Gene therapy

Gene transfer techniques are primarily involved in **Gene Therapy** where external or exogeneous or correct DNA are introduced inside the cell to replace or correct the damaged ones.

The direct DNA transfer methods are categorized into:

1. Physical gene transfer method
2. Chemical gene transfer method

Physical gene transfer method

A. Particle Bombardment

The Particle bombardment device, or 'gene gun', was developed so that they could easily penetrate the plant cell wall and the gene of interest can be introduced into the cell. Gene guns operate on the principle that under certain conditions, DNA becomes "sticky," readily adhering to biologically inert particles like gold. By accelerating this DNA-particle complex in a partially vacuum chamber and positioning the target tissue within the acceleration path, DNA is effectively introduced into the desired cell. The technique is widely used in *Agrobacterium* mediated transformation method.

B. Electroporation

Electroporation is another popular physical method for introducing new genes directly into the plant protoplasts using a specially designed machine called as electroporater. In this method, electric field is applied which makes the protoplast get temporarily permeable to DNA, and the desired gene is introduced.

C. Microinjection

Specially designed needles or micropipette is used to introduce exogeneous genes inside the target cell.

Chemical gene transfer method

This involves plasma membrane destabilizing and/or precipitating agents. Protoplasts are mainly used which are incubated with DNA in buffers containing PEG.

A. PEG mediated gene transfer

In this method protoplasts are isolated and a particular concentration of protoplast along with divalent cations are incubated for few minutes. PEG and divalent cations

destabilizes the plasma membrane of the plant protoplast and makes it permeable to DNA.

B. Calcium-Phosphate co-precipitation

DNA when mixed with calcium chloride solution gives rise to a co-precipitate. Along with it a heat shock is given which increases the efficiency of transformation of exogenous DNA.