



# SECONDARY METABOLITES

# INTRODUCTION

- \* Secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of an organism.
- \* Plant secondary metabolism produces products that aid in the growth and development of plants but are not required for the plant to survive.
- \* A common role of secondary metabolites in plants is defense mechanism i.e. used to fight from herbivores, pests and pathogens.
- \* In humans plant secondary metabolites are of great importance as they are used in many fields such as medicine, flavouring agent, dyes etc.



- \* Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group.
- \* It is believed that production of secondary metabolites is linked to the induction of morphological differentiation.
- \* Cultures are initiated by simply placing freshly cut sections from surface sterilized plant organs on a nutrient medium containing suitable hormones.
- \* Generally auxins and cytokinins are used for this purpose.
- \* On such a medium explant exhibit callusing which usually starts at the cut ends and gradually extends over the entire surface of tissue.
- \* There are 4 types of cultures involved:-
  - (i) Organized culture.
  - (ii) Disorganized culture.
  - (iii) Hairy root culture.
  - (iv) Immobilized cell culture.



# ORGANIZED TISSUE

- \* This refer to taking a specific part of a plant body and making them grow in appropriate medium to obtain healthy hybrid.
- \* Roots & shoot are mainly use for this technique.
- \* Cells that work together to perform a particular function are organized into Tissues.
- \* *Xylem Tissue, Ciliated Epithelial Tissue and Parenchyma Tissue.*



# DISORGANIZED TISSUE

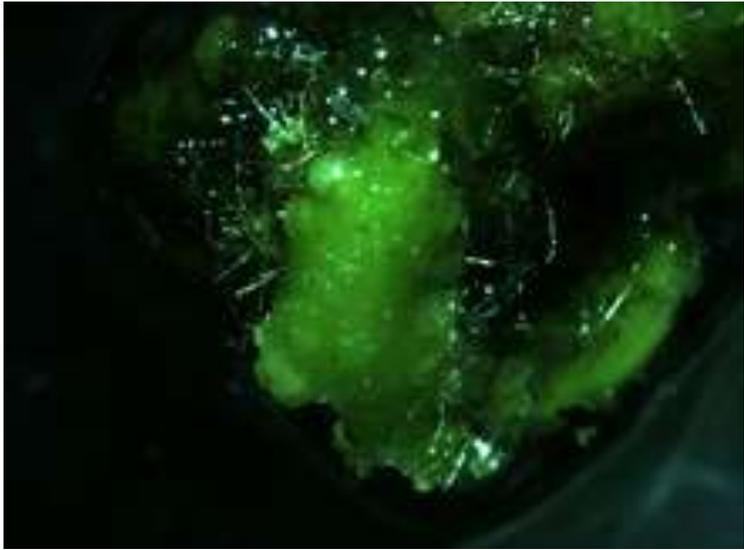
- \* The extra cellular growth in plants that leads to tough structure forms diorganized tissues.
- \* Generally callus formation leads to such disorganized tissues.
- \* **Plant callus** (plural *calluses* or *calli*) is a mass of unorganized parenchyma cells derived from plant tissue (explants) for use in biological research and biotechnology. In plant biology, callus cells are those cells that cover a plant wound.



# CALLUS CULTURE

- \* Callus formation is induced from plant tissues after surface sterilization and plating onto *in vitro* tissue culture medium.
- \* Plant growth regulators, such as auxins, cytokinins, and gibberellins, are supplemented into the medium to initiate callus formation or somatic embryogenesis.
- \* A callus cell culture is usually sustained on gel medium.
- \* Callus induction medium consists of agar and a mixture of macronutrients and micronutrients for the given cell type.





- \* Root Hair Cells are found in the roots of plants.
- \* Their role is to absorb water and minerals in the soil.
- \* They have a large surface area, due to their hair-like projections, which eases uptake.
- \* They also have a large amount of Mitochondria, which provide more energy for Active Transport.



# TECHNIQUES

## 1. Batch culture

- \* These are use for initiating single cell culture.
- \* Cell suspension are grown in 100-250 ml of flask, each containing 20-75ml of culture medium.
- \* The cultures are continuously propagated by routinely taking a small aliquot of suspension & transferring it to fresh medium.
- \* During the incubation period the biomass of suspension culture increases due to cell division and cell enlargement(continues up to certain limit).



## 2. Continuous culture

- \* A number of culture vessels have been design to grow large scale culture under steady state for long periods by adding fresh medium and draining out the used medium
- \* Continuous cultures may be closed types or opened type.
- \* In the closed type, the addition of fresh medium is balanced by outflow old medium and cells are collected mechanically and added back to the culture.
- \* In contrast, in open continues culture the in flow of medium is accompanied by a balancing harvest by equal volume of culture.



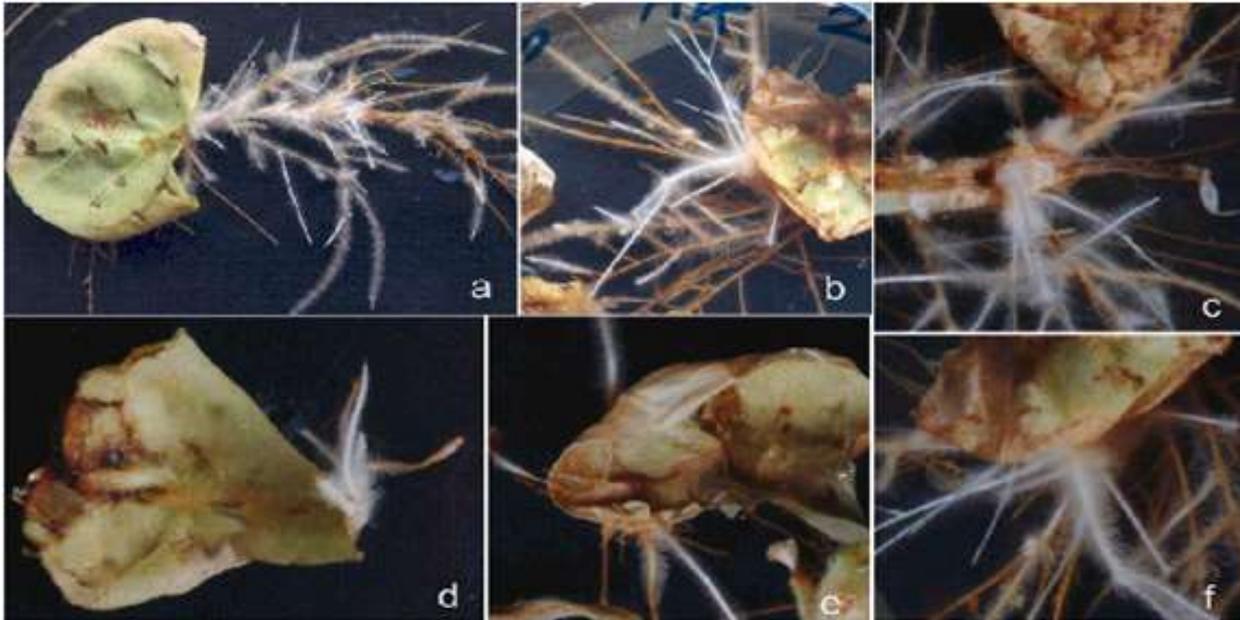
# HAIRY ROOT CULTURES

- \* Also called transformed root culture.
- \* It is a type of plant tissue culture that is used to study plant metabolic processes or to produce secondary metabolites or recombinant proteins, often with plant genetic engineering.
- \* It is the culture produced after the infection of explants or cultures by the gram negative soil bacterium Agrobacterium rhizogenes (contain Ri plasmids) can infect plant roots and cause them to produce a food source for the bacterium, opines and to grow abnormally.
- \* This process take the advantage of the naturally occurring hairy root disease in dicotlydons.



# PROPERTIES

- Genotype and phenotype stabilities.
- Autotrophy in plant hormones.
- Fast growth.
- High levels of secondary metabolite production.



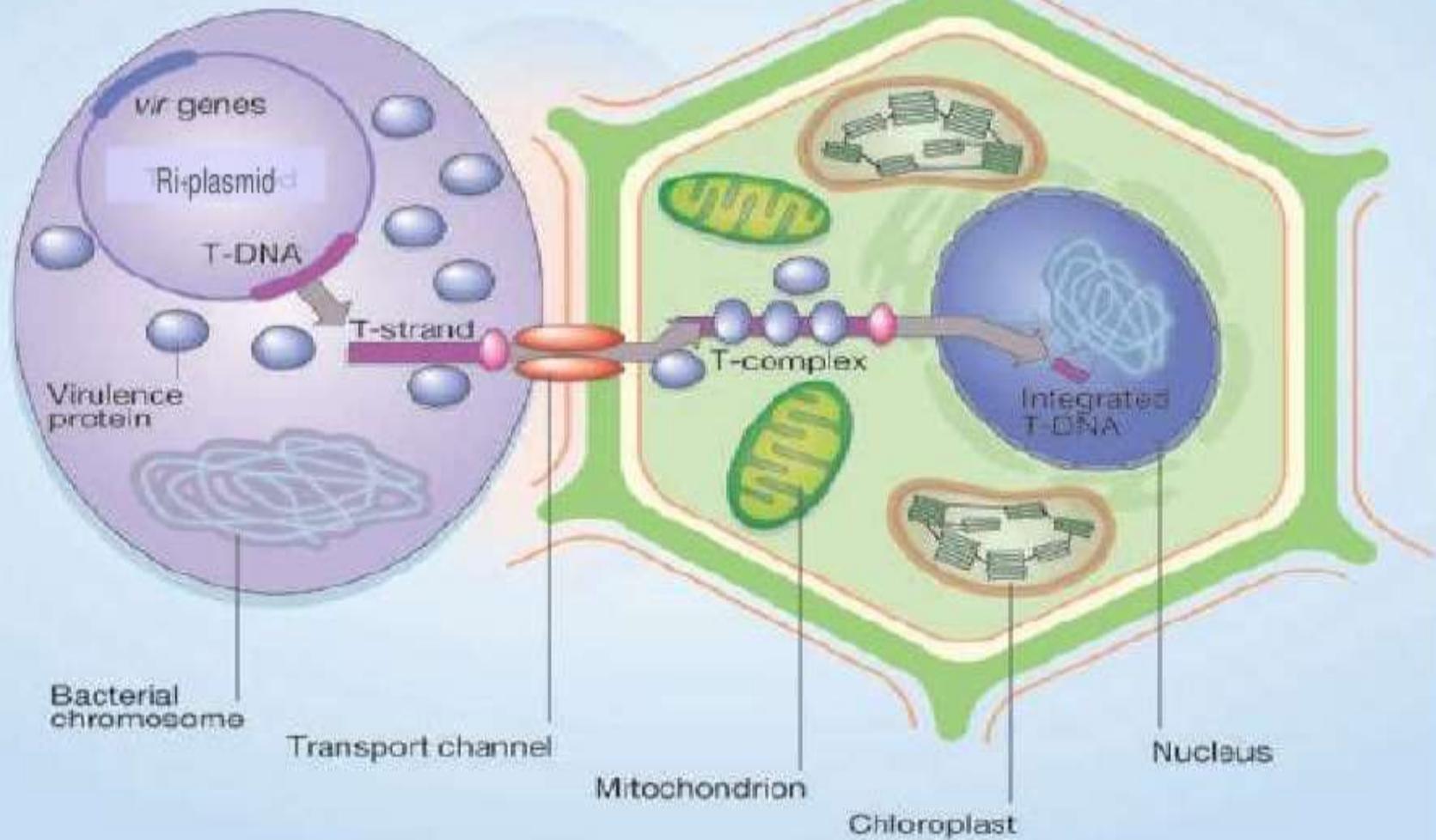
# PRODUCTION (IN-VIVO)

- \* Agrobacterium recognizes some signal molecules exuded by wounded plant cells and become attached to it.
- \* The bacteria contain the root inducing plasmid(Ri-plasmid).
- \* The bacteria genetically transfer the part of the Ri-plasmid called the transfer DNA(t-DNA) to the genome.
- \* Proliferate by increasing the rate of cell division(cytokinin expression) and cell elongation(auxin expression) to produce the hairy roots.
- \* Production of the opines which is the type unusual amino acids(octopine ,mannopine etc) which is used by the bacterium as a carbon, nitrogen and energy source.



*Agrobacterium* cell

Plant cell



# PRODUCTION(IN-VITRO)

- \* Explants are wounded and then inoculated with *Agrobacterium rhizogenes*.
- \* After 2 or 3 days, the explants can be transferred into solid media with antibiotics such as ceftioaxime ,vancomycin etc to kill or eliminate redundant bacteria.
- \* The hairy roots will be induced within a short period of time which varies from 1 week to over a month varying on different plant species.
- \* The decontaminated hairy root cultures can be subcultured on phytohormone free medium.



# ADVANTAGES

- Genetically and biosynthetically stable.
- High production of secondary metabolites.
- Can be grown under phytohormone free conditions.
- Fast growth which reduces the culture time.
- Easy to handle.



# APPLICATIONS

- \* Functional analysis of genes.
- \* Expressing foreign proteins.
- \* Production of secondary metabolites.
- \* May change the composition of metabolites.
- \* Can be used to regenerate a whole plant.
- \* May produce compounds which is not found in untransformed roots.
  
- \* Eg.(i) Quinine for malaria.  
(ii) Shikonin used for anti bacterial and anti ulcer agent.  
(iii) Berberine etc.



# IMMOBILIZED CELL CULTURE

## \* Need:-

(i) High cost due to slow growth

(ii) Low yield

(iii) Instability of selected lines

(iv) Low resistance of cells

(v) Intracellular accumulation of product

\* Cells are confined within reactor system preventing their entry into mobile phase which carries the substrate and product.

\* Firstly reported by Brodelius et. al 1979 in C. roseus and Daucus carota.

\* Immobilization involves 2 stages-

(i) Optimized for biomass production by suspension culture.

(ii) Optimized for product formation by immobilized cells.



# ADVANTAGES

- \* Prolonged use of biomass.
- \* Cell density in bioreactor increases 2-4 times.
- \* Simple bioreactor can be used.
- \* Separate cells from medium ,thus simplifying downstream processing.
- \* Uncouples growth & product formation without affecting growth.
- \* Provide stable production rate of 2<sup>nd</sup>ary metabolites.
- \* Minimizes fluid viscosity.



# METHODS OF CELL IMMOBILIZATION

- \* Cells are entrapped in gel or behind semi permeable membrane.
  - \* Some polymers used to entrap cells are alginate, agar, agarose & carrageenan.
  - \* Alginate has been widely used as it can polymerize at RT using  $\text{Ca}^{2+}$ .
  - \* Also introduction of semi permeable membrane in between cells & recirculating media , entraps cells at high density.
  - \* Cell immobilization on surface of inert support(eg. Fiberglass mat etc) has been used for 2<sup>nd</sup>ary metabolite production.
  - \* Cells adhere to immobilizing support matrix in 2 steps.
  - \* Surface immobilization promotes natural tendency of cells to aggregate, improving synthesis & accumulation of 2<sup>nd</sup>ary metabolites.
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- \* Advantage of this method is absence of physical restriction to mass transfer between culture medium & biomass surface.
- \* It is easy to monitor conditions, distribution & extent of biomass.
- \* Therefore, immobilized system should maintain viable cells over extended period and release the bulk of product into extracellular medium in a stable form.



# LIMITATIONS

- \* It may lead to decoupling of cell growth.
- \* Initial biomass must be produced in suspension culture.
- \* Secretion of product into the external medium is imperative.
- \* Extracellular degradation of products.
- \* Introduction of additional diffusion barrier by gel matrix.



# SUMMARY

- \* Secondary metabolism produces product that's aid in the growth and development of plants. But are not required for plant to survive.
- \* Secondary metabolism faulted the primary metabolite in plant.
- \* A common role of secondary metabolite in plants is defense mechanism.
- \* They also use to fight off herbivores pest and pathogens.



# BIBLIOGRAPHY

- \* Studies in plant science,5 by S.S. Bhojwani & M.K. Razdan
- \* <https://books.google.co.in/books>.
- \* <http://alevelnotes.com/cell-specialisation-and-organism-organisation/150>.
- \* Slideshare.net

