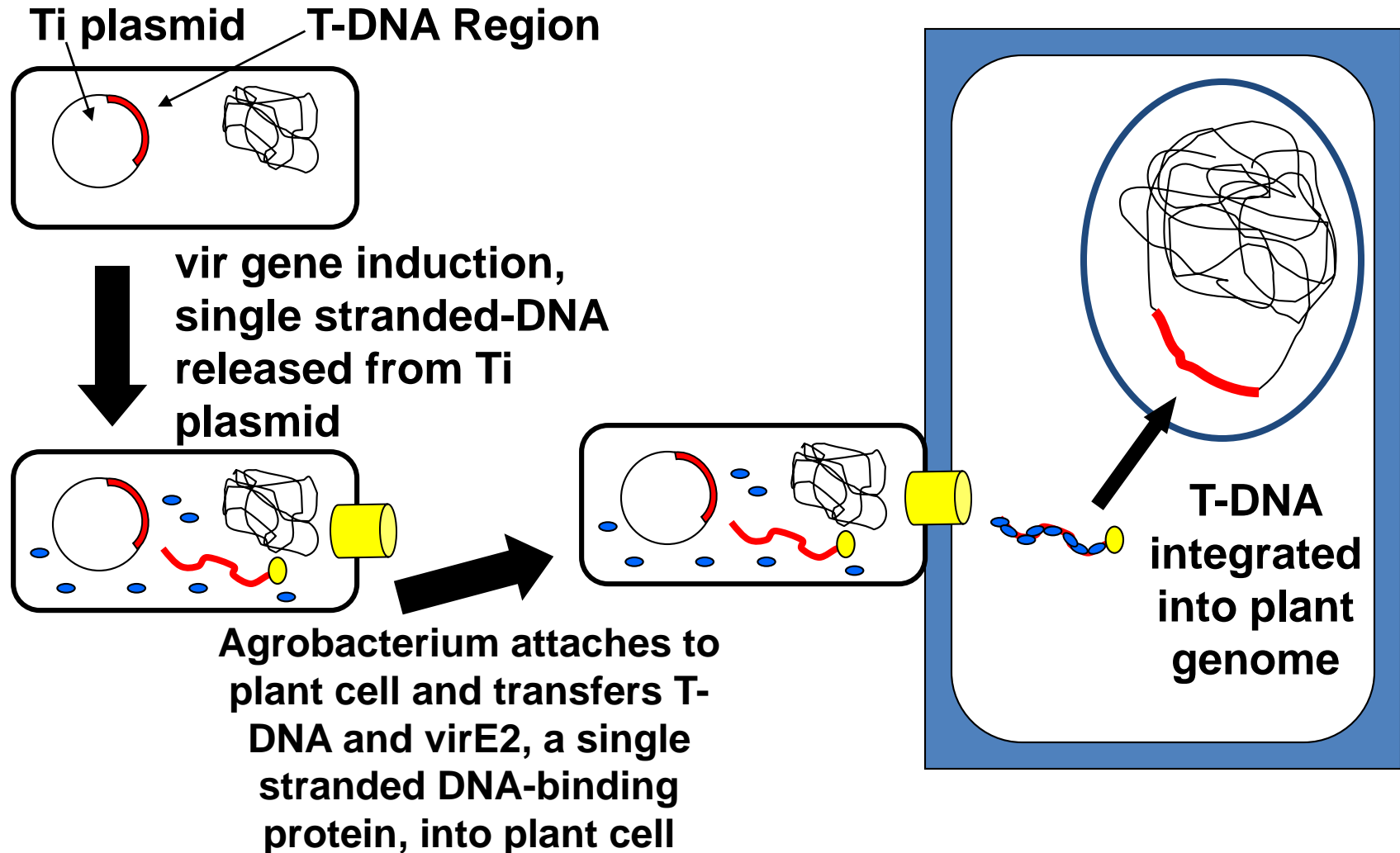
AGROBACTERIUM

THE NATURAL GENETIC ENGINEER

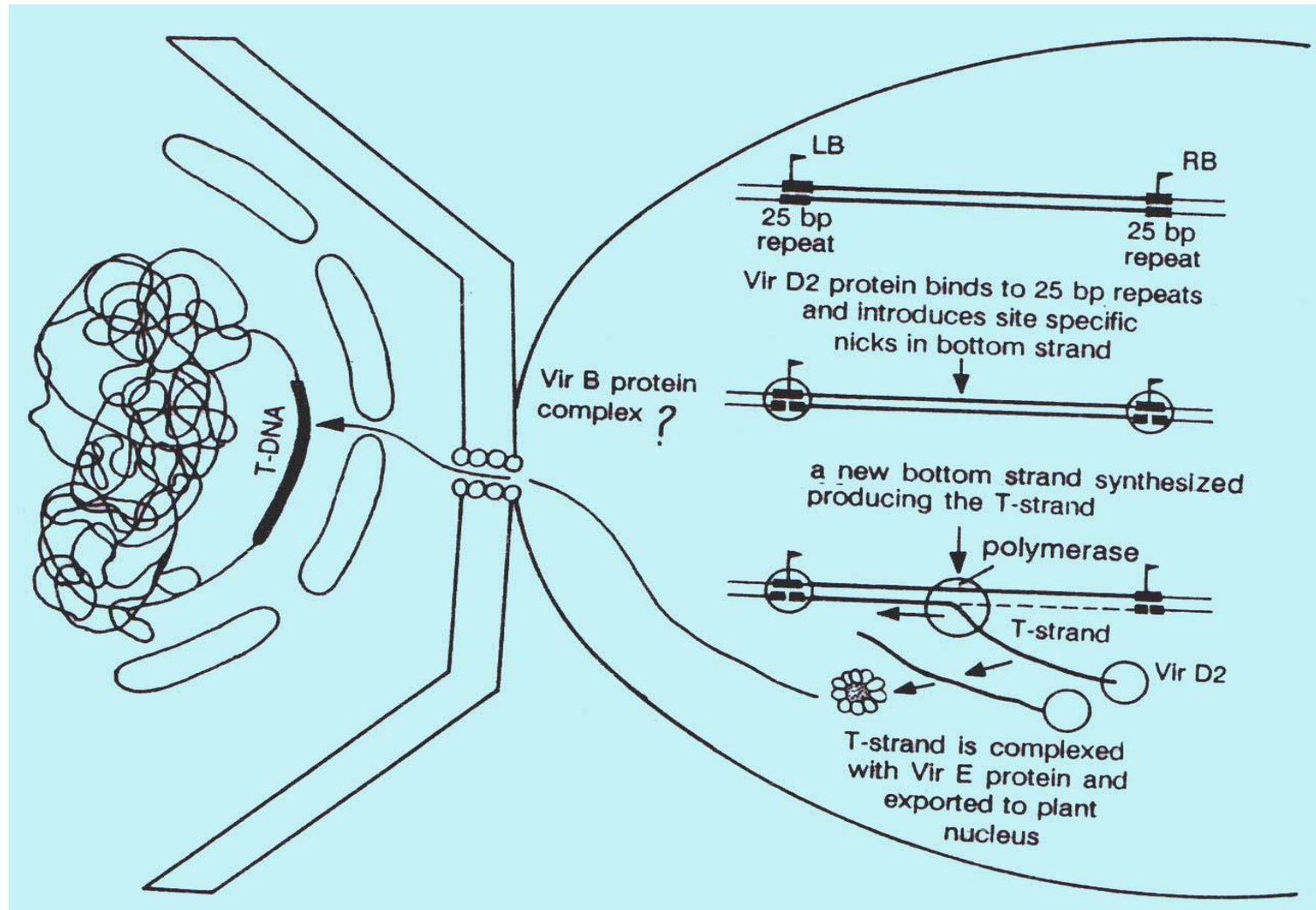
- The bacterium can transfer its own DNA into plants and modulates plant growth and development (causes crown gall / hairy root disease)
- Efficiently transforms many dicotyledonous plants
- Problematical with monocots
- Three different strains of *Agrobacterium* (*A. tumefaciens*, *A. rhizogenes* & *A. radiobacter*)



Agrobacterium tumefaciens plant infection and transformation



T-DNA TRANSFER & INTEGRATION



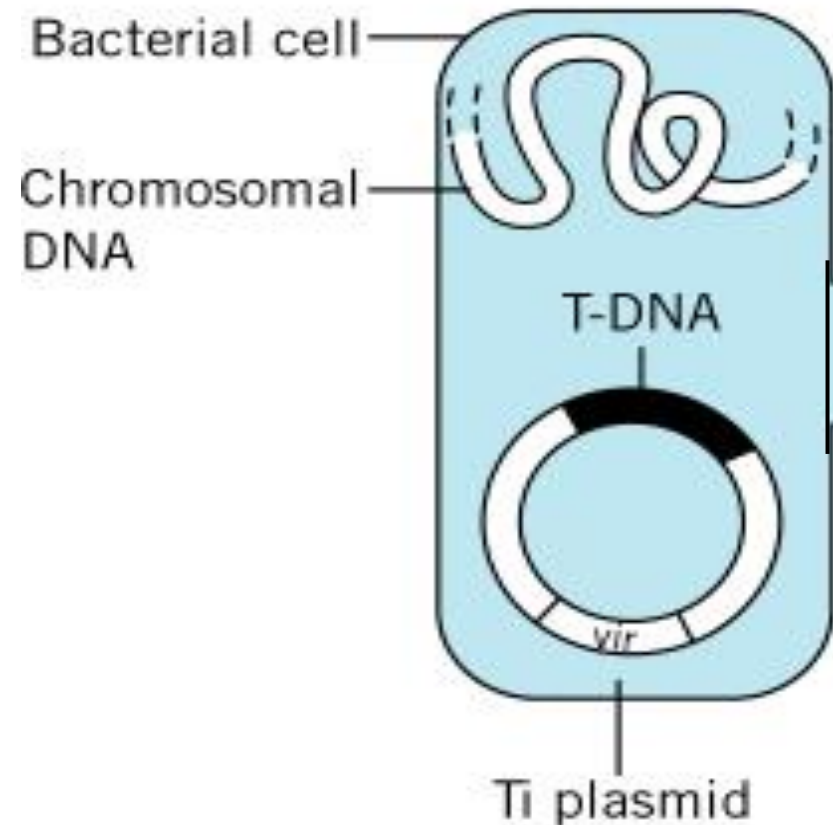
Agrobacterium contains Ti plasmid and T-DNA

***Agrobacterium* has two types of DNA:**

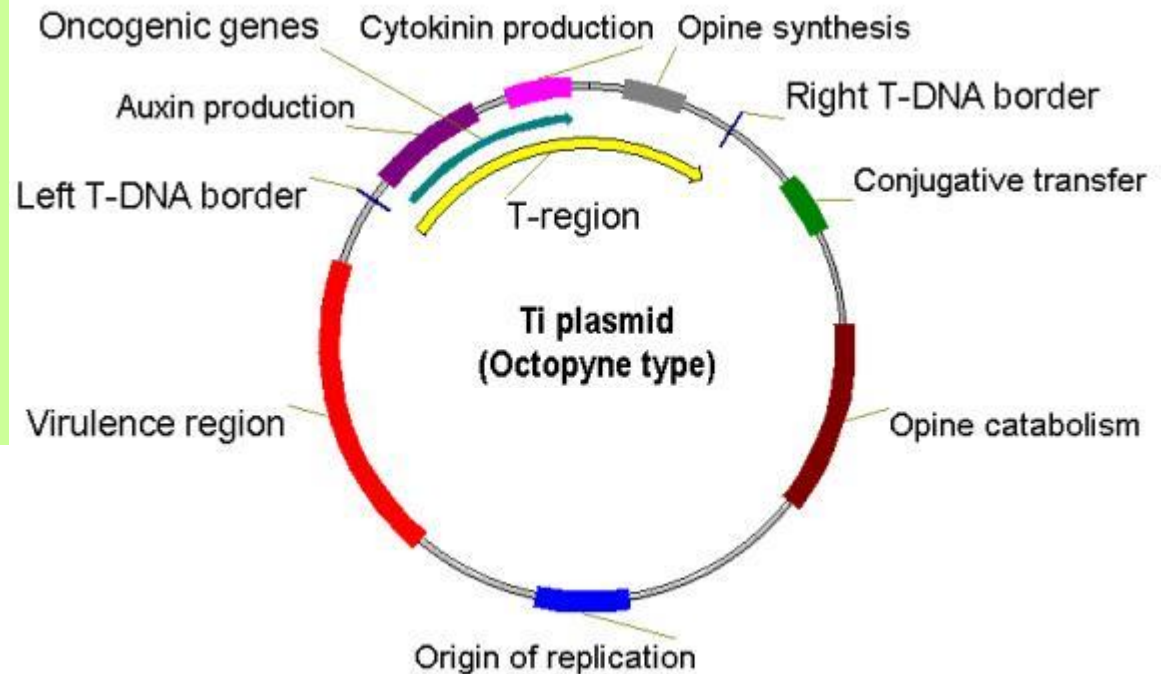
Chromosomal DNA
Ti plasmid

Ti plasmid:
Tumor inducing plasmid

T-DNA:
Transfer DNA, a portion of the Ti plasmid that is transferred into a host.



The Ti plasmid is key for *Agrobacterium*-mediated gene transformation



Important features on a Ti-plasmid:

Replication origin: required for DNA replication.

Virulence regions: genes required for T-DNA cleavage, binding, translocation, and possibly integration.

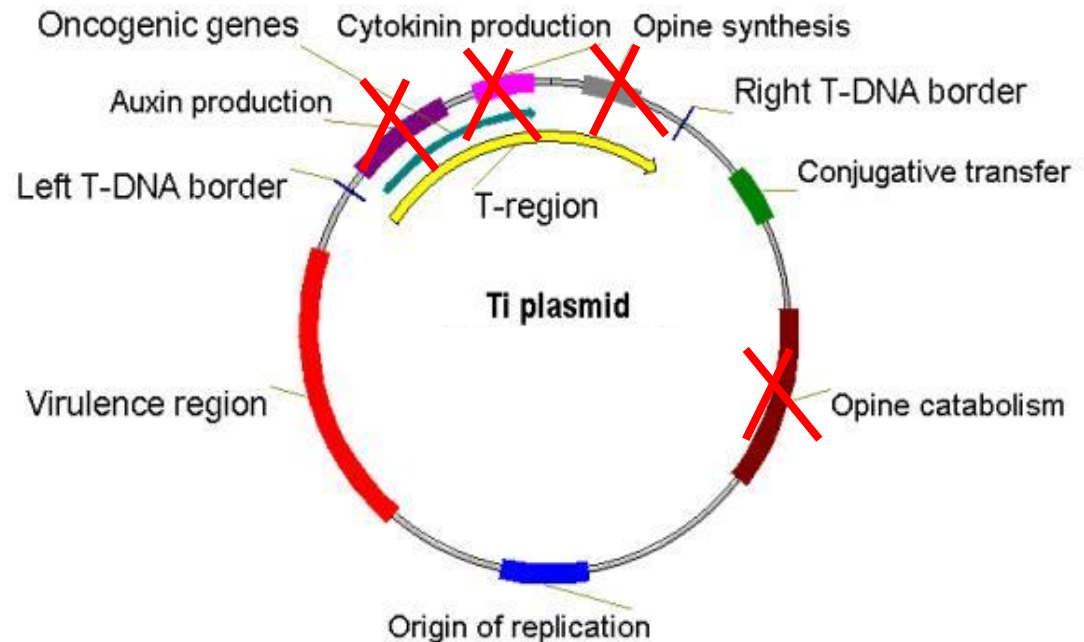
Onco genes: responsible for auxin and cytokinin production. They are required for tumor growth of the infected plant.

Opine metabolic genes: required for opine synthesis and catabolism, the nitrogen & carbon source for the survival of *Agrobacterium*.

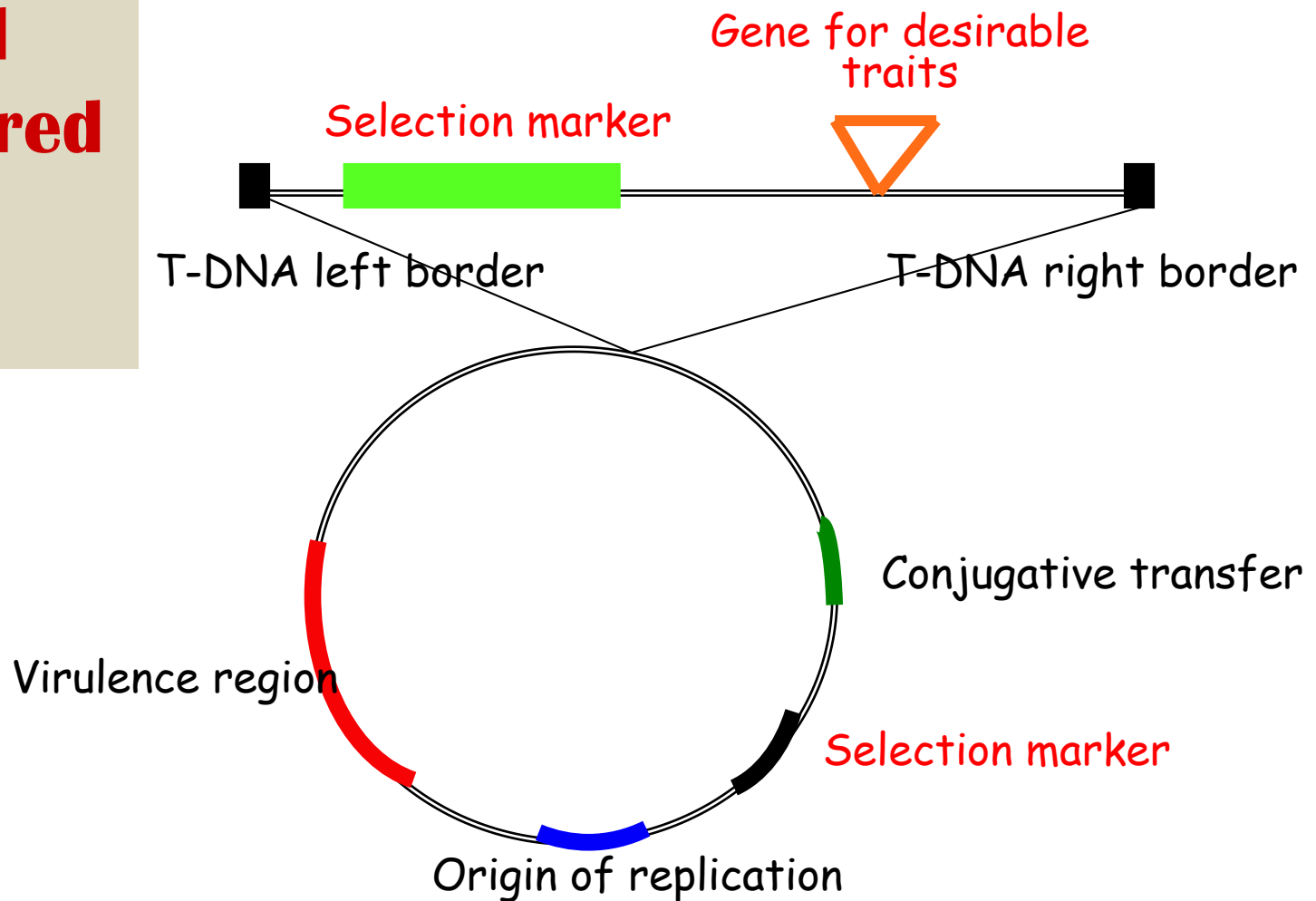
Engineering the Ti-plasmid

Binary vectors are derivatives of **Agrobacterium Ti-plasmid** that can replicate in both *Agrobacterium* and plant cells.

- Disarmed the onco-genes: no more tumor formation
- Disarmed opine metabolic genes: there is no need for bacterial growth during transformation.

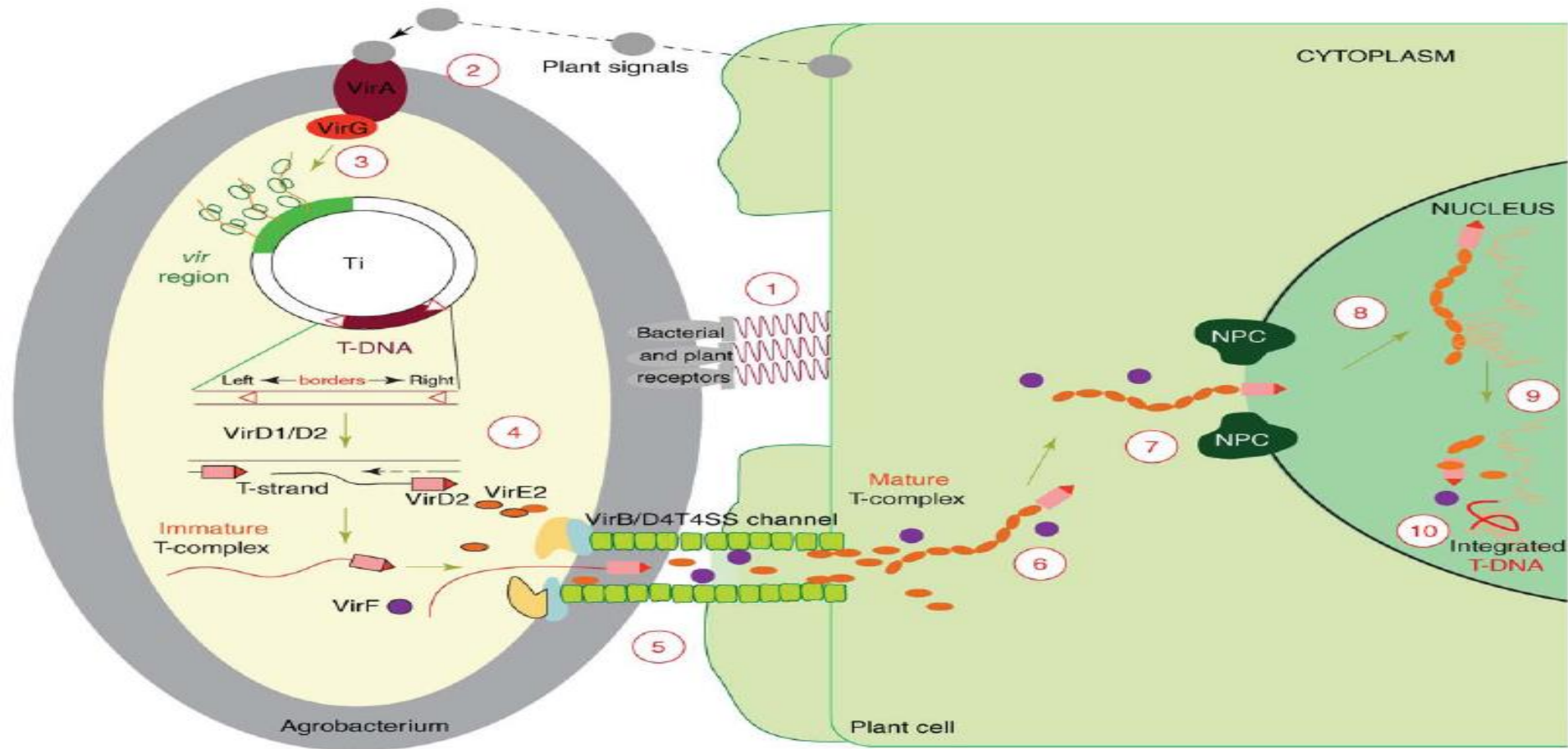


A typical Engineered binary vector

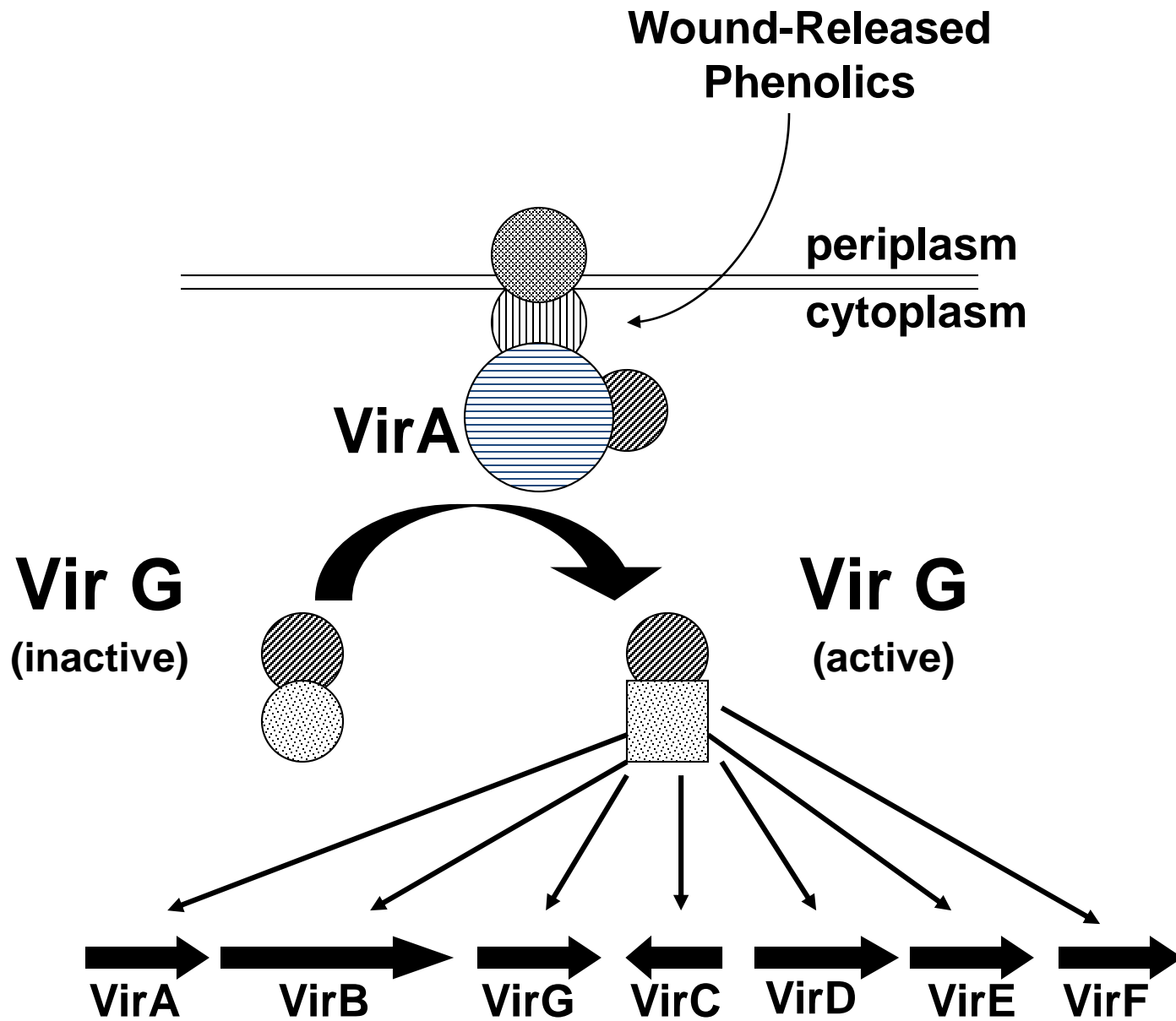


- Include the gene encoding desirable traits.
- Include the selection markers:
 - To tell the engineered *Agrobacterium* from non-engineered ones
 - To tell the engineered plants from the non-engineered ones.

A model for the *Agrobacterium*-mediated genetic transformation



Recognition and attachment, Vir genes expression by host signals, T-strand produce, T-complex export into host, Transport through cytoplasm and nuclear, T-DNA uncoating and integration.



Disarmed *Agrobacterium* strains and gene construct used for genetic transformation of *Dalbergia sissoo* Roxb.

Strain/ Construct	Antibiotic Marker	Characteristic	Reference
LBA4404	Rifampicin	Octopine type <i>Agrobacterium tumefaciens</i> strains containing plasmid pAL4404 (T-DNA deletion derivative of pTiAch 5)	Hoekema et al.,1983
EHA105	Rifampicin	Hyper-virulent succinamopine <i>Agrobacterium tumefaciens</i> strains containing plasmid pEHA105 (T-DNA deletion derivative of pTiBO542)	Hood et al.,1993

Timber Yielding Forest Trees

Sissu (*Dalbergia sissoo* Roxb.)

Indian Rosewood (*D. latifolia* Roxb.)

“Sajanapati” (*D. lanceolaria* L.)



Shimshapa

Shishu (*Dalbergia sissoo* Roxb.)

Chemical constituents : Leaves, flowers and immature pods contain Biochanin A, flavone-5-dihydroxy-6, 7- dimethoxyisoflavone

Uses : Root, bark and leaf in polyuria, chronic ulcer, leprosy, leucoderma, urinary bladder disorder. Leaves against eye infection & gonorrhoea, Roots astringent, Bark & Wood expectorant, antihelmintic & antipyretic.

Kushimshapa

Indian Rosewood (*Dalbergia latifolia* Roxb.)

Chemical constituents : Bark contains methyl dalbergin. Seeds contain coumarin sisafolin and rotenoid dalbinol

Uses : As for 'Shimshapa'



Goraksha (Kapotavanka)

Dalbergia lanceolaria (L.)

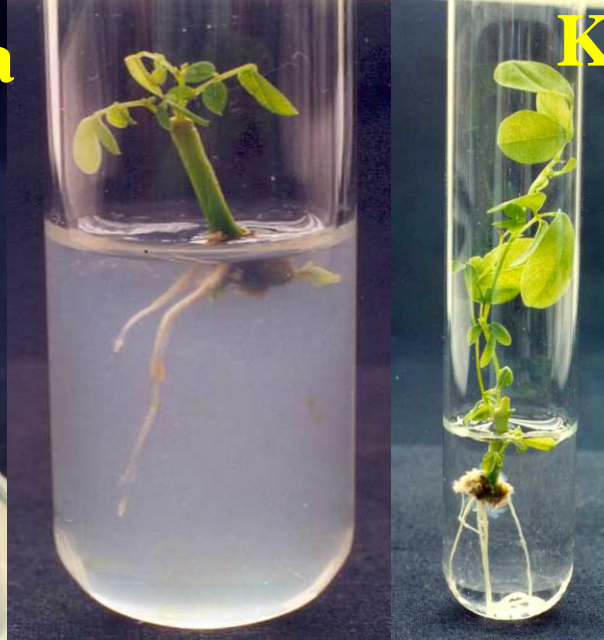
Chemical constituents : Root bark contains glucoside, Lanceolarin. Bark contains tanin

Uses : Decoction of bark used in dyspepsia. Seed oil in rheumatism, rheumatoid arthritis, anorexia

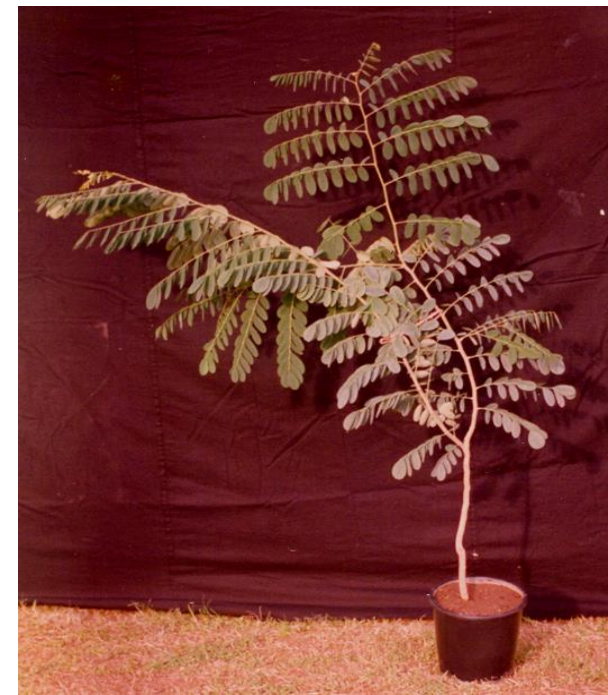
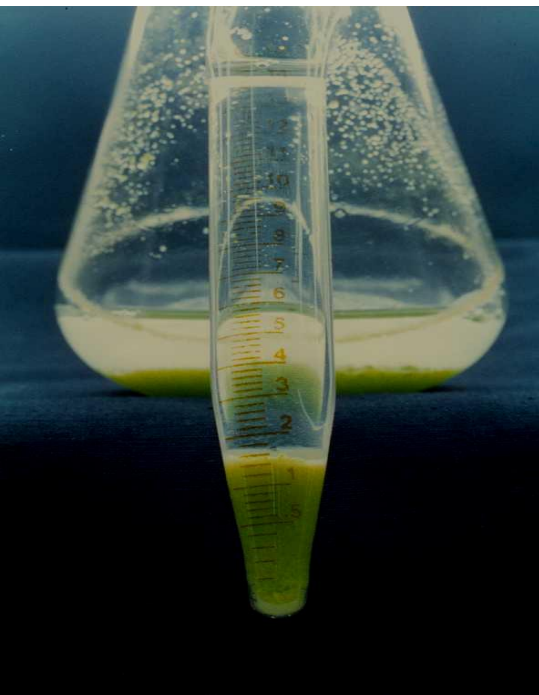
Shimshapa



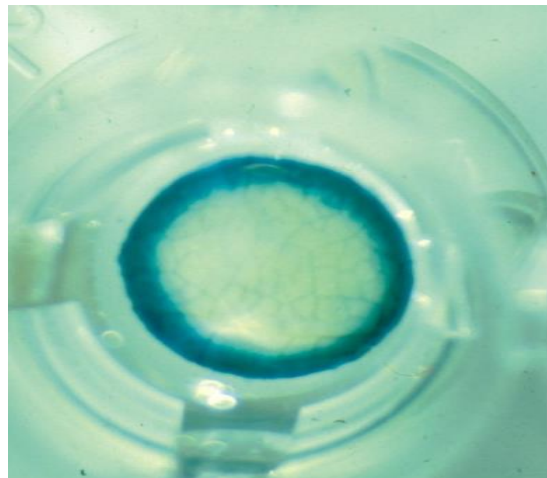
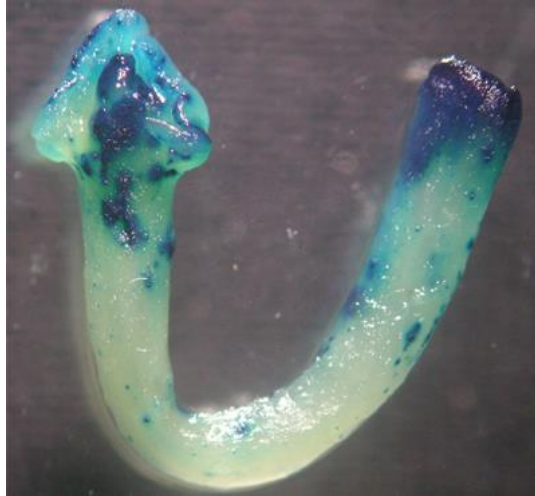
Kushimshapa



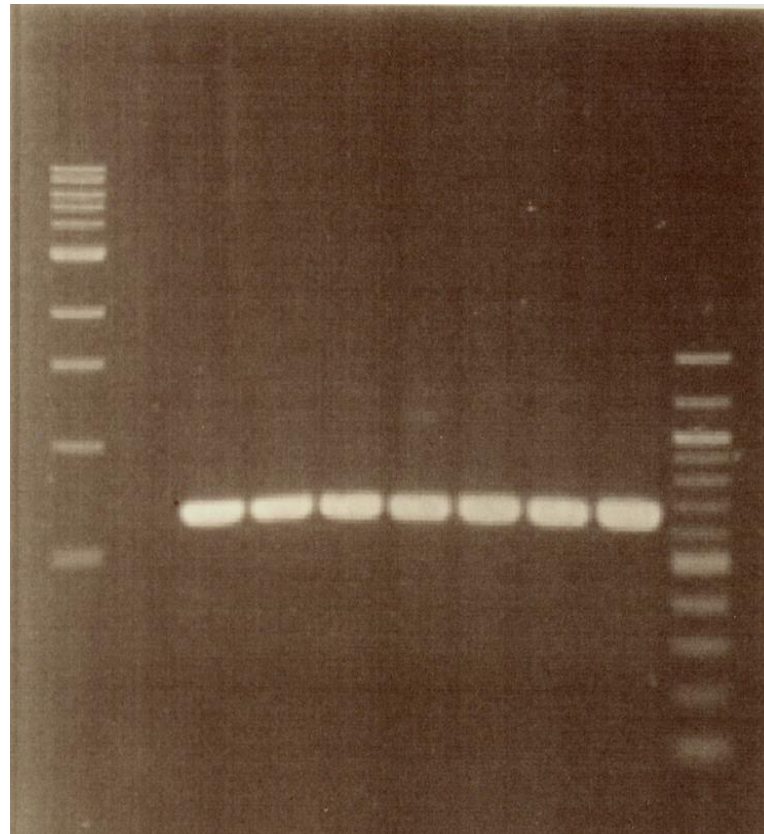
Goraksha (Kapotavanka)



Transient GUS expression in *D.sissoo*



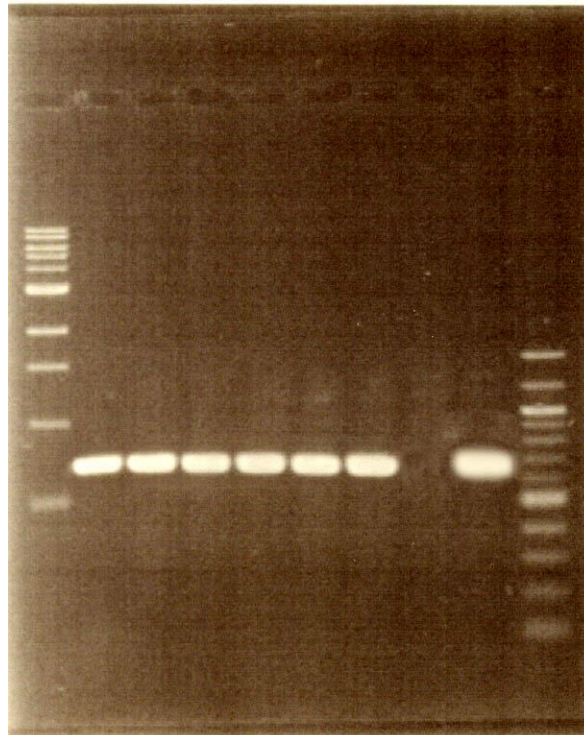
PCR amplification of **650 bp** fragment specifying *gus* transgene
in the leaf genomic DNA of putative transgenic lines of
Dalbergia sissoo



M1 1 2 3 4 5 6 7 8 M2

- M1 : DNA Marker
(1 kb ladder)
- M2 : DNA Marker
(100 bp ladder)
- 1: DNA from
untransformed shoot
(Negative Control)
- 2-8 : DNA from putative
transgenic lines

PCR amplification of 693 bp fragment specifying *nptII* transgene in the leaf genomic DNA of putative transgenic lines of *Dalbergia sissoo*



M1 1 2 3 4 5 6 7 8 M2

- M1 : DNA Marker (1 kb ladder)
- M2 : DNA Marker (100 bp ladder)
- 1-6 : DNA from putative transgenic lines
- ← 693 bp • 7: DNA from untransformed plant (Negative Control)
- 8 : Plasmid DNA of p35SGUSINT (Positive Control)

SUMMARY OF RESULTS

1. Epicotyl segments more suitable
2. Higher transformation efficiency recorded in LBA 4404
3. $OD_{600} \cong 0.3$ was ideal for infection
4. Hand pricking was most suitable for *Agrobacterium* infection
5. Infection period of 10 min was found most suitable
6. pH of 5.6 was found effective for both the explants
7. Cefotaxime ($500 \mu\text{gml}^{-1}$) was the most effective in eliminating out overgrown *Agrobacteria*
8. $100 \mu\text{gml}^{-1}$ kanamycin as selection antibiotic
9. GUS assay shows blue colouration
10. Putative transformed shoots rooted on $\frac{1}{2}$ MS + 1 mgl^{-1} IAA + 1 mgl^{-1} IBA + 1 mgl^{-1} IAA + kanamycin ($100 \mu\text{gml}^{-1}$).

Agrobacterium rhizogenes

the Natural Genetic Engineer for
Induction of Hairy Roots



Hairy root cultures as a source of
pharmaceuticals and novel
phytochemicals

Methodology

- Bacterial strain : **LBA9402, A4**
- Bacterial culture media: **YEB, MYA**
- Explant inoculation and rhizogenesis

- Plant material : *in vitro* and *in vivo*
- Mode of Infection: (i) **Direct injection**
(ii) **Pre-pricked & dipped**
(iii) **Explant unwounded**
- Co-cultivation Period : **1 - 3 weeks**

- Cloning and maintenance of transformed roots : **MS0 + Cefotaxime : 1-2 weeks**
: **MS0 : continued**
- PCR
- Opine assay

Plant Material for *Agrobacterium rhizogenes* – mediated Genetic Transformation of the Leafy Amaranth *Amaranthus tricolor*



- *In vitro*-germinated seedlings of var. 3 : red
- *In vitro*-germinated seedlings of var. 1 : green

Induction of transformed roots from leaf midribs



var. Red : Day 12



var. Green : Day 24

Induction of Hairy Roots in Genetically Transformed *A. tricolor*
following **INTERNODE** Co-cultivation with **LBA9402** Wild type
Strains of *Agrobacterium rhizogenes*



var. 3 : Green



var. 3 : Red

In vitro cloning of transformed roots on MS0



Long term objective

- The long-term objective of this work is to utilize such hairy root cultures as an effective renewable production system to solve the problems of
- i) plant-to-plant variation in the yield and quality of the desired compound.
- ii) Long period between planting and harvesting.
- iii) indiscriminate over-exploitation of the natural resources and
- iv) uncertainty over the supply of raw materials due to environmental and/or pathological constraints.

PUBLISHED PAPER

World J Microbiol Biotechnol (2012) 28:729–739
DOI 10.1007/s12274-011-0269-1

ORIGINAL PAPER

Hairy root cultures of butterfly pea (*Clitoria ternatea* L.): *Agrobacterium* × plant factors influencing transformation

S. S. Swain · L. Sahu · A. Pal · D. P. Barik ·
C. Pradhan · P. K. Choud

Future Directions

- **Multi-elemental analysis of root somaclones vis-à-vis donor plants and their callus/cell suspension cultures by EDXRF and EPIX techniques**
- **Phytochemical screening of root somaclones for secondary metabolites using TLC and HPTLC techniques**
- **Quality testing and certification of promising root somaclones and their products**

This will provide enough food and bring
“A Second Green Revolution”



GM crop may not be required for rich countries

-  There is plenty of food in developed countries. There is no compulsion for them to accept GM crops.
-  But in poor countries people suffer from malnutrition
-  They should adopt **“new agricultural avenues and knowledge-based agriculture”** to counteract their endemic poverty and feed their exploding population.

GM food has more nutritional qualities

**“Golden Rice” contains more vitamin-A,
which Prevents Blindness.**







Ingo Potrykus & Peter Beyer

GM crops will reduce application of chemical pesticides and herbicides.



b20-426340 fotosearch.com

More Research

-  However, more research and discussion is required before we accept or reject any new findings.
-  At the same time we should not be hurried.
-  Otherwise our experience with DDT may be repeated.
-  Paul Hermann Muller-the person behind DDT was awarded Nobel Prize. Now DDT is banned.

Novel Genes in GM Crops may bring more Biodiversity

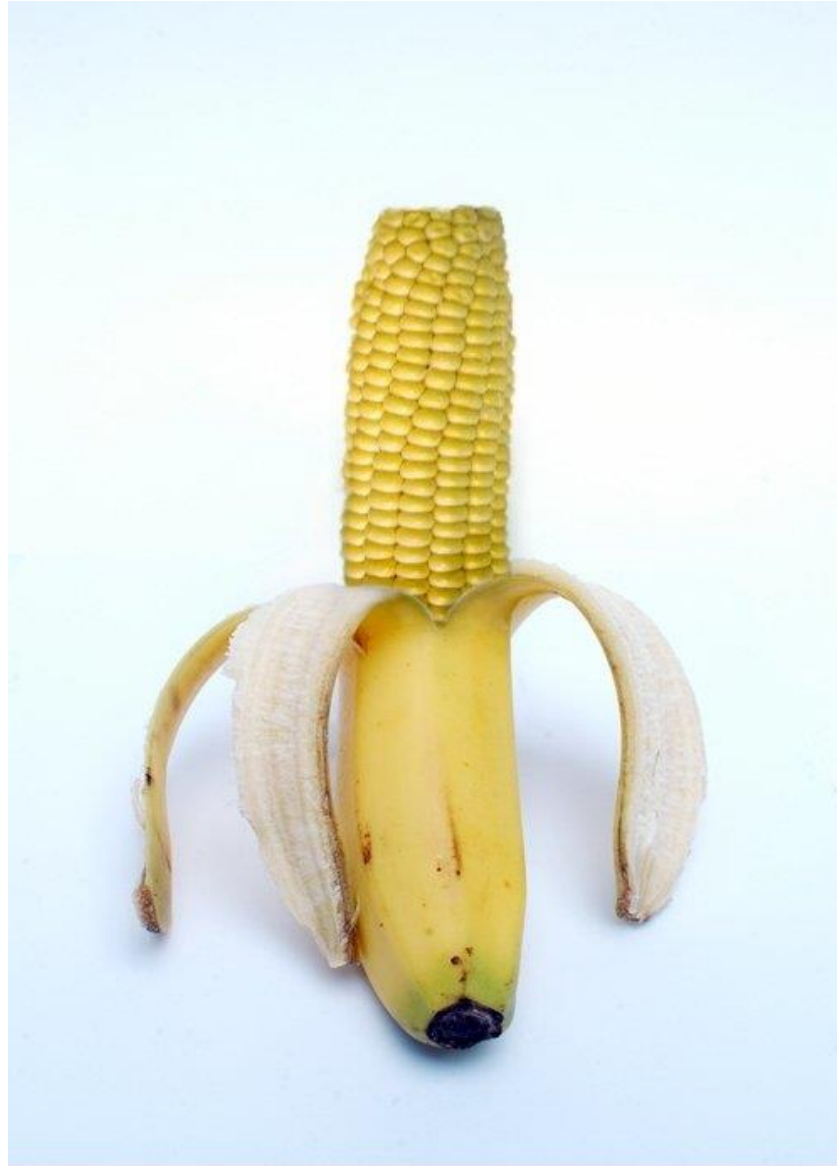


Days are not far away to produce

This



Or This





Thank you