Lecture 6: Plant Biotechnology:

Transgenic plants can be simply defined as plants that contain additional or modified genes that were introduced using specific physical or biological methods. The introduced DNAs or transgenes are typically very well defined and are precisely manipulated in the laboratory prior to delivery into the target plant cells. The methods for DNA introduction into plants cells are quite varied and are dependent largely on the plant selected for study and the background of the scientist performing the work. Over the years, tremendous efforts have been placed in development of gene introduction or "transformation" technology and, for many, if not most plants, the procedures have become almost routine. The efficiency of transgenic plant production is still being improved, and new methods for DNA delivery are still needed.

- The first trials of genetically engineered plants occurred in France and the USA in 1986, tobacco plants were engineered to be resistant to herbicides.
- The Flavr Savr tomato was a tomato engineered to have a longer shelf life. In 1995, Bt Potato was approved safe by the Environmental Protection Agency.
- Bt-Cotton is a genetically modified cotton which is resistant to pests.
- Golden Rice genetically modified to contain beta-carotene (a source of Vitamin A).
- Transgenic fruit obtained from pear and apple.
- A Blue Rose is a genetically modified Rose.
- In general, Genetic engineering has helped produce quicker and more predictable way of generating new cultivars. • Thus there are a number of ways by which the gene can be introduced into the cells. With the advent of molecular tools and technologies it is now comparatively easy to introduce gene into cells without losing its integrity and biological activity.
- For successful production of transgenic plants, plant cells, which have the ability to grow (differentiate) into whole plants, should be targeted. The ability of a single cell to grow into a whole plant is called totipotency, and the cell that is naturally totipotent is the fertilized egg. Although it is probably true that all plant cells have the potential to grow into whole plants, that potential has not yet been reached for most cells. At this point in transgenic plant history, scientists can regenerate plants only from specific cell types in most plants. With a few plants, many different cell types are more easily manipulated to grow into whole plants through the tissue culture process.

Successful production of genetically engineered plants is dependent on the coordination of DNA delivery with generation of a whole plant from the single cell, which is targeted for DNA introduction. An ideal target would therefore be the fertilized egg or even the pollen that gives rise to the fertilized egg. Unfortunately, these ideal targets do not appear to be responsive for almost all plants with the exception of the model plant, Arabidopsis thaliana. The next most suitable target for DNA delivery might be the shoot meristem, which gives rise to the aboveground parts of the plant. Although the meristem has been successfully targeted for DNA introduction, it is a complex multicellular structure, and the most appropriate target cells are located in the center of the structure, buried under quite a few cell layers.

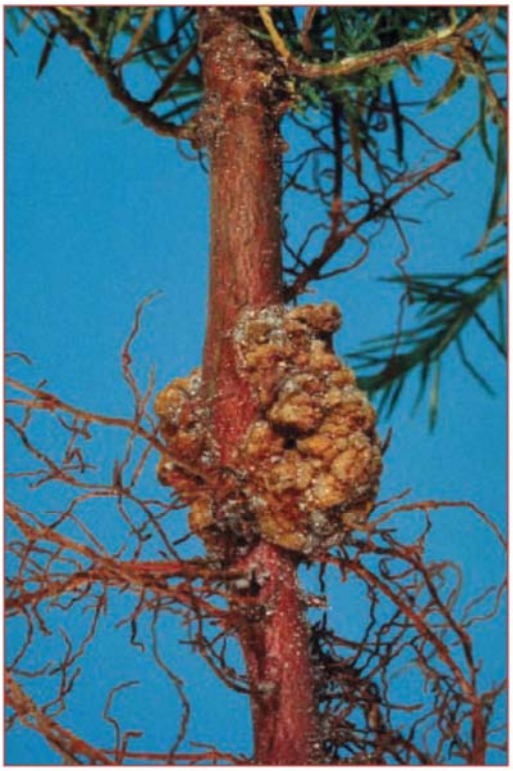
Methods of plant transformation:

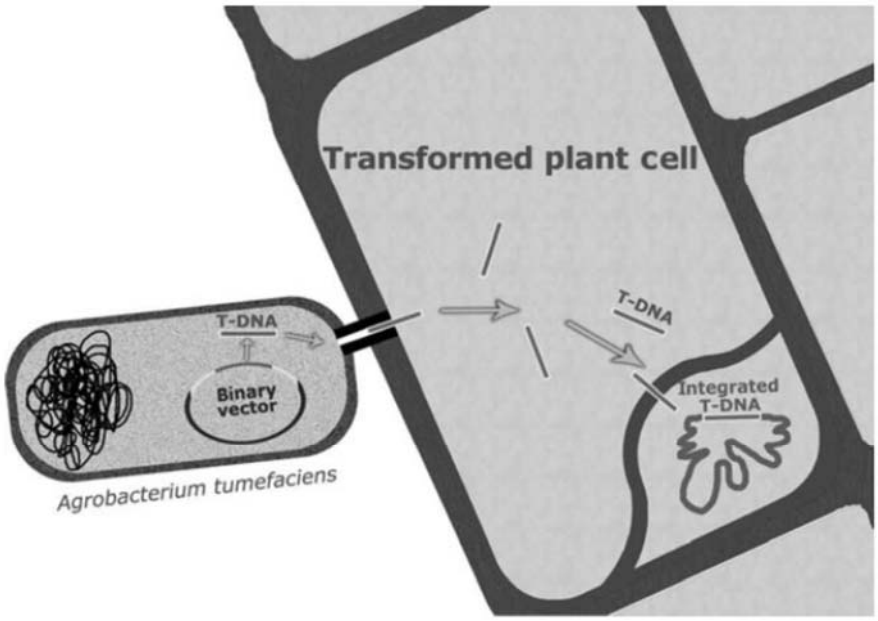
- • Indirect method :
- Agrobacterium mediated gene transfer.
- • Direct methods :
- particle gun/biolistic/ballistic method of DNA delivery.
- Chemical method; Polyethylene glycol (PEG).
- Calcium phosphate mediated gene transfer.
- Microinjection.
- Electroporation.
- Fibre mediated gene transfer.

Agrobacterium mediated gene transfer

- It is achieved by two ways are-
  - 1) Co-culture with tissue explants.
  - 2) *In planta* transformation.

Agrobacterium tumefaciens Characteristics: Soil born, 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 when bacterial DNA integrates into plant nuclear DNA. Have ability to transfer bacterial genes to plant genome. Following insertion of desired genes into bacterial DNA using recombinant DNA techniques, this system permits introduction of these new genes into plant DNA & gives a potential for genetic manipulation of plants. Attracted to wound site via chemotoxins in response to chemicals (Sugar and Phenolic molecules: Acetosyringone) released from damaged plant cells.





Vectors: the DNA carriers must have:

1. Origin of replication.
2. Antibiotic-resistant genes.
3. Allow the host to grow on selective media.
4. Can selectively amplify this specific vector in the host cell.
5. Multiple cloning sites. Allow insertion of foreign DNA.

▶ Tumor-inducing (Ti) -plasmid features: These are extracellular double stranded circular self replicating DNA in bacteria.

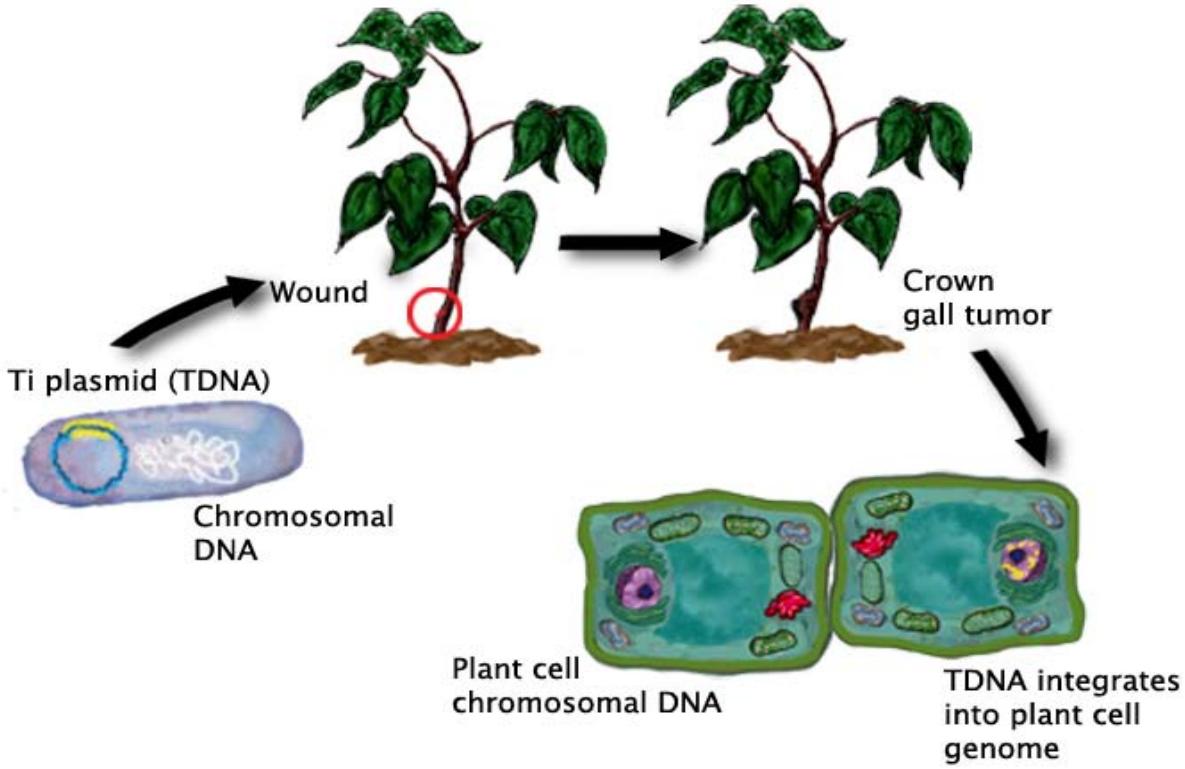
▶ Two strains of Ti-plasmid:

▶ 1-Octopine strains- Two T-DNA region: TL (14 kb) and TR (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. • Has required T-DNA.

Process of T-DNA transfer and integration 1. Signal recognition by *Agrobacterium*: *Agrobacterium* perceive signals such as sugar and phenolic compounds (Acetosyringone) which are released from plants when got wounded. 2. Attachment to plants cells: Two step processes: 1. Initial attachment via polysaccharide. 2. 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.

- 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*.
- *VirE2* protects this from nucleases. virB is essential for virulence which also has ATP-ase activity, therefore helps in deliver of T-DNA into plant cell, mostly through nuclear pore complex.
- 5. Transfer of the T-DNA and *vir* proteins into the plant nuclear localization: The SS T-DNA is immediately converted to double strand in nucleus by replication.
- The double strand T- DNA integrates at random site in the host cell genome by illegitimate recombination.

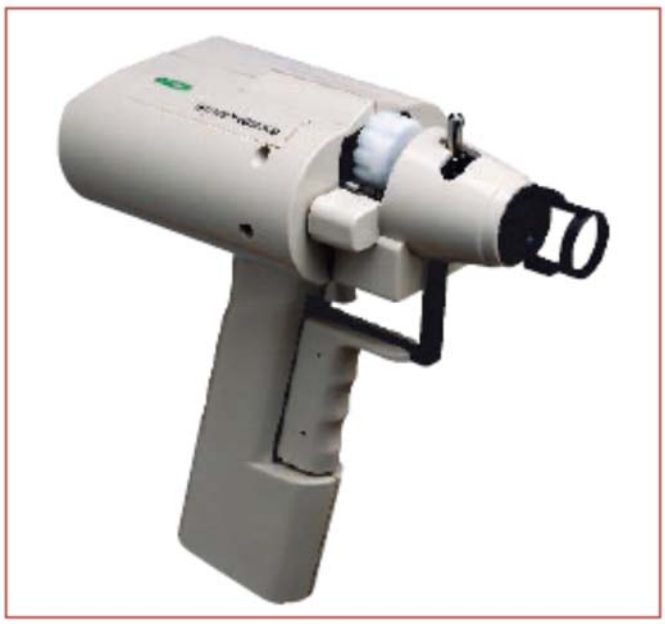


DIRECT METHODS

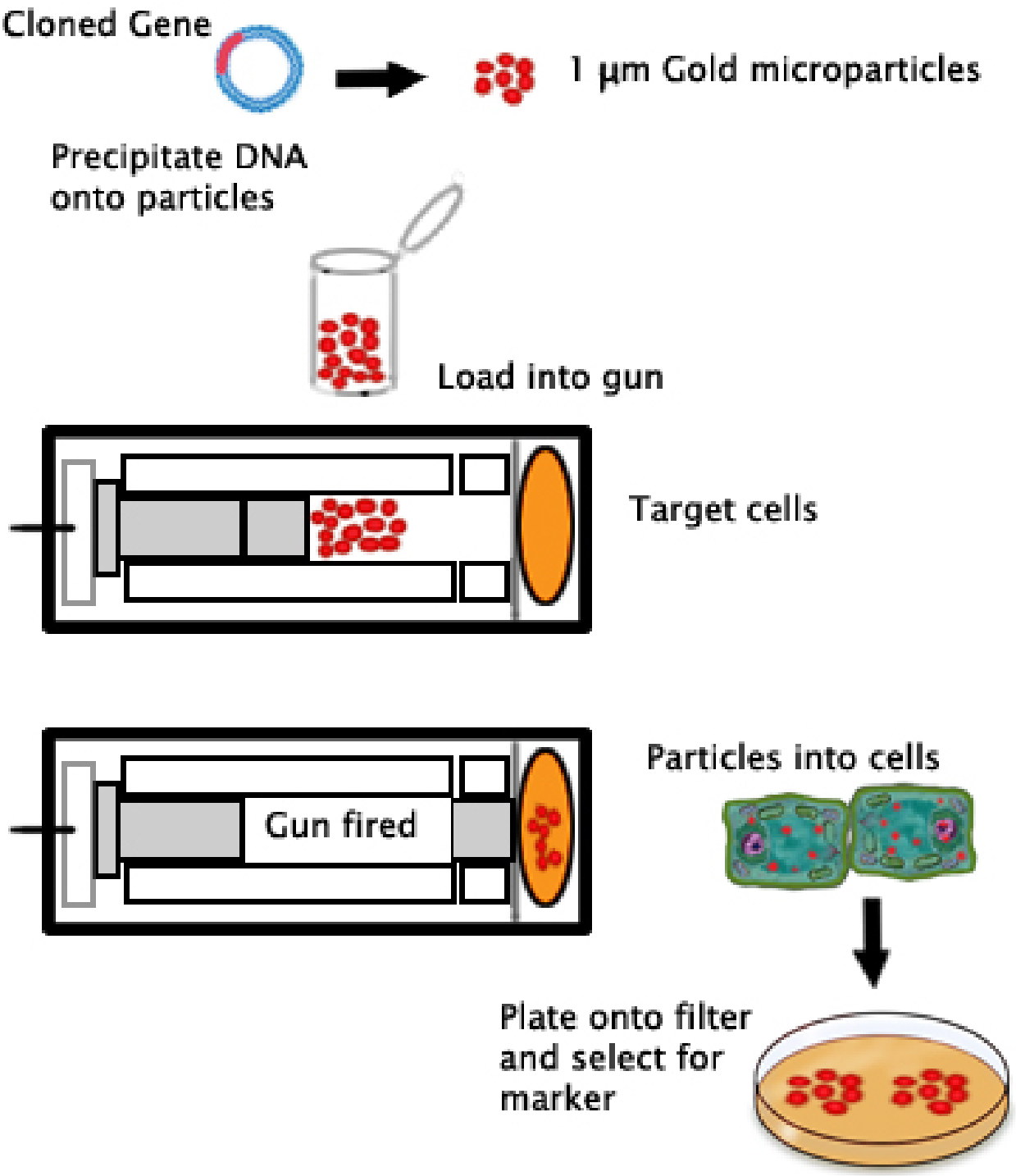
1. Particle gun method

- A gene gun or a biolistic particle delivery system, designed for plant transformation, It's a device for injecting cells with genetic information. The payload is an elemental particle of a heavy metal (tungsten/gold) called micro projectiles coated with plasmid DNA.
- Principle:
- The gold or tungsten particles are coated with the DNA that is used to transform in 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.

- Micro projectile bombardment or biolistic-mediated DNA transformation equipment (a) lab version (b) portable version
- Gene gun and system The biolistic system, the Biolistic PDS-1000. This instrument consists of the bombardment chamber connective tubing for attachment to vacuum source, and all components necessary for attachment and delivery of high pressure helium to the main unit (helium regulator, solenoid valve).



(a) (b)


Cloned Gene
1 µm Gold microparticles
Precipitate DNA onto particles
Load into gun
Target cells
Gun fired
Particles into cells
Plate onto filter and select for marker

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. • Eg: Rice, wheat, maize.

Electroporater

For electroporation, protoplasts are placed in a DNA solution between two electrodes and exposed to brief pulses of high-voltage current. The pulses cause pores to form in the membrane and the DNA then enters the cells.

- Fiber 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 10-100μm 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.