Physical delivery or DNA mediated gene transfer (DMGT)

Cereals, the most important food crops, were initially not amenable to *Agrobacterium* mediated gene transfer method. Further, in many crops including cereals and legumes, the tissue culture method for regeneration was not very successful initially.

For these reasons, researchers invented Physical delivery or DNA mediated gene transfer (DMGT) methods which are grouped according to the type of target cells.

Delivery of DNA to protoplasts only	Variety of explants (e.g. immature embryos, organ meristems, gametes, zygotes etc. could be used for DNA transfer
1. Chemically stimulated DNA uptake	4. Microinjection
2. Liposome mediated gene transfer	5. Macroinjection
3. Electroporation	6. Shooting with microprojectiles

Chemically stimulated DNA uptake by protoplasts

Chemicals like Polyethylene glycol (PEG) stimulated DNA uptake by protoplasts involves the use of high concentration (15-25%) of PEG, which will precipitate ionic macromolecules such as DNA and stimulates uptake of DNA by endocytosis without any gross damage to protoplast. This is followed by cell wall formation and initiation of cell division. Transformed cells can be selected later on a selection medium.

Microinjection

In microinjection process glass micropipettes each with 0.5-10 µm diameter tip are used for transfer of macromolecules into the cytoplasm or the nucleus of a recipient cell or protoplast. The recipient cells are immobilized on a solid support (cover slip or slide etc.) or artificially bound to a substrate or held by a pipette under suction. Often a specially designed micromanipulator is employed for microinjecting the DNA. Although, this technique gives high rate of success, the process is slow, expensive and requires highly skilled and experienced personnel.

Macroinjection

DNA macroinjection employing needles each with diameter greater than cell diameter, had also been tried. In rye (*Secale cereal*), a marker gene was macroinjected into the stem below the

immature floral meristem, so as to reach the sporogenous tissue leading to successful production of transgenic plants. Unfortunately, this technique could not be successfully repeated with any other cereal, when tried in other laboratories. Therefore, there is a doubt about the validity of earlier experiments conducted with rye.

Microprojectiles for gene transfer

Microprojectiles (heavy microparticles like tungsten or gold coated with DNA, 1-3 µm in diameter) are carried by a macroprojectile (or bullet), and are accelerated into target cells, where they penetrate the cell wall, leaving DNA to be incorporated. This technique results transfer of gene into many cells at a time. It has been successfully used for transformation in soybean, tobacco, maize, rice, wheat, oats etc. This method is universal in its application so that cell type, size and shape or the presence/absence of cell walls do not significantly alter its effectiveness and are utilized extensively irrespective of species and genotype.

Electroporation for gene transfer

Electroporation is based on the use of short electrical pulses of high field strength. These pulses increase the permeability of protoplast membrane and facilitates entry of DNA molecules into the cells, provided the DNA is in direct contact with the membrane.

Liposome mediated gene transfer

Liposomes are small lipid bags, in which large number of plasmids are enclosed. They can be induced to fuse with protoplast by endocytosis, using devices like PEG, and therefore have been used for gene transfer.

Advantages: 1. Protection of DNA/RNA from nuclease digestion, 2. Low cell toxicity, 3. Stability of nucleic acids due to encapsulation in liposomes, 4. High degree of reproducibility and, 5. Applicability to a wide range of cell types.

Calcium phosphate precipitation method

Foreign DNA can also be carried with the Ca^{++} ions, to be released inside the cell due to the precipitation of calcium in the form of calcium phosphate

Transformation using pollen or pollen tube, Incubation of dry seeds, embryos, tissues or cells in DNA, transformation by ultrasonication are also the possible methods applied for gene transfer, but none of these methods are absolutely fruitful.