



Transformation methods

random | [LINK](#)



Transformation:

- Transformation is the process by which genetic makeup of an organism is altered by the insertion of new gene(or exogenous DNA) into its genome .This is usually done using vectors such as plasmids.
- **The aim of producing transgenic plants is to-**
 - a) Improve crop yields.
 - b) Improvement of varietal trait.
 - c) Give cultivated plants more protection against their pests, parasites and harsh weather conditions.

History

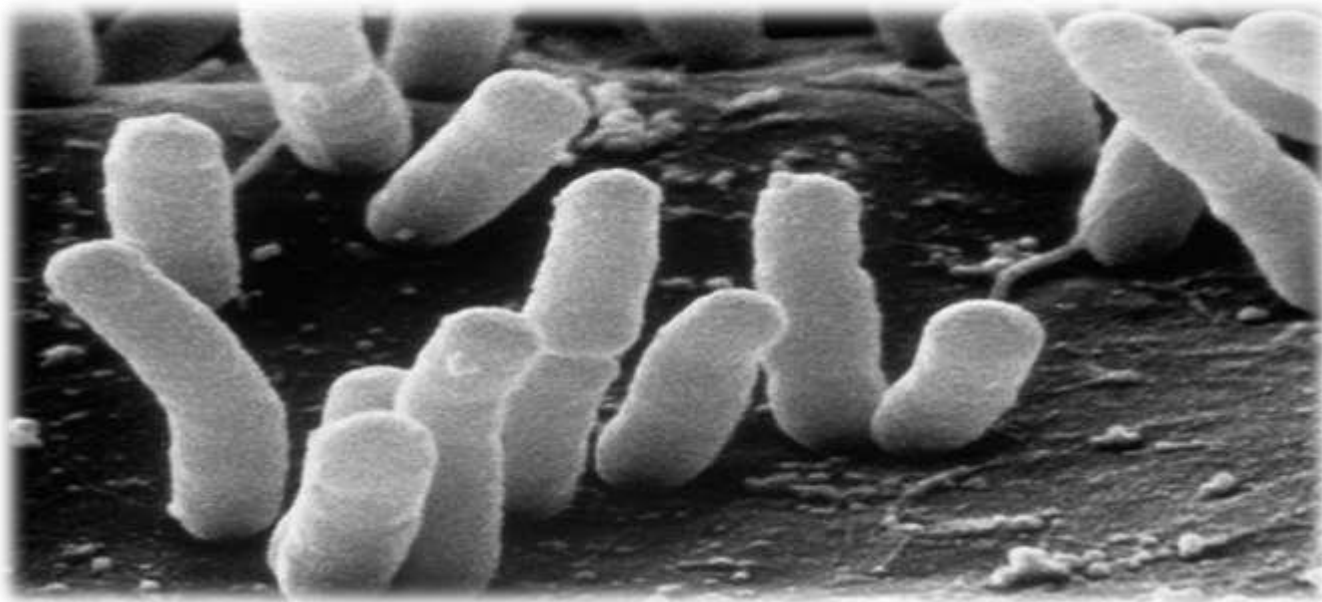
- Transformation was first demonstrated in 1928 by British bacteriologist [Frederick Griffith](#) in *Diplococcus pneumonia*.
- In 1944 this "transforming principle" was identified as being [genetic by Oswald Avery, Colin MacLeod, and Maclyn McCarty](#)
- Transformation using [electroporation](#) was developed in the late 1980s.
- Particle bombardment discovered (gene gun) by [John Sanford](#) in the 1980s.

Methods of plant transformation:

- **INDIRECT METHOD:** *Agrobacterium* mediated gene transfer.
- **DIRECT METHODS :**
 - ✓ Particle gun/biolistic/ballistic method of DNA delivery.
 - ✓ Chemical method; Polyethylene glycol (PEG)/protoplast fusion.
 - ✓ Microinjection.
 - ✓ Macroinjection.
 - ✓ Electroporation.
 - ✓ Lipofection; Liposome mediated gene transfer.
 - ✓ Fibre mediated DNA delivery.
 - ✓ LASER induced DNA delivery.
 - ✓ Pollen transformation.
 - ✓ DNA delivery via Growing pollen tubes.
 - ✓ Direct DNA uptake by mature zygotic embryos.

***Agrobacterium* mediated genetransfer**

- *Agrobacterium tumefaciens* : – A Natural genetic engineer.
- It is achived by two ways are-
 - 1) Co-culture with tissue explants. and
 - 2) In planta transformation.



Agrobacterium tumefaciens

Characteristics:

- Soil borne, gram – ve , rod shaped, motile bacteria found in rhizosphere.
- Encodes a large (~250kbp) plasmid called Tumor-inducing (Ti) plasmid, a vector which can transfer its T-DNA region into genome of host plants.
- Causative agents of “Crown gall” disease of dicotyledons.
- Have ability to transfer bacterial genes to plant genome.
- Attracted to wound site via chemotaxis in response to chemicals (Sugar and Phenolic molecules: like-Acetosyringone) released from damaged plant cells.

Vectors:

- **Vectors** -- the DNA carriers.

Must have:

Origin of replication.

Antibiotic-resistant genes.

- Allow the host to grow on selective media. Can selectively amplify this specific vector in the host cell.

Multiple cloning sites.

Allow insertion of foreign DNA.

Ti-plasmid features:

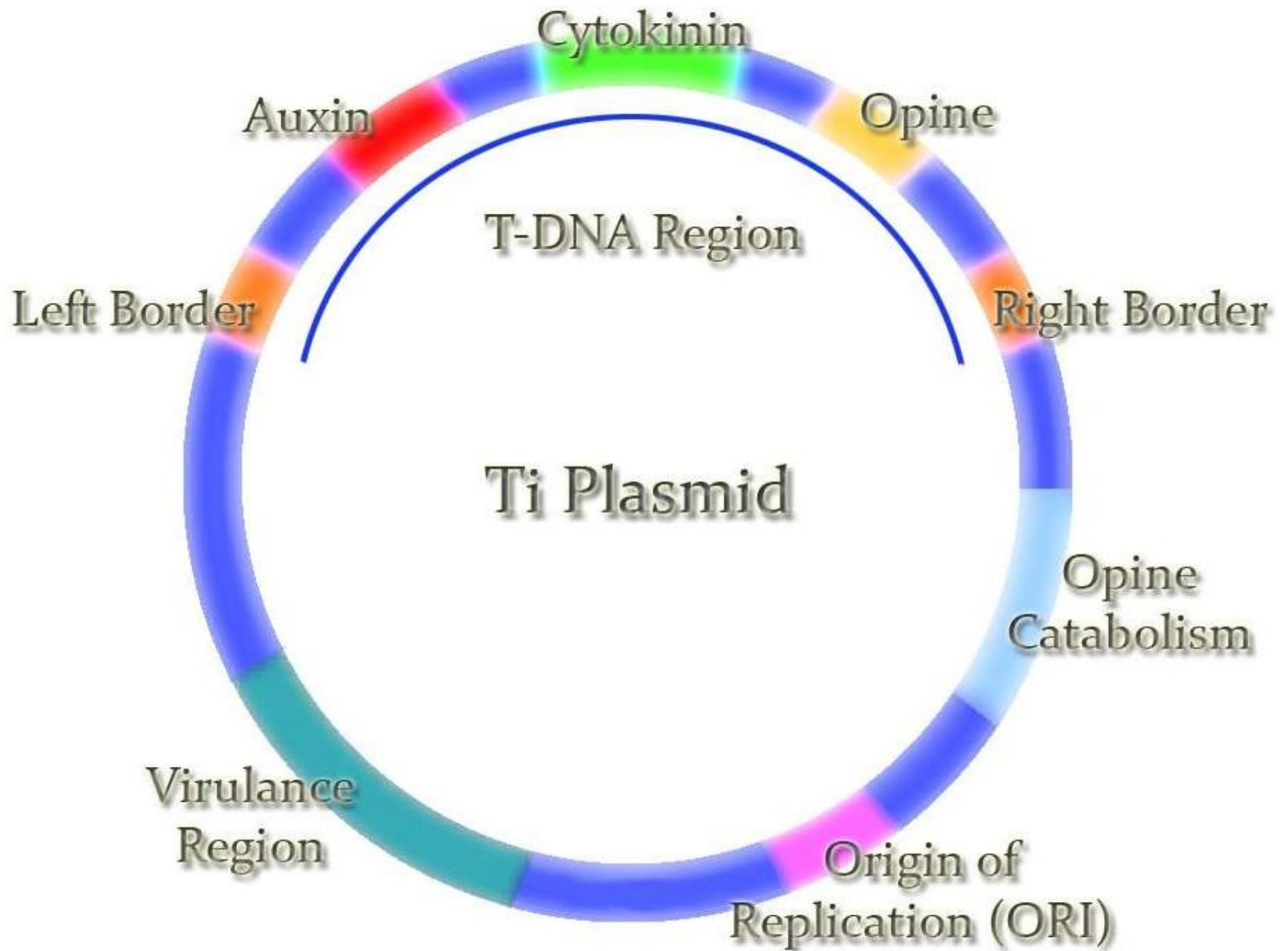
These are extracellular double stranded circular self replicating DNA in bacteria.

Two strains of Ti-plasmid:

- 1 **Octopine strains-** Two T-DNA region: T_L (14 kb) and T_R (7 kb).
- 2 **Nopaline strains-** contain one T-DNA region(20 kb) .
 - Size is about 200 kb.
 - Has a central role in Crown-gall formation.
 - Our required T-DNA.
 - Also other regions.

Organisation of t-dna.

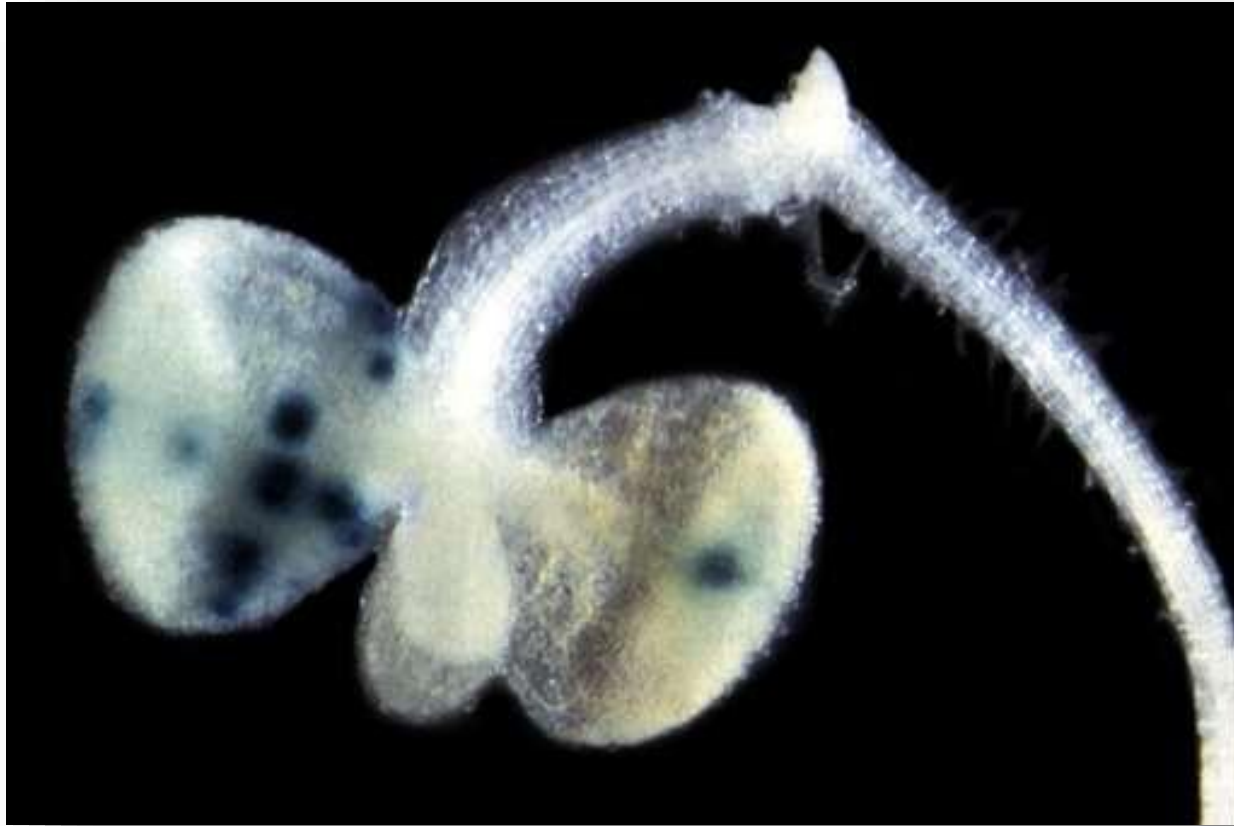
- IAAM encodes-tryptophan monooxynases.
- IAAH-Indole acetamide hydrolase.-these two constitutes auxin biosynthesis.
- IPT-precursor for cytokinin synthesis.
- Contains one or more T-DNA region that is integrated into the genome of host plants of size~ 12 to 24 kb.
- Left and right border sequence(24-bp) which will be transferred into genome of host plant.
- Contain a vir region ~ 40 kb ,at least 8~11 vir genes.



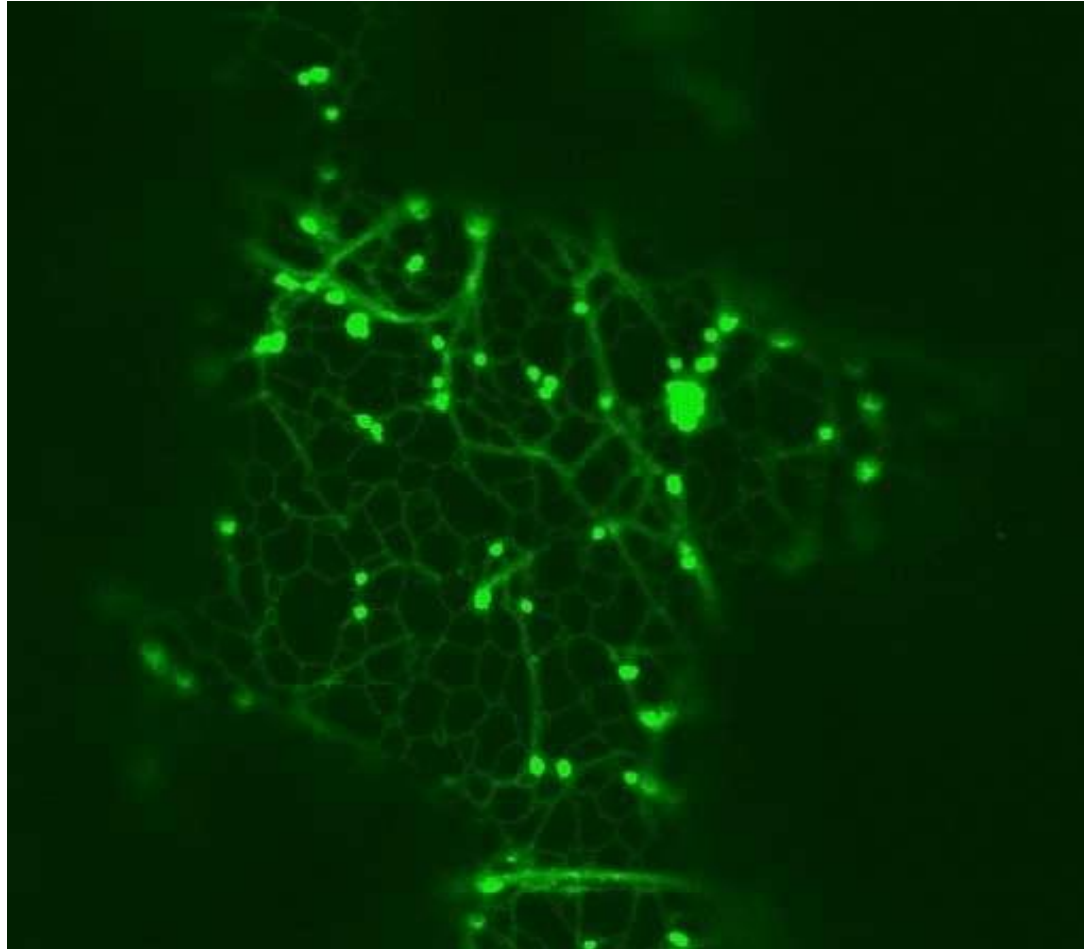
Visible markers:

- **B-glucuronidase (GUS)**
- Gives a blue colour from a colourless substrate (*Xglu*) for a qualitative assay.
- Also causes fluorescence from Methyl Umbelliferyl Glucuronide (*MUG*) for a quantitative assay.
- **Green Fluorescent Protein (GFP)**
- Fluorescent green under UV illumination
- Non-destructive

B-glucuronidase (GUS) based selection:



**Selection based on GFP reporter gene
(green fluorescent protein):**



Process of T-DNA transfer and integration;

1. Signal recognition by *Agrobacterium*:

-*Agrobacterium* perceive signals such as sugar and phenolic compounds (Acetosyringon) which are released from plants when got wounded.

2. Attachment to plants cells:

Two step processes:

i) Initial attachment via polysaccharide.

ii) Mesh of cellulose fiber is produced by bacteria.

Virulence genes are involved in the stable attachment of bacterial cells to the plants cells.

3. *Vir* gene induction:

VirA senses phenolics and subsequently phosphorylating there by activating *VirG*.

VirG then induces expression of all the *vir* genes.

4. T-strand production:

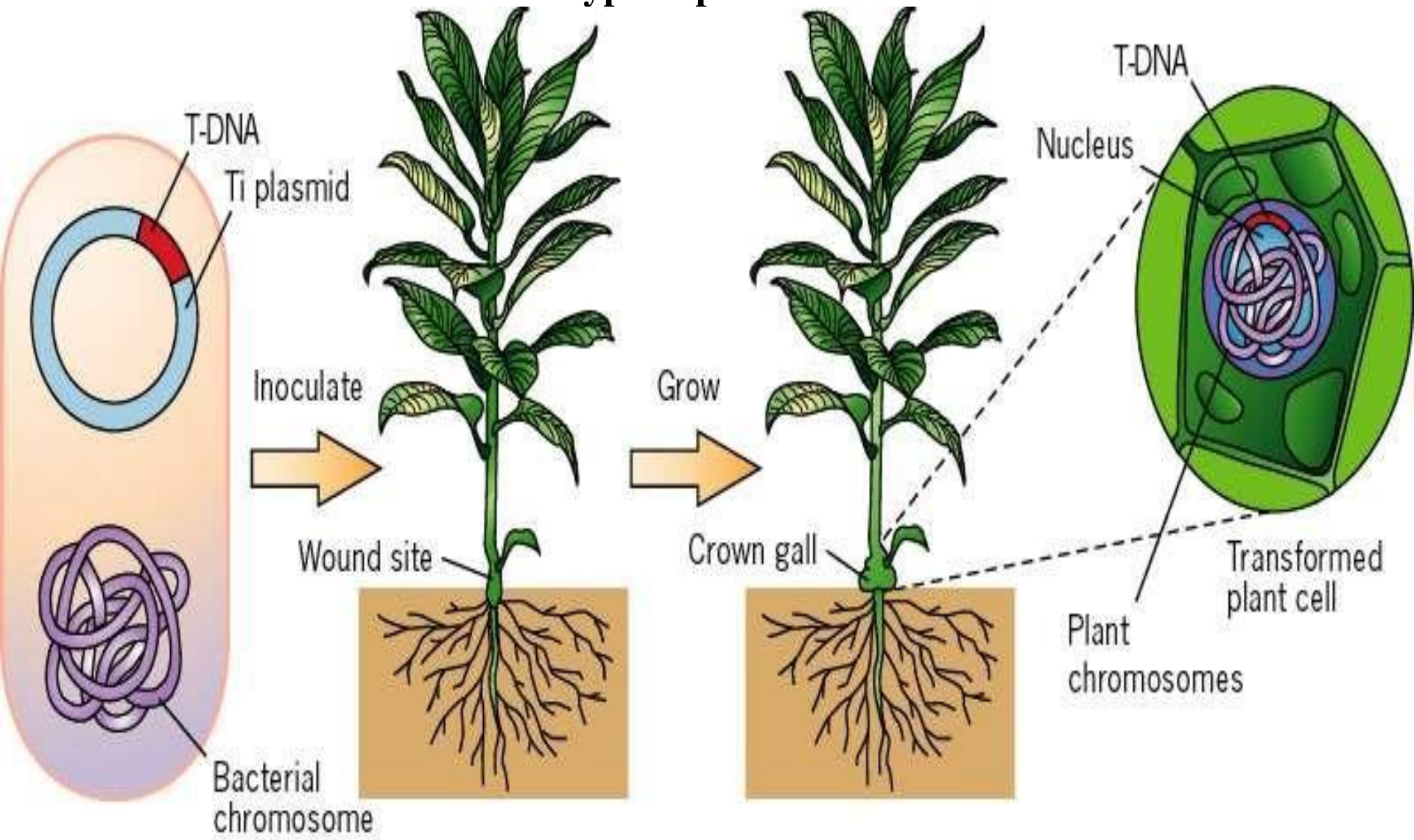
- ✓ The virD1 as topoisomerase activity which binds to RB and relaxes the super coiling which facilitates the action of virD2(as endonuclease activity),
- ✓ It nicks the RB & covalently binds to 5' end. Similarly the 3' end produced at the site of nick, serves as a primer. As a result single strand of T-DNA is displaced by virE.
- ✓ The T-DNA is again nicked at LB to generate ssT-DNA copy. VirE2 protects this from nucleases. virB is essential for virulence which also as ATP-ase activity, therefore helps in deliver of T-DNA into plant cell, mostly through nuclear pore complex.

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5. Transfer of the T-DNA and Vir proteins into the plant nuclear localization:

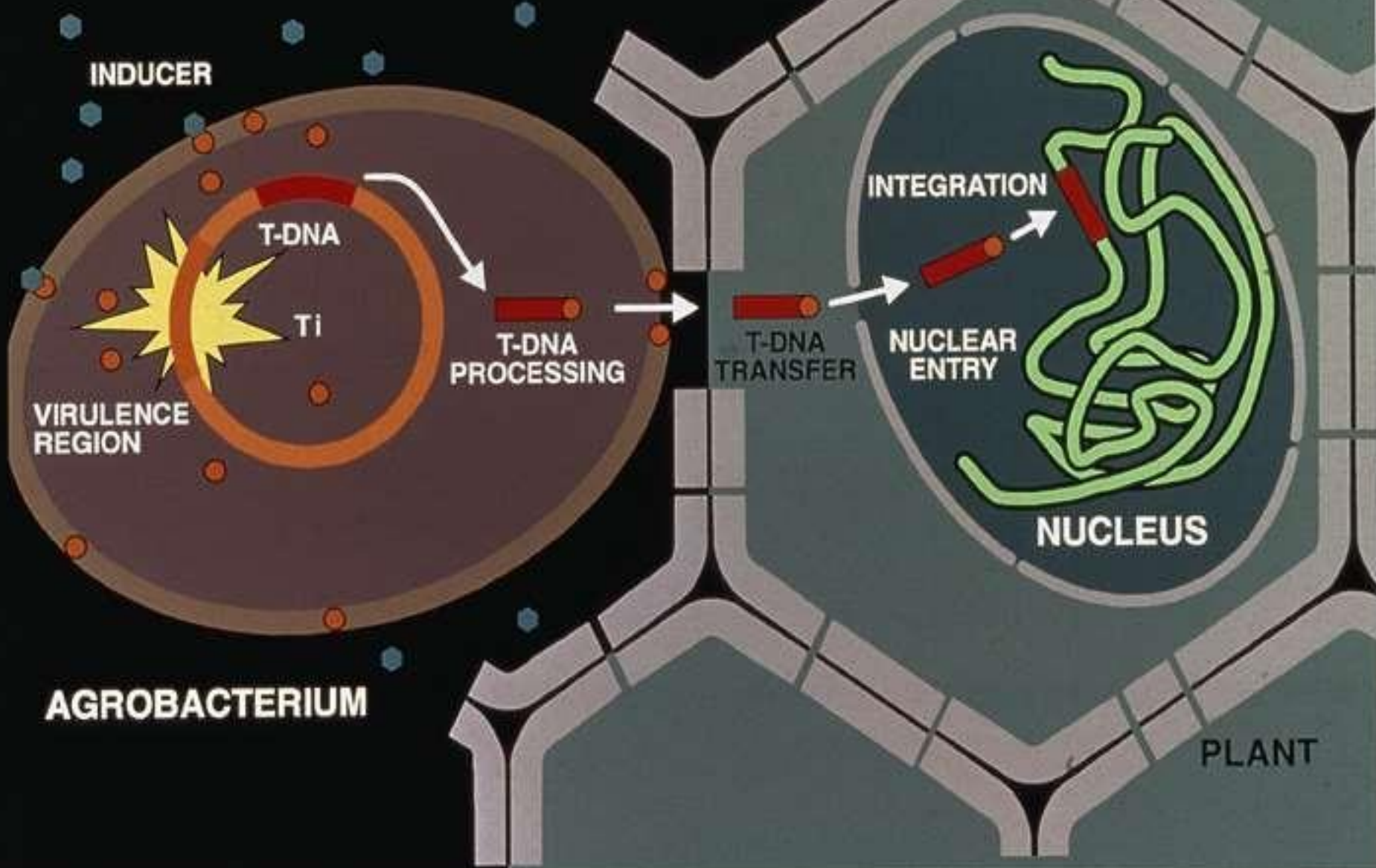
- ✓ The ssT-DNA is immediately converted to ds in nucleus by replication.
- ✓ The dsT- DNA integrates at random site in the host cell genome by illegitimate recombination.

Transformation of plant cells by *Agrobacterium tumefaciens* harboring a wild-type Ti plasmid:



Agrobacterium tumefaciens

PASSAGE OF T-DNA FROM AGROBACTERIUM INTO PLANT NUCLEAR DNA



Agrobacterium tumefaciens



DNA containing gene for desired trait



1
Insertion of gene into plasmid using restriction enzyme and DNA ligase



2
Introduction into plant cells in culture



3
Regeneration of plant

T DNA carrying new gene within plant chromosome



Plant with new trait

Practical application of *Agrobacterium*-mediated plant transformation

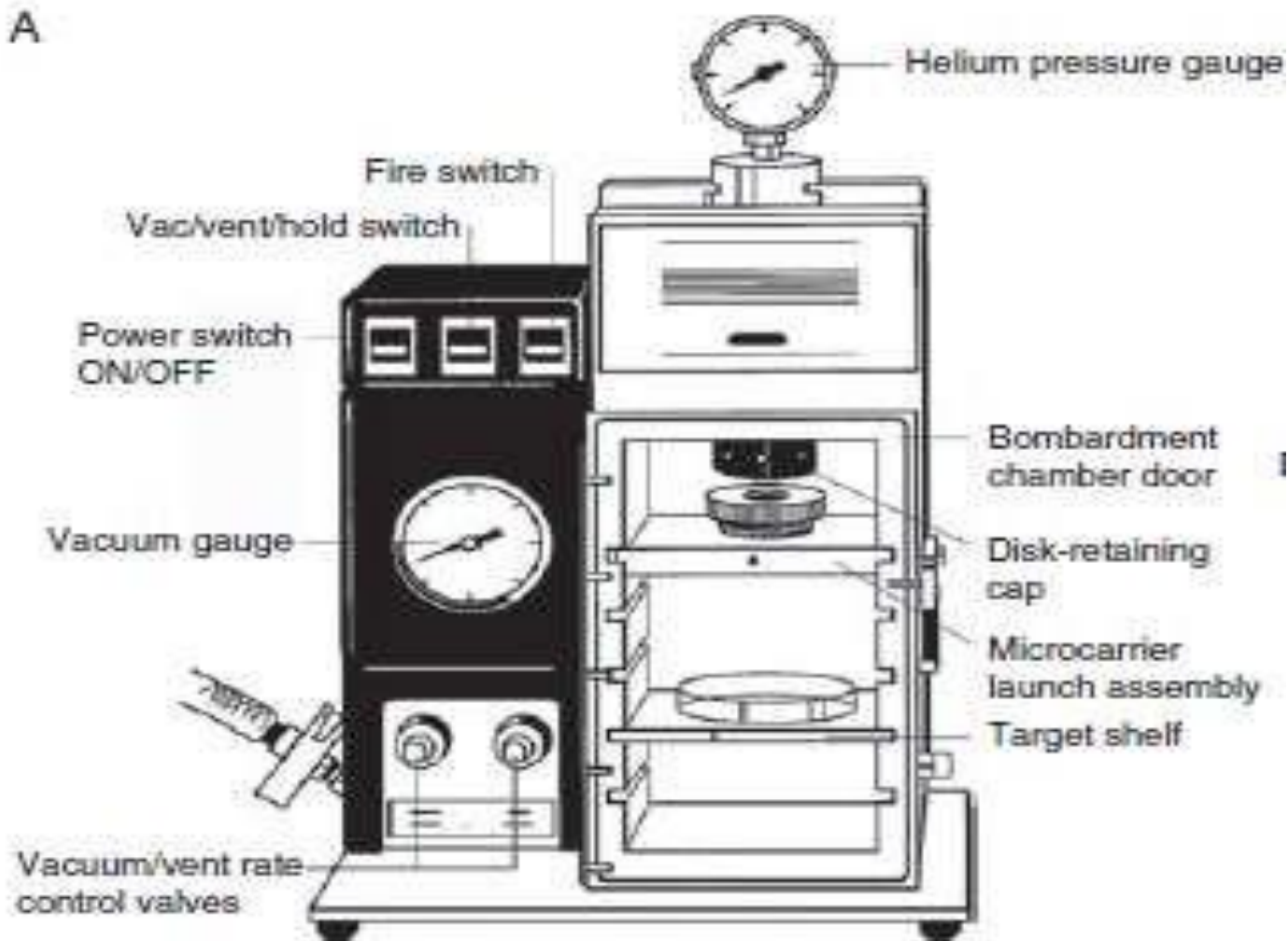
- *Agrobacterium* mediated transformation methods are thought to induce less rearrangement of the transgene.
- Lower transgene copy number than direct DNA delivery methods.

DIRECT METHODS

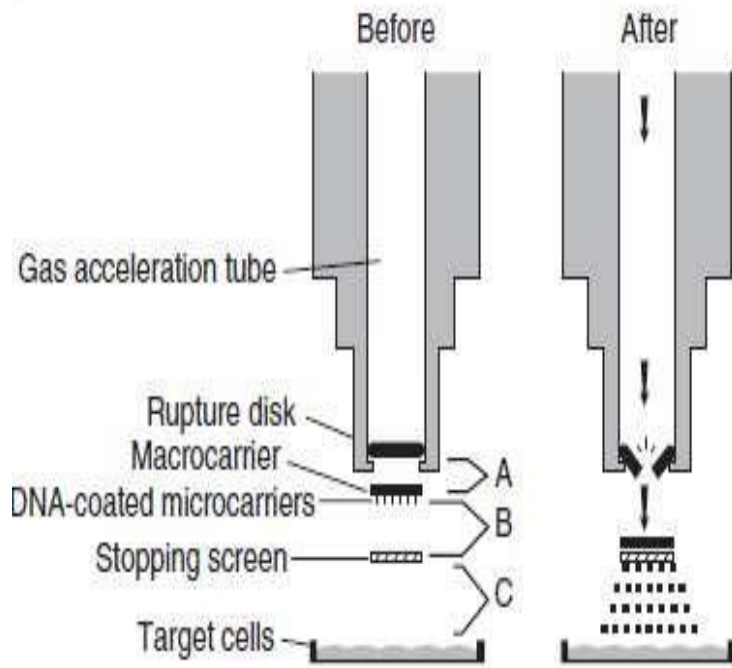
1. PARTICLE GUN METHOD.

- ✓ A **gene gun** or a **biolistic particle delivery system**, designed for plant transformation, is a device for injecting cells with genetic information. The payload is an elemental particle of a heavy metal(tungston/gold) called microprojectiles coated with plasmid DNA.

A



B



Principle:

- ✓ The gold or tungsten particles are coated with the DNA that is used to be transform the plant tissue.
- ✓ The particles are propelled at high speed into the target plant material where the DNA is released within the cell and can integrate into the genome.
- ✓ **Two types of plant tissues are used for particle bombardment:**
 - a) Primary explants that are bombarded and then induced to become embryogenic.
 - b) Proliferating embryonic cultures that are bombarded and then allowed to proliferate further and subsequently regenerate.

How the PDS-1000/bombardment System Works :

- ✓ The sample to be transformed is placed in the bombardment chamber, which is evacuated to sub atmospheric pressure.
- ✓ The instrument is fired; helium flows into the gas acceleration tube and is held until the specific pressure of the rupture disk is reached.
- ✓ The disk bursts, and the helium shock wave drives the macrocarrier disk (which carries the coated microparticles) a short distance toward the stopping screen.
- ✓ The stopping screen retains the macrocarrier, while the microparticles pass through the screen into the bombardment chamber and penetrate the target cells

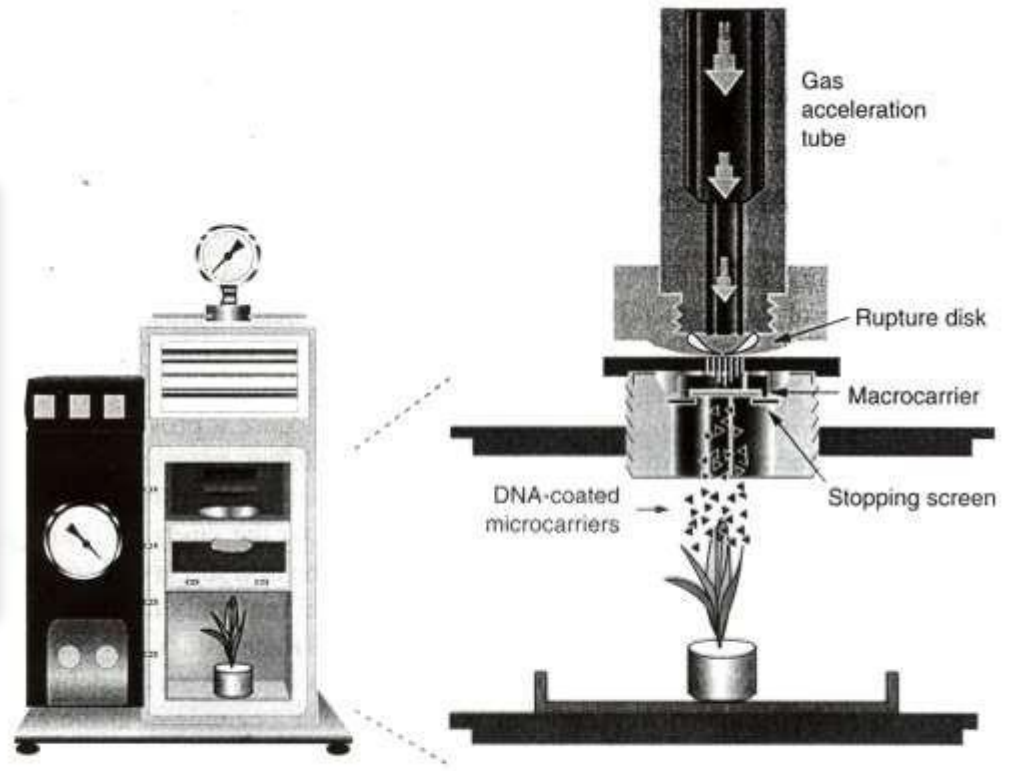
- **The launch velocity of microcarriers** depends on a number of adjustable parameters:
 - ✓ The helium pressure (rupture disk selection, 450–2,200 psi),
 - ✓ The amount of vacuum, the distance from the rupture disk to the macrocarrier,
 - ✓ The distance from the microcarrier launch assembly to the stopping screen,
 - ✓ The distance between the stopping screen and target cells.
 - ✓ Adjusting these parameters allows you to produce a range of velocities to optimally transform many different cell types.

(a)

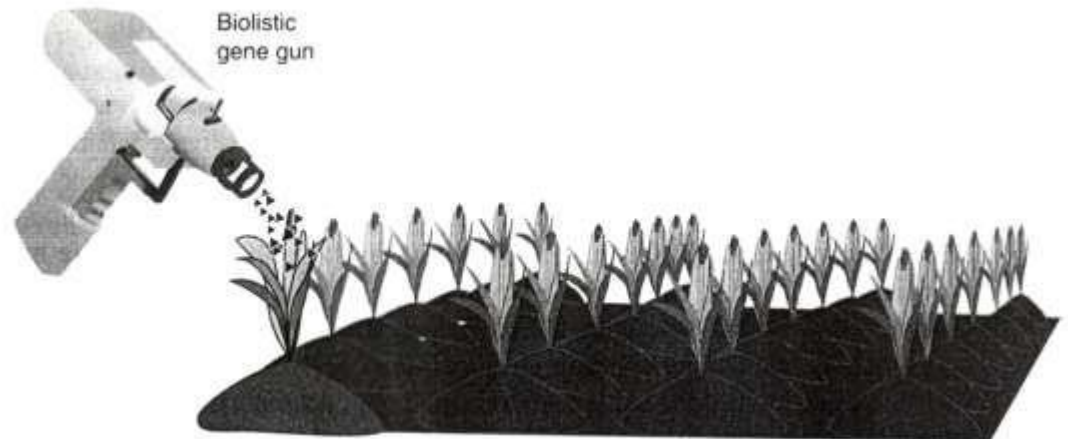
Microprojectile bombardment
or biolistic-mediated DNA
transformation equipment

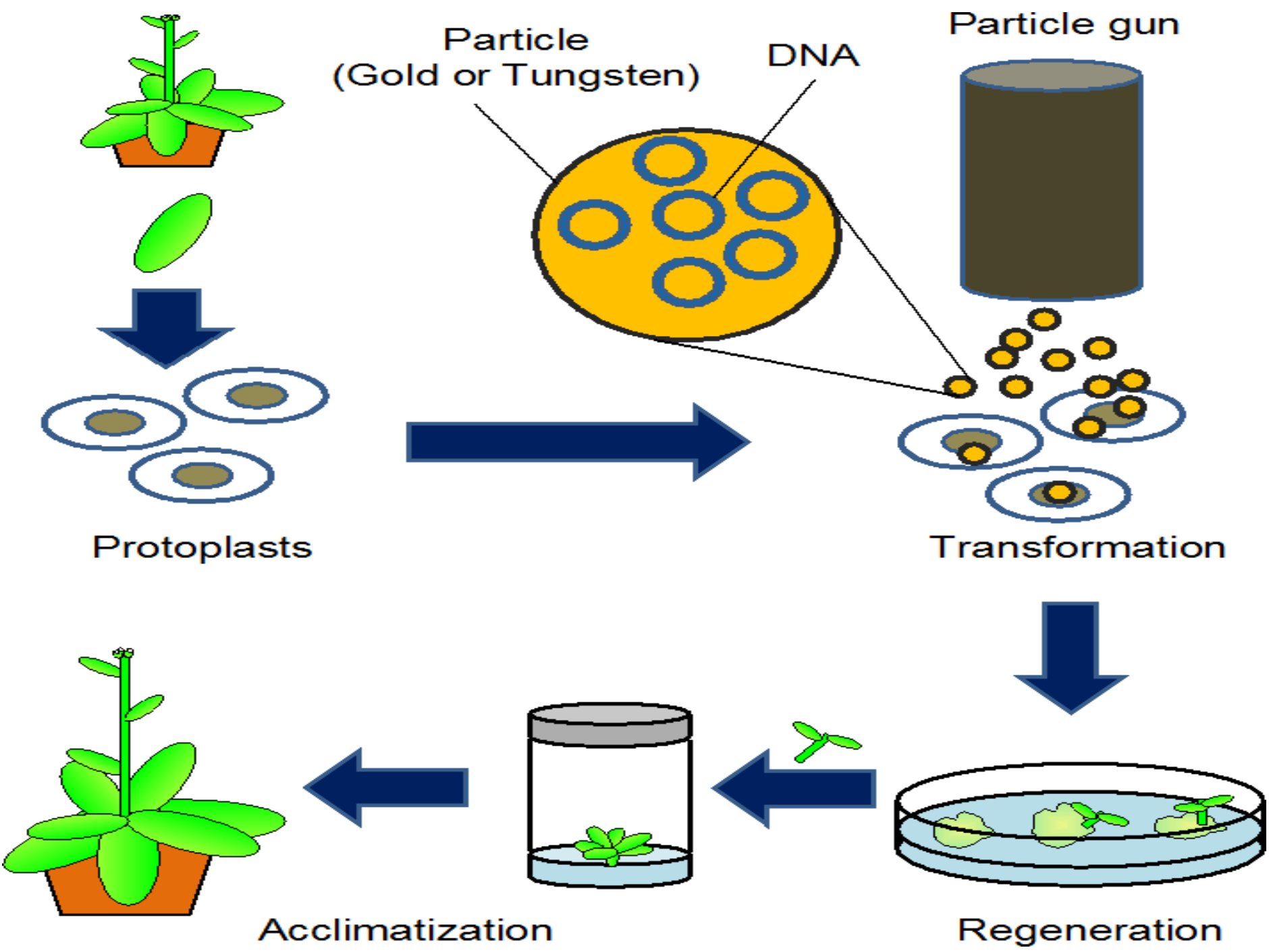
(a) lab version

(b) portable version



(b)





Polyethylene glycol (PEG) mediated transformation - the chemical method.

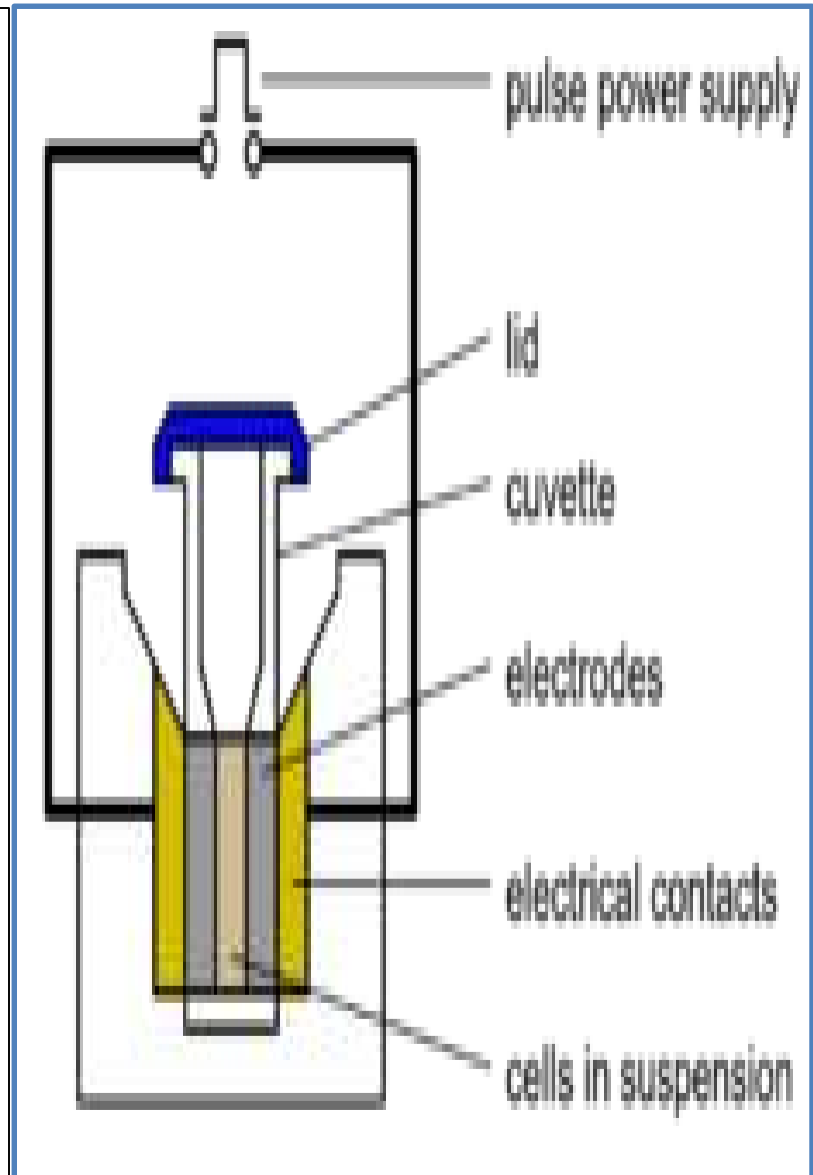
- ✓ Plant protoplast can be transformed with naked DNA by treatment with PEG in the presence of divalent cations .
e. g., Calcium.
- ✓ PEG and divalent cations destabilize the plasma membrane of the plant protoplast and rendered it permeable to naked DNA.
- ✓ DNA enters the nucleus and integrates into the host genome.

Disadvantage and advantages of PEG:

- Regeneration of fertile plants from protoplasts is a problematic for some species bcoz of secondary metabolites accumulation or any compounds.
- The DNA used for transformation is also susceptible to degradation and rearrangement.
- Despite the limitations,
- The technique have the **advantage** i.e., protoplast can be isolated and transformed in number of plants species.

Electroporation:

- It can be used to deliver DNA into plant cells and protoplasts.
- The genes of interest require plant regulatory sequence.
- Plant materials is incubated in a buffer solution containing DNA and subjected to high-voltage electric pulse.
- The DNA then migrates through high-voltage-induced pores in the plasma membrane and integrates into the genome.
- It can be used to transform all the major cereals particularly rice, wheat, maize.



Advantages and disadvantages:

- Both intact cells and tissue can be transformed.
- The efficiency of transformation depends upon the plant materials, electroporation and tissue treatment conditions used for transformation.

Disadvantage:

- Only 40 to 50% incubated cells receive DNA.
- ~50% of the transformed cells can survive.

Fibre mediated DNA delivery-Whiskers

- Plant materials (Cells in suspension culture, embryos and embryo-derived callus) is introduced into a buffer containing DNA and the silicon carbide fibers which is then vortexed.
- The fibers (0.3-0.6 μm in diameter and 10-100 μm long) penetrate the cell wall and plasma membrane, allowing the DNA to gain access to the inside of the cells.
- This method appears to be widely applicable, and is the most rapid and inexpensive, provided stable integrations are achieved.

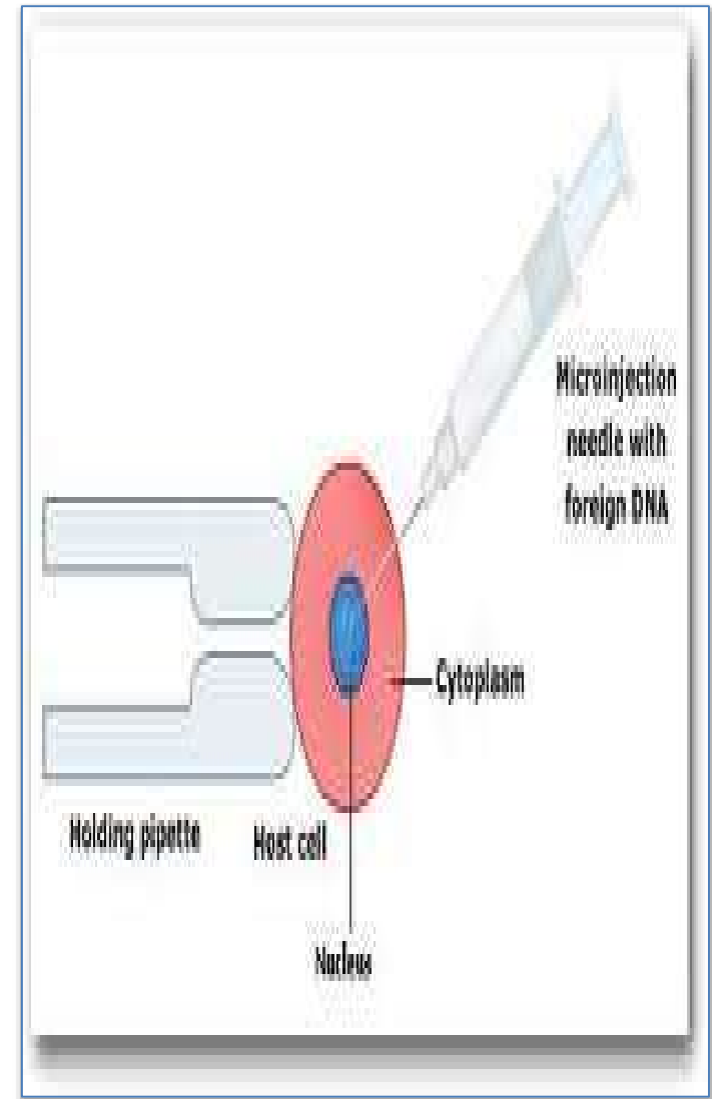
Disadvantages :

- ✓ The drawbacks of this technique relate to the availability of suitable plant material and the inherent dangers of the fibers, which require careful handling.
- ✓ Many cereals, produce embryonic callus that is hard and compact and not easily transformed with this technique.
- ✓ Despite the some disadvantages, this method is recently used for successful transformation of wheat, barley, and maize without the need to cell suspension.

Microinjection

➤ Microinjection techniques for plant protoplasts utilize a holding pipette for immobilizing the protoplast while an injection pipette is utilized to inject the macromolecule.

➤ In order to manipulate the protoplasts without damage, the protoplasts are cultured for from about 1 to 5 days before the injection is performed to allow for partial regeneration of the cell wall.



- It was found that injection through the partially regenerated cell wall could still be accomplished and particular compartments of the cell could be targeted.

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Macro injection;

- The injection of plasmid DNA(or uncloned native DNA) into the lumen of developing inflorescence using a hypodermic syringe called macroinjection.

Laser induced DNA delivery.

- LASERs punctures transient holes in cell membrane through which DNA may enter into the cell cytoplasm.
- But, There is no information/report on gene expression or stable integration.

Advantages and disadvantages of direct gene transfer :

- ✓ **Advantage-** Widespread use of transformation of cereal crops that initially proved difficult to transformation with *Agrobacterium*.
- ✓ **Disadvantage-** They tend to lead higher frequency of transgene rearrangement and higher copy number. This can lead to high frequency of gene silencing.

Bibliography(references);

- Biotechnology-expanding horizons; B D SINGH. Fourth revised edn, 2012 (page no.395-402).
- www.google.com
- www.Kirkhousetrust.com
- www.ejbiotechnology.info