B) Direct Gene Transfer:

The term direct transfer of gene is used when the foreign DNA is directly introduced into the plant genome. Direct DNA transfer methods rely on the delivery of naked DNA into the plant cells.

The direct gene transfer can be broadly divided into two categories.

A. Physical gene transfer methods—electroporation, microinjection, particle bombardment.

B. Chemical gene transfer methods—Poly-ethylene glycol (PEG)-mediated, diethyl amino ethyl (DEAE) dextran-mediated, calcium phosphate precipitation.

(A) Physical Gene Transfer Methods:

1. Electroporation:

This method involves suspension of plant protoplasts in a suitable ionic solution containing linearized recombinant plasmid DNA. This mixture is then exposed to low voltage-long pulses or high voltage-short pulses for the desired number of cycles. The electrical pulses are thought to induce transient pores in the plasma lemma through which the DNA molecules are incorporated. Treated protoplasts are then cultured to obtain cell colonies and plants. This method is called electroporation (i.e. introduction of DNA into plant cells by making minute pores in the plant cell membrane).

2. Microinjection:

Microinjection is a direct physical method involving the mechanical insertion of the desirable DNA into a target cell. The target cell may be the one identified from intact cells, protoplasts, callus, embryos, meristems, etc. Microinjection is used for the transfer of cellular organelles and for the manipulation of chromosomes.

The technique of microinjection involves the transfer of the gene through a micropipette (0.5-10.0 pm tip) into the cytoplasm/nucleus of a plant cell or protoplast. While the gene transfer is done, the recipient cells are kept immobilized in agarose embedding, and held by a suction holding pipette.

As the process of microinjection is complete, the transformed cell is cultured and grown to develop into a transgenic plant. In fact, transgenic tobacco and *Brassica napus* have been developed by this approach. The major limitations of microinjection are that it is slow, expensive, and has to be performed by trained and skilled personnel.

2. Particle Bombardment Gun Method (Biolistics):

Particle bombardment is the most effective method for gene transfer, and creation of transgenic plants. This method is versatile due to the fact that it can be successfully used for the DNA transfer in mammalian cells and microorganisms.

The micro projectile bombardment method was initially named as biolistics by its inventor Sanford (1987). Biolistics is a combination of biological and ballistics. In this method, 1-2 µm gold or tungsten particles coated with DNA are shot into the plant cells using a helium pressure particle gun device. Important crop plants like, wheat, rice and maize have now been transformed by this method.

(B) Chemical Gene Transfer Methods:

Polyethylene glycol (PEG)-mediated transfer:

DNA molecules can be forced to enter into the host genome only in those cells which do not possess cell walls. The naked plant protoplasts are mixed with molecules of linearized plasmid DNA containing the foreign gene. The two are mixed in a transformation medium rich in Mg^{2+} ions in place of Ca^{2+} ions, following which 20% polyethylene glycol (PEG) solution is added. After the treatment, the PEG concentration is reduced and Ca^{2+} concentration is enhanced. It promotes the frequency of transformation.

The chemical method involving transformation in presence of polyethylene glycol is convenient and simple but there are, however, some disadvantages:

1) Many cells are so sensitive that the chemical method cannot be applied whereas some cells die during the treatment.

2) This method is not perfect because many treated cells do not contain any transfer DNA.

3) Sometimes the foreign DNA is degraded in the cytoplasm before reaching the nucleus.