JUST AGRICULTURE
multidisciplinary e-Newsletter

Methods of Plant Transformation

Ashok Kumar
Ph.D. Scholar
Division of Plant Breeding and Genetics
Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu

ARTICLE ID: 116

Introduction

- The process of introducing DNA into living cells is called transformation.
- Introduced DNA is viral DNA, the process is called transformation.
- Transgene is a foreign gene or genetic material that has been transferred naturally or by any of several genetic engineering techniques from one organism to another.
- The plant whose genome is altered by adding one or more transgenes is known as transgenic plants.

➢ Methods of Plant Transformation

(I) Biological methods

- Agrobacterium-mediated gene transfer
- Plant virus vectors

(II) Physical methods

- Electroporation
- Micro projectile
- Microinjection
- Liposome Fusion

(III) Chemical methods

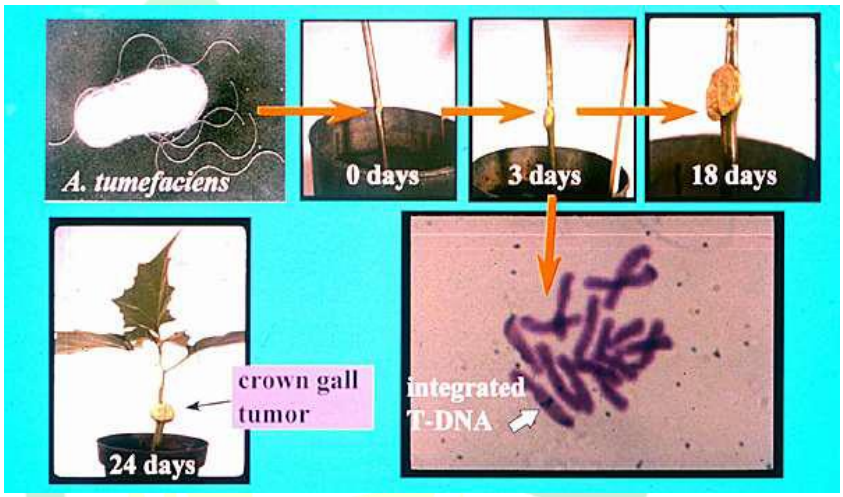
- Polyethylene glycol mediated
- Diethylaminoethyl dextran mediated

(I) Biological methods

JUST AGRICULTURE
multidisciplinary e-Newsletter

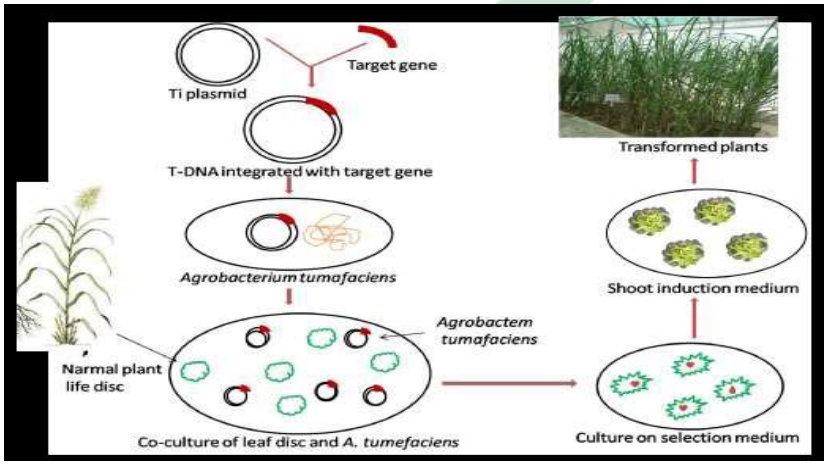
(a) Agrobacterium-mediated gene transfer

- Agrobacterium is treated as nature's most effectiveplant genetic engineer.
- *A. tumifaciens*infectswounded or damaged plant tissues results in the formation of a plant tumor called crown gall.
- The bacterium releases Ti plasmid into the plant cell cytoplasm which induces crown gall.



Agrobacterium-mediated gene transfer

Transformation technique using agrobacterium mediated gene transfer



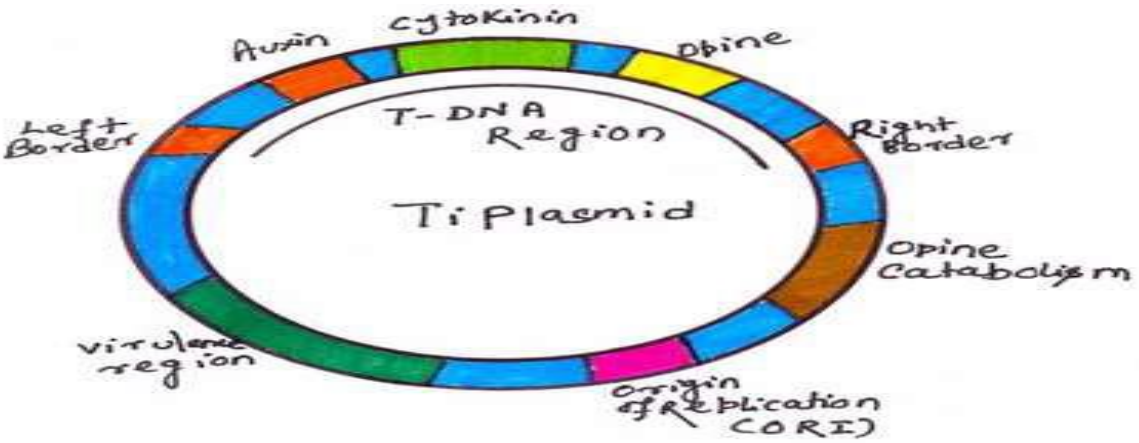
JUST AGRICULTURE
multidisciplinary e-Newsletter

- **Organization of Ti plasmid:-**The Ti plasmid has three important regions;

(i) T-DNA region

(ii) Virulence region

(iii) Opine catabolism region



The size of the Ti plasmid is approx. 200 kb.

There is an ore region that is responsible for the origin of DNA replication.

(b) Plant virus vectors

Plant viruses are considered efficient gene transfer agents as they can infect the intact plants and amplify the transferred genes through viral genome replication. It has some limitations like that the vast majority of plant viruses have a genome, not of DNA but RNA. Two classes of DNA viruses are known to infect higher plants:

(i) Caulimovirus

They contain circular dsDNA and are spherical. The calicivirus group has around 15 viruses & among these Cauliflower Mosaic Virus (CaMV) is the most important for gene transfer.

(ii) Geminivirus

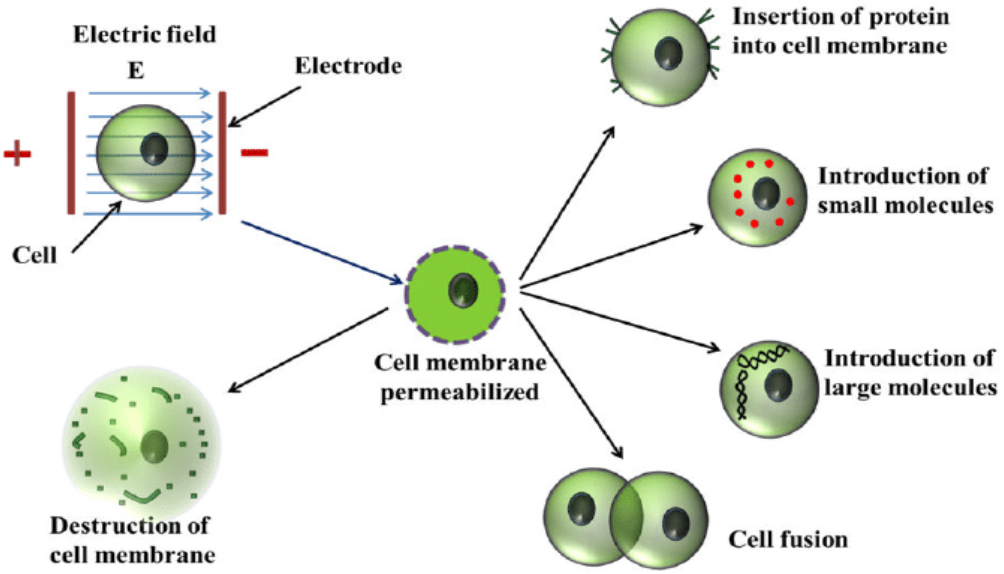
JUST AGRICULTURE
multidisciplinary e-Newsletter

They contain one or two circular ssDNA. They are particularly interesting because their natural host includes plant sizes such as maize & wheat. They could therefore be a potential vector for these and other monocots.

(II) Physical gene transfer methods

(a) Electroporation

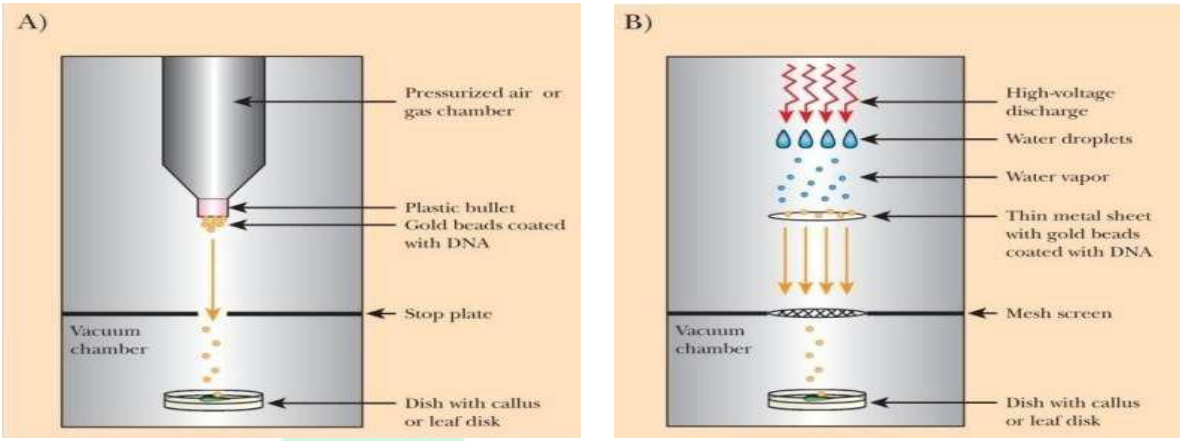
Electroporation involves the creation of pores in the cell membrane using an electric pulse of high field strength. If DNA is present in the buffer solution at sufficient concentration, it will be taken up through these pores.



(b) Particle bombardment

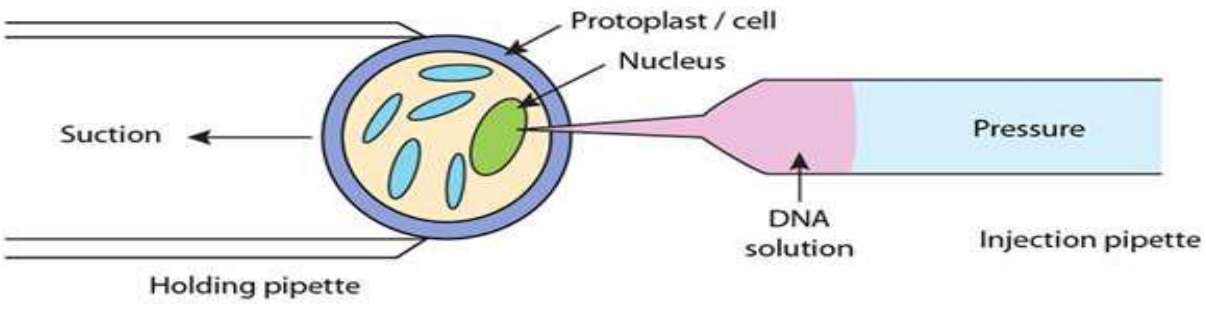
- It is also known as microprojectile bombardment, biolistic, gene gun, etc.
- Foreign DNA is coated with high-velocity gold or tungsten particles to deliver DNA into cells.
- This method is widely being used because of its ability to transfer foreign DNA into mammalian cells and microorganisms.

JUST AGRICULTURE
multidisciplinary e-Newsletter



(c) Microinjection

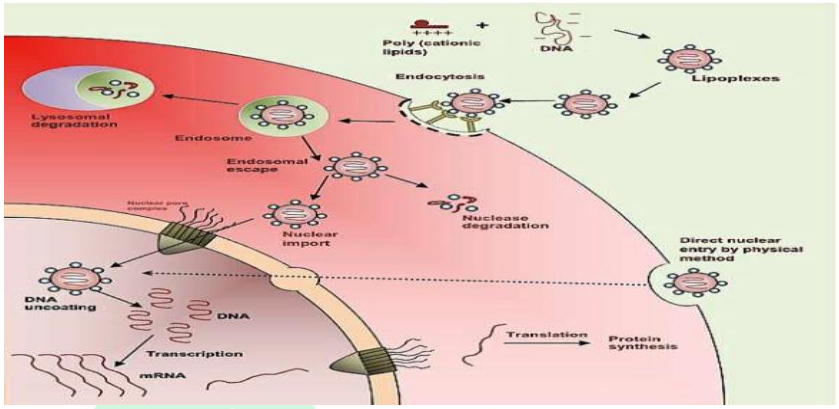
- Microinjection is a direct physical method involving the mechanical insertion of the desirable DNA into a target cell.
- The technique of microinjection involves the transfer of the gene through a micropipette into the cytoplasm or nucleus of a plant cell or protoplast.
- The most significant use of this is the introduction of DNA into the oocyte and the eggs of animals, either the transient expression analysis or to generate transgenic animals.
- The major limitations of microinjection are that it is slow, expensive has to be performed by trained and skilled personnel.



(d) Liposome mediated transformation

JUST AGRICULTURE
multidisciplinary e-Newsletter

Liposome-mediated transformation involves the adhesion of liposomes to the protoplast



surface, its fusion at the site of attachment, and the release of plasmids inside the cell.

(III) Chemical gene-mediated transfer

(a) Polyethylene glycol-mediated transformation

- Polyethylene glycol (PEG), in the presence of divalent cations, destabilizes the plasma membrane of protoplasts and renders it permeable to naked DNA.

- A large number of protoplasts can be simultaneously transformed.
- This technique can be successfully used for a wide variety of plant species.

It has certain limitations:

- The DNA is susceptible to degradation and rearrangement.
- Random integration of foreign DNA into the genome may result in undesirable traits.
- Regeneration of plants from transformed protoplasts is a difficult task.

(b) Deaedextran mediated transfer

- The desirable DNA can be complexed with a high molecular weight polymer diethyl aminoethyl (DEAE) dextran and transferred.
- The major limitation of this approach is that it does not yield stable transformants.

Marker genes for plant transformation:

JUST AGRICULTURE
multidisciplinary e-Newsletter

Marker genes are introduced into the plant material along with the target gene. The marker genes are of two types:

(i) Selectable marker genes- The selection is based on the survival of transformed cells when grown on a medium containing a toxic substance (antibiotic, herbicide, antimetabolite). This is because the selectable marker gene confers resistance to toxicity in the transformed cells, while the non-transformed cells will get killed.

Some of them are given below:

- Antibiotic resistance genes (Hygromycin phosphotransferase, hpt gene)
- Antimetabolite marker genes (Dihydrofolate reductase, dhfr gene)

(ii) Reporter genes-An assay for the reporter gene is carried out by estimating the quantity of the protein it produces or the final products formed.

Some of the important ones are given below:

- Opine synthase (ocs)
- Bacterial luciferase (lux A)
- Firefly luciferase (luc)