SGTB Khalsa College, University of Delhi
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Department of Botany

Title of the Paper: Economic Botany and Biotechnology (Theory) : BTPE-3
Course Name: B.Sc. (Life Sciences) 3rd Year, Semester: VIth
Faculty Names: Dr. Gurpreet Kaur (GP)

Topic : Direct methods of DNA delivery

I. Particle gun bombardment/Biolistic/microprojectile gun

1. 1-2um tungsten or gold particles coated with DNA is used for plant transformation
2. The particles are accelerated to high velocities which enable their entry into plant cells or nuclei.
3. This particle acceleration is achieved by using a device which uses pressurized helium gas .
4. components of the device
   - gas acceleration tube,
   - stopping screen
   - rupture disc
   - macrocarrier through which particles coated with DNA reach the target cells.
   - chamber for creating a vacuum during particle acceleration a
   - After creation of vacuum, pressurized helium gas is released in the acceleration tube which breaks the rupture disc. This generates helium shock waves which accelerate the DNA-coated microprojectiles . These microprojectiles are stopped by a stopping screen, they pass through the screen and get embedded in the cells which are below the stopping screen

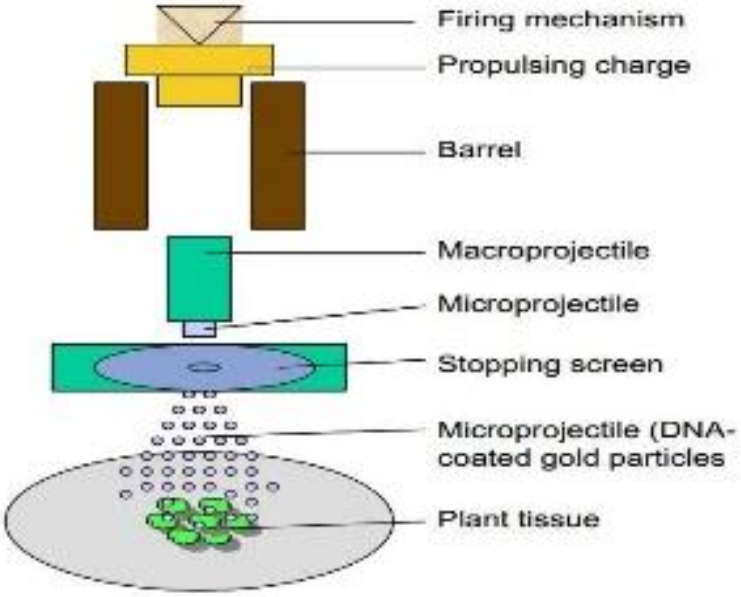
Advantages

1. In plant species this can be used to transform almost all tissues eg shoot apical meristem, pollen, leaves, immature and mature embryos
2. It's a quick method
3. chloroplast transformation has also seen great success with particle bombardment.

4. Monocots don’t respond to *Agrobacterium*-mediated DNA delivery, so in monocot, this technique is successful

Limitations :

1. Biolistics introduces DNA randomly into the target cells. Thus the DNA may be transformed into whatever genomes are present in the cell, be they nuclear, mitochondrial etc
2. Gene may be get inserted in multiple copies in either the same or different locations of the genome



Microinjection

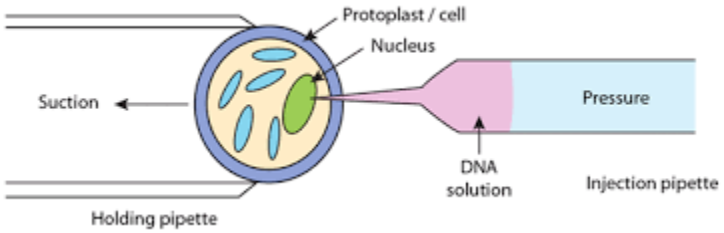
1. micropipette is used to deliver DNA; needle, roughly 0.5-5umin diameter, specially designed to penetrate cell membrane or nuclear membrane
2. involves use of specialized optical microscope setup called micromanipulator.
3. Cells to be microinjected are placed in a container . A holding pipette holds a target cell at the tip when gently sucked. The tip of the micropipette is injected through the membrane of the cell. Contents of the needle are delivered into the cytoplasm and the empty needle is taken out.

advantages

1. This technique is now established as one of the most flexible technique for introducing DNA and even RNA into living cells.
2. Specific cells can be isolated and transformed individually

Limitation :

1. microinjecting naked DNA directly into cytoplasm has been shown to have high chances of degradation
2. microinjection is a laborious procedure. Only one cell can be injected at a time, and many injections are required before getting a successful transgene expression.
3. Time consuming process



Electroporation

1. involves use of short electrical pulses of high field strength
2. this increases the permeability of cell membranes by changing the transmembrane potential
3. There is disruption of the lipid bilayer …allows transfer of molecules across the cell membrane via nano-size pores.
4. Thus, It causes the uptake of DNA into protoplasts by making plasma membrane permeable to macromolecules
5. The protoplasts and foriegn DNA are placed in a buffer solution between 2 electrodes and short pulses of high intensity current is passed through it.
6. Electric field/pulses creates transient pores in the membrane through which DNA diffuses inside the cells and the pores re seal once the current removed
7. There are a number of factors that can influence the efficiency of gene electrotransfer, such as: temperature, parameters of electric pulses, DNA concentration, electroporation buffer used, cell size and the ability of cells to express transfected genes

Advantages

1. This method has been successfully in wide range of plant species
2. Very less amount of DNA needed

Disadvantages

1. There are chances of cell damage
2. There may be transport of non specific molecules into and out of the cell (rather than only DNA moving in)