Mohanlal Sukhadia University, Udaipur
Third Year B.Sc. Botany
Theory

PAPER – II1

Molecular Biology and Biotechnology

Unit 3 - History of Plant Tissue Culture

Submitted by

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Plant Tissue Culture

- Plant tissue culture broadly refers to the *in vitro* cultivation of all plant parts (leaf, stem, root, flower, bud etc.) under aseptic conditions on a nutrient culture medium of known composition
- An important tool in both basic and applied studies as well as in commercial application:

- True-to-type (production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits)
- The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds
- Year-round production
- Germplasm conservation
- Production of disease free plants
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- **Ex plant** (The excised piece of differentiated tissue or the organ which is used for culture is called as explant like embryos, young leaf, bud, etc.)
- **Callus** (The undifferentiated mass of cells, cells are meristematic in nature)
- **Suspension Culture** (Defined as the culture of cell and cell aggregates suspended in a liquid medium)

Historical Development in Plant Tissue Culture

- **Gottlieb Haberlandt**
- **Father of plant tissue culture**
- **Concept of Totipotency**

"Theoretically all plant cells are able to give rise to a complete plant"

Experiments on *Lamium purpureum* and *Eichhornia crassipes*, the epidemis of *Ornithooalium* and epidermal hairs of *Pulmonaria Mollissima*

Experimented with isolated photosynthetic leaf cells and other functionally differented cells and was unsuccessful, but nevertheless he predicted that one could successfully cultivate artificial embryos from vegetative cells

German Academy of Science in **1902** He opined that, to my knowledge, no systematically organized attempts to culture isolated vegetative cells from higher plants have been made. Yet the results of such culture experiments should give some interesting insight to the properties and potentialities that the cell, as an elementary organism, possesses. Moreover, it would provide information about the interrelationships and complementary influences to which cells within a multicellular whole organism are exposed

❑ Kotte and Robbins

In 1922 progress was made in culturing plant tissue aseptically

Kotte (German)

- ✓ **Kotte** worked with excised root tips such as pea and maize placing these in a variety of nutrients which contained the salts of Knop's solution, glucose and several nitrogen compounds such as asparagine, alanine and meat extract.
- ✓ Obtained growth of root tips for a periods of up to 2 weeks but he did not subculture.

Robbins (American)

- ✓ **Robbins** on the other hand maintained maize roots in vitro for longer periods by sub-culturing but with time the growth of the cultures decreased and the cultures were lost.
- ✓ Used yeast extract in his cultures but his choice of maize was unfortunate

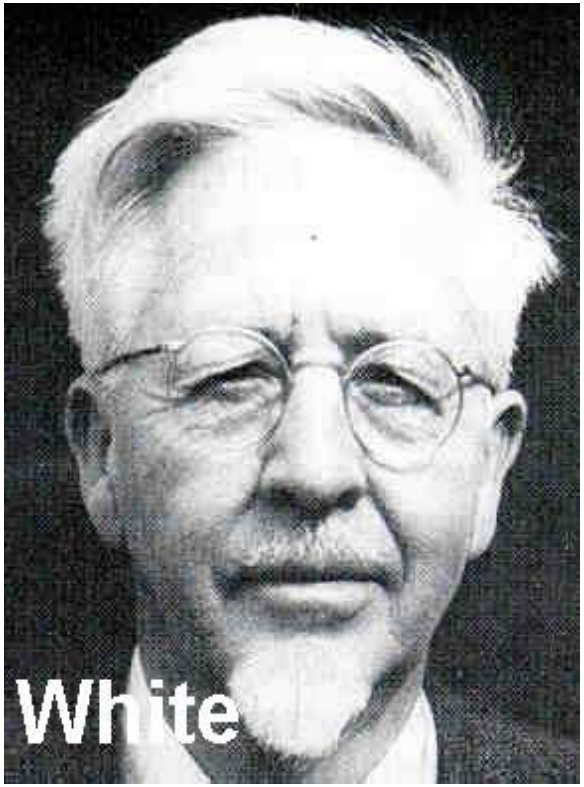
❑ White

The first successful experiment to maintain growth and cell division in plant cell culture was conducted by White (1934) who established cultures of isolated tomato roots under aseptic conditions

White's medium was simple, containing only sucrose, mineral salts and yeast extract, which supplied vitamins

The cultured roots maintained their morphological identity as roots with the same basic anatomy and physiology

This happened only because excised plant organs on nutrient media are capable of synthesizing hormones necessary to maintain cell division.



❑ Gautheret and Nobecourt

The in vitro cultivation of plant tissues for indefinite periods of time was first time achieved in 1939, working with carrot (*Daucus carota*)

This achievement was due to a fortunate choice of plant material as both are considered to be relatively easy to culture now in comparison with many other species but this in no way negates the magnitude of their efforts

Nobecourt (1949) reported un differentiated callus, produced from a small fibrous root of a carrot, had been grown continuously through successive transfer for nine years

Van Overbeck (1941) and co-workers demonstrated for the first time the stimulatory effect of coconut milk, which was similar to embryo sac fluid, on embryo development and callus formation in *Datura*.

This proved a turning point in the field of embryo culture, for it enabled the culture of young embryos which failed to grow on a mixture of mineral salts, vitamins, amino acids and sugar

❑ F.C. Steward

British botanist and plant physiologist

In 1948, Steward set out to study the behaviour of mature cells, isolated from carrot roots, when cultured in sterile nutrient culture media (liquid endosperm, or coconut water), using specially designed flasks and the rotating 'Steward' wheel.
He unequivocally demonstrated that plant cells are totipotent, carrying the genetic information to enable them to develop into complete plants, often by embryogenesis, if given the right chemical stimuli in the correct order – thus vindicating an earlier prophecy that this would be so

His most important scientific contribution came in **1958**, when he established that plants could be totally regenerated from one cell. The finding revolutionised the world of plant cell biology, establishing for the first time that plant cuttings and shoots were no longer required to propagate hybrids and create mutations but that individual plant cells contained all the necessary information to regulate the entire plant organism

❑ J. Reinert

- ✓ Somatic embryogenesis “1958” Steward, Mapes and Mears
- ✓ Culturing undifferentiated parenchyma tissue of carrot root in complex medium containing sugar, salt and coconut milk
- ✓ Histological analysis showed bipolar embryo formation in the system, which produced plants on proper medium. This had proved the development of a complete plant from mature undifferentiated parenchyma and in other words ‘the totipotency’ of cells

❑ Skoog and Miller

- ✓ 1948 Skoog & Tsui – Auxin and adinine promotes callus growth and bud formation
- ✓ 1955 Skoog discovered “Cytokinin” Promote cell division
- ✓ 1957 Skoog and Miller “auxin-cytokinin ratio used in culture media determines the degree of shoot and/or root formation in tissue culture
  - A high ratio of cytokinin to auxin favor's shoot production, whereas a high auxin to cytokinin ratio favor’s root production.
  - Intermediate levels of both hormones enhance callus formation

❑ Georges Morel

- ✓ G. Morel was among the first to culture monocotyledonous tissues
- ✓ in 1950, they obtained the indefinite growth of monocotyledonous tissues such as ***Gladiolus***, ***Iris*** and ***lily*** on the medium enriched with natural extract (coconut milk, yeast extract)
- ✓ He developed the method of meristem culture for the elimination of viruses and the micro-propagation of orchids and discovered the two unique opines of crown gall tissues
- ✓ Morel and Martin (1952) developed meristem culture technique and recovered Dahlia shoots, free from viruses, by meristem tip culture
- ✓ In 1955, they recovered virus free potato

❑ E. C. Cocking

- ✓ In 1960s, for the first time a method of isolation of protoplasts in large quantities was developed using enzymes obtained from ***Myrothecium*** fungus
- ✓ They obtained protoplasts from root tips for ***Lycopersicon esculentum*** using cellulase produced by the fungus
- ✓ Bhojwani and Cocking 1972 were also successful in isolating protoplasts from pollen grains and pollen mother cells. They used protoplasts for understanding tobacco mosaic virus infection and multiplication in plant cells

- In India, studies on tissue culture started in mid 1950 at the Department of Botany, University of Delhi under the directions of Professor P. Maheshwari
- ✓ This was beginning of experimental embryology

❑ S. Guha Mukherjee and S.C. Maheshwari

- ✓ First time of the development of haploids through anther and pollen culture
- Prof. Sipra Guha Mukherjee worked on regeneration of plants and mechanism of regeneration involving various enzymes, membrane phospholipids and second messengers

❑ Indra K. Vasil and Vimla Vasil

- ✓ The landmark work of Vimla Vasil on culture of single isolated cells of tobacco has opened new applications of cell cultures in genetics, morphogenesis and vegetative propagation and proved the totipotency of cell
- ✓ Single cells of hybrid tobacco callus were grown in micro-chambers in the absence of any other cells, in fresh liquid medium containing coconut milk. These produced a colony of 50-75 cells in 10-25 days.
- ✓ Upon transfer from the micro-chambers to agar medium, cell clumps produced callus in 3 months, which ultimately produced complete plantlets (Vasil V. and Hildebrandt, 1965)