

# **Plant Tissue Culture and their techniques**

# Plant Tissue Culture and their techniques



# What is plant tissue culture?



**Plant tissue culture** is a technique of growing plant cells, tissues, organs, seeds or other plant parts in a sterile environment on a nutrient medium





# HISTORY

1902	The idea of the totipotency of plant cell was given by Haberlandt
1937	White first time established successful root culture of tomato
1941	Vanoverbeek used coconut milk for growth and development of young <i>Datura</i> embryos
1957	Stog and Kasper demonstrated the role of auxin and cytokinin on root and shoot formation in tobacco — tissue
1962	Murashige and Skoog introduced the medium for tobacco culture
1987	Isolation of Bt. gene from bacterium <i>Bacillus thuringiensis</i>

# Fundamental principles:



PTC depends upon

1. Totipotency :- It is the ability of plant cells to regenerate into a whole plant
2. Plasticity :- It is the ability of plants to alter their metabolism, growth and development to best suit their environment

# Explant

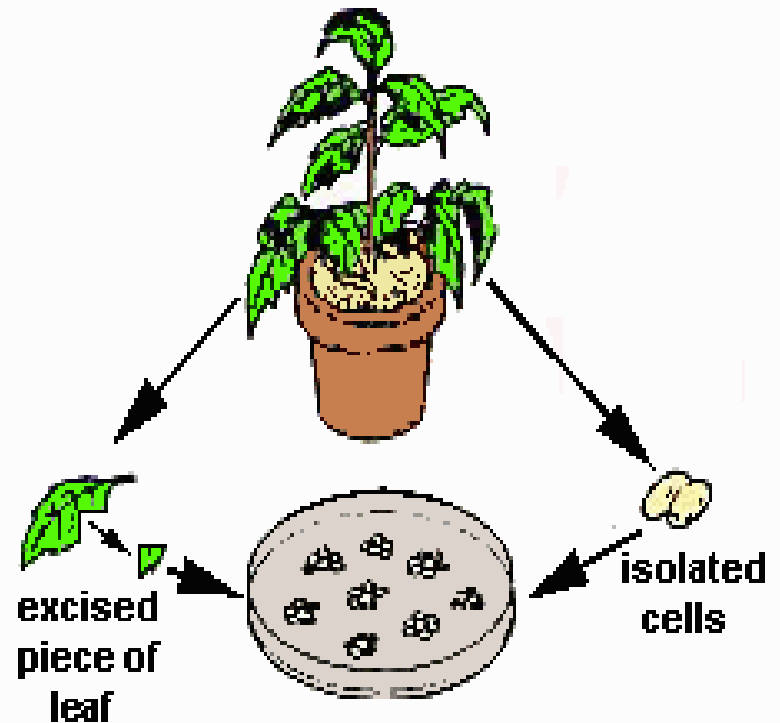


Plant tissue cultures are generally initiated from multicellular tissue fragments, called explants, obtained from living plants. Explants may originate from a wide range of plant tissues, such as...

leaf, stem, root, petiole, hypocotyl, cotyledon, embryo, or meristem

# Selection of explant

- The explant is selected it is either haploid or diploid explant
- The plant growth can be achieved in two ways:
  - 1.shoots directly by appropriate media
  - 2.BY somatic embryogenesis



# Sterilization



- Sterilization Methods Used in Tissue Culture Laboratory - All the materials, e.g., vessels, instruments, medium, plant material, etc., used in culture work must be freed from microbes



# STERILIZATION TECHNIQUES



- sterilization is achieved by one of the following approaches:
- (i) dry heat treatment
- (ii) flame sterilization
- (iii) autoclaving
- (iv) filter sterilization
- (v) wiping with 70% ethanol
- (vi) surface sterilization.

# Culture media



- Explants are then usually placed on the surface of a solid culture medium, but are sometimes placed directly into a liquid medium, when cell suspension cultures are desired.
- **Culture media** are generally composed of inorganic salts plus a few organic nutrients, vitamins and plant hormones.



- As cultures grow, pieces are typically sliced off and transferred to new media (subcultured) to allow for growth or to alter the morphology of the culture.



# MAJOR TYPES OF MEDIA



- **White's medium** - is one of the earliest plant tissue culture media
- **MS medium** - formulated by Murashige and Skoog (MS) is most widely used for many types of culture systems
- **B5 medium** - developed by Gamborg for cell suspension and callus cultures and at present it's modified form used for protoplast culture
- **N6 medium** - formulated by Chu and used for cereal anther culture
- Nitsch's medium developed by Nitsch and Nitsch and used for anther culture

# Steps in plant tissue culture technique

- Selection of plant
- Isolation of explant
- Sterilization of explant
- Inoculation of explant

Incubation

Initiation of callus

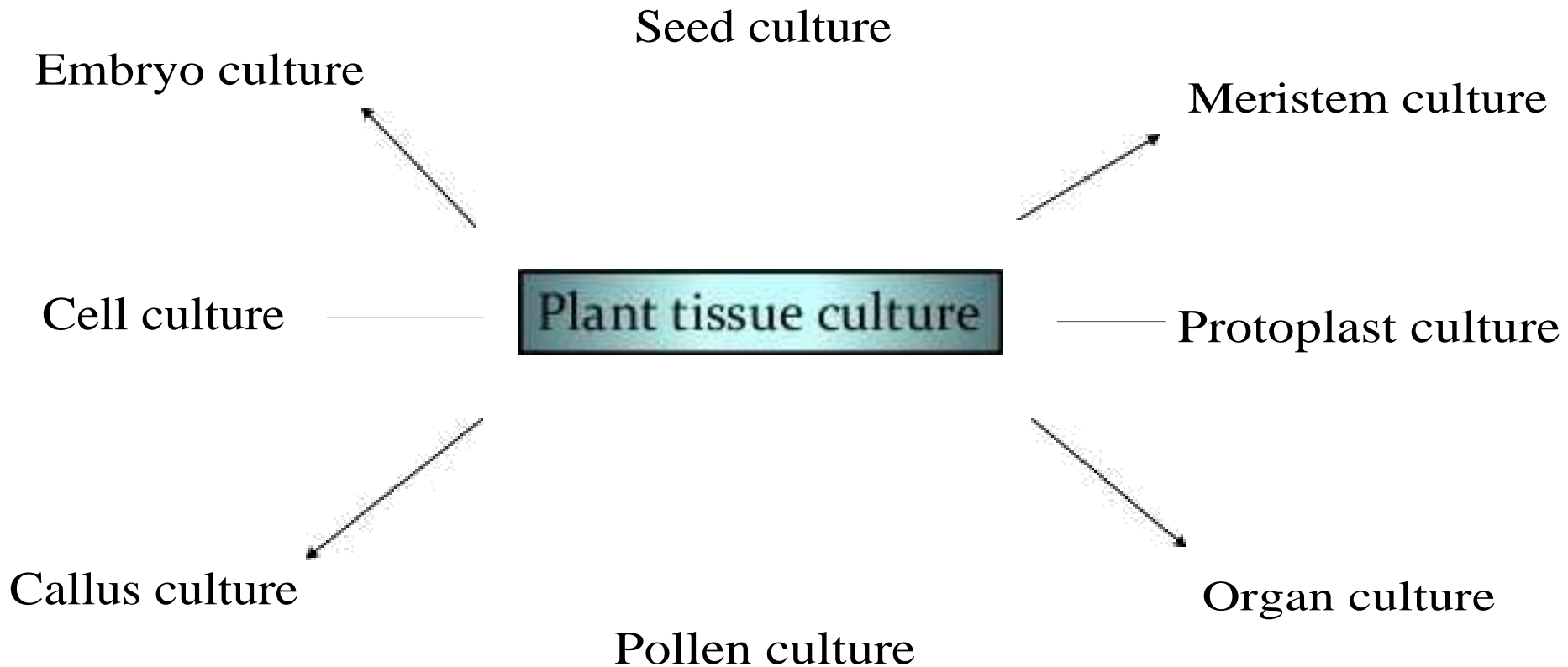
Sub culturing

Regeneration

Hardening

Transfer of plantlets to Green house or open field

# TYPES OF PTC



# Factors Affecting Tissue Culture Efficiency



Plant regeneration from tissue culture varies with the following parameters:

- plant species,
- genotype within the species,
- source of the cultured tissue,
- age and health of the donor plant,
- nutrient medium, other factor



# Plant Tissue Culture Applications



- The commercial production of plants used as potting, landscape, and florist subjects
- To conserve rare or endangered plant species.
- To screen cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance.
- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals.

# Plant Tissue Culture Applications



- To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.
- To produce clean plant material from stock infected by viruses or other pathogens.
- Production of identical sterile hybrid species can be obtained

# Difference in PTC& Animal culture



- It grow on at specific temperature i.e. normal temperature for human is 37°c.
- Carbon dioxide is also require.
- It needed proper change in media otherwise cells will not grow properly.
- Animal cells needed protein and hormones for proper development

# References



- Barcelo, P., Rasco-Gaunt, S., Thorpe, C. and Lazzeri, P. (2001). Transformation and gene expression. In *Advances in botanical research, volume 34: Biotechnology of cereals* (ed. P. R. Shewry, P. A. Lazzeri and K. J. Edwards), pp. 59–126. Academic Press, London.
- Dahleen, L. S. (1999). Donor-plant environment effects on regeneration from barley embryo-derived callus. *Crop Science*, **39**, 682–5.
- Dahleen, L. S. and Bregitzer, P. (2002). An improved media system for high regeneration rates from barley immature embryo-derived callus cultures of commercial cultivars. *Crop Science*, **42**, 934–8.
- Dodds, J. H. and Roberts, L. W. (1995). *Experiments in plant tissue culture*. Cambridge University Press, Cambridge.

