Pharmacognosy and Phytochemistry

Introduction-Part 4

B. Pharm. Semester-1

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Learning Outcomes

At the end of this lesson, students will be able to explain

- **Plant Biotechnology**
- **Callus Culture**
- **Plant Growth Regulators**
- **Organ culture and regeneration of plants**
- **Suspension culture**
- **Factors affecting plant tissue and cell culture**

Objective

The objective of this course is to give to the students of pharmacy the basic knowledge about the Medicinal Plants and discussion of Medicinal Plants according to their uses and their effects upon the different organs of the body.

Plant Biotechnology

Plant tissue and cell culture:-

- Cultivation of a plants on an artificial medium was first described in 1939.
- It is an important technique in several areas:-

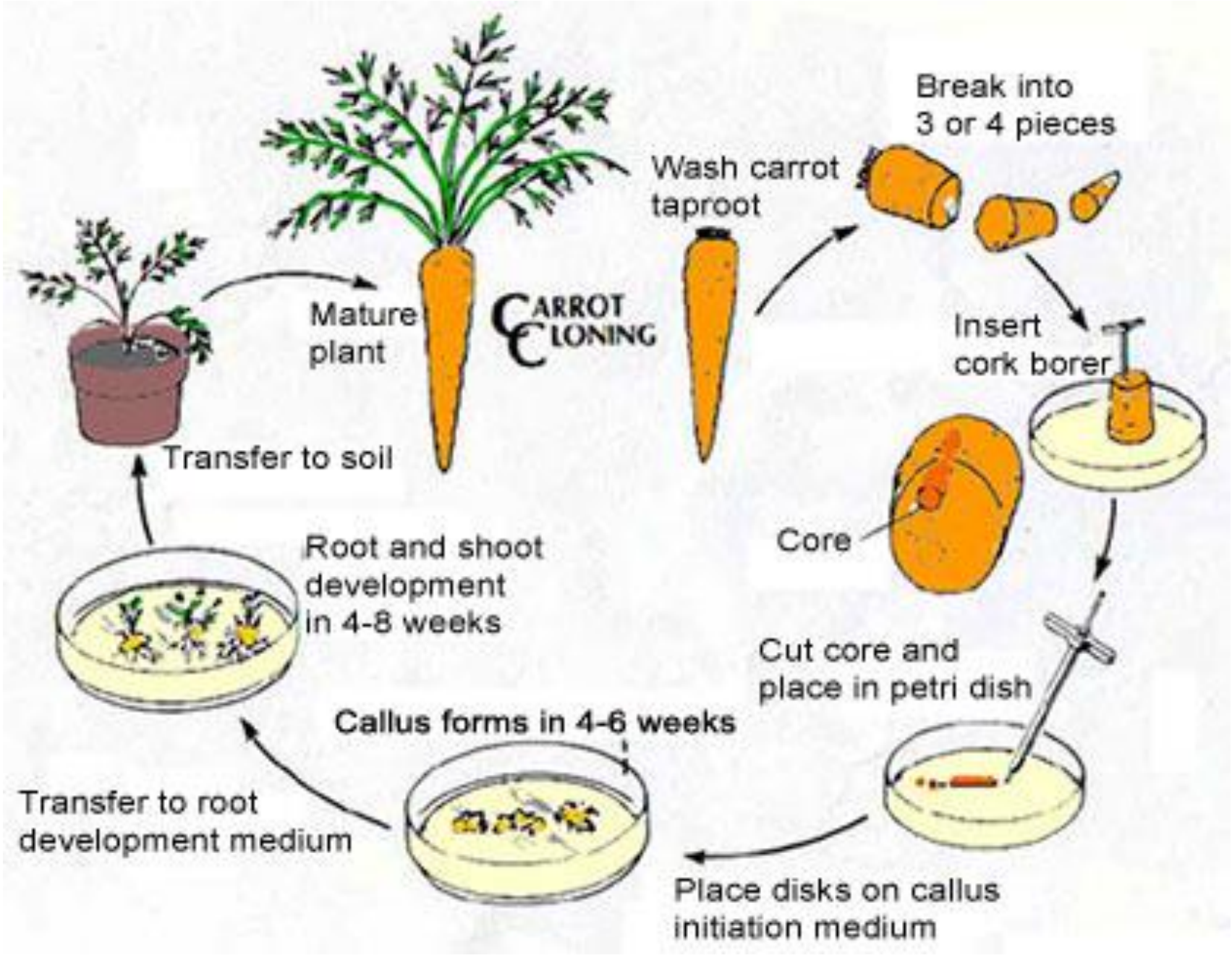
1. Commercial production of ornamental plant.
2. Plant breeding.
3. Studies in the biosynthesis of secondary metabolites.

- The basis for plant tissue and cell culture is that each cell contains all the genetic information relating to the whole plant. It is thus possible to start with a single cell which is allowed to multiply by division and form tissue of loosely attached cells called a callus.

Callus Culture

- **Callus culture** can be started from a **single cell** or small **piece of plant** tissue such as Tips of stems or Roots or Leaves or Cotyledon from very young plants.
- Tissues from the ovary or stamens, and parts of the cambial zone of root or stems. The plant part taken to establish a culture is termed the explant.
- The surface of the explant must be sterilized using sodium hypochlorite.
- It is left for 5 to 30 minutes, then the explant is rinsed with sterile water and transferred to the sterile growth medium: Agar gel in a Petri dish. And Flask.

Callus Culture

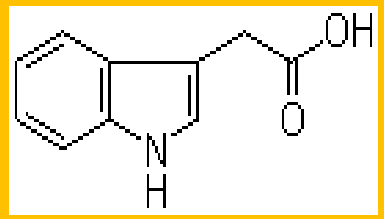
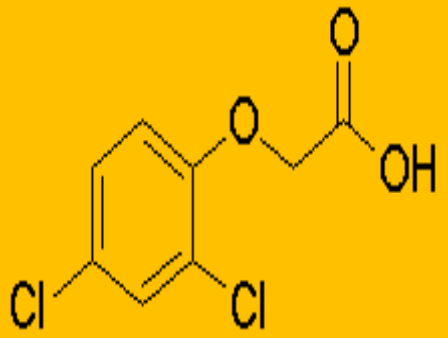
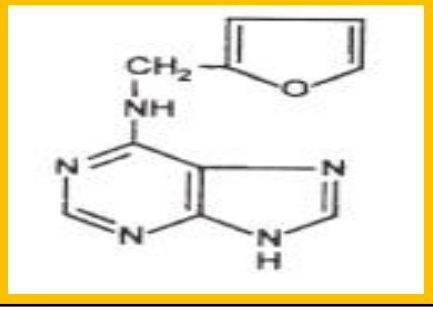


Culturing of plant

Nutrient of tissue culture

- The medium must contain **salts**, **nitrogen**, **organic carbon source**, **vitamins** which cannot be synthesized by the callus tissue.
- Ammonium nitrate (NH_4NO_3), arginine, L-aspartic acid, L-asparagine, glycine, L-glutamic acid and L-glutamine **are the source of nitrogen**.
- Some growth regulators should be used such as auxins and cytokinins.
- Auxins stimulate lengthwise growth of stems and have the opposite effect on the root (**swelling**).
- **Cytokinins promote cell division**.
- Callus are usually stored in the dark.

Plant Growth Regulators

Indole-3-acetic acid [IAA]= Natural auxin (plant growth promotor)	
2,4-dichlorophenoxy acetic acid [2,4-D]= Artificial auxin (plant growth promotor)	
Kinetin = Natural cytokinin (cell division hormone)	

❑ Natural auxins:

- Indole-3-acetic acid (IAA).
- α-naphthaleneacetic acid (NAA).

❑ Artificial auxins:

- 2, 4-dichlorophenoxyacetic acid (2,4-D).

❖ Natural cytokinins:

- Kinetin.
- 2iP.
- Zeatin.

Organ culture and regeneration of plants

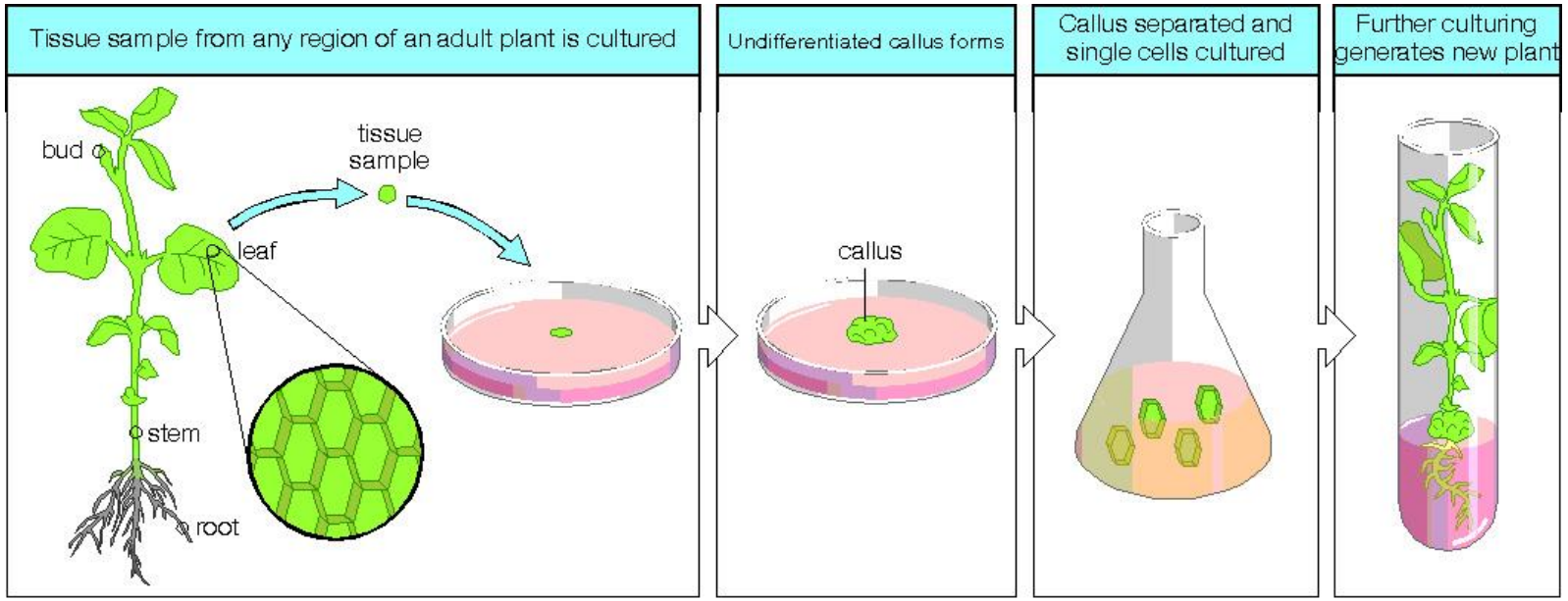
- Plant organs are developed depending on the balance between the concentrations of auxin and cytokinin.
- The salt concentration in the medium and the light conditions are also important.
- Shoots فسائل are regenerated in presence of light and a medium of high osmolarity containing chelating agents e.g. EDTA. {compounds that are capable of binding metals ; cations particularly, Ca^{2+} . ROLE: binding to calcium preventing clumping of cells grown in liquid suspension, or detaching adherent cells}. The auxin should be lower than the cytokinin.

Suspension culture

➢In callus cultures, where cells are in contact with one another, they form an unorganized tissue. In suspension culture the cells are free in the medium or form small aggregate.

➢ The same medium can be used as for a callus culture, the only difference being that the agar is omitted.

➢Suspension cultures grow much faster than callus cultures, because of the ease with which nutrients access the growing cells.



Factors affecting plant tissue and cell culture

➢**Temperature**: the optimum temperature for in vitro growth of plant cells is in the range **25 -30 °C**.

➢**Aeration:** plant cells in a culture are **aerobic** and must therefore have access to oxygen in order to prevent infection by microorganism.

➢ Also, exchange of gases with the outside environment must be permitted.

➢ For flask culture, the flask is usually closed with a plug of cotton or wool, which permits exchange of gases but prevents microorganisms from entering the flask. The flask is placed on a shaker, which keeps the uptake of oxygen.

Factors affecting plant tissue and cell culture

➢**Light:** Plant cells in tissue culture normally do not carry out photosynthesis, it is therefore necessary to control the light conditions with respect to **intensity**.

➢The **irradiation** needed to allow plant tissue or cell culture to develop is **much lower** than that need by normal living plants.

➢The best light source for tissue culture is the **fluorescent lamp**.

➢The influence of light on plant culture has been studied at levels of cellular tissues and organs.

Factors affecting plant tissue and cell culture

- Thus, callus growth and shoot initiation can be either enhanced or inhibited depending on the **wave length** and **irradiance (amount of energy/unit area)**.
- High near UV {**visible to birds, insects and fish**} and blue light have been found to **inhibit** the growth of callus culture.
- **Red light** can cause both enhancement or inhibition of callus growth depending on the species.
- **Example:** *Digitalis lanata* when exposed to light for 2-3 periods of 15 minutes, showed that the cardiac glycosides content of the exposed culture was about **10 times higher** with each period.

REFERENCES

Textbooks:

1. **Trease And Evans Pharmacognosy, 16th Edition, 2019, Author: William C Evans, Publisher: Elsevier, ISBN: 978-8131261187.**
2. **Textbook of Pharmacognosy and Phytochemistry 2nd Edition, 2019, Authors: B. Shah, A. N. Kalia, Publisher: Elsevier, ISBN: 978-978-9386217738.**
3. **Medicinal Natural Products: A Biosynthetic Approach, 2nd Edition, 2002, Author: Paul M Dewick, Publisher: John Wiley and Sons Ltd, ISBN: 0471496405.**

Supplementary book:

Fundamentals of Pharmacognosy and Phytotherapy. A Guide for Health Care Professionals by Carol A. Newal, Linda A. Anderson and J. David Phillipson. (2010). the Pharmaceutical Press, London, UK; ISBN: 0 85369-474-5.