Chapter-8

Genetic Transfer Methods: Gene Gun, PEG techniques and *Agrobacterium* mediated.

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ABSTRACT

The ability to transfer genes from one organism to another has been made possible in recent years by the gene transfer technique. These days, gene technology is developing at an incredible rate and is used in a wide range of fields, from food to health, from plants to animals. The ongoing development of gene transfer techniques altered the course of crop modification and led to important breakthroughs in agricultural output, crop protection, and crop enhancement. The process of finding and introducing new genes to already-existing elite cultivars is known as genetic engineering, and it has completely changed the crop improvement pathway. Numerous techniques have been devised to introduce the gene into plant cells, and ongoing endeavors have been undertaken to enhance its effectiveness. There are advantages and disadvantages to both direct and indirect gene transfer strategies. Constant work was done to remove obstacles and create a simple, prestigious, and eco-friendly gene transfer

technique. Agrobacterium-mediated gene transfer and the gene gun transformation approach have shown promising results in recent years. The Agrobacterium gene transfer technique is particularly popular for introducing foreign genes into a wide variety of plant species. The methodology in question, as well as the advantages and disadvantages of the different approaches, were briefly covered together.

KEYWORDS: Gene Gun, Microinjection, PEG, *Agrobacterium*, Plant Biotechnology

INTRODUCTION

In genetic engineering, molecular biology, and biotechnology, gene transfer techniques are essential instruments. In order to investigate gene function, create genetically modified species, and further gene therapy, they make it possible to introduce foreign genetic material into cells. Gene transfer is the process of changing a cell or organism's genetic composition by transferring genetic material, such as DNA or RNA. This method is essential in domains such as gene therapy, biotechnology, and genetic engineering. It allows researchers to create genetically modified organisms (GMOs), conduct research on gene functions, and treat a variety of diseases. The development of novel cultivars that will increase production stability and quality, protect the environment, provide consumers with nutritional benefits, and provide new medications and vaccines are the main goals of plant biotechnology.

TYPES OF GENE TRANSFER

Gene Transfer occurs in two primary ways-

1. **Vertical Gene Transfer: -** The natural transfer of genetic material from parent to child during reproduction is known as vertical gene transfer. In both sexual and

asexual reproduction, it is the typical mode of inheritance.

2. **Horizontal Gene Transfer: -** The transfer of genetic material between species that are not related by parent-offspring is known as horizontal gene transfer, or HGT. Particularly prevalent in bacteria, HGT is important for the development of novel characteristics and the spread of antibiotic resistance.

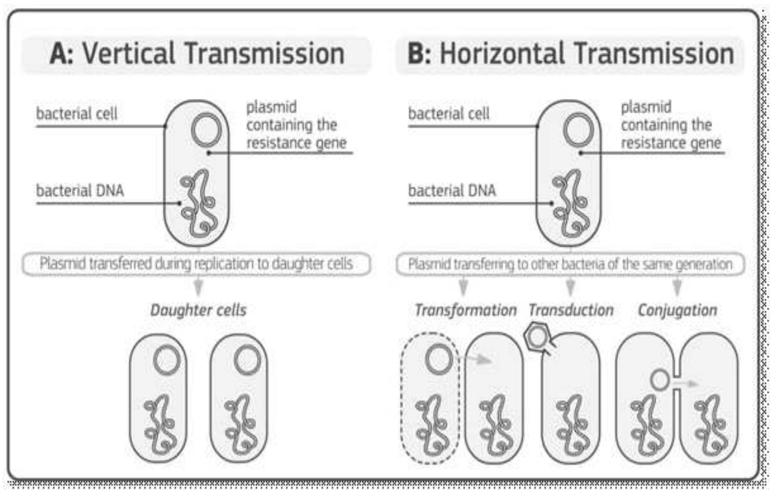

Figure 1. Types of gene transfer.

Plant biotechnology's use of this gene transfer system and its tactics has produced plants that are resistant to pests and diseases, delayed the senescence of leaves, modified the composition of fatty acids, produced flowers with a variety of colors and patterns, developed storage traits, and produced plants that are resistant to stress.

IMPORTANCE OF GENE TRANSFER

A. Provide resistance against viruses and acquire insecticidal resistance

B. To strengthen the plant to grow against bacterial diseases

C. Develop the plants to grow in draught
D. Engineering plants for nutritional quality
E. Make the plants to grow in various seasons
F. Herbicide resistant plant can be made
G. Resistance against fungal pathogens
H. Engineering of plants for abiotic stress tolerance
I. Delayed ripening can be done

TYPES OF GENE TRANSFER

1. Physical gene transfer methods

a) **Electroporation:** An electric field is used to improve the permeability of the cell membrane, allowing DNA to enter. Protoplasts are typically used in plant cell electroporation, but macromolecule mobility is constrained by thick plant cell walls.

The plant material is exposed to high voltage electrical impulses while being cultured in a buffer solution containing the target or desired foreign DNA. The protoplast membrane develops tiny, transient holes as a result of the electric current, which allow DNA to flow through.

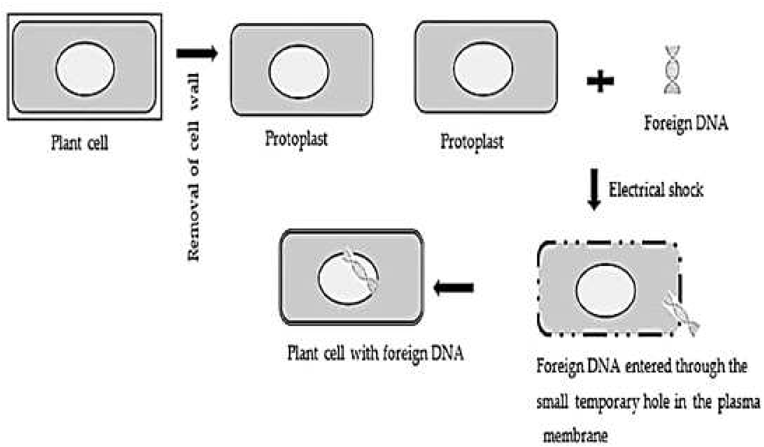

Figure 2. Electroporation.

The foreign DNA enters the cell and merges with the host genome, causing a genetic modification. The protoplasts are subsequently grown to become whole plants. This technique can be used to agricultural species when protoplast regeneration is possible.

b) **Microinjection**: A direct physical technique called microinjection involves mechanically inserting the desired DNA into a target cell. The targeted cell could be found in embryos, callus, meristems, protoplast etc. Microinjection is used to transfer cellular organelles and manipulate chromosomes. Using capillary glass micropipettes (0.5–10.0 pm tip), the DNA solution is injected straight into the cell with the help of micromanipulators of a microinjection assembly. Protoplasts are more suitable for microinjection than cells due to their lack of cell walls. Protoplasts are typically trapped in agarose, on polylysine-coated glass slides, or by being held under suction with a micropipette. After microinjection is finished, the altered cell is grown and expanded to create a transgenic plant. For example, this method has been used to create transgenic *Brassica napus* and tobacco.

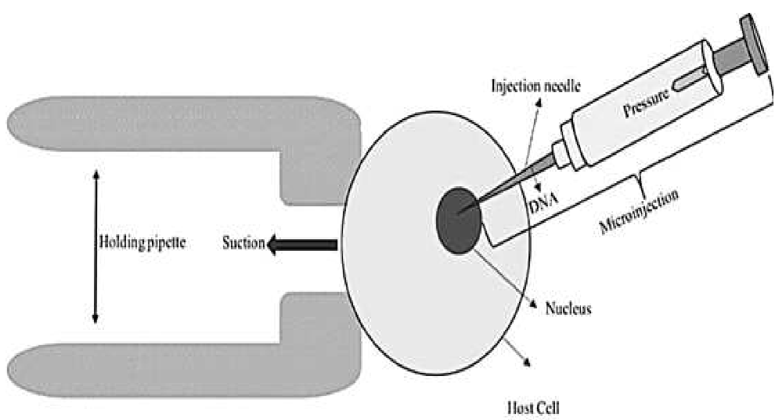

Figure 3. Microinjection Method.

c) **Gene Gun/ Biolistic transfer method**: A technique for introducing foreign DNA into plant cells is particle bombardment. The best method for transferring genes and producing transgenic plants is using gene gun. The fact that this technique can be applied to the effective transfer of DNA in both microbes and mammalian cells makes it adaptable. Sanford (1988) coined the term "biolistics" for the microprojectile bombardment approach. Biolistics is a blend of biological and ballistic concepts. Foreign DNA coated with tiny 0.2–0.7 ìm gold (or tungsten) particles is used in the transformation process to enter the target plant cells. After being put into a particle gun, the coated particles are rapidly accelerated by-

- By using pressurized helium gas
- By electro static energy released by a droplet of water exposed to a high voltage

The target could be plant cell suspensions, callus cultures, or tissues. The projectiles penetrate the membranes and cell walls of plants. The surface of the micro projectiles releases transgenes as they enter the cells for subsequent integration into the chromosomal DNA of the plant.

Utilizing plants for bombardment: Plant tissue comes in two varieties that are frequently utilized for particle bombardment:

1. Primary explants that are capable of being bombarded and then made to become embryo genic and regenerate.
2. Proliferating embryonic tissues that can be exposed to high concentrations in cultures before being permitted to grow and regenerate.

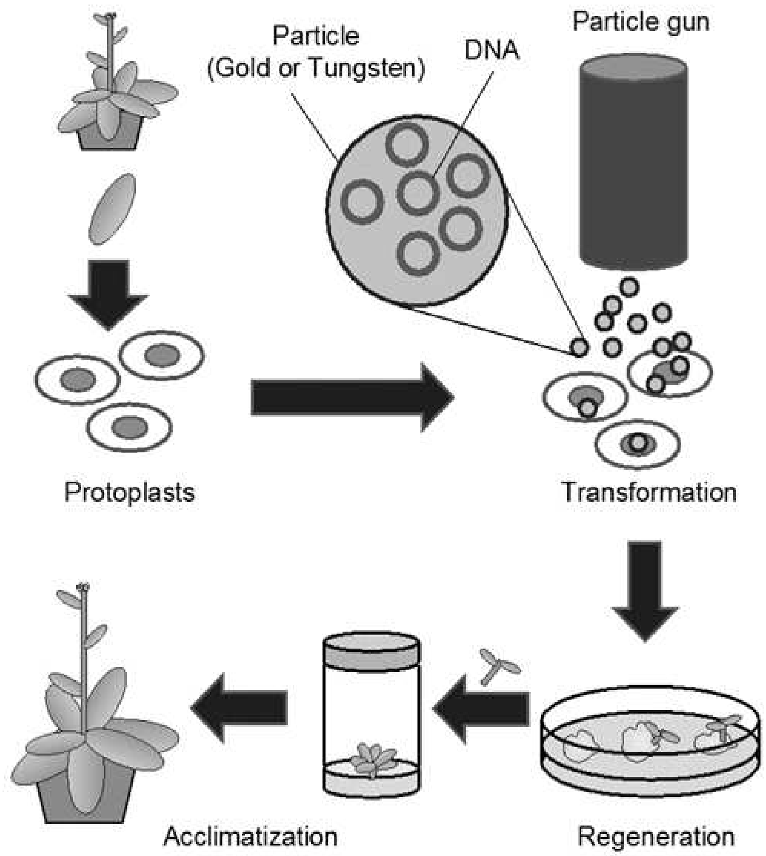

Figure 4. Gene Gun Method.

During the integrative phase, additional transgene integration can occur at or near the starting location. In the end, particle bombardment is usually linked to a high copy number at a single location. Plant regeneration may benefit from this kind of single locus.

FACTORS AFFECTING BOMBARDMENT

- **Nature of micro particles:** Inert metals such as tungsten, gold and platinum are used as micro particles to carry DNA. These particles with relatively higher mass will have a better chance to move fast when bombarded and penetrate the tissues.

- **Nature of tissues/cells:** The target cells that are capable of undergoing division are suitable for transformation.
- **Amount of DNA:** The transformation may be low when too little DNA is used. On the other hand, too much DNA may result is high copy number and rearrangement of transgenes. Therefore, the quantity of DNA used should be balanced.
- **Environmental parameters:** Many environmental variables are known to influence particle bombardment. These factors (temperature, humidity, photoperiod etc.) influence the physiology of the plant material, and consequently the gene transfer. It is also observed that some explants, after bombardment may require special regimes of light, humidity, temperature etc.

2. Chemical gene transfer methods

a) **Polyethylene glycol (PEG)-mediated:** Polyethylene glycol (PEG)-mediated transmission in the presence of divalent cations (Ca2+), polyethylene glycol (PEG) destabilizes the plasma membrane of protoplasts, rendering it ineffective permeable to naked DNA. DNA enters the nucleus of protoplasts and integrates with their genome. The process comprises isolating and suspending protoplasts, adding plasmid DNA, and slowly adding 40% PEG-4000 (w/v) in mannitol and calcium nitrate solution. When this mixture is incubated, protoplasts undergo transformation.

Advantages of PEG-mediated Transformation

i) This technology successfully transforms many protoplasts at once.

ii) It can be used to a variety of plant species.

Limitations of PEG-mediated transformation

i) The DNA is susceptible for degradation and rearrangement.

ii) Random integration of foreign DNA into genome may result in undesirable traits.

iii) Regeneration of plants from transformed protoplasts is a difficult task.

b) **Diethyl amino ethyl (DEAE) dextran-mediated:** The desirable DNA can be complexed with a high molecular weight polymer diethyl amino ethyl (DEAE) dextran and transferred. The efficiency increased to 80% when DMSO shock is given. The major limitation of this approach is that it does not yield stable trans-formants.

c) **Calcium phosphate precipitation:** The DNA is allowed to mix with calcium chloride solution and isotonic phosphate buffer to form DNA-calcium phosphate precipitate. When the actively dividing cells in culture are exposed to this precipitate for several
hours, the cells get transformed. The success of this method is dependent on the high concentration of DNA and the protection of the complex precipitate. Addition of dimethyl sulfoxide (DMSO) increases the efficiency of transformation.

3. Agrobacterium mediated gene transfer:

The Agrobacterium system was the first effective plant transformation system, leading to a breakthrough in plant genetic engineering in 1983. The Agrobacterium is naturally occurring gram negative soil bacterium with two common species *A Tumifacience* and arhizogenes there are known as natural gene engineers for their ability to transform plants. Tumifacience produces crown galls, while rhizogenes causes hairy root infections. These bacteria have large plasmids known as root-inducing (Ri plasmid) and tumor-inducing (Ti plasmid), respectively. *A. tumefaciens'* inherent, special aptitude is largely responsible for the development of plant transformation techniques.

The Ti plasmid's transformation targets two key segments: T DNA and viral area. The T DNA portion of the Ti plasmid is transported to plant cells and integrated into the nuclear genome of cells. T DNA transfer is facilitated by virs genes, which are located in another region of the Ti plasmid. Modified Ti plasmids are created by removing unwanted Ti genes and replacing them with a foreign gene (e.g., antibiotic resistance) and a closely connected selectable marker gene. Genes inserted into plasmid cysts' T DNA region are transmitted to the plant genome. T DNA is often incorporated in low copy numbers per cell. A. *tumifacience* can only transfer genes to injured plant parts and has a limited host range. It can infect approximately 60% of gymnosperms and angiosperms. Agrobacterium-mediated transformation is preferred for dicotyledonous plant species with well-established regeneration systems. However, Agrobacterium-mediated gene transfer is not effective for monocotyledons.

In general, most of the Agrobacterium-mediated plant transformations have the following basic protocol:

a. Development of Agrobacterium carrying the co-integrate or binary vector with the desired gene
b. Identification of a suitable explant e.g. cells, protoplasts, tissues, calluses, organs
c. Co-culture of explants with Agrobacterium
d. Killing of Agrobacterium with a suitable antibiotic without harming the plant tissue
e. Selection of transformed plant cells
f. Regeneration of whole plants

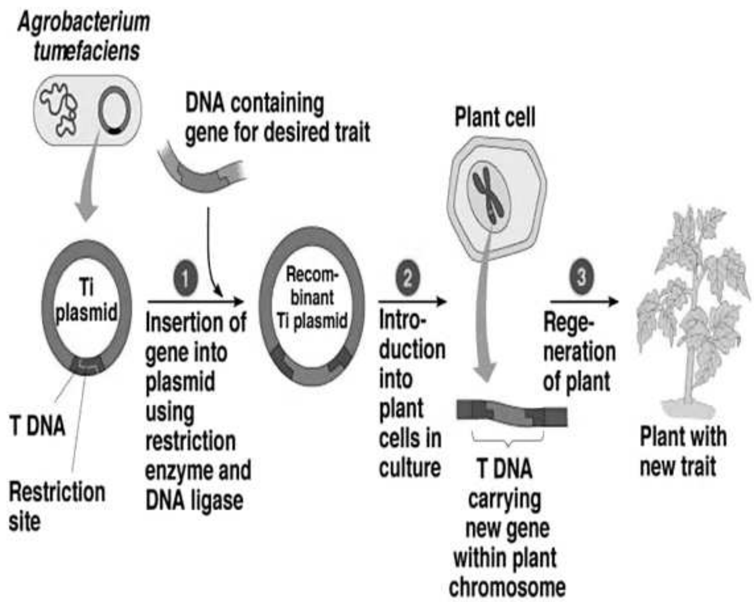

Figure 5. Agrobacterium mediated gene transfer.

ADVANTAGES

- It is a natural form of gene transfer.
- Agrobacterium can infect plant cells and tissues.
- Agrobacterium can transmit large DNA fragments efficiently
- T DNA integration is a relatively accurate process
- Gene transfer stability is excellent
- Transformed plants can be regenerated effectively.

LIMITATIONS

- Host specificity: There is a limitation of host plants for Agrobacterium, since many crop plants (monocotyledons e.g. cereals) are not infected by it.
- Inability to transfer multiple genes: The cells that regenerate more efficiently are often difficult to transform, e.g. embryonic cells lie in deep layers which

are not easy targets for Agrobacterium.

- Soma clonal variation
- Slow regeneration

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