# Protoplast isolation

**Protoplast:**

- Protoplast, also known as naked plant cell refers to all the components of a plant cell devoid of cell wall thus surrounded only by plasma-lemma made up of pectin.
- The cell wall exerts wall pressure on the protoplast preventing excessive water uptake and bursting of the cell.
- Cell wall can be removed from explant tissue mechanically or enzymatically and then protoplast can be isolated. The isolated protoplast have the ability to fuse with each other irrespective of their origin.
- Protoplast offers possibility of efficient and direct gene transfer to plant cells or to generate fusion hybrids. By using a single protoplast, a whole plant can be regenerated.
- Protoplast yield and viability depends on the type of tissue material from which it is isolated and the methods of isolation used.



- **Protoplast can be isolated from:**
  - Mesophyll cells
  - Young microspores
  - Pollen grain calli
  - Embryonic and non-embryogenic suspension cultures

**Protoplast isolation methods:**

- Before isolation of protoplast, the source material; in case of in vivo source, should be properly surface sterilized.
  Protoplasts are very fragile single cells, which must be maintained in an osmotically and nutritionally balanced medium to prevent lysis.

There are majorly 2 methodologies used in protoplast isolation.

1. **Mechanical method**

- In 1892, Klercker had demonstrated protoplast isolation from *Stratiotes aloides* by placing cells in suitable plasmolyticum. Once the plasmolysis is complete, the damaged plasmolysed cells were cut through knife mechanically to release protoplasts.